# DEVELOPMENT OF NOVEL

# THERAPIES FOR GUT DYSBIOSES

**A Thesis Submitted For the Degree**

**Of Doctor of Science**

**By**

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# DECLARATION

I certify that the work in this thesis has not previously been submitted as part of requirements for a higher degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me.

Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student: _____

Date: 11 July 2016

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The work that led to this Thesis was carried out over 30 years at the Centre for Digestive Diseases starting with the establishment of the clinic on 16th October, 1984 and was completed in early 2015. The work was carried out during routine clinical sessions, after hours, often on weekends, on international travel where writing was so much easier, but in particular during stimulating sessions with numerous staff members, Researchers, Research Managers of the clinic and in particular, Professor Robert Clancy – Professor of Pathology, Faculty of Health and Biomedical Sciences, University of Newcastle. I am indeed grateful to Professor Clancy for his invaluable support, guidance, ideas and help with the construction of the writing. I'd also like to thank Professor Ian Charles, the head of **i3** Institute for his help and support in progressing the work through discussion session.

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## ABSTRACT

This thesis reports results of 30 years of systematic clinical investigations carried out by the author in the general area of "*infection-driven inflammation*". The Centre for Digestive Diseases (CDD) endoscopy clinic was founded by the author in 1984 closely coinciding with Drs Barry Marshall and Robin Warren reporting gastric spiral bacteria with pathogenic role in ulcer disease. CDD was able to source gastric biopsies with ***Helicobacter pylori***(HP) from numerous patients with ulcer disease creating the environment necessary for HP research. Hence, *Section 1* of the Thesis describes systematic studies which asked questions such as 'which is the best therapy', 'what is the role of acid suppression' and the 'role of cigarettes'? This culminated in the development of '*triple therapy*', the first clinically effective HP cure commercialised later as *Helidac*.

Success in HP research soon led to the treatment of the candidate infective cause of Crohn's disease called ***Mycobacterium avium subspecies paratuberculosis*** (MAP). This comprised of three antibiotics which were active intracellularly - another 'triple therapy' akin to HP treatment. However, curing HP could be achieved in 7 days and ulcer healing in 6 weeks whereas MAP – one of the slowest growing bacterial pathogens – required treatment for many months to heal the bowel. So in *Section 2* of the Thesis the effect of Anti-MAP therapy on healing Crohn's disease was studied. Funding from the Broad Foundation supported culture and PCR of MAP while an Israeli company, RedHill Biopharma has funded current pivotal trials with a view to an FDA approval.

*Section 3* of the Thesis describes research in Faecal Microbiota Transplantation (FMT). FMT was first used at CDD in 1988 in a patient with indeterminate colitis - the first patient world-wide treated for colitis and cured. Generally ***Clostridium difficile*** infection (CDI) is treated with FMT at CDD with a cure rate exceeding 95%. Subsequently over 6,000 FMT treatments were carried out and new indications for FMT were discovered, both within and outside of the GI tract. These included ulcerative colitis, irritable bowel syndrome, constipation, MS, arthritis, acne, and others, reported in enclosed publications. CDD work in FMT contributed to the global growth of microbiome research and culminated in the creation of the lyophilised encapsulated FMT.

Each of the three Sections has addressed "*infection-driven inflammation*" in different body regions. It is hoped that reported findings in this Thesis will lead to new therapies that will result in significant clinical improvements.

# INTRODUCTION AND STRATEGY UNDERPINNING RESEARCH PRESENTED IN THIS THESIS

The clinical studies comprising the body of work presented in this thesis reflect a continuous research program directed by myself within the Centre for Digestive Diseases (CDD) over a 30-year period in developing solutions to manage infective dysbioses. Though the Centre provides a *private clinical service* outside of a public hospital or university structure, it also houses a *dedicated research department* aimed at improving outcomes in patients with gastrointestinal disease. The work conducted has focused on the development of novel therapies for major gastrointestinal diseases including peptic ulcer disease, Crohn's disease (CD) and *Clostridium difficile* (*C. difficile*) infection. These areas of study were determined by the oftentimes near-desperate requirement for new, effective treatments. This has facilitated a unique pattern of information disclosure through the publication of abstracts and 'special publications' from a series of international meetings and presentations.

The history of clinical innovation at the CDD, particularly as applied to novel therapies, has frequently been borne out of clinical observation initially of single case, or small population studies. These studies initially focused on proof-of-concept 'idea-stage' therapies. Such therapies included approved out-of-patent antibiotics, as in the case of anti-Helicobacter 'triple therapy', or homogenised faecal preparations, which were originally of little interest to the pharmaceutical industry. Based on these studies, patents were filed which primarily related to various antibiotic combinations, bacterial compositions of faecal flora, and methods of preparation and use of microbiota extracts. These patents are listed at the end of each Section. Larger, more costly and powerful studies ensued, and initial concepts were translated into lower risk proposals considered more appealing to the 'pharma' industry (provided a patent therapy was involved).

Originating with *Helicobacter pylori (H.pylori)* the research examined the concept of an underlying mucosal infection, a concept which created an important paradigm shift in medicine. The importance of such contributions to medicine at critical time points is reflected in two seminal papers published by the Centre for Digestive Diseases, achieving recognition as two of the 10 most quoted papers in the Medical Journal of Australia during the journal's 90$^{th}$ anniversary of publication.[1-3]

**Table 1**. The Medical Journal of Australia top 10 articles, by citation analysis

| | |
|---|---|
| *Number 1 (888 citations)* | Cade JFJ. Lithium salts in the treatment of psychotic excitement. *Med J Aust* 1949; 2: 349-352.[4] |
| *Number 2 (766 citations)* | Marshall BJ, Armstrong JA, McGechie DB *et al.* Attempt to fulfil Koch's postulates for pyloric campylobacter. *Med J Aust* 1985; 142: 436-439.[5] |
| *Number 3 (523 citations)* | Marshall BJ, McGechie DB, Rogers PA *et al.* Pyloric campylobacter infection and gastroduodenal disease. *Med J Aust* 1985; 142: 439-444.[6] |
| *Number 4 (299 citations)* | Derrick EH. "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Med J Aust* 1937; 2: 281-299.[7] |
| *Number 5 (267 citations)* | Swan C, Tostevin AL, Moore B *et al.* Congenital defects in infants following infectious diseases during pregnancy. *Med J Aust* 1943; 2: 201-210.[8] |
| *Number 6 (203 citations)* | George LL, Borody TJ, Andrews P *et al.* Cure of duodenal ulcer after eradication of *Helicobacter pylori. Med J Aust* 1990; 153: 145-149.[3] |
| *Number 7 (170 citations)* | Trautner EM, Morris R, Noack CH *et al.* The excretion and retention of ingested lithium and its effect on the ionic balance of man. *Med J Aust* 1955; 2: 280-291.[9] |
| *Number 8 (169 citations)* | Bower C, Stanley FJ. Dietary folate as a risk factor for neural-tube defects: evidence from a case–control study in Western Australia. *Med J Aust* 1989; 150: 613-619.[10] |
| *Number 9 (167 citations)* | Wilson RMcL, Runciman WB, Gibberd RW *et al.* The Quality in Australian Health Care Study. *Med J Aust* 1995; 163: 458-471.[11] |
| *Number 10 (166 citations)* | Borody TJ, Cole P, Noonan S *et al.* Recurrence of duodenal ulcer and *Campylobacter pylori* infection after eradication. *Med J Aust* 1989; 151: 431-435.[2] |

The timing of such publications is often essential to not only the evolution of ideas, but also the establishment of new therapeutic platforms. For example a letter reporting 55 case presentations to the Medical Journal of Australia[12] formed the foundation for Faecal Microbiota Transplantation (FMT) therapy which later featured so prominently in the treatment of *C. difficile* colitis.

**Table 2.** Three areas of dysbiosis and gut diseases which comprise the broad sections included in this thesis

| Infecting Organism | Clinical Condition | Novel Therapy |
|---|---|---|
| 1. *Helicobacter pylori* | Peptic Ulcer Disease | First effective eradication therapy = Triple Therapy |
| 2.*Mycobacterium avium subspecies paratuberculosis* (MAP) | Crohn's Disease | Effective combination anti-bacterial therapy = Anti-MAP Treatment |
| 3. Defined – *Clostridium difficile* and Undefined dysbiosis | (i) *Clostridium difficile* infection (CDI)<br><br>(ii) Irritable bowel syndrome (IBS)<br><br>(iii) Ulcerative colitis | Faecal Microbiota Transplantation : Cures CDI<br><br>Controls - IBS<br><br>? Cures Ulcerative Colitis (UC) |

Molecular techniques have opened the world to the extraordinary complexity of the gut microbiome, their role in health and disease, and the equally complex host response that communicates with the microbiome. The gut microbiome is the aggregate of all gut microbial species with over 100 trillion ($10^{14}$) organisms including over 500 identified species in 70 divisions. From a genetic standpoint, around 100-fold more genes are present in the human gut than the human body, emphasising the relevance of the gut flora to our genetic landscape, and our biology. Bacterial numbers in the gut represent a continuum that increases in both concentration and complexity as the intestinal contents pass along the gastrointestinal tract. For example, few bacteria are present in the upper gastrointestinal tract, approximately $10^2 – 10^3$/ml acid-resistant bacteria, a requirement for survival. In the mid-gut, these numbers increase to approximately $10^{10}$/ml. While in the colon, in addition to 'acid-resistant' bacteria from above, other species include lactobacilli, streptococci, enterobacteriaceae as well as anaerobic species such as *Bacteroides, Veillonella, Fusobacterium* and *Clostridium* species are present taking the numbers towards $10^{13}$/ml.[13]

Hence the central theme of the entire work is focused on improving patient care by eradicating or suppressing infecting organisms in the three areas covered in the Thesis - **a.** *Helicobacter pylori* which is largely confined to the gastric mucosa; **b.** *Mycobacterium avium ss paratuberculosis* which is more broadly located including the intestinal epithelial cells but also circulation macrophages; and **c.** *Clostridium difficile* and other unknown pathogens in the gut microbiome treatable by FMT. Hence, the common theme of this Thesis is the management of a **dysbioses** in these three locations within the body.

# - SECTION 1 -

## *HELICOBACTER PYLORI*

### A. *HELICOBACTER PYLORI* ERADICATION AND HEALING OF PEPTIC ULCER DISEASE WITH ANTIBIOTICS - SIGNIFICANT CONTRIBUTIONS IN THE *H. PYLORI* FIELD

**(a) OVERVIEW AND CONTENT – Major Question: "What therapy will reliably eradicate *H. pylori* in most, if not all, patients?" – The Development of 'Triple Therapy'**

In 1984, our studies of *Helicobacter pylori (H. pylori)* infection and peptic ulcer disease began, soon after Robin Warren and Barry Marshall published in the Lancet in 1983[6] the finding of a spiral bacterium in the mucosal specimens of patients with gastritis. Later, Warren reported the presence of bacteria in 77% of gastric ulcers and 100% of duodenal ulcers, in contrast to 50% of gastric mucosa in normal patients.[14] These disease associations led them to state, "the bacteria were present in almost all patients with active chronic gastritis, duodenal ulcer and gastric ulcer, and thus may be an important factor in the aetiology of these diseases" though no causal relationship had been established.[14]

In reality, and then unknown to these researchers, the presence of a spiral bacterium in the stomach had been accurately described nearly 100 years earlier by Walery Jaworski[15] a Polish physician. However, as the publication was in Polish and not publicized in the west, the significance of this original finding remained unknown for many years. Interestingly, Jaworski already postulated that this spiral bacterium, which he called *vibrio rugula,* caused ulcer disease and stomach cancer. Later, Warren and Marshall would be awarded the Nobel Prize for Physiology and Medicine in 2005 for their culture of *H. pylori* and its link to gastritis and peptic ulcer.[16] Thagard (2000),[17] reviewing the discovery and process of acceptance of the *H. pylori* bacteria as a causal factor in peptic ulcer disease, identified three phases in the discovery period, culminating in a 1994 US National Institutes of Health Consensus Development Panel to conclude that "*Helicobacter pylori* infection is strongly associated with the predominant forms of peptic ulcer disease and appears to play an important contributory role in their pathogenesis" and recommend that antibiotics be used in their treatment.

In the following decade, the proposed causation hypothesis was hotly debated and controversial. Marshall's submitted abstract to the meeting of the Australian Gastroenterology Society announcing the discovery of *H. pylori,* was rejected.[16] Early studies by Marshall showed symptom improvement with one or a combination of two antibiotics in some but not all patients, and an attempt to satisfy Koch's postulates for causation between *H. pylori* and gastritis, fell short of proving *H. pylori* was the underlying cause of peptic ulcers.[5] The intense interest in this topic was reflected by the publication of over 3,000 articles on *H. pylori* within 5 years of Warren and Marshall's Lancet paper. Thagard summarised the circumstances succinctly: "the question of whether ulcers can be cured by antibiotics converted immediately into a search for the most effective antibiotics."[17] Much later, in 1990, general consensus acknowledged that *H. pylori* infection was the most common cause of active chronic gastritis, and that *H. pylori* was present in the vast majority of patients with duodenal ulcers.[3] However it was *recognised that the strongest argument for causation would be to eradicate H. pylori in virtually all patients treated using an antibiotic regimen, to document healing of ulceration*, and an absence of duodenal ulcer recurrence. Such a therapy was the most pressing agenda item in peptic ulcer disease research. The aim at the time was *to differentiate the healing of duodenal ulcers following eradication of H. pylori, from acid suppression using H2-receptor blockade (such as Tagamet or Zantac), which had a duodenal ulcer recurrence rate of 70 to 90% within 12 months of effective healing.* By the late 1980's, no efficient and reliable method to eradicate *H. pylori* had been developed outside of our CDD clinic, where by late 1984 the bismuth 'Triple Therapy' was already in use.[18] Marshall *et al.* (1986) had proven that some antibiotics could reverse gastritis as early as 1986 using bismuth alone, or in combination with tinidazole or amoxicillin.[19] These 'dual therapy' observations by Marshall were followed with numerous attempts from other groups to eradicate *H. pylori* using both single- and dual combination therapies, all with low eradication rates, high recurrences, and uncertain conclusions regarding its association with duodenal ulcer causation. This was exemplified by a study Marshall *et al.* (1988) conducted of 100 subjects with both duodenal ulcer and *H.* pylori using bismuth alone in patients with and without persistent *H. pylori* infections.[20] Seven-year follow-up in this population revealed an active duodenal ulcer in 20% of *H. pylori*-positive patients versus 3% of *H. pylori*-negative patients.[21] Similar results were reported by others using sub-efficacious antibiotic strategies.[22,23]

Historically, this was the uncertain therapeutic landscape at the time we published two key papers in 1989 and 1990 stating that the absence of an effective strategy capable of durable eradication in >90% of patients made assessment of 'cure' doubtful.[2,3]

(i) Recurrence of Duodenal Ulcer and *Campylobacter pylori* Infection after Eradication. Borody TJ, Cole P, Noonan S, *et al. Med J Aust* 1989; 151: 431.[2] It was in this preliminary report of a 'Triple Antibiotic Therapy' comprising bismuth, tetracycline and metronidazole, that coined the term 'Triple Therapy' and it entered the vernacular of gastroenterologists worldwide. An earlier report of these findings was published in 1987, establishing a priority date.[24] Biopsy from 100 consecutive *H. pylori*-positive patients with either duodenal ulcer or non-ulcer dyspepsia, at 8 weeks confirmed eradication in 94% of patients, with repeat endoscopic biopsy at 1 - 3 years revealing that 100% of those patients who had achieved eradication had not experienced a *H. pylori* recurrence. *Given the 3 year follow-up of 'Triple Therapy' in these patients, this publication dated the first development of 'Triple Therapy' at CDD in 1984.*

(ii) Cure of Duodenal Ulcer after Eradication of *Helicobacter pylori*. George LL, Borody TJ, Andrews P, *et al. Med J Aust* 1990; 153: 145-149.[3] Armed with an effective eradication therapy it was now possible to reliably examine the notion of 'cure'. The 'Triple Therapy' detailed above (Appendix 2 – Schedule A) was administered to 78 *H. pylori*-positive subjects with duodenal ulcers. The duodenal ulcers healed in 100% of patients and *H. pylori* was no longer detectable by endoscopic biopsy in 96% of patients (75/78) at 4 weeks post-antibiotics. At year 1, 97% (71/73) of patients remained free of *H. pylori* and free of duodenal ulcer; at year 2, 100% (57/57); at year 3, 100% (34/34); and year 4, 100% (15/15) were cured of *H. pylori* and duodenal ulcer. No duodenal ulcers were detected in *H. pylori*-negative patients followed for up to 4 years. On endoscopic examination, previously distorted duodenal caps (due to ulceration) had returned to their near-normal shape in 80% of patients by two years after treatment. It was concluded that duodenal ulcer disease would not recur if the patient remained free of *H. pylori*.

In 1992, Graham *et al.,*[25] using the same 'Triple Therapy' regimen, confirmed our earlier findings, that persistent *H. pylori* infection was the best predictor of recurrence. $H_2$ receptor blockade healed duodenal ulcers but, as expected, exhibited no effect on *H. pylori* infection. As such, 95% of patients

in this $H_2$ receptor group developed ulcer recurrence. Furthermore, no patient with *H. pylori* eradication in the 'triple therapy' cohort became re-infected. Graham's subsequent editorials further substantiated CDD's findings that *H. pylori* ulcer disease could now be cured (though the data for gastric ulcer remained 'probable').[26] Furthermore, Graham clearly articulated that "the first truly successful therapy - that is one with a reliable ≥90% eradication rate - consisted of bismuth, tetracycline, and metronidazole for 14 days" was developed by Borody at the CDD.[27] And indeed all subsequent effective eradication therapies consisted of various triple, not dual, therapies.

**(b) PAPERS IN SUPPORT OF DSc. SUBMISSION – (Major Question: "What therapy will reliably eradicate *H pylori* "- Details of therapy development)**

**PAPER 1.1**

Recurrence of Duodenal Ulcer and *Campylobacter pylori* Infection after Eradication. Borody TJ, Cole P, Noonan S, *et al. Med J Aust* 1989; 151: 431.[2]

At the time of publication in 1989, it was accepted that "*C. pylori* infection is the most common cause of active chronic gastritis," however any causal role in peptic ulceration was as yet unproven. Based on widely held beliefs at the time, the notion that ulcers should be treated with antibiotics was met with broad skepticism (and any role in 'non ulcer dyspepsia' was even less clear). It was acknowledged that a major stumbling block to establishing a causal relationship was the lack of a 'reliable eradication therapy'. At the time, studies with an antibiotic plus a bismuth compound had provided poor long-term results.[19] A program, commencing in 1984, had previously been embarked upon at the CDD to screen a variety of 'triple therapy' regimens. A total of 36 combinations were assessed in a total of 180 patients (5 patients per group) before the most promising candidate composition was selected late in 1984 – a regimen containing bismuth plus two antibiotics.[18] Interim results detailing the most efficacious of these combination therapies were published in 1986, and in more detail by 1987.[24] Later, long-term data on ulcer relapse was available (up to 37 month follow-up), and a preliminary abstract appeared in Gastroenterology in 1988 describing the long-term results of the 'selected' eradication therapy.[28] The therapy, consisting of **bismuth-tetracycline-metronidazole** 'Triple Therapy' (Appendix 2 – Schedule A), was trialed in 100 consecutive *H. pylori*-positive subjects (with either duodenal ulcer or non-ulcer dyspepsia). The treatment regimen consisted of colloidal bismuth subcitrate and tetracycline hydrochloride each for 4 weeks, with metronidazole for the first 10 days. Endoscopic biopsy 8 weeks post-treatment demonstrated a 94% *H. pylori* eradication rate. Of sixty-four patients with *H. pylori*-negative biopsies at 8 weeks undergoing repeat endoscopy, 94% (60/64) remained *H. pylori*-negative at 12-37 months post-therapy. Those with duodenal ulceration, *and in spite of continued smoking*,[29] exhibited long-term healing and restoration of inflamed mucosa to normal. Side effects of therapy were common but mild in nature. The short-term efficacy of this *H. pylori* eradication triple therapy was quickly confirmed by others.[30] By the early – mid 1990's the permanency of eradication following 'Triple Therapy' was confirmed by both Marshall[31] and Graham[19] .

Conclusion: Antibiotic triple therapy achieves long-term eradication of *H. pylori* in over 90% of treated patients.

Comment: This was an un-blinded study and a placebo control group was not used, based on overwhelming published evidence which showed that one- and two-drug regimens studied failed to achieve long-term eradication in >80% of subjects (an arbitrary cut-off reflecting 'acceptable' treatment efficacy). In those with duodenal ulcer, H2-receptor blockade was initially used to 'heal' the ulceration as this was the 'standard of care' and H2-receptor blockers had no impact on *H. pylori* colonization(see review of Paper 1.2). Patients then entered the 'Triple Therapy' study, This development of an effective eradication therapy made it possible, for the first time, to determine whether *H. pylori* was the primary aetiological agent of duodenal ulcer disease (see Paper 2). The CDD 'Triple Therapy' was officially reviewed by a working party at the 1990 Sydney World Congress of Gastroenterology and recommended as the most effective therapy combining either colloidal bismuth subcitrate (CBS) or bismuth subsalicylate four times daily, tetracycline HCl 500mg four times daily, and metronidazole three times daily for 14 days.[20]

**PAPER 1.2**

Cure of duodenal ulcer after eradication of *Helicobacter pylori*. George LL, Borody TJ, Andrews P, *et al. Med J Aust* 1990; 153: 145-149.[3]

By the end of the 1980's, spiral bacilli found in gastritis were recognised as a new species (*Helicobacter pylori*) and accepted as the most common cause of gastritis. Though present in the vast majority of patients with duodenal ulcers, a causal role remained unproven.[32] Preventing ulcer recurrence following *H. pylori* eradication using a highly effective therapeutic regimen was considered the best assessment of 'cure'.

Our research (Paper 1.1) developing 'Triple Therapy', which attained long-term eradication in >90% of patients for the first time, made such a study possible. This study is the subject of Paper 1.2.

At the time, contemporary therapy with H2-receptor antagonists (H2-RAs) reported a duodenal ulcer recurrence rate of 70 – 90% at 12 months.[33] This high rate of recurrence was accepted dogma at the time. The 'Triple Therapy' study recruited 82 patients with recurrent duodenal ulcers, or those with ulcers resistant to H2-RA therapy. The patients selected purposefully

representing the most difficult treatment group to evaluate the scientific rigor of the therapy. Following four weeks of H2-RA therapy, patients commenced 4 weeks of triple therapy comprising **bismuth-tetracycline-metronidazole** (Appendix 2-Schedule A). Follow-up endoscopy at 4 weeks showed that all patients had their ulcers healed, with *H. pylori* eradication confirmed in 75 subjects (96%). Annual progress assessment demonstrated that 97% (71/73) of patients remained free of both duodenal ulcer and *H. pylori*. In subsequent years, 100% of patients remained free of both duodenal ulcer and *H. pylori* at year 2 (57/57); year 3 (34/34); and year 4 (15/15). Endoscopic examination of duodenal caps showed progressive return to normal, with resolution of minor scarring and stricture reversal by two years. Continued smoking did not affect ulcer cure. This study identified *H. pylori* infection as the primary aetiological agent in duodenal ulcers, and demonstrated that factors known to influence recurrence of duodenal ulceration (such as gastric acid production and smoking) only operated in the presence of *H. pylori* infection.[29] A breakthrough was finally achieved in altering the natural history of duodenal ulcer disease.

Conclusion: It was concluded that, for the first time, in those with *H. pylori*-positive duodenal ulcer, eradication of *H. pylori* cured ulceration. Comment: This study represented the final link in the *H. pylori* chain of pathogenicity – gastritis – and finally duodenal ulcer. This study would not have been possible had it not been for the earlier development of a highly effective eradication therapy used in this study, 'triple therapy'. An open-label study design was selected, based on established literature reporting >90% duodenal ulcer recurrence following therapy with H2-antagonist medication. The subject population chosen were considered extremely challenging – with recurrent or treatment-resistant duodenal ulcers [see Fig. 2 in Paper 1.2 –11% 'resistant' to H2-RA blockage; 73 patients (89%) having recurrence of ulceration at 1 – 10 months (mean 4.2 months)]. This dramatic difference in treatment outcome, as seen in Fig. 2, is reinforced when compared against published duodenal ulcer relapse rates in a further 208 patients, of which 92% had relapsed by 4 years.[3]

Commercial Result: The 'Triple Therapy' invention was commercialised by Procter and Gamble and continues to be marketed as Helidac® in the US through 2015 (Granted US patent 5,196,205 [Appendix 3, Figure 2b. Helidac]).

## B. STRATEGIC DEVELOPMENT OF A MANAGEMENT PROGRAMME FOR *HELICOBACTER PYLORI*-RELATED DISEASE

### (a) OVERVIEW AND CONTENT – Major Question: "Given the effectiveness of Triple Therapy, can it be optimised to avoid Eradication Failure?" – The Development of "Quad Therapy"

Following the initial studies demonstrating the success of 'Triple Therapy,' others quickly confirmed its efficacy.[23,25,34] However it soon became evident that 1-2 weeks of treatment was required for optimal efficacy, and that it was more difficult to re-treat those who had initially failed eradication and addition of omeprazole to our 'Triple Therapy' was found to improve eradication. By 1992, a proton pump inhibitor (PPI) – omeprazole – was being used to enhance the efficacy of triple therapy for *H. pylori* eradication as discovered at CDD where Aust. Patent Application, PI8438 was filed Oct 12, 1987 (Appendix 1) describing triple therapy plus the addition of a proton pump inhibitor. This newly developed 'quad therapy' was then applied to those patients at the Centre who had previously failed eradication with 'Triple Therapy'- as these were the most difficult patients to achieve ulcer cure.[35] Later in the same year, 4 years after the filing of our provisional patent application, Hosking *et al.* (1992) confirmed that the addition of omeprazole enhanced our Triple Therapy, achieving a 95% cure rate in a controlled trial.[36] These high eradication rates were also published by our Centre using a 12 day course of omeprazole + Triple Therapy to achieve an eradication rate of 97.6% in a controlled trial (Appendix 2 – Schedule B).[37]

In 1994 with the arrival of an acid-stable macrolide, clarithromycin, Bazzoli *et al.* (1994),[38] introduced a simplified one week course of clarithromycin, tinidazole and added omeprazole – a double therapy plus omeprazole - with a reported efficacy of 95% - confirmed by subsequent studies (86% - 95% efficacy).[39,40] Later amoxicillin was substituted for tinidazole and this became the 'Legacy Triple Therapy' (Appendix 2-Schedule C).[41] This combination became the basis of the convenient "Losec HP-7" and later "Nexium HP-7" therapeutic strategies in Australia. Over time, and in the absence of a third antibiotic, increased levels of resistance to clarithromycin developed, and falling efficacy concerns were raised regarding the appearance of growing numbers of people failing eradication – an undesirable outcome. By this time the proton pump inhibitor had become an integral component of *H. pylori* regimens and had been shown to enhance eradication in single and double antibiotic regimens, particularly when combined with clarithromycin and a nitroimidazole.[38] During this time, CDD had continued to refine the original triple therapy regimen aimed at reducing

the dosage and duration of therapy, ultimately demonstrating that a 12-day course of (Appendix 2-Schedule B): bismuth subcitrate (108 mg); tetracycline (250 mg); and metronidazole 200 mg, was as effective ($p<0.07$) as the original formulation and dosage but with fewer side effects ($p<0.01$).[42] Results from a subsequent double-blind controlled trial comparing triple therapy (modified-dose and duration plus a H2-receptor antagonist - famotidine) versus an identical triple therapy, but with a proton pump inhibitor (omeprazole) in place of the H-2 receptor antagonist, showed eradication improved from 89% to 97.6% ($p = 0.006$) in the omeprazole group.[37] Between 1992 and 2000 our group published several reviews and invited commentaries related to optimal primary therapy, and the reduction of the 'pool' of patients with resistant *H. pylori*. We advised the use of 'salvage therapy' for those with failed initial eradication therapy. [43 44 45 46 47 55]

These publications present the substantial contribution made by our group towards the burgeoning secondary problem of *eradication failure* in patients following initial treatment of *H. pylori* (i.e., a strategy to reduce the number of patient becoming 'eradication failures' and to develop a highly effective eradication therapy for these patients. Early 'in vitro' studies of antibiotic 'resistance' profiles did not correlate well with effective eradication regimens. It was equally clear that those failing primary eradication had increased drug-resistant organisms. This was particularly evident following 'triple therapy' with clarithromycin/metronidazole/proton pump inhibitor, with half of those failing to eradicate *H. pylori* developing resistance to both antibiotics ('double resistance'), and 70% developing secondary resistance. However, careful analysis of studies using either omeprazole/amoxicillin, or clarithromycin or bismuth-based triple therapies also showed that the difficulty in curing *H. pylori* could not be explained simply in terms of drug-resistance. We concluded that this 'resistance' was either due to an 'effect of therapy' or a 'selection' for defective host factors. Our group collaborated with the Newcastle Mucosal Group to study the pattern of cytokine secretion from both gastric biopsies and whole blood cultures, and found a significant defect in the eradication failure groups' capacity to produce IL-4 (a surrogate for a Th2 response).[48] We argued that employing 'failing' therapies with less than 90% primary eradication outcomes whilst reserving the most effective eradication therapy (classical 'triple therapy' plus a proton pump inhibitor) as 'rescue therapy' for those with one or more eradication failures (as had been recommended by two international consensus conference), was contributing to an increasing number of drug-resistant *H. pylori* cases, and creating a significant but avoidable medical challenge. Indeed recent opinion stated the "vast majority (of reviews) show unacceptably low treatment

success for 'legacy therapy' with only 18% exceeding 85% and ~ 60% failing to reach 80% eradication." It was urged not to "use 'legacy triple therapy' consisting of a PPI, clarithromycin and amoxicillin unless it has been proven to be highly effective locally (e.g. eradication > 90%)".[41] Hence, the argument to use 'Quad Therapy' (TT plus PPI) as a 'first-line' treatment[45,47,49] was based on the fact that:

(i) Quad therapy was the most effective drug combination for *H. pylori* eradication, with >90% efficacy. Subjects with metronidazole resistance still had an eradication rate > 95%.

(ii) The treated population was not exposed to clarithromycin, which was costly, and associated with pseudomembranous colitis at times. This also made an effective anti-*H pylori* 'rescue' antibiotic available for those few not responding to 'Quad Therapy.'

We later treated 130 *H. pylori*-positive patients who had failed one or more treatments with a modified rifabutin-containing novel triple therapy (Appendix 2-Schedule D).[50] Patients were treated with lower dose rifabutin, and higher dosages of PPI and amoxicillin given more frequently for 12 days. The overall eradication rate was 91-96%, albeit higher when more amoxicillin was used.

At the turn of the century, 'Quad Therapy', as developed by our group (TT+PPI) was the only regimen to regularly achieve greater than 90% eradication rates when used first (Appendix 2-Schedule B). This led us to conclude that 'Quad Therapy' should be used as first-line rather than 'rescue' therapy, thus reducing the number of patients in the community with resistant organisms.[43,45] Quad Therapy could then be followed in patients with 'eradication failure' patients using our novel 'rifabutin-based' rescue therapy (Appendix 2- Schedule D).

Commercial Result: 'Quad Therapy' was commercialized by Axcan Pharma and marketed as Pylera® in the US (Appendix 1. Borody US patent 5,476,669) and more recently in Europe. It was produced as a single capsule product co-prescribed with a proton pump inhibitor.[51,52] (Figure 2b. Pylera® product).

There still remained a need to develop a 'rescue' strategy for patients who had failed Quad Therapy; particularly a second-line therapy aimed at overcoming the increasing problem of clarithromycin resistance with Triple Therapy. In 2000, Perri *et al.* (1992) demonstrated high *in vitro* sensitivity of *H. pylori* to rifabutin.[53] Several studies showed clinical benefit, with a mean eradication rate of 71% using a high rifabutin (300 mg/d) dose in a triple therapy regimen given for one week.[54] Following extended dosing studies we developed a 12-day regimen using low dose of rifabutin (150 mg/day), combined with high doses of amoxicillin (1.5 G TDS) and pantoprazole (80 mg TDS)

(Appendix 2- Schedule D). This was confirmed as highly effective in subjects who had previously failed one or more eradication attempts, achieving eradication in 96% of patients, with only mild side effects in 40% of patients.[50]

Conclusion: In this section, a period of over 20 years of continuous clinical research into *H. pylori* is summarised in the context of contemporary thinking. In recent times, no area of medicine has attracted more attention, reflected such a radical shift in accepted wisdom, or altered the landscape of medicine in a more dramatic fashion. In this intensely competitive environment, the studies and reviews from our group have contributed much towards current knowledge and have served to shape popular opinion and management strategies. Successful eradication of *H. pylori* resulted in the near- disappearance of peptic ulcer and its complications in clinical practice – a condition that had dominated medicine for decades. Our early studies focused on the development of a safe and effective therapy capable of eradicating *H. pylori* in ≥90% of patients – this enabled, for the first time, studies to be conducted demonstrating duodenal ulcer cure (see Section 1 above). Subsequently, attention shifted to combating the new and increasing challenge of antibiotic resistance and eradication failure experienced with dual antibiotic regimens containing clarithromycin and proton pump inhibitors administered for short periods. To counteract this problem, 'Quad Therapy' was developed by adding a proton pump inhibitor to our bismuth-based triple therapy (Appendix 2 – Schedule E), which led to >90% *H. pylori* eradication in those undergoing first-line treatment. The treatment was also effective in those failing one or more previous attempts to cure *H. pylori* infection. Results of the published Quad Therapy trials have been summarised.[45] However, failure to eradicate *H. pylori* could not be explained by antibiotic resistance alone, and likely occurred because of an associated 'host deficit.' Study of the host pattern of cytokine response (using mucosal organ cultures and whole blood cultures) indeed showed an impaired Th2 response to *H. pylori* antigen (we had previously shown that recruiting a Th2 response was a characteristic of successful eradication). These observations were most consistent with the theory that a number (perhaps 20%) of *H. pylori*-infected patients had a limited capacity to contribute host protecting mechanisms to the clearance of infection. Consequent selection of resistant bacteria in those patients with host deficits contributed to a sub-group of patients progressively less likely to respond to antibiotics. Our group presented the opinion that rather than viewing quad therapy as a 'last attempt' rescue therapy, it should be used as first-line therapy, ensuring cure in over 90% of patients whilst minimising the expansion of this 'failed

therapy' group. A 'last-line' therapy was therefore required for the minority of patients who had failed eradication with 'Quad Therapy'. Early studies with rifabutin provided encouraging results – pre-existing regimens using rifabutin, which had high adverse effects, were modified by halving the rifabutin dose (and therefore the side effects) and increasing the amoxicillin and proton pump inhibitor doses to bolster efficacy. Using this modified approach, a 96% eradication rate was achieved in 130 patients *who had one or more prior failed eradication attempt.* This regimen of first-line quad therapy, followed by a rifabutin-based rescue therapy in the small number of patients who failed 'Quad Therapy', meant approximately 1% of all patients infected with *H. pylori* would fail eradication attempts and remain infected. Even in the unlikely event this was to occur, clarithromycin-based 'rescue' therapies could still be used.

**(b) PAPERS SUBMITTED IN SUPPORT OF D.Sc. SUBMISSION**

**PAPER 1.3**

Use of high efficiency, lower dose triple therapy to reduce side effects of eradicating *Helicobacter pylori*. Borody T, Brandl S, Andrews P, *et al. Am J Gastroenterol* 1994; 89: 33-38.[42]

A decade following the discovery of *H. pylori* as a cause of gastritis, great strides had been made in the acceptance of *H. pylori* eradication as the primary aim of ulcer management. The sheer number of patients with ulcer disease and the emergence of more sophisticated diagnostic tools had shifted eradication management largely away from specialists to primary practitioners who required a simplified treatment strategy with minimal toxicity. No first-line eradication therapy had been described that was superior to our bismuth-based triple therapy. However, many patients complained of nausea and antibiotic-associated side effects with the therapy and the four weeks of treatment adversely affected compliance. In 1991, the importance of intra-luminal drugs for achieving mucosal concentrations relevant to the eradication of *H. pylori* was demonstrated.[55] We studied a strategy of reducing the duration (to 14 days) and dose of tetracycline and metronidazole, but increasing the dosage frequency to five time per day (compared to the current regimen of 4 times per day) – with an aim of maximising continual mucosal concentrations, whilst simultaneously reducing side effects. The prospective randomised controlled trial recruited 466 symptomatic *H. pylori*-positive patients. Of the 413 available for analysis, eradication rates in the 4x/day group were 92% (196/213) versus 96% (202/210) in the 5x/day group ($p = 0.07$). Side effects were significantly less frequent in the 5x/ day group ($p < 0.01$).

Comment: This study confirms both the success of our 'classic' bismuth-based 'triple therapy' and that reducing the length of the protocol to a more frequent dosing schedule but lower individual dosages (of tetracycline and metronidazole), has good compliance, fewer adverse events and highly efficient eradication (≥ efficient as the original formulation).

**PAPER 1.4**

Omeprazole enhances efficacy of triple therapy in eradicating *Helicobacter pylori*. Borody TJ, Andrews P, Fracchia G, *et al. Gut* 1995; 37: 477-481.[37]

This is a pivotal paper in the development of *H. pylori* eradication therapy for two reasons:

(i) The paper underpins the development of 'quad' therapy which gained particular importance as a recommended 'first-line therapy', aimed at minimising the increasing number of 'eradication failures.'

(ii) The first clear comparative paper indicated an additive value of a proton pump inhibitor (omeprazole) over H2 receptor antagonists, in combination with antibiotics. Our group had established a priority date for the potential value of adding omeprazole to the 'classical' triple therapy (1992) in a study of subjects who had failed to eradicate with prior therapy. In this group, 78% (35/45) of patients achieved eradication. This study also showed that metronidazole resistance (in vitro) was unrelated to outcome [see Paper1.5: H. pylori eradication failure (EF) – further treatment possibilities. Borody TJ et al. Gastroenterology 1992;102].[35]

Several other groups described successful eradication with regimens adding omeprazole to bismuth-based triple therapy,[36,56] however these did not show that replacing an H2 receptor antagonist with a proton pump inhibitor provided a significant advantage in a controlled trial.[30, 47] Our study, using the 'low-dose' modification to the classical triple therapy (Appendix 2- Schedule B) as the original therapy, recorded a 97.6% eradication level with the addition of omeprazole.

Comment: The combination of 'low-dose' triple therapy with omeprazole attained near-100% *H. pylori* eradication. In a prospective, randomised un-blinded, single centre study, a significantly higher percentage of patients taking triple therapy plus omeprazole (97.6%; 122/125) achieved eradication versus triple therapy plus famotidine (89%; 110/124; p = 0.006). This comparative study established 'quad therapy' as the most reliable 'first-line' eradication therapy.

**PAPER 1.6**

Nizatidine in combination with amoxicillin and clarithromycin in the treatment of *Helicobacter pylori* infection. Talley NJ, Chang FY, Wyatt JM, Adams S, Lau A, Borody TJ, *et al. Aliment Pharmacol Ther* 1998; 12: 527-532.[57]

This multicenter study focused on a contributing role for H2 receptor antagonists as several earlier studies suggested similar eradication rates for clarithromycin-containing dual antibiotic regimens using either a proton pump inhibitor or an H2-receptor antagonist study[58] relied on historical controls showing that dual antibiotic regimens eradicated bacteria in about 60%, to suggest that single or double dose arms of H2-receptor antagonist gave similar levels of eradication in the 70% range: this was less than many reported studies using proton pump inhibitors.

**PAPER 1.7**

*Helicobacter pylori* eradication with doxycycline-metronidazole-bismuth subcitrate triple therapy. Borody TJ, George LL, Brandl S, *et al. Scand J Gastroenterol* 1992; 27: 281-4.[59]

In an attempt to further simplify antibiotic treatment for *H. pylori,* doxycycline was substituted for tetracycline HCl, with its advantages of less frequent dosing and extra-renal excretion. However doxycycline was found to be less effective than tetracycline HCl (65%, 22/34; versus 92%, 36/39, p = 0.004) and had a greater nausea adverse effect.

Comment: The value of this study was to again confirm the value of 'traditional' bismuth-based triple therapy (Appendix 2- Schedule A), and to emphasise the value of tetracycline HCl.

**PAPER 1.8**

Possibilities for Helicobacter pylori suppression/eradication. Borody TJ. *Eur J Gastroenterol & Hepatol* 1992; 4: 537.[60]

**PAPER 1.9**

Treatment of patients with failed eradication – a personal view. Borody TJ and Shortis NP. *Helicobacter pylori*: Basic Mechanisms to Clinical Cure. Lancaster: Kluwer 1996; 357-365.[47]

**PAPER 1.10**

What is the optimal therapy for *Helicobacter pylori* eradication? Borody TJ. The Esophago-Gastric Junction: 420 Questions, 420 Answers. John Libbey Eurotext 1996; 653-656.[61]

**PAPER 1.11**

Eradication therapies for *Helicobacter pylori.* Borody TJ, Shortis NP, Reyes E, *et al*. *J Gastroenterol* 1998; 33: 53-56.[43]

**PAPER 1.12**

Treatment failures and secondary resistance to antibiotics: A growing concern in *Helicobacter pylori* therapy. De Boer W, Borody TJ. *Digest Liver Dis* 2000; 32: 673-675.[46]

**PAPER 1.13**

*Helicobacter pylori* eradication failure – 'salvage' therapies needed. Borody TJ. *Ital J Gastroenterol Hepatol* 1998; 30: 375-377.[44]

**PAPER 1.14**

Quadruple should be first-line therapy for *Helicobacter pylori* infection. Borody TJ. *Helicobacter pylori:* Basic Mechanisms to Clinical Cure. Netherlands: Springer 2000; 623-629.[45]

This series of 7 invited reviews trace the evolution of *Helicobacter pylori* management over the decade of the 1990's. In particular, the publications focused on the burgeoning problems of treatment failure and antibiotic resistance, and the subsequent increase in eradication failures. This led to the positioning of 'Quad Therapy' as a first-line, rather than rescue, treatment.

**PAPER 1.15 – Major Question: "What effective 'Rescue Therapy' can be offered to those patients who fail first-time therapy?" - The Development of the Rifabutin-Based Rescue Therapy**

Efficacy and safety of Rifabutin-containing 'rescue therapy' for resistant *Helicobacter pylori* infection. Borody TJ, Pang G, Wettstein AR, *et al. Aliment Pharmacol Ther* 2006; 23(4); 481 – 488.[50]

The development of this rescue therapy has in part been described above. One hundred and thirty patients who had failed to eradicate *H. pylori* following open-label administration of the standardised "Nexium HP-7" regimen of esomeprazole, clarithromycin and amoxicillin (Appendix 2-

Schedule C) were included. Patients were treated for 12 days with rifabutin (150 mg / day), amoxicillin 1 gm or 1.5 gm TDS and pantoprazole 80 mg TDS (Appendix 2- Schedule D). The results reported:

(i) An eradication rate of 90.8%;

(ii) A higher dose of amoxicillin provided a higher overall eradication rate (96.6%)

(iii) *In vitro* metronidazole and/or clarithromycin resistance did not impact eradication; and

(iv) Mild adverse events in 40% of patients, with no haematological complications. No patient withdrew from the study due to side effects.

A recent review of rifabutin use in refractory *H. pylori* infection examined the various questions often posed by physicians regarding the use of rifabutin in this capacity and summarised the wealth of evidence supporting the effectiveness and need for rifabutin use in this 'rescue' role.[62]

Comment: The study, reported on in Paper 1.15, identified an effective eradication therapy unaffected by *in vitro* resistance to clarithromycin. This strategy was developed from earlier, less developed regimens, which had little supporting data.

Conclusion: Rifabutin-based therapy as second-line 'rescue therapy' for patients failing early eradication treatment complements quad-based primary therapy strategy.

Commercial Result: the 'Rifabutin Salvage Therapy' was commercialised by Redhill Biopharma Ltd after acquiring Borody Patent (Appendix 1 - US 6,489,317, filed April 30th, 1998 as PP3253) from Giaconda Ltd, a publically listed ASX company which Borody and others progressed through an IPO to commercialise several CDD inventions. Redhill Biopharma Ltd is set to conclude pivotal trials using this product, currently termed Heliconda™, aimed to be submitted for NDA application to the FDA early in 2016.

**PAPER 1.16**

Impaired host immunity contributes to *Helicobacter pylori* eradication failure. Borody T, Ren Z, Pang G, *et al. Am J Gastroenterol* 2002; 97(12): 3032-3037.[48]

**PAPER 1.17**

Circulating T-cell response to *Helicobacter pylori* infection in chronic gastritis. Ren Z, Pang G, Lee R, Batey R, Dunkley M, Borody T, *et al. Helicobacter* 2000; 5(3): 135-141.[63]

**PAPER 1.18**

Gastric carcinoma: T-cell response and vascularity. Ren Z, Pang G, Clancy R, Chen Li L, Lee CS, Batey R, Borody T, *et al. J. Gastroenterol & Hepatol* 2001; 16: 142-148.[64]

These series of studies were performed in conjunction with the Newcastle Mucosal Immunology Group. The premise was to determine the mucosal response to *H. pylori* at a molecular level in an attempt to elucidate possible treatment outcome determinants distinct from *H. pylori* pathogenicity. Of particular interest was: to what extent did defective host protection contribute to eradication failure? The collaborative study also examined host mechanisms in response to *H. pylori* colonisation and, if any shift occurred following successful eradication with standard eradication therapy (Paper 1.17). Prior to this, the immune effector parameters studied had largely consisted of blood T-cell proliferative responses, which have provided oftentimes confusing results, potentially as a result of methodology (reviewed in Paper 1.17) and uncertainty that *H. pylori* infection was not present within the crypts of *H.pylori*-negative gastritis. Our study (Paper 1.17) attempted to avoid conflicting results by standardising assays using whole blood cultures. A reproducible and specific increase in INF-$\gamma$ secretion was observed in *H. pylori* positive subjects following antigen stimulation, while IL-4 secretion was undetectable, reflecting a dominant and inducible Th1 response. However, following successful eradication, a shift in cytokine balance was found with an increase in IL-4 secretion. It was not clear whether this 'shift' towards a Th2-Th1 (or Th0) balance was due to eradication, or contributed to eradication in the presence of antibiotics. These results provided a background to studies in patients with failed eradication (Paper 1.16). A total of 63 subjects were studied. First the validity of using blood cultures was assessed comparing cytokine secretion with secretion from gastric mucosal organ cultures ($R2 = 0.55 / 0.62$; $p < 0.001$). Significantly lower levels of IL-4 were detected in whole blood stimulated or not stimulated with *H. pylori* antigen, from subjects with eradication failure compared with subjects in whom *H. pylori* was successfully eradicated ($p < 0.05$) or in subjects with untreated infection ($p < 0.05$) or no infection. No significant differences were noted for INF-$\gamma$. Lower levels (short of significance) of anti-*H. pylori* antibody in those failing to eradicate *H. pylori*, may reflect a Th2 defect and contribute to resistance. (See paper 1.24 on the use of IgG2 antibody for diagnoses).[65]

In the third study (Paper 1.18), cytokine patterns were studied in subjects with gastric dysplasia and carcinoma, in an attempt to better understand the host-parasite relationship link with gastric cancer. Major differences were noted in terms of cytokine secretion from mucosal 'organ

cultures' – a reversal of the dominant 'Th1' pattern characteristic of uncomplicated *H. pylori* positive gastritis, was found in gastric cancer, irrespective of *H. pylori* status. Those with dysplastic mucosa had an intermediate pattern retaining INF-$\gamma$ secretion, but also secreting IL-4 (see Fig. 3).

Comment: These studies were the first to demonstrate a consistent pattern of cytokine secretion from gastric mucosa and whole blood in relation to infection with *H. pylori*, the shift in pattern associated with effective eradication, a persistent defect associated with failed eradication, and a progressive distortion of these patterns as gastritis progressed through dysplasia to carcinoma. These studies were performed in collaboration with the Newcastle Mucosal Immunology Group; those related to failed eradication were conceived and coordinated by our clinical research group. With respect to therapeutic eradication of *H. pylori*, recognition of a mucosal immune defect (in approximately 20% of infected subjects) influenced our thinking with respect to strategy for eradication – a mucosal defect would encourage clinical failure of marginally effective therapy, with consequent appearance of resistant organisms of relevance to both the individual and the community. Our view that the 'best available' antibiotic regimen should be used as 'first-line' therapy to overcome any host deficits was reinforced by these considerations.

Conclusion: Consistent patterns of cytokine secretion were detected in whole blood and/or mucosal cultures, reflecting *H. pylori* infection, successful and failed eradication and progression of gastritis to carcinoma. Recognition of a 'mucosal defect' in generating a Th0 (or balanced) pattern of secretion influenced thinking towards our strategy for *H. pylori* eradication.

## C. ANCILLARY STUDIES RELEVANT TO *HELICOBACTER PYLORI* INFECTION AND ERADICATION

### INTRODUCTION

The preceding *major questions* [Sections 1(A) a and b; 1(B); Paper 1.15] detailing the development of effective 'first-line' and 'rescue' therapies have been answered above. However during the research efforts into eradication therapies, numerous significant questions were generated which will be discussed in a summarised manner. Following the description of spiral bacteria in human gastric mucosa and their association with gastritis and peptic ulcer disease, interest intensified into examining all infection aspects relevant to the diagnosis and management of *H. pylori*-associated disease. Topics of particular clinical interest included:

(a) optimal diagnosis – endoscopic, serologic, and urease-related
(b) dynamics of infection recurrence – recrudescence vs reinfection
(c) mucosal changes due to *H. pylori* infection
(d) smoking and ulcer relapse
(e) adverse effects of Triple Therapy
(f) *H. pylori*-negative ulcers
(g) Duodenal stricture as a complication of duodenal ulcer

The contributions from the CDD relevant to these areas follow.

### (a) OPTIMAL DIAGNOSIS: Question: "How can we improve the diagnosis of *H. pylori* infection*?"*

**PAPER 1.19**

*Campylobacter pyloridis* gastritis I: Detection of urease as a marker of bacterial colonization and gastritis. Hazell S, Borody TJ, Gal A, *et al. Am J Gastroenterol* 1987; 82: 292-296.[66]

**PAPER 1.20**

*Campylobacter pyloridis* gastritis II: Distribution of bacteria and associated inflammation in the gastroduodenal environment. Hazell SL, Hennessy WR, Borody TJ, *et al. Am J Gastroenterol* 1987; 82(4): 297-300.[67]

**PAPER 1.21**

The use of serology to diagnose active *Campylobacter pylori* infection. Mitchell HM, Lee A, Berkowicz J, *et al. Med J Aust* 1988; 149: 604-609.[68]

**PAPER 1.22 [Abstract]**

Failure to use combined tests may miss *Helicobacter pylori* (HP) diagnosis. Borody TJ, Andrews P, Shortis NP, *et al. Am J Gastroenterol* 1994; 89: 1348.[69]

**PAPER 1.23**

Evaluation of whole blood antibody kit to detect active *Helicobacter pylori* infection. Borody TJ, Andrews P, Shortis NP, *et al. Am J Gastroenterol* 1996; 91: 2509-2512.[70]

**PAPER 1.24**

Evaluation of anti-*Helicobacter* pylori IgG2 antibody for the diagnosis of *Helicobacter pylori* infection in western and Chinese populations. Ren Z, Borody TJ, Pang G, *et al. Aliment Pharmacol Ther* 2005; 21; 83-89.[65]

**PAPER 1.25 [Abstract]**

Diagnostic Value of Fundic Red Spots in *Helicobacter pylori* (Hp) Diagnosis. Borody T, Wettstein A, Finlayson S, *et al. Am J Gastroenterol* 2012; 107(S1): S59.[71]

**PAPER 1.26 [Abstract]**

Rapid and Superior Diagnosis of *H. pylori* Infection by $^{14}$C-Urea Heliprobe$^{TM}$. Borody T, Wettstein A, Campbell J, *et al. Gastroenterology* 2008; 134(Suppl1): A329.[72]

Comment: The diagnostics research addressed topics such as endoscopic appearance, biopsy urease testing and serology, with the urea breath test becoming available later. These studies were commenced in the early stages of *H. pylori* research, when mucosal urease was not yet well-characterised as a diagnostic indicator, and endoscopic detection was the only means available, making serology an attractive option. *H. pylori's* marked urease activity, including in human gastric mucosa (previously thought to be secreted by mucosal cells), was demonstrated within a year of

discovery. The specificity of high level urease activity led to the development of two key diagnostic assays: the urea breath test and rapid point-of-care diagnostic assay for urease activity using gastric punch biopsies. Clinically useful 'breath tests' were developed in the late 1980's[73] but it would be some time before such tests became widely available. Such non-invasive assays were impractical at that time as epidemiological tools or 'immediate' office diagnoses.

Our clinical group, working with the School of Microbiology (University of New South Wales) and the Mucosal Immunology Group (University of Newcastle), assessed the clinical value of the first assays to demonstrate a useful clinical role of, respectively, a 'yes/no' mucosal biopsy-based assay for urease and a serum antibody assay, and a 'near patient' 'yes/no' test for antibody using a finger-prick blood test. The mucosal biopsy-based assay (Papers 1.19 and 1.20) reacted to the presence of ammonia which was hydrolyzed in the presence of *H. pylori* from urea, detected by pH change – it was 100% specific for *H. pylori* and 90% sensitive after 18 hours of incubation (75% of patients tested positive for a reaction within one hour). The test was formulated for routine use in the endoscopy unit, and was commercially developed. The speed of hydrolysis correlated with histological characteristics, in studies that included 376 patients. A second study correlated distribution of *H. pylori* with histology, finding a close topographic association (Paper 1.20).[67] Two primary patterns of colonisation were identified: antral colonisation extending proximally to the body, or specific areas of chronic-only gastritis induced by other agents.

Antibody testing research began in earnest from 1986 onwards[74] but great variation of sensitivity and specificity were noted and no diagnostically-validated assay was available. This was due in part to poor antigen preparations, often encountering cross-reactivity to *C. jejuni.* Cross-reactivity made clarity surrounding the positivity/negativity cut-off point difficult. Absorption of PNG serum with *C. jejuni* confirmed this point, halving the incidence of seropositivity (56% to 25%). The absorption assay was tested in 189 Australian subjects with dyspepsia and achieved 100% sensitivity and 94% specificity.[68] Using a rapid 'whole blood assay' with an improved antigen, 82% sensitivity and 91% specificity was achieved from 71 *H. pylori*-positive patients (of 203 subjects).[70] However, five patients were considered to be 'false negative' on endoscopic tests for *H. pylori* due to confounding factors (e.g. recent antimicrobial use, extensive metaplasia). Upon correctly including these five patients in the calculation, sensitivity rose to 89% and specificity to 91%. Using the same antigen (from the *H. pylori* strain NCTC 11637), an attempt to increase sensitivity and specificity as well as to use in saliva as an assay was found to be valuable in assessing successful

eradication (Paper 1.23). Regional variation was demonstrated and some value noted for an IgG2 *H. pylori* antibody assay, though no clear practical advantage over the IgG assay was found. In Paper 1.22 another important clinical use of the antibody test was to improve diagnostic power through the use of a combination of urease, histology, culture and serology. Each test contributed to the attainment of a 'gold standard' of accuracy of diagnosis, particularly in the era of PPI's.

In paper 1.25, a new endoscopic indicator was presented which detected the presence of *H pylori* infection in the mucosa.[71] The endoscopic appearance of distinctive 'red spots', particularly in the fundus, turned out to be extremely useful in the era of PPI's, where urease testing and urea breath tests may both provide false-negative results. In these situations, the appearance of classic 'red spots' could reveal a patients' positivity. Following the eradication of *H. pylori,* the 'red spots' were seen to disappear. This novel endoscopic indicator was researched over many years, with long-term data collection, and assessments into its sensitivity and specificity in detecting *H. pylori* infection. It was observed that while these 'spots' are not uniformly observed in all *H. pylori* infections, they exhibited considerable diagnostic utility when present. In this sense, the 'red spots' indicator is similar to that of the endoscopic pattern of antral nodularity, which is considered highly predictive of *H. pylori* infection.[75] As it remains a 'work-in-progress', an abstract only is available to date.[71]

In paper 1.26, a novel mucosal Urease Test device is described. The original urease test, the CLO-Test, was developed by Marshall and commercialised by Pharmacia Upjohn. However it had a number of disadvantages. By design, the incubation medium is solidified by including a gelling agent. This medium also contains urea, a buffer, and phenol red which turns pink when ammonia is released from urea by the action of bacterial urease, causing a pH rise above 6.0. The system is buffered so that contaminated specimens do not cause a rise in pH and a false-positive result, however it requires refrigeration and protection to prevent dehydration of the gel. When specimens are first inserted into the CLO-Test gel they may have a slight pink tinge due to the presence of blood or alkaline bile. The analyst is required to record the specimens' initial appearance. A positive test is recorded only if the pink color increases in intensity or area. With most *H. pylori* infections now occurring in developing countries with little funding, higher temperatures and few refrigeration methods, the utility of the CLO-Test becomes compromised. Such improvements to these issues were included in the simplified, low-cost, dry Urease Test developed at CDD. It is simple to use and can withstand temperature extremes. It has commercial

applications and is described in CDD patents (Appendix 1 – US patents 6,649,360 and 20130273583).

Comment: These endoscopic diagnostic methods and the collaborative serology studies were pivotal in improving diagnostic accuracy and helped establish gastric urease and serological assays as standard diagnostic procedures, each with their own niche or role in the management of *H. pylori* infection.

**(b) INFECTION RECURRENCE AFTER *H. PYLORI* ERADICATION**

**Question: "What is the chance of my catching Helicobacter again?"**

**PAPER 1.27**

*Helicobacter pylori* reinfection rate in patients with cured duodenal ulcer. Borody TJ, Andrews P, Mancuso N, *et al. Am J Gastroenterol* 1994; 89: 529-532.[76]

Comment:

The study of *H. pylori* reinfection extended upon earlier short-term studies focusing on duodenal ulcer 'cure' following effective *H. pylori* eradication. Ninety-four subjects who had been previously treated 4-8 years earlier for *H. pylori* infection, underwent re-screening using the $^{14}$C-urea breath test. Those patients who were positive on breath test underwent further endoscopic examination for confirmation of infection. Only 2 patients (2.2%) were found to be *H. pylori* positive, providing an effective re-infection rate of 0.36% per patient per year. One patient exhibited normal mucosa, the other had duodenitis without ulceration. The timing of this study was significant (1994) as doubt still remained at that time as to whether eradication could 'cure' duodenal ulcers and 'change the natural history' of the disease,[77] with the strongest evidence of 'cure' following patients up to 6 years (see Paper 1.2).[3]

This publication addressed the common question following *H. pylori* eradication – is reinfection likely? This is not only significant from a patient perspective, but is also of relevance to the clinician who is now able to reassure the patient using published data. In 1994, using long-term follow-up data up to 8 years post-*H pylori* eradication, we described an extremely low reinfection rate of 0.36%/ year.[76] Later published series regarding reinfection generally had short follow-up periods, which became problematic if the follow-up data included the first 12 months post-treatment. We

now know that recrudescence or regrowth of the occult, original non-eradicated *H. pylori,* which was often included in the data, provided a false, high 'reinfection rate'. Results of our work were confirmed in 2006 by a similar, albeit larger, study conducted by Cameron *et al.* using a 1.5-14 year follow-up period.[78] Appropriately, this group also removed the first year post-treatment 'recrudescence' cohort and published a reinfection rate of 0.4%/year, confirming our original findings 12 years earlier.

**(c) MUCOSAL CHANGES DURING *H. PYLORI* AND AFTER ITS ERADICATION**

**Question: "Could pre-cancerous mucosal changes improve after eradication of *H pylori?*"**

**PAPER 1.28 [Abstract]**

Triple Therapy of *C pylori* can reverse hypochlorhydria. Borody T, Noonan S, Cole P, *et al. Gastroenterology* 1989; 96: A53.[79]

**PAPER 1.29**

Apparent reversal of early gastric mucosal atrophy after triple therapy for *Helicobacter pylori*. Borody TJ, Andrews P, Jankiewicz E, *et al. Am J Gastroenterol* 1993; 88: 1266-1268.[80]

**PAPER 1.30 [Abstract]**

Occult *Helicobacter pylori* infection: Detection and Treatment. Borody TJ, Pearce L, Shortis NP, *et al. Gastroenterology* 1996; 110(4): 68.[81]

**PAPER 1.31**

Eradication of *Helicobacter pylori* may not reverse severe gastric dysplasia. Borody TJ, Clark IW, Andrews P, *et al. Am J Gastroenterol* 1995; 90: 498-499.[82]

Comment: The 1.28 abstract demonstrates a lesser-known, minor pathogenic component *H. pylori* induces in its environment to promote its survival – hypochlorhydria – a deficiency in hydrochloric acid production – and how effective eradication successfully reverses this low acid secretion, normalising the pH to 1-2. This environment contributes to the bacterial production of nitrosamines and carcinogenesis. Therefore, the earlier in life *H. pylori* is eradicated the fewer the pre-cancer mucosal changes which develop. In the 1.29 publication, we reported on the early

reversal of gastric dysplasia following *H. pylori* eradication. In response to *H. pylori* infection, mucosal changes develop, and patients become predisposed towards cancer development (adenocarcinoma and MALT lymphoma). In pre-cancerous mucosal changes such as intestinal metaplasia and atrophy, the mucosal environment becomes less hospitable for *H. pylori* survival. As such, *H. pylori* rarely becomes detectable in these settings. Serology testing therefore becomes crucial to detecting the 'vestige' of *H pylori* infection this context. In 1.30 the importance of serology is demonstrated when other diagnostic methods have been unable to detect the low level of infection. Serology titres are also shown as the monitoring method for the eradication of occult *H. pylori* and subsequent diminution of precancerous mucosal changes.

In 1.31 the mucosal changes had progressed to an irrevocable level, and in spite of successful eradication of the carcinogenic bacteria, intra-mucosal gastric carcinoma developed.

**(d) RELEVANCE OF SMOKING ON DUODENAL ULCER RELAPSE/CAUSALITY**

**Question: "Does smoking contribute to the cause of duodenal ulcer disease in the absence of *H pylori*?"**

**PAPER 1.32**

Smoking does not contribute to duodenal ulcer relapse after *Helicobacter pylori* eradication. Borody TJ, George LL, Brandl S, *et al. Am J Gastroenterol* 1992; 87(10): 1390-1393.[29]

Comment: Smoking had long been linked to less favorable clinical outcome, poorer response to treatment and higher relapse rates in patients with duodenal ulcers. The development of triple therapy which was able to reliably eradicate *H. pylori*, provided an opportunity to examine smokers' response to therapy and relapse characteristics. Of 197 patients who were enrolled in the study, 80 (41%) were smokers. In smokers with duodenal ulcers, after eradication of *H. pylori* ulcers were shown to not recur. This indicated that smoking alone is not contributory to duodenal ulcer relapse.

**(e) THE ADVERSE EFFECT PROFILE OF TRIPLE THERAPY**

**Question: Will the side effects be too severe for the cure of ulcer disease to be used generally?**

**PAPER 1.33 [Abstract]**

Adverse effects of triple therapy. Borody TJ, Brandl S & Andrews P. Recent advances in chemotherapy. Munich: Futuramed Publishers 1991; 632-633.[83]

**PAPER 1.34**

Proposal for use of a standard side-effect scoring system in studies exploring *Helicobacter pylori* treatment regimens. De Boer WA, Thys JC, Borody TJ, *et al. Eur J Gastroenterol & Hepatol* 1996; 8(7): 641-643.[84]

Comment: The adverse effect profile of various potential eradication regimens was of importance, particularly when viewed in the context of the required therapy duration for many regimens. This profile was anticipated as likely affecting compliance and treatment selection. The incidence and characteristics of side effects of the three eradication regimens developed by our group (triple therapy, quad therapy, and later rifabutin-based rescue therapy not addressed her) were generally minor (and later reduced further by the addition of a PPI) and detailed in publications listed. However the lack of a standard reporting system limited the ability to relatively compare adverse event profiles. The collaborative paper (1.34) served to address this topic and identify the major works in the field and the international collaboration between CDD and these groups that existed by the mid 1990's.

**(f) ULCERS WITHOUT *HELICOBACTER PYLORI***

**Question: "What are the causes of ulcers where *H. pylori* is absent?"**

**PAPER 1.35**

*Helicobacter pylori*-negative gastric ulcer. Borody TJ, Brandl S, Andrews P, *et al. Am J Gastroenterol* 1992; 87: 1403-1406.[85]

**PAPER 1.36**

*Helicobacter pylori*-negative duodenal ulcer. Borody TJ, George LL, Brandl S, *et al. Am J Gastroenterol* 1991; 86(9): 1154-1157.[86]

Comment: In the 1980's, study of peptic ulcer disease was dominated by investigation and management of *H. pylori* infection. In 1990 we designed an epidemiological study to assess the mechanisms responsible for "*H. pylori* negative" duodenal and gastric ulcers. In 302 consecutive patients with duodenal ulcer, 284 (94%) had *H. pylori* gastritis. All but one of the 18 subjects

identified as "*H. pylori* negative" had an identifiable factor: Non-steroidal anti-inflammatory drugs (8), recent intake of antibiotics (4), infection with *Gastrospirillum hominis* (now *Helicobacter heilmanni)* (2), Crohn's disease (2), and penetrating pancreatic cancer - being the main factors. While this study serves as a central reference point in the changing patterns of *H. pylori* infection, it also emphasizes that duodenal ulcer in the absence of *H. pylori*, at the time of publication, signals unusual pathology such as NSAIDs or pancreatic cancer, for example. Such ulcers now require further investigation including biopsy of the ulcer edge, pancreatic imaging, and Crohn's disease investigations, distinct from recent antibiotic and NSAID use history.

Similarly, causes of "*H. pylori* negative" gastric ulcers had not been systematically studied. Of 115 consecutive patients with endoscopic diagnosis of a gastric ulcer, 71 (62%) had *H. pylori* infection – of this group 47 (66%) had no other detectable cause, 21 (30%) regularly took non-steroidal anti-inflammatory drugs and 3 (4%) had a carcinoma. Of the 44 "*H. pylori* negative" patients, 29 (66%) were taking non-steroidal anti-inflammatory drugs, 2 (5%) had malignant disease, and 13 (30%) had no identifiable cause. Thus of all these gastric ulcers 11% were 'idiopathic.' Again this study provided a useful baseline to examine change over time as *H. pylori* infection becomes less common, but importantly it *reinforces the need to identify the cause of gastric ulcers*, as this may dictate treatment choice. Unlike duodenal ulcers, malignancy is the feared complication of *H. pylori*-infected gastric ulcers.

**(g) DUODENAL CAP STRICTURE COMPLICATION**

**Question: "Can duodenal cap stricture spontaneously open after *H pylori* cure?"**

**PAPER 1.2 [discussed above]**

Cure of duodenal ulcer after eradication of *Helicobacter pylori*. George LL, Borody TJ, Andrews P, *et al. Med J Aust* 1990; 153(3): 145-149.[3]

**PAPER 1.38**

Panel Discussion: Short-term and Long-term consequences of gastritis and duodenitis. Axon AR, Borody T, Dixon M, *et al. Eur J Gastroenterol & Hepatol* 1992; 4(Suppl 2): S53-64.[87]

**PAPER 1.39 [Letter]**

Re: W. de Boer: Gastric Outlet Obstruction and *Helicobacter pylori*. Borody TJ, Bampton P, Moont M, *et al. Am J Gastroenterol* 1997; 92(9): 1576-7.[88]

Comment: During the *H. pylori* pandemic last century, duodenal bulb stricture, a complication of duodenal ulcer, often called for balloon dilatation and surgical intervention.[89] As early as 1990 (Paper 1.2) our group began observing slow, progressive, and spontaneous dilatation of duodenal cap strictures following *H. pylori* eradication. This was later brought to the key opinion leaders in this field (Paper 1.38 )[87] during a published 'Panel Discussion'. Later, a formal letter was published in the American Journal of Gastroenterology (Paper 1.39)[88] in response to Perng *et al.* (1996) to minimise unnecessary surgical intervention.[89] In essence, it was our group which first identified the progressive opening of duodenal strictures after eradication of *H. pylori.*

## D. DISCUSSION OF SECTION 1

This section assembles peer-reviewed papers which record contributions to the management and understanding of *Helicobacter pylori* disease between 1984 and 2006.

At the core of these clinical studies has been the *development of three therapeutic strategies* that have enabled the establishment of a benchmark for primary eradication, in excess of 90%. The original regimen was a bismuth-based antibiotic mix ('triple therapy") followed by "quad therapy" which included a proton pump inhibitor to a dose/duration modified triple therapy.

With 'triple therapy' available as the first reliable treatment to eradicate *H. pylori* in over 90%, this provided a *tool to prove for the first time the cure of duodenal ulcers,* with a very low recurrence rate.

More recently the "*rescue therapy" based on rifabutin*, attained eradication in up to 96% in the failed eradication group. An international discussion occurred as to whether the most effective regimen be used as 'first-up' or 'rescue' therapy, with our group strongly supporting the best available therapy (bismuth-based quad therapy) as initial treatment. This view was based on concern for an increasing pool of drug-resistant bacteria in the community and our observations of a mucosal immune deficit in those patients who fail first-line eradication therapy. A 2014 treatment review for *H. pylori* by Professor David Graham was quoted as stating that, over 30 years, increasing bacterial resistance (specifically to clarithromycin) has rendered most 'short term' strategies obsolete, and that bismuth-based 'quad' therapies are the recommended first-line treatment, with rifabutin-based therapy a valuable 'escape' therapy. Studies from our group refined the original bismuth-based 'triple therapy' and demonstrated an additive benefit with omeprazole.

Finally numerous clinical observations are listed as *ancillary achievements* which were largely possible due to the availability of an effective *H. pylori* therapy.

Many questions remain unanswered – these include clarification of the factors encouraging progression to dysplasia and carcinoma, and relationship with non-ulcer dyspepsia.

## APPENDIX 1 – PATENTS EMERGING FROM THIS WORK

**1. TRIPLE THERAPY: US PATENT # 5,196,205 [HELIDAC]**

This is a composition matter patent describing the combination of bismuth, tetracycline and metronidazole. Commercialized as a compliance-enhancing pack by Procter&Gamble. [Figure 2a. Appendix 3]

**2. QUAD THERAPY: US PATENT # 5,476,669 [PYLERA]**

This is a composition and use patent describing among others, the combination of triple therapy above with added proton pump inhibitors. Commercialized by Axcan Pharma. [Figure 2b. Appendix 3]

**3. RIFABUTIN RESCUE THERAPY: US PATENT # 6,489,317 [HELICONDA]**

This is a composition and use patent describing combination of rifabutin, amoxicillin, and a PPI. Commercialized by RedHill Biopharma obtaining rights from Giaconda Ltd, the Australian ASX listed public company raised via an IPO to commercialize CDD intellectual property.

**4. UREASE TEST KIT: US PATENT # 6,649,360. ALSO US APPLICATION # 20130273583**

This is an application of a refinement of the granted ‘360 patent the latest version of the product is described in the US Application. Not yet commercialized. [Figure 3. Appendix 3]

## APPENDIX 2 ANTIBIOTIC SCHEDULE

| Antibiotic Schedule | Strength | Dose | Duration | Strength | Dose | Duration | Strength | Dose | Duration | Strength | Dose | Duration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Colloidal bismuth subcitrate | | | Tetracycline hydrochloride | | | Metronidazole | | | | | |
| | 108mg | qid | 28 days | 250mg | qid | 28 days | 200mg | qid | 10 days | | | |
| B | Colloidal bismuth subcitrate | | | Tetracycline hydrochloride | | | Metronidazole | | | Omeprazole | | |
| | 108mg | 5 times/ day | 12 days | 250mg | 5 times/ day | 12 days | 200mg | 5 times/ day | 12 days | 20mg | b.d. | 12 days |
| C | Clarithromycin | | | Amoxicillin | | | Omeprazole | | | | | |
| | 500 mg | bd | 7 days | 1000 mg | bd | 7 days | 20 mg | bd | 7 days | | | |
| D | Rifabutin | | | Amoxicillin | | | Pantoprazole | | | | | |
| | 150mg | daily | 12 days | 1-1.5g | t.d.s. | 12days | 80mg | t.d.s. | 12 days | | | |
| E | Bismuth subcitrate | | | Tetracycline hydrochloride | | | Metronidazole | | | Omeprazole | | |
| | 108mg | 5 times/ day | 12 days | 250mg | 5 times/ day | 12 days | 200mg | 5 times/ day | 12 days | 20mg | bd | 12 days |

## APPENDIX 3


**Figure 2.a.** Helidac® therapy


**b.** Pylera® therapy

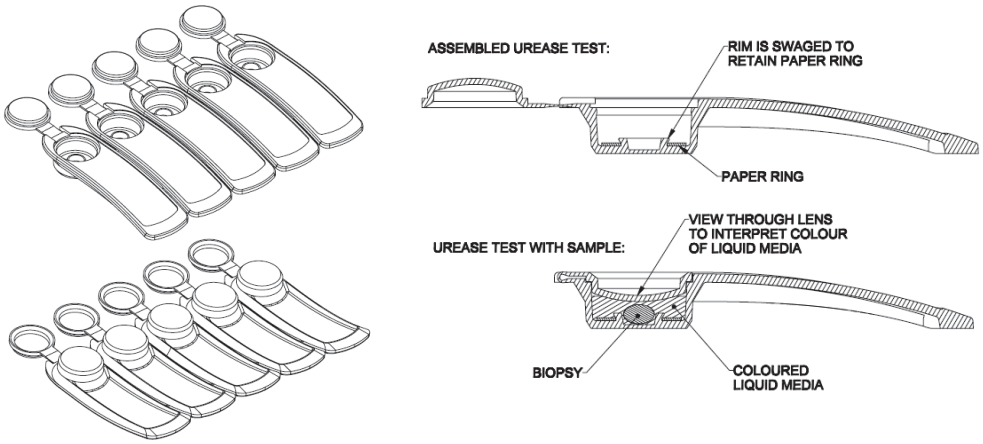


**Figure 3.** Urease Test Kit

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# SECTION 2

## CROHN'S DISEASE (CD) AND *MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS* (MAP)

### A. DEVELOPMENT AND PROLONGED TREATMENT OF CROHN'S DISEASE PATIENTS WITH A NOVEL 'ANTI-MAP' THERAPY

**(a) OVERVIEW AND CONTENT: Major Question: "What Anti-MAP therapy will heal CD in majority of patients when administered for a prolonged period?" – The Development of 'Anti-MAP Therapy'**

The work summarised in this section followed the critical studies conducted on *H. pylori*, and comprised an extension of the notion that gastrointestinal inflammation is microbial-driven, and therefore eradication of the putative pathogen would theoretically lead to disease cure. As with *H. pylori* and peptic ulcer disease, the hypothesis that a specific pathogen was responsible for Crohn's Disease (CD) was counter to the accepted theory of causality at the time. Podolsky (2002) best summarised this theory, concluding that "inflammatory bowel disease is thought to result from inappropriate and ongoing activation of the mucosal immune system by the presence of normal luminal flora".[1]

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) was first identified in 1895 as the cause of chronic inflammation of the intestine in cattle. (Johne HA & Frothingham L. Ein eigenthuemlicher Fall von Tuberculose beim Rind. Deutsche Zeitschrift fur Tiermedicine und Pathologie 1895;21:438-54 – Quoted in Ref 2). Since first described, infection and disease have been mainly found in domestic livestock but MAP can also cause disease in a number of animal species, including primates.[2] Soon, interest into MAP as a possible infectious agent in CD gained momentum when Chiodini *et al.* cultured MAP from three patients with Crohn's Disease.[3] Since then, a wealth of

studies have detected MAP involvement in human intestinal inflammation in CD, by long-term culture and from PCR detection.[4-6]

Early studies pointed to CD improvement in patients treated with newer antibiotics targeting atypical Mycobacteria, which became available due to the HIV complications.[7] However due to the difficulties with reproducibility using current detection methods and MAP positivity in non-CD patients, controversy remained as to its association with CD or whether this pathogen was merely an "innocent" bystander in CD.[8] At the onset of these studies, available data were limited by our knowledge of the pathogen at that time. Early Anti-MAP therapies had been developed but were limited to anti-TB drugs, were 'double therapies', or were not standardised, and had only been subject to short-term follow-up. No pathogenic mechanisms were available outside of the animal model of Johne's Disease, which differed in some aspects from the disease in CD. The studies conducted by our Centre were designed to address these issues:

(i) to determine whether protracted therapy with multiple antibiotic regimens constructed to clear MAP infection, induced profound reversal of mucosal inflammation or cure of CD, and

(ii) to measure whether therapy targeting MAP infection down-regulated surrogate markers of a sequence of events that linked MAP with mucosal inflammation.

**PAPER 2.1**

Treatment of severe Crohn's disease using antimycobacterial triple therapy – approaching a cure? Borody TJ, Leis S, Warren EF, *et al. Digest Liver Dis* 2002; 15(34): 29-38.[9]

First published in abstract form in 1998[10] and 2000[11], this publication sought to report long-term observations in patients with severe CD treated with triple macrolide-based antimycobacterial therapy, consisting of rifabutin, clarithromycin and clofazimine for up to 54 months of therapy.[9] The rationale for therapy selection was explained, as instructed by recent *in vitro* and *in vivo* experience with treatment of mycobacterium avium complex (MAC). Contemporary data on the presence of MAP (detected by PCR) and antibody to MAP in CD, were also discussed. The key points from this open and prospective study included:

(i) *selection of subjects with severe obstructive/penetrating disease* and active inflammation, not responding to standard therapy (salazopyrin and analogues, cytotoxic drugs and corticosteroids), i.e., a patient population with well-documented severe disease not responding to

standard therapy were included in the intervention study, with patients acting as their own controls.

(ii) *Follow-up data extending for up to 54 months.* Eight of the 12 patients (66.7%) experienced complete clinical remission – 6 of the 8 (75%) had complete histological remission [deep mucosal healing]. Of these 6 patients, four were able to cease all treatment, 3 of whom remained in complete remission for up to 26 months. In five patients, strictures in the terminal ileum dilated and the bowel returned to normal, and of the patients in partial or complete remission, the Harvey Bradshaw activity index fell from 13.4 to 0.5 ($p < 0.001$) after 52 – 54 months.

DISCUSSION

Review of earlier anti-tuberculosis drug regimens trialed between the 1970's and 1990's provided conflicting results, which we now know were only partially effective against intracellular atypical mycobacteria. Several important additional clinical observations were made in this publication including the complete reversal of tight CD strictures, healing of deep ulcers and disappearance of pseudopolyps, a hallmark of inflammation. The reversal of disease activity in patients who had failed years of standard therapy, and the extended duration of remission *with the use of antibiotics*, supported a bacterial aetiology in Crohn's Disease, and that this combination anti-mycobacterial therapy positively altered the natural history of Crohn's disease.

Further controlled studies were required to consolidate the value of combination antibiotic therapy, and to link CD pathogenesis to MAP infection. Unlike *H. pylori* trials, anti-MAP trials were much more complex, using expensive drugs for prolonged periods of time, which required external funding not then available.

**PAPER 2.4**

Anti-mycobacterial therapy in Crohn's Disease heals mucosa with longitudinal scars. Borody TJ, Bilkey S, Wettstein AR, *et al. Digest Liver Dis* 2007; 39: 438-444.[12]

This study extended the experience base using combination antibacterial therapy in 52 subjects and provided details of a unique pattern of repair of full thickness bowel wall lesions in CD. These observations were initially reported as an abstract [paper 2.5].[13]

A subset of 34 patients treated with this combination of antibiotics between 6 months and 9 years led to remission with a reduction in the Crohn's Disease Activity Index to >70 in each patient.

Withdrawal of the antibiotic treatment in patients with complete remission led to a recurrence of disease over months or years [paper 2.6] indicating the presence of a slow-growing pathogen.[14]

The primary purpose of this publication was to demonstrate the unique repair process experienced in those patients treated with anti-mycobacterial antibiotics. Of the 39 patients who underwent a follow-up colonoscopy, 22 (56%) healed, with a scarring process including the appearance of branching, linear and raised scars, which appeared to disappear with time (**Figure 1**).

The macroscopic scarring observed correlated with marked reduction of inflammation in mucosal biopsies. Within this population of treated subjects, 6 had fistulae linked to colonic Crohn's Disease and had had at least 12 months follow-up. Four of 6 on combination antibiotic therapy closed their fistulas (most were also curetted to encourage closure) [paper 2.7].[15]

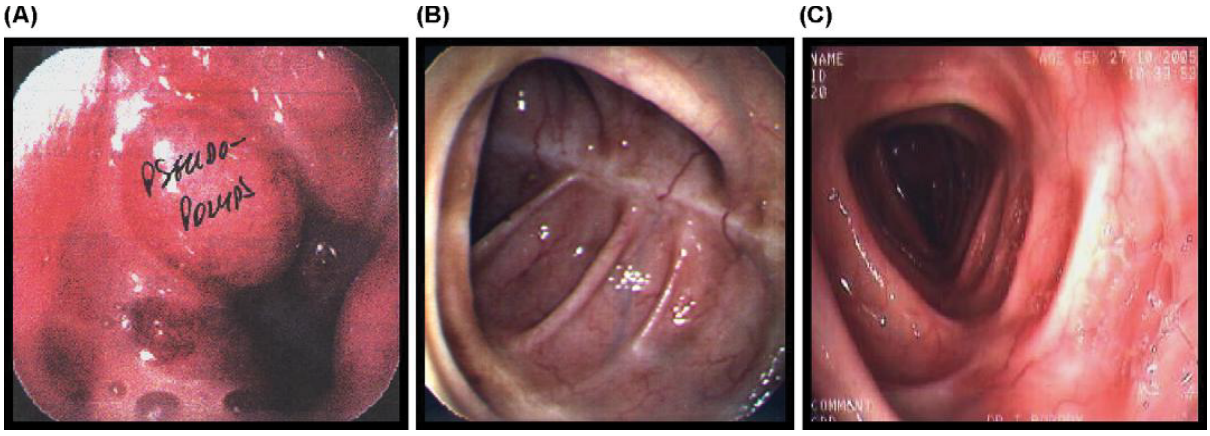



**Figure 1.** Regression of pseudopolyps in the descending colon in a patient on anti-MAP therapy. (A), Before anti-MAP therapy – severe inflammation with contact bleeding and pseudopolyps; (B), 1 year on anti-MAP therapy – elevated longitudinal scarring with haustrations pointing to the scar; (C), 2 years on anti-MAP therapy – The scar has now become a faint line lacking distinct elevation from the mucosal wall, suggesting progressive softening of scar tissue.

**PAPER 2.14** [Recently submitted Abstract]

Antibiotics only for 'Treatment-Naïve' Crohn's Disease. Borody TJ, Ramrakha S, Sharma R, *et al.* Submitted to the Am College of Gastroenterol Annual Meeting. Oct 2015.[16]

This protracted study is the natural progression towards the use of Anti-MAP therapy being used only in patients previously not exposed to any CD treatment. The idea is to prevent development of MAP resistance to Anti-MAP antibiotics. It took several years to accrue the 7 patients as newly-diagnosed CD is rare to see in clinical practice, most patients being referred at a time when they

have failed most therapies. Even with these small numbers of CD patients it is clear that rapid and profound healing occurs with MAP antibiotics alone, indicating clearly that CD is Mycobacteria-driven. The Results and Table below are taken from the Abstract as is the pre- and post-treatment photograph of a patient colon. (**Figure 2.**) The next phase of work in the MAP area is to enlarge the observations below to a formal controlled trial as envisaged in the 'Conclusions' below in italics – Anti-MAP needs to trialed as 'first line' treatment of CD vs 'standard-of-care' anti-inflammatory and generally immune-suppressive treatment, to better appreciate the primacy of antibiotics in CD.

**Results:** Mean ($\bar{x}$) CDAI score decreased significantly from 354.4(SEM 28.8) prior to treatment to 64.1(SEM 7.3) - $p < 0.001$ (CI=95%) in the seven patients (3 male; 4 female; 12-31 y). All patients achieved remission (CDAI<150) between 4-21 weeks (Table). Follow-up colonoscopy showed healed ulcers and mucosa often replaced by a fine white scars, disappearance of contact bleeding and histological deep mucosal healing.

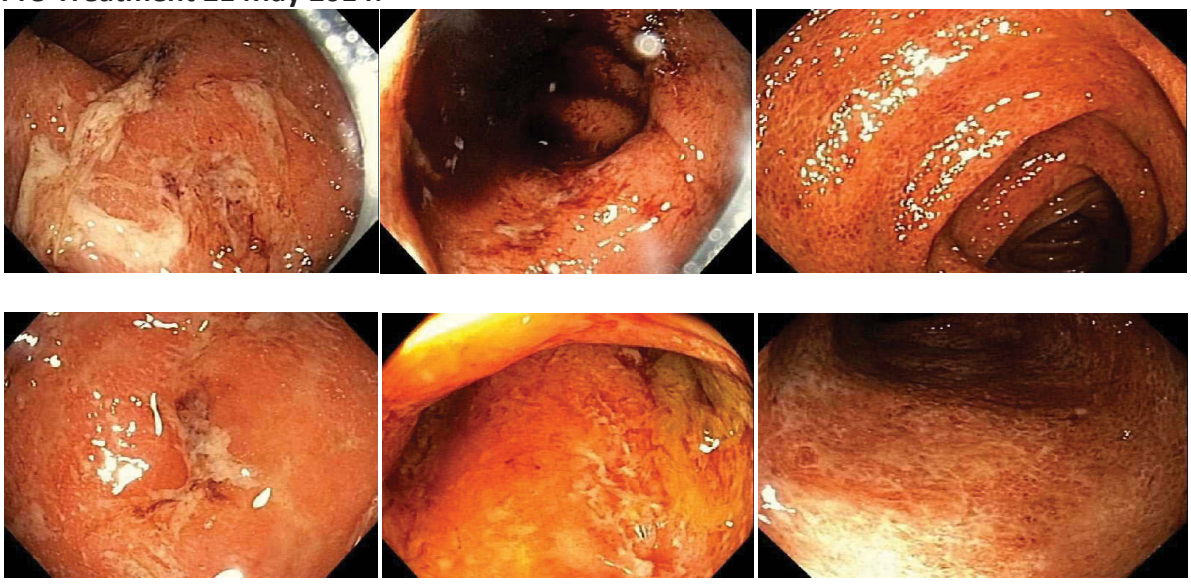
**Table 1.** Mean and Standard error of Mean (SEM) Indicators of Crohn's disease healing

| | **Pre Treatment** | **Post Treatment** |
|---|---|---|
| **CDAI** | 354.4 (SEM= 28.8) | 64.1 (SEM= 7.3) |
| **CRP (mg/l)** | 40.3 (SEM=19.0) | 3.4 (SEM=0.7) |
| **ESR (mm/hr)** | 16.4 (SEM=3.9) | 10.6 (SEM=4.0) |
| **Ferritin (ug/l)** | 73 (SEM=36.3) | 121.7 (SEM=40.2) |
| **Haemoglobin (g/l)** | 126.1 (SEM=4.8) | 138.9 (SEM=5.8) |

The $\bar{x}$ and SEM values were calculated to n=7. The before and after values for ESR, CRP, Ferritin and Haemoglobin were taken from the final blood results and compared to the initial. CDAI scores were calculated at the initial consultation and compared to the first CDAI calculated that identified remission.

**Conclusions: 1.** Rapid healing to remission in CD in all patients using **antibiotics alone** suggests CD is infection-dependent. **2.** *Our results suggest the need for an RCT comparing initial therapy in CD with Anti-MAP antibiotics vs 'standard-of-care' treatment.*

**Pre-Treatment 21 May 2014:**



**Post-treatment 18 March 2015:**

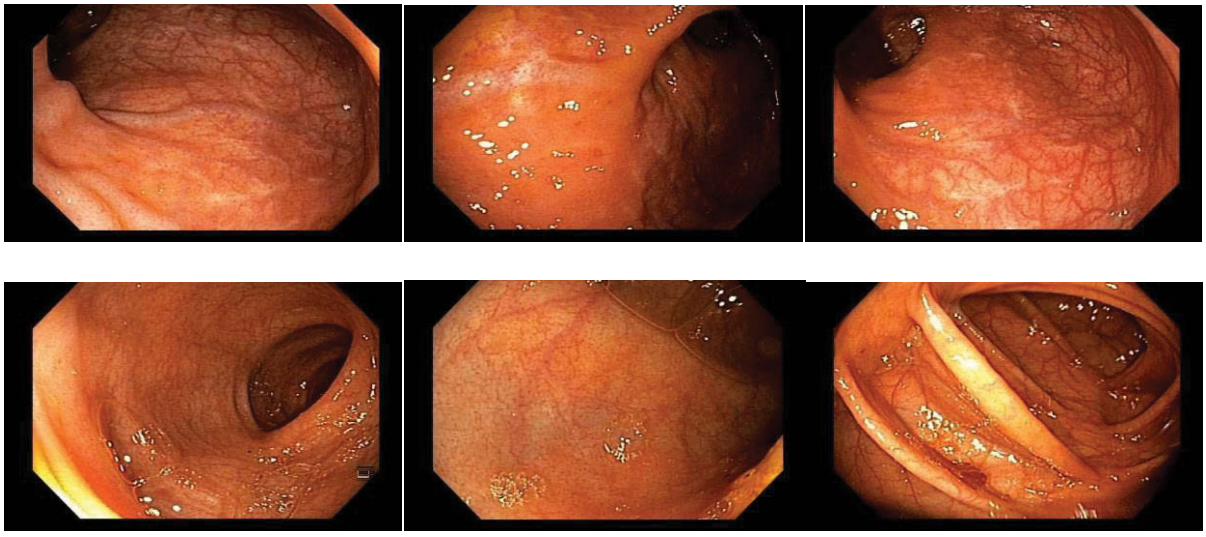


**Figure 2.** Pre and post-treatment appearance of colonic mucosa showing the complete healing of CD involved colon after the use of anti-MAP antibiotics only.

## B. ANTI-MAP AND MARKERS OF INFLAMMATION

**(b) Study of Surrogate Markers of Inflammation. Major Question: "Will therapy of CD with Anti-MAP down-regulate surrogate markers of mucosal inflammation?"**

**PAPER 2.8**

Selective Th2 pattern of cytokine secretion in mycobacterium avium subspecies paratuberculosis infected Crohn's Disease. Ren Z, Turton J, Borody T, *et al. J Gastroenterol Hepatol* 2007; 23: 310-314.[17]

A role for MAP in the pathogenesis of CD remained contentious despite mounting clinical data demonstrating a significant clinical response to antibiotics targeting intracellular atypical mycobacteria (data and discussion above). If MAP were to contribute to the transmural inflammation characteristic of CD, shifts in host immunity and inflammation parameters would be expected to correlate with the presence of MAP. This study set out to ask that question, with respect to the pattern of cytokine secretion from circulating T lymphocytes. Several main points of study are emphasized:

- A large number of subjects with CD (46), disease controls (52), and normal controls (18) were studied to ensure sufficient power to analyse the impact of MAP presence on cytokine secretion profiles.
- The method used to study the pattern of cytokine secretion, detected secretion of cytokines from CD4 +ve T cells committed to that pattern, with secretion amplified by ligation of CD40L on the surface of the T cell by CD40 expressed on the surface of platelets contained in the culture.
- Significantly higher levels of IL-4 and IL-2 (each $p < 0.05$) were found to correlate with MAP-positive CD compared with MAP-negative CD.
- Both MAP-positive CD patients and controls (both 'diseased' and 'normal'), possessed similar levels of secreted cytokines compared to MAP-negative CD patients.
- Cytokine secretion levels in MAP-negative CD did not differ from control groups.

DISCUSSION

A significant skew in circulating CD4+ T cells was observed in MAP-positive CD patients towards a Th2 pattern. This is the first evidence supporting a pathogenic impact of infection with MAP, thus a role in promoting active CD.

A second study performed at the CDD asked whether the presence of MAP within CD mucosa-influenced the pattern of cytokine secretion, using weighed biopsies as "organ cultures."

**PAPER 2.9**

Molecular evidence of mycobacterium avium subspecies paratuberculosis (MAP) in Crohn's Disease correlates with enhanced TNF-2 secretion. Clancy R, Ren Z, Turton J *et al. Digest Liver Dis* 2007; 39: 445-451.[18]

The aim of this study was to further evaluate whether the presence of MAP contributed to the pathogenesis of CD by promoting cytokine secretion within the gut mucosa. Again, large numbers of patients with CD (63), disease controls (98) and normal controls (74) were studied to ensure appropriate power of the study. Important findings were:

- Only 24% of those with CD had MAP detected – this was not significantly greater than controls (11% - 15%). However, these numbers may have been affected by current or previous antibiotic therapy.
- TNF$\alpha$ was the only significantly increased cytokine in CD compared to control groups.
- In all control groups, there was no difference in cytokine secretion between MAP-positive and MAP-negative patients.
- In CD, MAP-positive patients secreted twice the amount of TNF$\alpha$ ($p < 0.05$) than MAP-negative patients. [TNF$\alpha$ secretion in MAP-positive patients on combination antibiotic therapy, was 40% less than in untreated patients who were MAP positive (not significant)].

## DISCUSSION OF SECTION 2

Based on our previous studies of *H.pylori*, which proved the direct and incontrovertible relationship between infection and inflammation using 'triple therapy' antibiotic strategies, similar studies of therapeutic regimens in CD began in the mid 1990's. The modern argument contending that MAP was a specific aetiological agent in CD presented an attractive research hypothesis. At this time, the gut microbiota was suspected of playing a pivotal role in the pathogenesis of CD, but whether this commensal bacteria contribution was 'specific' or 'non-specific', was vigorously argued. The notion that CD resulted from an abnormal reaction to commensal bacteria, gained acceptance after 2000, not because of persuasive evidence in its favour, but rather due to a lack of definitive evidence supporting a critical role for 'specific' bacteria (in particular, MAP).

Support for MAP as an aetiological agent in CD were derived from four lines of evidence:

(i) MAP, the known causative agent of Johne's Disease in cattle and other animals, was postulated as a model for human disease a century ago.[19]

(ii) MAP had been isolated from CD and had then been successfully transmitted to goats by Chiodini *et al*. (1984), thereby fulfilling Koch's postulates.[20]

(iii) MAP was not originally readily isolated from CD tissue, nor could it be identified using standard stains. However, following detection of a specific insertion element (IS900) in MAP DNA, Sanderson *et al.* (1992) was able to detect MAP in CD tissue.[4] These data are important as they reflect an 'average' of more recent results: non-inflammatory gut disease 12%; chronic ulcerative colitis 4%, CD 65%. The dilemma was, that by 2000, there were also studies that had failed to detect PCR-positive CD subjects. Technical difficulties with this new detection method were numerous and heterogeneous, with variations in sensitivity and specificity, which were later clarified by Bull *et al.* (2003).[6] One particular obstacle was the focal nature of infection – variable PCR results were obtained from multiple biopsies taken together while others using *in situ* hybridization with IS900, and found MAP clustered within granulomas. In our studies we optimized sensitivity and specificity by (a) pooling 2 – 3 biopsies and (b) sequencing PCR positive product.

(iv) Early studies using antibiotic regimens, selected due to *in vitro* and *in vivo* sensitivities effective against *M. avium,* were promising but difficult to assess, due to poorly

characterised patients, limited dosage and restricted drug combination regimens, and a lack of substantive follow-up.

There were three main outcomes of our work in CD. Clinical studies confirmed the value of triple therapy (clofazimine, rifabutin, and clarithromycin) in the doses used administered for ≥6 months, enabled several conclusions. First, approximately 50% of patients treated with the antibiotic combination obtained complete remission, included profound mucosal healing, rarely seen before. However, prolonged remission was dependent upon ongoing antibiotics therapy, with treatment cessation often resulting in clinical relapse within months or several years. This similar phenomenon is often observed with TB, due to the slow-growing nature of mycobacteria, and particularly fits with our knowledge of the growth pattern of *mycobacterium avium paratuberculosis* – considered to be the slowest replication bacterial species known. The degree of disease response based on a particular pattern of scar formation, healing of fistulae, and the degree of clinical remission based upon reduction in the CDAI, surpassed our previous experience with accepted 'anti-inflammatory' therapy and provided further support in favor of the infectious theory of CD. Secondly, specific changes in cytokine secretion in CD patients were skewed to the presence of MAP, characterised by a switch towards a Th2 phenotype and increased mucosal secretion of TNF$\alpha$, the first demonstrations of an association between MAP infection and molecular pathways of mucosal inflammation. Thirdly, we became a participant in an international discussion which was important in tracing the evolution of ideas regarding the role of 'specific' infection in the pathogenesis of CD and the role for combination antibiotic therapy targeting intracellular mycobacteria. These ideas and discussions are best summarised by review of a series of papers contributed to by our group:-

## C. INTERNATIONAL REVIEW DISCUSSION PAPERS

PAPER 2.10 MAP-associated Crohn's Disease, MAP, Koch's postulates, causality and Crohn's Disease Chamberlin W, Borody TJ, Naser S. Dig & Liver Dis 2007; 39: 790 – 794.[21]

PAPER 2.11 Primary treatment of Crohn's Disease: Combined antibiotics taking centre stage. Chamberlin W, Borody TJ, Campbell J. Rev Clin Immunol 2001; 7(6): 751 -760.[22]

PAPER 2.12 Mycobacterium avium ss paratuberculosis-associated diseases. Piecing the Crohn's Disease puzzle together. Gitlin L, Borody T, Chamberlin W, *et al*. *Clin Gastroenterol* 2012; 46: 649 – 655.[23]

PAPER 2.13 The many faces of Crohn's Disease: Latest concepts in etiology. Campbell J, Borody T, Leis S. *Open J Int Med* 2012; 2: 107 – 115.[24]

The publications borne from the international review reflected a shift in thinking towards the notion of MAP as a unique and obligatory pathogen and an important 'driver' of inflammation within a complex framework. At the commencement of the clinical studies outlined above (Papers 2.1 & 2.4),[9] [12] MAP detection PCR methodology was not readily available to the CDD, limiting interpretation of clinical results. Subsequent MAP detection studies (Papers 2.8 & 2.9)[17,18] on our patient population demonstrated that MAP detection was focal in mucosal biopsies, that exposure to MAP was common in both normal and disease controls, but that MAP presence was associated with a distortion of cytokine secretion patterns, including an increase in mucosal secretion of TNF$\alpha$. Further, TNF$\alpha$ secretion in MAP-positive CD patients was reduced in patients when anti-MAP therapy was administered. Taken together, and with recent observations of impaired macrophage function and genetic abnormalities targeting macrophage function and antigen handling, our initial hypothesis was amended to: "CD results from impaired handling of those intracellular bacteria (such as MAP) that require efficient clearance from the macrophages." MAP was found to contribute at a cellular level by:

(i) Switching the dominant 'bias' of cytokine paths away from a Th1 response, that normally operates to maintain optimal phagocyte function

(ii) Being an obligate intra-phagocyte parasite, MAP was capable of inducing an excessive and inappropriate pro-inflammatory cytokine profile (e.g., TNF$\alpha$) once taken up into already defective macrophages.

(iii) Enhancing mucosal permeability through inappropriate IL-4 secretion (a marker of a Th2 response) enabling further influx of gastrointestinal microbiota into the mucosal space.

The current views and studies using 'triple anti-MAP therapy' following the results outlined above, are further examined in the 'discussion papers' (2.10 – 2.13). [21-24] These studies led to the conduct of a global multi-centre trial to assess the practical value of 'anti-MAP triple therapy' as a therapy for patients with CD. The next stage to follow, Anti-MAP as 'First-Line' CD therapy.

# APPENDIX 1 - PATENTS EMERGING FROM THIS WORK

**1. ANTI-MAP TRIPLE THERAPY PATENT - US PATENT # 6,277,836 [MYOCONDA]**

A composition matter patent describing the combination of rifabutin, clarithromycin and clofazimine. Licensed to RedHill Biopharma Ltd, Israel. Pivotal multicentre trials are now continuing to achieve FDA-registered Anti-MAP Crohn's Disease therapy. [ClinicalTrials.gov Identifier NCT01951326]

**2. ANTI-MAP IMMUNISATION THERAPY. US PATENT # 6,551,632**

An immunisation composition and use, however it will not be commercialized at this stage. Assigned to RedHill Biopharma Ltd.

**3. ANTI-MAP TRIPLE THERAPY IN A SINGLE CAPSULE. US PATENT # 8,343,511 [MYOCONDA]**

A composition matter patent describing the combination of rifabutin, clarithromycin and clofazimine, further refined as a single capsule with a new composition to minimise interaction between the three components in the capsule. Licensed to RedHill Biopharma Ltd, Israel. Pivotal multicentre trials are now continuing to achieve FDA-registered Anti-MAP Crohn's Disease therapy using this capsule. This is the same trial as described above - two patents cover this product [ClinicalTrials.gov Identifier NCT01951326].

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# - SECTION 3 -

## FAECAL MICROBIOTA TRANSPLANTATION (FMT)

Data presented in Sections 1 and 2 of the thesis explored the notion that specific infectious pathogens were responsible for two particular gut diseases – *Helicobacter pylori* (duodenal ulcer) and *Mycobacterium avium paratuberculosis* (Crohn's Disease [CD]), respectively. Antibiotic treatment provided the method of hypothesis testing, with the observed clinical outcomes measured.

This section will cover studies, initiated in 1988, which serve to address the problem encountered in clinical practice regarding the lack of treatment response patients with *C. difficile* infection, Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD) exhibit towards standard therapies. Learning from previous experience that long-standing infection is a valid marker of chronic infection, we generated the hypothesis "that the fundamental defect in antibiotic-related diarrhea (with or without *C. difficile*), irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD) is an occult bacterial infection within the gut microbiota, with a co-existing deficiency in regulatory bacteria." The significance of this hypothesis was that therapy aimed at transplanting beneficial "normal" donor gut microbiome would suppress pathogenic dominance, restore the previous deficient components of the microbiome, and minimise the associated inflammation. In 1958, the first review of two case patients with pseudo-membranous colitis (presumed *C. difficile* infection) treated with an infusion of homologous "normal" faecal bacteria emerged, which resulted in cure of both patients.[1, 2] In May of 1988, at CDD the first patient was treated successfully with FMT, and by 1989, we reported the first data from 55 FMT-treated patients.

**PAPER 3.1**

"Bowel flora alteration: A potential cure for inflammatory bowel disease and irritable bowel syndrome?" Borody TJ, George L, Andrews P, *et al. Med J Aust* 1989; 150: 604.[3] In the study, 55 patients were treated with FMT and then followed up. At the time of follow-up, 20 patients were in complete symptomatic remission, 9 were in partial remission and 26 remained unchanged. Symptomatic improvements were noted in IBS, ulcerative colitis (UC) and CD, as well as *C. difficile* infection. Prior to treatment, the index colitis patient had patchy, indeterminate colitis and

constitutes the first reported colitis patient treated with FMT. Following FMT, the patient has remained in remission for 26 years. This is indeed a profound remission, which, given the prolonged period of follow-up may represent the earliest documented cure of colitis.[4]

As these results were published in 1989, the rampant epidemic of *C. difficile*-associated enteritis associated with the appearance of hyper-virulent toxigenic strains such as $NAP_1$ / $B_1$ / 027 could not have been predicted.[5] During the height of the epidemic, in the US alone mortality reached 300 patients a day,[6] with up to 3 million cases diagnosed per year.[7] The underpinning cause of this *C. difficile*-associated syndrome was the unrelenting use of antibiotics over 60 years with damage to the human intestinal microbiome. Markers of this damage are now well recognized, and include reduction of *Bacteroidetes* and *Firmicutes* species in those with clinical infection associated with *C. difficile,*[8] supporting our hypothesis 20 years prior.

## A. DEVELOPMENT AND OPTIMISATION OF FAECAL MICROBIOTA TRANSPLANTATION METHODOLOGY

Throughout the next 26 years of FMT at the CDD clinic improvements to the FMT material and administration methods took place to optimize efficacy in the various indications.

Considerable methodological development occurred to improve and maximize efficacy of colonic re–colonisation. This included refining donor stool blending, filtering, and volume adjustments. Administration via enema, transcolonoscopically and via naso-jejunal route were also examined. The use of a cultured bacteria was also considered, with early studies reporting a defined cultured faecal bacterial consortium of *Bacteroidetes, Clostridia and E coli.*

**PAPER 3.2**

"Modification of the colonic microflora using probiotics: The way Forward?" Pearce L, Bampton PA, Borody TJ, *et al. Gut* 1997; 41(Suppl 3): A63.[9]

**PAPER 3.3**

"Chronic constipation (CC) may be reversed by 'Bacteriotherapy." Andrews PJ, Barnes P, Borody TJ, *et al. Gastroenterology* 1994; 106: A459.[10]

By 2000 the concept of a multi-component cultured, defined microbiota in an encapsulated form had been considered in print:

**PAPER 3.4**

"Flora Power – Fecal bacteria cure chronic *C. difficile* diarrhea." Borody TJ. *Am J Gastroenterol* 2000; 95: 3028–3029.[11]

Discussions regarding the refinement of guidelines surrounding donor selection were held internationally, and a consensus paper published by an international committee set the parameters, which are now accepted worldwide.

**PAPER 3.5**

"Treating Clostridium difficile with Fecal Microbiota Transplantation." Bakken JS, Borody TJ, Brandt LJ, *et al. Clin Gastroenterol Hepatol* 2011; 9: 1044-1049.[12]

The trans-colonoscopic infusion method remained the preferred route of FMT administration as reviewed in the following publication:

**PAPER 3.6**

"Fecal microbiota Transplantation: Techniques, applications, and issues." Borody TJ, Campbell J. *Gastroenterol Clinics North Am* 2012; 41: 781 – 803.[4]

Later an instructional paper describing the methodology in detail was published aimed at nurses, the primary providers of the treatment.

**PAPER 3.7**

Fecal Microbiota Transplantation: A "How to" Guide for Nurses. Leis S, Borody TJ, Jiang C *et al.* Collegian 2014; 280: 1-7.[13]

Currently, as of December 2014, a lyophilised filtered donor microbiota capsule formulation has been developed at CDD (**Figure 1**) for patients with relapsing CDI to allow daily oral dosing, thereby avoiding the need for sedation and colonoscopy. It successfully eradicated CDI in the first 3 patients treated to date.

**Figure 1**. Early example of encapsulated lyophilised filtrate product used successfully to cure CDI at CDD.

## B. STUDIES IN IMPLANTATION, FMT INDICATIONS AND CLINICAL OBSERVATIONS AFTER FMT

The next stages in the development of a clinically acceptable and useful FMT product involved documenting evidence of implantation, extending potential treatment indications, collection of secondary efficacy observations after FMT, and finally, the development of a lyophilised product.

### 1. IMPLANTATION

Implantation of the donor fecal microbiome phenotype was important to demonstrate:

**PAPER 3.8**

"Durable Alteration of the Colonic Microbiota by the Administration of Donor Fecal Flora." Grehan MJ, Borody TJ, Leis S, *et al. J Clin Gastroenterol* 2010; 44: 551-561.[14]

Patients in this study were followed for 24 weeks following an antibiotic plus lavage technique to facilitate clearance of the resident microbiota in an attempt to reduce colonisation resistance. Patients were then administered donor FMT. Using molecular identification methodology, durable implantation of the donor microbiome phenotype was identified up to 24 weeks (study end). Subsequent papers followed which confirmed the implantation of donor microbiota following FMT.[8]

### 2. TREATMENT INDICATIONS

**Treatment of Relapsing *Clostridium difficile***

Relapsing *Clostridium difficile* Infection (R-CDI) is the primary indication for FMT and consistently achieves >95% cure with a single, or 2 FMT infusions. Numerous abstracts and papers have been published from CDD for this indication. One of the earliest champions of FMT in its recorded history, CDD treated its first patient using FMT in 1988. The dramatic impact FMT has had on the clinical outcome in *C. difficile* infection (CDI) is reflected in the results gained at the CDD, which provided international guidance in this area:

**PAPER 3.9**

"Fecal Microbiota Transplantation (FMT) in the treatment of recurrent *C. difficile* infection." Borody, T, Leis S, Pang G, *et al. Up to Date* 2014.[15]

The publication documents the Centre's clinical experience of 81 patients who had failed standard antibiotic therapy for recurrent CDI and underwent FMT. Of 81 patients, 75 (93%) achieved prolonged eradication of *C. difficile*. The published international experience at that time using fecal retention enemas was approximately 80% eradication, as discussed in this paper. Given our success, numerous publications followed on FMT in CDI.

**PAPER 3.4**

"Flora Power – Fecal bacteria cure chronic *C. difficile* diarrhea." Borody TJ. *Am J Gastroenterol* 2000; 95: 3028–3029.[11]

**PAPER 3.10**

"Bacteriotherapy using Fecal Flora." Borody T, Warren EF, Leis S, *et al. J Clin Gastroenterol* 2004; 38: 475-483.

**PAPER 3.11**

"Faecal bacteriotherapy for chronic *C difficile* syndromes." Borody TJ, Leis SM, Chongnan J, *et al. J Gastroenterol Hepatol* 2003; 18(Suppl): B8.[16]

**PAPER 3.12**

"Endoscopic Fecal Microbiota Transplantation: "first-line" treatment for severe *Clostridium difficile* infection." Brandt, L, Borody, T, Campbell J. *J Clin Gastroenterol* 2011; 45: 655 – 657.

**PAPER 3.5**

"Treating *Clostridium difficile* with Fecal Microbiota Transplantation." Bakken JS, Borody TJ, Brandt LJ, *et al. Clin Gastroenterol Hepatol* 2011; 9: 1044-1049.[12]

**PAPER 3.13**

"Fecal microbiota transplantation: A new standard treatment option for *C. difficile* infection." Borody TJ, Brandt LJ, S, *et al. Expert Rev Anti Infect Ther* 2013; 11: 447 – 449.[17]

**PAPER 3.14**

"Could fecal microbiota transplantation cure all *Clostridium difficile* infections?" Borody TJ, Peattie D, Kapur A. *Future Microbiol* 2014; 9: 1-3.

**Treatment of Ulcerative Colitis**

In 1988 the first patient was treated at the Centre with Fecal Microbiota Transplantation. This patient had 'indeterminate' colitis and achieved induction of remission and histological resolution of previous inflammation following only two consecutive FMT infusions (see full description in Paper

3.6).[4] This observation also formed part of a published letter in 1989 describing 55 patients previously treated at the Centre with FMT. The letter announcing resolution of clinical disease following FMT included patients with chronic ulcerative colitis (See Paper 3.1).[3] The resident gut flora in IBD patients had long been implicated in the classic relapsing presentation of inflammatory bowel disease (IBD), with 'cross-reactive autoimmune' mechanisms believed to be involved.[18] However the exact nature of any precise relationship and proof of connection remained controversial. In the same year as our initial publication, Bennet *et al.* (1989) published his personal experience of treating his own recalcitrant UC with large volume retention enemas of donor bacterial flora. He reported documenting prolonged clinical and histological remission of his ulcerative colitis.[19] The first clear evidence of FMT providing prolonged remission in a series of patients with UC was published by from our Centre in 2003:

**PAPER 3.15**

"Treatment of Ulcerative Colitis using Faecal Bacteriotherapy". Borody TJ, Warren EF, Leis S, *et al. J Clin Gastroenterol* 2003; 37: 42-47.[20]

This study documented the treatment of six patients with Ulcerative Colitis using retention enemas of normal stool for five consecutive days. All patients achieved complete remission by 4 months, and maintained remission for a follow-up period of 1-13 years without clinical, colonoscopic or histological evidence of active disease during this time, and without the need for maintenance medication. A number of review articles and opinion pieces from CDD described various aspects of FMT in UC as listed below. The discovery of *the need for repeated FMT infusions* in this disease was a CDD achievement, resulting in the prolonged remission/cure of UC (**Figure 2**). Unfortunately, unlike CDI where >95% of patients achieve cure, it was found that not all patients with UC achieved remission. As such, investigations to improve the clinical results remain ongoing.

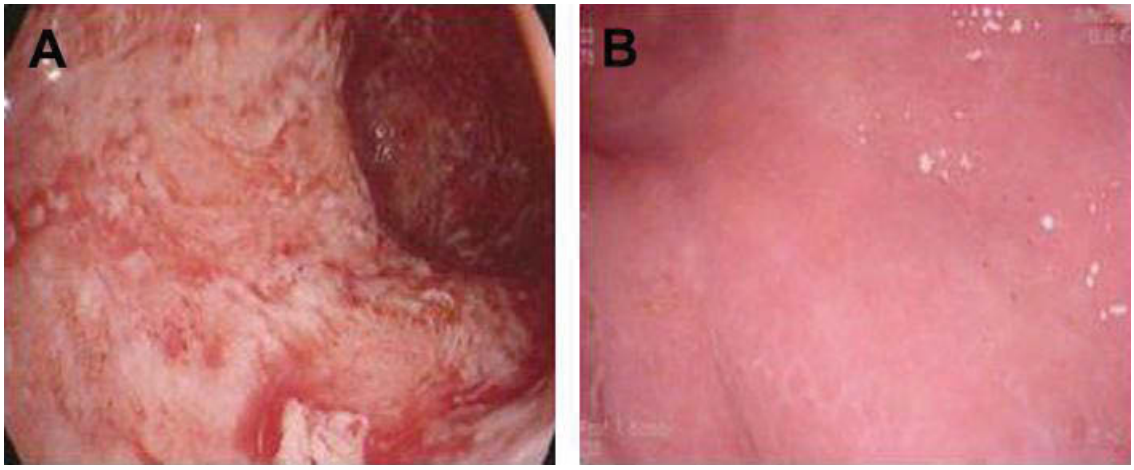


**Figure 2.** Example of before and 4 years after initial repeated FMT treatment in a patient with chronic relapsing distal colitis. Example of prolonged histological 'remission' and possibly cure of UC.

**PAPER 3.16**

"Fecal microbiota transplantation and emerging applications." Borody TJ, Khoruts A. *Nature Rev Gastroenterol Hepatol* 2012; 9: 88-96.[21]

**PAPER 3.17**

"Therapeutic Potential of Fecal Microbiota Transplantation." Smits LP, Bouter KE, de Vos WM, *et al. Gastroenterology* 2013; 145: 946-953.[22]

**PAPER 3.18**

"Therapeutic faecal microbiota transplantation: Current status and future developments." Borody TJ, Brandt LJ, Paramsothy S. *Curr Opin Gastroenterol* 2014; 30: 97-105.[23]

**PAPER 3.19**

"Fecal microbiota transplantation: indications, methods, evidence and future directions."
Borody TJ, Paramsothy S, Agrawal G. *Curr Gastroenterol Rep* 2013; 15: 337.[24]

More recent experience with treating UC and CD with FMT is discussed in the review papers listed above (and especially 3.6), which emphasize:

- A significant difference between patients with *C. difficile* enteritis, which responds to a single infusion, and UC, which requires repeated treatments with FMT.

**PAPER 3.20**

"Fecal Microbiota Transplantation in ulcerative colitis: Review of 24 years' experience."

Borody TJ, Wettstein A, Campbell J *et al. Am J Gastroenterol* 2012; 107: S665.[25]

This publication reviews the substantial experience gained from our Centre over the 24 years we have been treating patients with UC. In this review of 62 patients, 92% of patients achieved response, with 68% achieving complete clinical remission defined as a 0 – 1 score on a modified Powell-Tuck Index. Twenty four percent of patients achieved partial remission, defined as a $\geq$ 2 point decrease in score, while 8% of patients were 'non-responders.' In the 21 patients with documented follow-up colonoscopy (mean period 33 months), 57% displayed a normal mucosa complete with normal histology. Experience indicating the need for repeated FMT infusions in UC has dictated a FMT treatment schedule at the Centre as follows: Transcolonoscopic FMT infusion on Day 1, followed by daily enemas for 14 days. This is progressively stepped down to one FMT infusion per week for 14 days, and colonoscopic review every 12 weeks. An important observation was the existence of a delayed healing process which would be seen for up to 33 months - in a large subset of patients.

The currently accepted view of UC pathogenesis emphasizes the importance of the fecal microbiome, and the profound distortion of this microbiome in UC towards a more pathogenic flora, with reduction in species such as *Bacteroidetes, Bifidobacteria* and *Clostridium* IXa and IV groups. These collective changes of microbiome, or a dysbiosis, may possibly occur as a consequence of a pathogenic infection and resulting disease process, leading to an inhospitable environment and disruption to normal down-regulation of inflammation. It may also be feasible that the inability to achieve a cure in UC points to a distinct mechanism separate from that operating in CDI, with different pathogenic mechanisms such as biofilm production, and pathogen persistence within crypt architecture or intracellular residence beneath the biofilm.

It is still feasible that the difficulty in achieving in UC a cure with 1-2 infusions as can be achieved in CDI, is the presence of a yet-to-be-described pathogen histologically visible in the biofilm, the crypts, and intracellular locations under the biofilm.[26] Commensal bacteria may also be entering colonic epithelial cells and induce pro-inflammatory cytokine secretion: a possible pathogenic mechanism of ulcerative colitis,[27] which can also be seen *in-vivo* using confocal laser endomicroscopy.[28] These advancements in our understanding of UC pathogenesis support the

notion that an occult pathogenic bacterial infection drives the characteristic inflammatory hallmark of the disease. This is further supported by the observation of UC response to FMT which re-introduces a complete stable community of gut micro-organisms aimed at manufacturing antimicrobial substances which eradicate locally invasive organisms and repairing and/or replacing the disrupted native microbiota. A multi-centre randomized controlled study initiated at the CDD is currently underway (FOCUS Trial, ClinicalTrials.gov Identifier NCT01896635) aimed at rigorously investigating the effectiveness of FMT in UC. Through serendipitous clinical observation, often in patients with coexisting CDI and UC, other potential indications for FMT have been uncovered. Some of these are summarised below.

**Treatment of Crohn's Disease**

The first patient with Crohn's Disease to be treated with FMT reported in world literature occurred in 1989 and was included in our MJA publication (see **Paper 3.1**).[3] At the time of publication, this 31 year old patient with ileal CD remained in a 4 month remission at the time of publication. Other cases were reported including that of a 46y old female with severe CD and CDI treated with a single, large volume FMT (See **Figure** 3). Other cases were reported by our group (**Paper 3.6**;[4] **Paper 3.18**;[23] **Paper 3.19**)[24] and an invited commentary published on Crohn's Disease and FMT (see **Paper 3.21**).[29] Meanwhile other groups had commenced study into the role of FMT in CD.[30-34] Research into this field has accelerated, with these early observations reported originally from the CDD being followed by researchers internationally, and multicentre trials commencing by various groups.

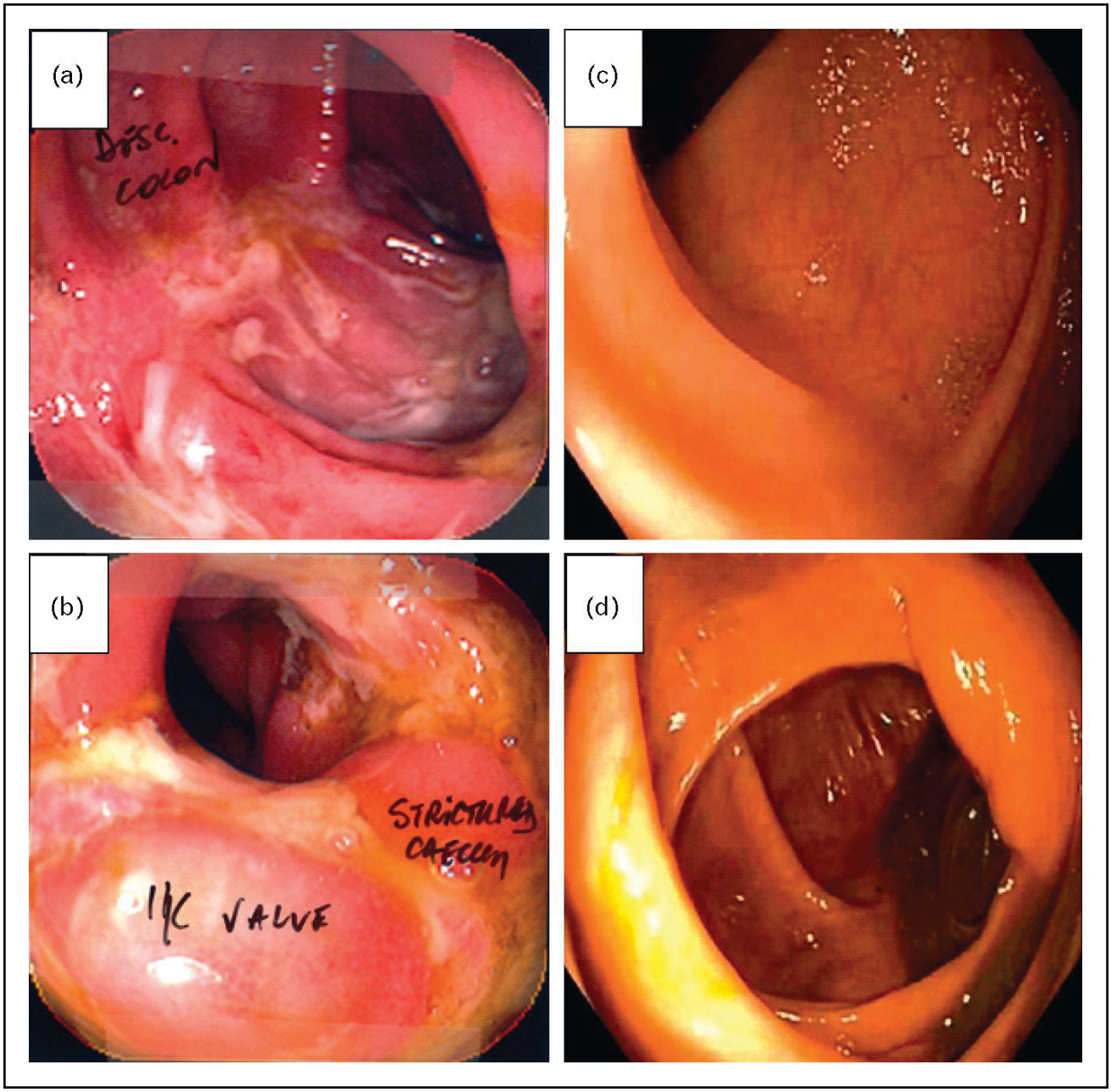


**Figure 3.** Crohn's colitis before and after FMT. A 46-year-old woman with a 2-year history of Crohn's colitis was treated with a single, large volume nasojejunal infusion of FMT over 6 h for concurrent CDI. (a, b) Descending colon and caecum (respectively), pre-FMT. (c, d) Descending colon and caecum (respectively), 12 years post-FMT. Stricture completely normalized. On no active CD treatment.

**Treatment of Diarrhoea-predominant- and Constipation-predominant IBS**

In 1989 we reported the first documented case of a constipation patient who was treated successfully with FMT in 1988 (see **Paper 3.1**).[3] In 1992 we published a detailed case report of a patient with severe constipation, melanosis coli and documented anorectal dysmotility. Not only did the constipation resolve, but repeat manometric and motility studies quantitatively showed reversal of previous dysmotility (**Paper 3.22**).[10] Our experience using FMT in constipation continued to expand, and in 1994 we reported on 27 patients with chronic, severe constipation who were treated with cultured 'bacteriotherapy' product. Eighty five percent of patients obtained measurable symptomatic improvement, including ease of defecation, and elimination of bloating and abdominal pain.

Approximately 85% of these patients no longer required laxatives at the time of the clinical review for this paper (**Paper 3.23**).[35] By 1995, we published the long-term follow-up of 45 patients with severe chronic constipation (**Paper 3.24**). Eighty-nine percent (40/45) of patients experienced substantial improvement with respect to defaecation, bloating and abdominal pain – an improvement that persisted in 60% at review at 9 – 19 months (**Paper 3.24**).[36] This revelation that the human gastrointestinal microbiome plays a leading role in the pathogenesis of constipation is a substantial medical breakthrough first reported from the CDD. Most medical practitioners considered constipation to occur as a result of a low fibre or water intake, and lack of exercise. The novel concept of the intestinal microbiota driving motility is slowly gaining recognition.

The use of FMT in diarrhoea-predominant IBS was also described in this early 1989 report (**Paper 3.1**)[3] and constitutes the most commonly treated indication for FMT use at the CDD FMT Clinic. The patients treated often have severe diarrhoea refractory to standard treatments, however they do not have detectable pathogens including *Clostridium difficile.* From our experience the majority [>75%] of patients respond positively to repeated FMT infusions and are able to markedly reduce their diarrhoea frequency and associated symptoms. This response indicates the presence of an undetectable pathogen or pathogens driving the diarrhoea symptoms in this form of IBS. In 1997 an attempt was made to use a defined consortium of freshly cultured human flora bacteria in 51 patients to perform FMT in IBS including diarrhoea-predominant IBS. A substantial improvement in symptom scale was achieved with prolonged improvement in 65% of patients, as reported in the paper below.

**(PAPER 3.2)**

"Modification of the colonic microflora using probiotics: The way forward?"

-Pearce L, Bampton PA, Borody T, *et al*. *Gut* 1997; 41(Suppl 3): A63.[9]

Subsequent reports confirmed these observations (**Paper 3.25**).[23, 37] A controlled trial in diarrhoea-predominant IBS of orally delivered FMT capsules is currently in progress (NCT 02328547) by Brandt's group in New York.

Study of patients with constipation / diarrhoea-predominant IBS drew attention to the gut/brain axis and the potential for therapy in autoimmune disease due to coincidental observations of improvement following FMT in systemic diseases. Such studies are preliminary and predominantly case reports, however they emphasise directions for future research and highlight the importance of the gut microbiome to not only innate gut function, but also systemic physiology, and the potential for interventional therapy in these disorders. The far-reaching nature of this therapy could not have been envisioned in the 1980's when faecal matter was considered a waste for disposal and the role of the gut microbiota remained largely undiscovered. Such studies and directions are summarised in **Paper 3.6**.[4]

Early studies from CDD that defined the way forward in other indications also include:

(i) **Paper 3.26** Myoclonus dystonia: "Myoclonus dystonia-affected by GI microbiota." Borody T, Rosen DM, Torres M, *et al. Am J Gastroenterol* 2011; 106: 5352.[38]

(ii) **Paper 3.27** Multiple Sclerosis: "Fecal Microbiota Transplantation in Multiple Sclerosis." Borody T, Leis S, Campbell J, *et al. Am J Gastroenterol* 2011; 106: S352.[39]

(iii) **Paper 3.28** Autoimmune Thrombocytopenia: "Reversal of idiopathic thrombocytopenia purpura (ITP) with fecal microbiota transplantation." Borody TJ, Campbell J, Torres M, *et al. Am J Gastroenterol* 2011; 106: 5352.[40]

(iv) **Paper 3.29** Fatigue as an Illness: "The GI Microbiome and its role in Chronic Fatigue Syndrome: A summary of Bacteriotherapy." Borody T, Nowak A, Finlayson S. *ACNEM Journal* 2012; 31: 3-8.[41]

These case reports are important in clinical research as they identify areas of potential leads for definitive controlled studies (themselves difficult and costly). The 'fatigue' study included two groups – those with disturbed gut function and those with normal gut function. The important

observation in this study was that reduction in level of fatigue was only seen in those with gut symptoms (which also resolved with FMT).

**DISCUSSION OF SECTION 3**: In this section, studies pivotal to the development of FMT as a therapeutic product for clinical application, are described. They begin with *C. difficile*-associated enteritis, then progress to IBS and later to inflammatory bowel disease. In parallel there has been the increasing recognition of the critical role played by the gut microbiome in control of gut function, immune and inflammatory responses, metabolism and its role in the gut-brain axis. Just as the scientific studies have to date been preliminary, so too has the use of FMT as a blunt therapeutic instrument. This is reflected in the very early days of FMT use in the manipulation of the gut-microbiome in attaining benefit in a range of gut diseases (described above).

While CDI resolves in most patients with 1-2 FMT infusions , the requirement of *repeated FMT infusions* in conditions such as UC, CD and various IBS variants, in part led to the development of a lyophilised oral format of the FMT therapy to enable ongoing dosing delivered daily with a considerable bacterial volume of $10^9$ or $10^{10}$ . In late 2014, work at CDD has culminated in such a preliminary capsule product which contained 5 X $10^9$ bacteria per capsule. It contained ultra-filtered donor stool suspension with high viability after freeze-drying. This product is therefore capable of delivering the full spectrum of the gut microbiome as does crude, homogenized stool. The cure of the first relapsing CDI patient using this product was achieved in December 2014 using $4X10^{10}$ bacteria. Further two patients with CDI were cured in early 2015. This successful result provides a useful indicator that future modification or restoration of the damaged human microbiome will become a markedly simplified oral therapy. Future use of such a product delivering the microbiota in an enteric-coated capsule to the distal jejunum - instead of the use of repeated enemas of FMT - will permit longer term delivery of normal microbiota to the lumen of the bowel to create a milieu of more normalised diverse composition. As such, it opens the door to potentially modifying numerous disorders characterized by a deficient or superinfected gut microbiome, so reaping clinical improvement.

# APPENDIX 1 - PATENTS ARISING FROM THIS RESEARCH

1. **FAECAL TRANSPLANT PATENT - US PATENT # 6,645,530. [Expired]**

   This patent describes transplantation of normal microbiota into the bowel of a person with symptoms cause by an enteric infection.

2. **TREATMENT OF GI DISORDERS WITH FAECAL OR BACTEROIDES/E.COLI COMPOSITION- US PATENT # 5,443,826 [Expired]**

   This patent described the use of cultured as well as filtered human flora.

3. **PROBIOTIC RECOLONISATION THERAPY. US PATENT # 8,460,648 [Currently Active] – Plus Continuations filed. USSN 61/729,994 and USSN/62/140,035**

   Composition patent describing a mix of spore-containing *Clostridia* plus *Collinsella* human-derived bacteria for e.g. CDI. Several Divisional patent applications ensued. This patent was licensed to CIPAC Therapeutics and funded for commercialisation by a Venture Capital group.

4. **COMPOSITIONS FOR FECAL FLORA TRANSPLANTATION AND METHODS FOR MAKING AND USING THEM AND DEVICES FOR DELIVERING THEM. US PATENT APPLICATION # 2013/0195804 [Published - Going through Grant Process]**

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# - SECTION 4 -

## GENERAL DISCUSSION

This thesis comprises the collection of studies conducted within the Centre for Digestive Diseases (CDD) by the applicant, over a 30-year period. The themes connecting these studies include closely related concepts that:

(i) Inflammatory conditions affecting the gut wall can be driven by components of the microbiota, which by definition are designated 'pathogens.'

(ii) Along the gut continuum, the microbiota becomes increasingly dense and complex in both its constitution and its interactive role with the host.

(iii) As with clinical infection elsewhere, pathogen-provoked inflammation [eg *H pylori* and MAP] within the gut wall reflects the outcome of interaction between the pathogen and its host. In the gut, this concept is modified by the recognition that the 'host' now must be recognized as including the intestinal microbiome, essentially a giant 'extracorporeal organ.'

(iv) This large 'organ' in itself can be infected by pathogens and affect the tissues of the 'host'.

(v) An essential focus of research is the development of therapeutic regimens aimed at correcting dysbioses, with the use of these therapeutic strategies aimed also at furthering an understanding of the pathogenesis of disease. A summary of this work is contained in Table 1.

**Table 1.** Dysbioses along the gastrointestinal tract and therapies developed to treat them

| ORGAN | DISEASE | Aetiology | Intervention | Therapy Outcome | Scientific Outcome |
|---|---|---|---|---|---|
| **STOMACH** | Peptic ulcer disease | Single pathogen (*H. pylori*) | • Triple therapy<br>• Quad therapy<br>• Rescue therapy | Effective eradication therapy for *H. pylori* | Eradication cures duodenal ulcer |
| **ILEUM / COLON** | Crohn's Disease | Contributory pathogen (MAP) | Anti-MAP triple therapy | Establish value of anti-MAP therapy to control not cure disease | MAP promotes inflammation |
| **COLON** | • *C. difficile*<br>• IBS<br>• IBD<br>• Systemic disease | Defined and Undefined pathogen(s) | Faecal Microbiota Transplantation (FMT) | Control / cure:<br>• Specific pathogen (*C. difficile*)<br>• IBS<br>• IBD | Normalised microbiome; suppresses occult pathogens |

This oversimplified table reflects a transition related to complexity, from a situation in the gastro-duodenum with its scant resident colonising bacteria, where a single pathogen (*H. pylori*) causes a precise pathology (duodenal ulcer), through one where a bacterium known to cause chronic gut infection in animals (MAP) in a region of the gut with a more complex resident microbiome contributes in part to disease in man (CD), to a third circumstance where the microbiome itself becomes the therapeutic agent directed against pathogen(s) and inflammation within the colonic microbiota and mucosa.

Our studies of *H. pylori* provided clear results which were confirmed in a timely manner by key clinical groups around the world. Two major discoveries included the development of effective therapeutic regimens, enabling a study into the relationship of *H. pylori* and duodenal ulcer, ultimately demonstrating that eradication of *H. pylori* equated cure of duodenal ulcers. Collaborative studies with the mucosal immunology group at the University of Newcastle using cellular and molecular technologies on blood and mucosal samples, provided further understanding of the importance of impaired host immune responses, mechanisms operating in failure to eradicate *H. pylori,* and helped unravel mechanisms of progression to dysplasia and carcinoma. These latter findings, together with additional studies by the Newcastle group using molecular methods in subjects with non-ulcer dyspepsia, provided a basis for novel diagnostics to detect aberrant *H. pylori* infection.

The studies of MAP and CD reflect the inordinate complexity of the relationship between the microbiome and host mucosa, and what constitutes a 'pathogen'. Our studies clearly demonstrate that while on the one hand both those with CD and normal/disease controls have similar exposure to MAP, those with CD have a distorted T-cell response, evident in both blood and mucosa. This response is an enhanced secretion of TNF$\alpha$ (a cytokine that contributes to mucosal inflammation). The clinical studies confirmed a clinical response in most subjects with severe disease, but showed disease relapse in most patients if triple antibiotic therapy was ceased. A discrepancy was that while most appeared to respond to ‘anti-MAP therapy,’ only 20 – 30% had MAP detected in their mucosal biopsies. This discrepancy may at least in part be due to MAP being a focal infection or the detection method required greater tissue volume to optimize detection, or the need to cease anti-MAP therapy at the time of study, making correlation of microbiology with clinical disease difficult. Ongoing clinical studies may clarify these relationships, but much careful work is needed. Recent

studies showing genetically influenced defects in macrophage-dependent clearance in CD adds to our understanding of roles played by MAP in CD. MAP is an intracellular bacterium contained largely within macrophages, which if defective, could promote an excessive and inappropriate inflammatory response (as was demonstrated), contributing to mucosal inflammation. Studies are planned to combine anti-MAP triple therapy with immune-stimulating vaccine.

The third aspect of study of microbiota and gut inflammation began with the idea that specific pathogens (e.g., *C. difficile*) could be eradicated by replacing an 'abnormal microbiome' with full spectrum microbiota using Faecal Microbiome Transplantation. Thus, normal colonic microbiota itself became the therapeutic agent. Prior to our first published studies, only several case reports had appeared, in subjects with *C. difficile*-associated pseudomembranous colitis. Our hypothesis was that the 'normal' colonic microbiota had - as a primary regulatory role - the capacity to eradicate and prevent emergence not only of identified 'specific pathogens' but also 'cryptic pathogens' (i.e., not currently recognized due to the great complexity and only superficial knowledge of the gut microbiota). These 'cryptic pathogens' could directly influence gut function (as may occur in IBS) or indirectly cause chronic inflammation in subjects with genetic defects affecting mucosal clearance of bacteria (as may occur in CD), or by driving cross-reactive autoimmune destruction of the mucosal epithelium (as may occur in a subset of chronic ulcerative colitis). This approach to the use of FMT as a therapeutic tool (albeit a necessarily blunt one) is noted in our initial report which included patients with *C. difficile*, CD, ulcerative colitis and IBS (**Paper 3.1**). Subsequent studies in the CDD provided substantive support to this hypothesis, while confirming that 'transplanted' biota persisted in the recipient host. Numerous studies (reviewed in attached papers) conclusively agree with eradication of *C. difficile* in over 90% of patients receiving FMT. The important observation that FMT can induce protracted remission (or even cure) in ulcerative colitis, as suggested by our published data, is being tested in our current Australian multisite study, and further refinements are planned. Less data are available to assess the value of FMT as a routine therapy in CD, though a proportion of treated CD patients enter a protracted remission. Currently a study is planned in CD to combine anti-MAP antibiotics with bacteria from a normal colonic biota, selected for their capacity to act as polyclonal mitogens (and thus enhance phagocytic activity within the mucosa). This novel approach represents progression from our initial approach using "FMT," to the use of selected components of the microbiota to suppress mucosal inflammation. The study reported on subjects with chronic fatigue represents a control of systemic

symptoms, with limited studies reported also suggesting a relationship between 'pathological microbiota' and structural neurological lessons. Large studies will be needed to gain definitive answers to these important questions.

An important observation in the chronic fatigue study is that only those subjects with gut symptoms recorded remission of fatigue following FMT. This again links systemic response with a gut dysbiosis and provides a valuable 'marker' for selection in future studies. The future for FMT will be dominated by careful and well-powered trials on one hand, and improved selection of faecal donors. A contribution to donor selection and faecal preparation is published but this process will change dramatically as more is known about components of faeces contributing desired outcomes. Perhaps the greatest challenge is to develop a surrogate method of assessing value of a putative therapeutic faecal preparation (or components thereof), to facilitate current individual therapy and future component preparations. This is more attainable in disorders where the pathogen is known, as with *C difficile* but less so in conditions where it is cryptic, e.g. IBS.

## CONCLUSION

The assembled clinical studies in this Thesis focus on the development of more effective management strategies, but in their own way provide a framework for future studies asking more basic questions about the gut microbiome and the relationship to its host, as well as contributing to understanding pathogenesis. For example, recent interest in CD has been in recognition of genetic defects clustered around the proteins involved in autophagy, and identifying the larger role played by autophagy as it intersects innate and adaptive immunity. These ideas bring into focus the critical role of antigen presentation, in triggering the inappropriate and excessive Th1-dependent inflammation within the mucosal compartment characteristic of CD. These ideas support a special place for MAP which binds to Toll-like receptors and is intimately involved in the autophagic process. This contemporary concept underpins the importance of the correlative studies of MAP using mucosal biopsy cultures and whole blood cultures, showing significant enhancement of TNF secretion and Th2 'switching' in MAP +ve subjects with CD. The importance of these findings is reinforced with specificity for both microbe and disease diagnosis. In CD, any normal communication between the APC and the intestinal microbiome breaks down because of defects in proteins designated to process microbes. It is likely that key roles in communication between the intestinal microbiome and the innate immune mechanisms within the mucosal compartment, will be explored in the future, with CD seen as an outcome of defective communication. As the language of communication is identified, selective manipulation of the microbiome will make current FMT a blunt instrument of the past! Selective impact by host immune mechanisms on components of the microbiome will play key roles in defining both normal and pathological environments. Recent studies showing a shifting mutation rate *in H. pylori*, may underpin evolution of urease-negative forms adapted to chronically inflamed gastric mucosa. With the molecular tools now available, the results of studies will remain only as good as the clinical studies that provide the framework for any study on causation.

The three areas of clinical research within the CDD over the last 30 years described above, have contributed significantly to therapeutic advances in the major inflammatory challenges in gastroenterology. These include:-

1. Development of definitive eradication therapy for *H. pylori* (initial therapy, treatment of resistant H pylori and 'rescue therapy').

2. Provision of effective ‘anti MAP’ therapy and platform in CD and,

3. Pioneer work in FMT with leadership in *C. difficile* eradication and broadening target options/indications to IBS and IBD among others.

In each area careful and practical contributions have been made in treatment development. Also in each of these three areas, collaboration with academic immunology and microbiology units has contributed to the better understanding of mechanisms.

The strategy of learning from clinical observation, trialing logical therapy and progressing to definitive controlled studies is defined. This was completed with *H. pylori* eradication, it is currently being completed in a multi-centre antibiotic study for MAP in CD (Redhill Biopharma). Future FMT trials will move forward as with current ulcerative colitis - FOCUS Trial, but also encapsulated product trials in CDI, Autism, IBS, IBD, and others fully funded by the new Venture Capital Group.