HELICOBACTER PYLORI: ITS ROLE IN GASTRIC LYMPHOMA AND OTHER CURRENT ISSUES

Medical Grand Rounds

University of Texas Southwestern Medical School

Walter L. Peterson, M.D.

July 28, 1994

INTRODUCTION

Helicobacter pylori was "discovered" in 1983 when Warren and Marshall, two Australian investigators, reported spiral organisms in mucosal biopsies of patients with chronic active gastritis (1). First named *Campylobacter* ("curved rod") *pylori*, its name was changed to *Helicobacter pylori* (*H. pylori*) when biochemical and genetic characterization of the organism showed that it was not a member of the Campylobacter genus. There are now seven known gastric helicobacters (Table 1).

Table 1. Known gastric helicobacters and their hosts.

H. mustelae	Ferrets
H. felis	Cat, dog
H. nemestrinae	Pig-tailed macaque
H. acinonyx	Cheetah
H. rappini	Sheep, dogs
H. heilmani*	Humans, cats, dogs
H. pylori	Humans

*Also known as Gastrospirillum hominis

The organism is a slow-growing, microaerophilic, motile, gram negative rod whose most striking biochemical characteristic is abundant production of urease. *H. pylori* is very sensitive *in vitro* to low pH. However, if the organism is able to escape the effects of acidic gastric juice, it burrows through the mucus layer and colonizes the surface epithelium of gastric mucosa, where the pH is near neutrality. It is found only on gastric epithelium (i.e., stomach and areas of gastric metaplasia in Barrett's epithelium or the duodenal bulb) and virtually never penetrates the cell. It nevertheless elicits robust inflammatory and immune responses which are life-long unless the organism is eradicated. Arguably the most common human bacterial infection, *H. pylori* gastritis is associated with peptic ulcer disease, gastric adenocarcinoma, and gastric lymphoma.

DIAGNOSIS OF *H. pylori* INFECTION

Diagnostic tests for *H. pylori* may be divided into those which do or do not require endoscopically-obtained mucosal biopsies (Table 2) (3,4).

Table 2. Diagnostic tests for *H. pylori*

REQUIRE ENDOSCOPY

Tests	Sensitivity	Specificity	Comments
Histology	93-99%	95-99%	Expensive
Rapid Urease Test	89-98%	93-98%	Inexpensive
Culture	77-92%	100%	Expensive, technically challenging

DO NOT REQUIRE ENDOSCOPY

Tests	Sensitivity	Specificity	Comments
Serology			
Multi-well Lab ELISA	88-99%	86-95%	Inexpensive, Unsuitable for follow-up
Quick Office Tests	94-96%	88-95%	Inexpensive, very rapid Unsuitable for follow-up
Urea Breath Tests	90-100%	89-100%	^{14}C-urea gives small radiation exposure, best for follow-up, not yet marketed

Mucosal biopsies may be used for histological demonstration of the organism (5) or determination of the presence of urease by a urea slide test (e.g., CLO-Test). If urease is present in the mucosal biopsy, it will split the urea into ammonia and carbon dioxide. The ammonia will raise the pH of the medium and change the color of a pH sensitive indicator (phenol red) from yellow to red. This test may require several hours to become positive. Culture is the least sensitive of the direct techniques, perhaps because of the fastidious growth characteristics of the organism; its primary use is for research studies in which the susceptibility of *H. pylori* to antimicrobial agents is desired.

Noninvasive methods include serological tests and urea breath tests. Chronic *H. pylori* infection produces a circulating antibody response that can be detected readily by ELISA tests (6,7). Serological tests are almost as sensitive and specific as biopsy-based methods and have been adapted for rapid use in the office (4). Serological tests are not useful for confirming eradication of *H. pylori* infection. Antibody titers decrease only slowly over a period of 6 to 12 months and remain positive in a substantial proportion of patients in whom eradication of the organism has been confirmed by other tests (8). Emerging data suggest that antibodies to *H. pylori* may also be detected in saliva or gingival transudate.

Other non-invasive means of detecting *H. pylori* are the urea breath tests (UBT), which are not yet commercially available. Here, urea labeled with either ^{13}C or ^{14}C is ingested with a liquid meal (9). If urease is present, labeled carbon dioxide will be split off and absorbed into the circulation where its presence can be determined by analysis of expired breath. This test has virtually 100% positive predictive value and about 95% negative predictive value. Small numbers of organisms which can be detected by direct examination of gastric tissue may not produce enough urease to be detected by the UBT. ^{13}C labeled urea has the advantage of not being radioactive but requires a mass spectrometer for analysis; ^{14}C can be measured with a simple gamma counter but does subject an individual to a small dose of radioactivity. Once they are formally approved and marketed, such tests may become the preferred means of evaluating the success of eradication therapy in clinical practice.

A minimally-invasive means to obtain tissue has been developed by Drs. Cryer and Feldman, colleagues at the Dallas VAMC. With this test biopsy forceps are passed through a modified nasogastric tube positioned either in the gastric body or antrum. Biopsy material can thereby be obtained without the need for endoscopy.

EPIDEMIOLOGY

PREVALENCE IN HEALTHY INDIVIDUALS

The prevalence of *H. pylori* in healthy individuals varies enormously depending upon age and country of origin (Figure 1). In developed countries, there is a clear age-related increase in prevalence (10-12) whereas in developing countries most children are infected by age 10 (13). In developed countries, prevalence varies among different ethnic

groups of similar socioeconomic status (14) (Figure 2). The explanation for these observations has both environmental and genetic components.

Figure 1. Age-related prevalence of *H. pylori* in healthy subjects in developed and developing countries.

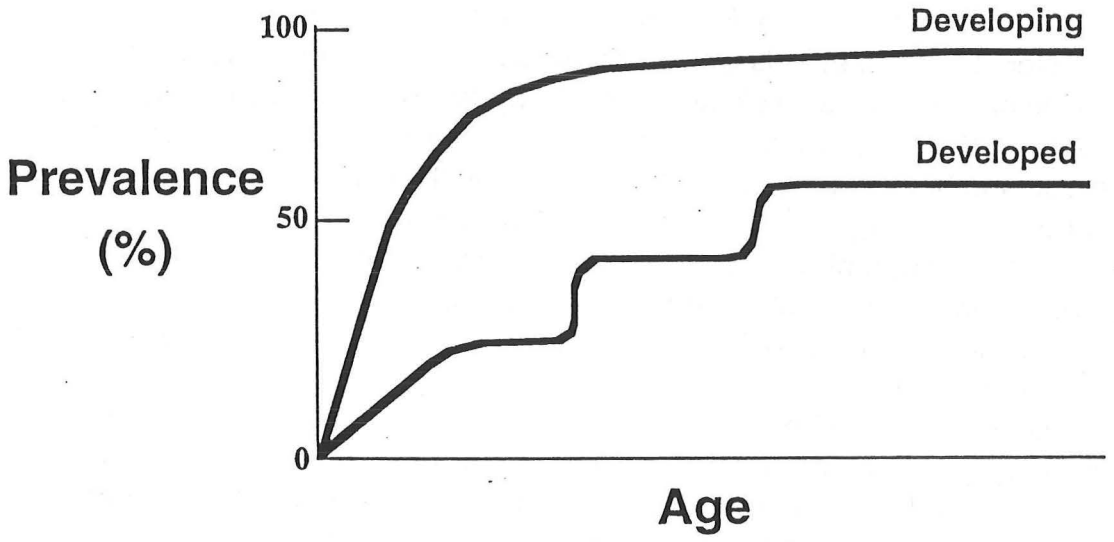
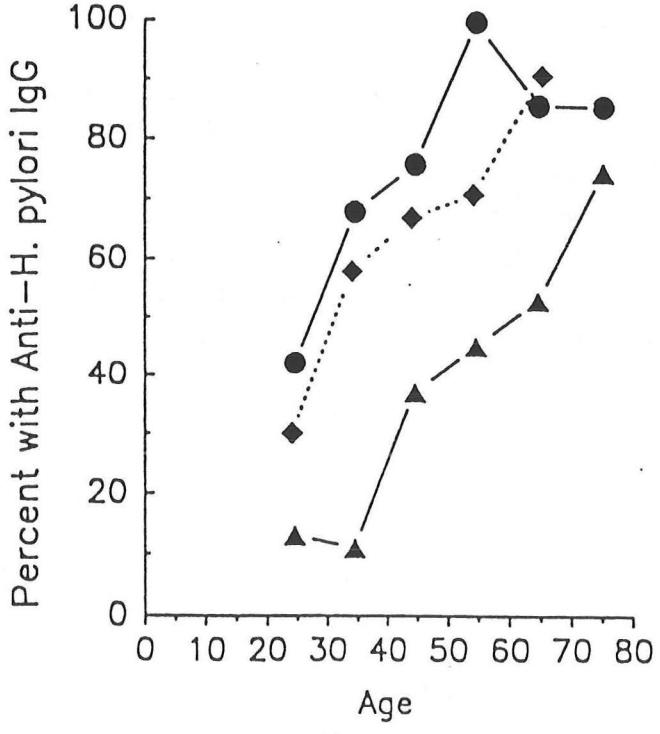


Figure 2. Age-related sero-prevalence of *H. pylori* in blacks (closed circles), hispanics (closed diamonds), and whites (closed triangles) in Houston. From reference 14.



Environmental Factors

H. pylori infection occurs primarily during childhood, with acquisition during adulthood occurring only about 0.3% - 0.5%/year (15-17). The major risk factor for infection is the socioeconomic status of the family during childhood (18-22) (Table 3). Smoking and alcohol consumption have no effect on the prevalence of *H. pylori* (22).

Table 3. Odds ratios for *H. pylori* seropositivity in adulthood as a function of living conditions during childhood. From Reference 18.

Persons per room During Childhood	Odds ratio for Hp as adult
<0.7	1.0
0.7 - 0.99	1.36
1.0 - 1.29	4.04
≥1.3	6.15

Table 4. Sero-prevalence of *H. pylori* in adult blacks and hispanics in Houston as a function of social class during childhood. I = lowest, V = highest social class. From Reference 21.

		Social Class Now	
		II,III	IV, V
Social Class In Childhood	I	81%	100%
	II, III	56%	40%
	IV, V	29%	0%

As the socioeconomic status of individuals and countries has risen, the prevalence in younger generations has declined (23). Improvement in socioeconomic status among blacks and hispanics in the United States has lagged behind other ethnic groups and is one explanation why the overall prevalence of *H. pylori* in these groups is higher (Figure

2). Socioeconomic conditions have improved even more slowly, or not at all, in developing countries, which explains the continuing high rate of infection in young people.

Genetic Factors

Two recent studies lend credence to a genetic predisposition to infection with *H. pylori* (24,25). In one study from Belgium, Caucasian children had a significantly lower seroprevalence of *H. pylori* infection than did non-Caucasian children matched for age and socioeconomic status (24). In another, monozygotic twins reared apart or together had a higher rate of concordance of infection than did age-matched dizygotic twins (25).

TRANSMISSION OF INFECTION

H. pylori has never been found in traditional environmental reservoirs such as water, insects, pets, or farm animals. Thus, the organism almost certainly is transmitted from person-to-person. Support for this concept comes from studies of children in institutions of custodial care, where prevalence if higher than expected (26), and from studies of families in which there is at least one child (27-29). Family members of a child infected with *H. pylori* are more likely to be infected than family members of an uninfected child, at times with an genetically-identical organism (27,28) (Table 5). In another study, spouses and children of an infected parent were more likely to be infected with *H. pylori* than spouses and children of an uninfected parent (29). There is no concordance of infection in couples without children (30).

Table 5. Seroprevalence of H. pylori in family members of children with and without H. pylori. From Reference 27.

	H. pylori Positive Children	H. pylori Negative Children
Sibs	18/22 (82%)	5/37 (14%)
Mothers	15/18 (83%)	2/17 (12%)
Fathers	10/16 (62%)	6/16 (38%)

The most likely mode of transmission of *H. pylori* is fecal-oral and the organism has now been cultured from stool (31,32). Shedding of bacteria into the stool may be enhanced by drugs which raise gastric pH (33). Evidence of *H. pylori* in dental plaque has been found by culture (34,35) and by reverse transcription PCR (36). Thus, oral-oral transmission cannot be totally excluded. Indeed, one group which may be at higher relative risk for infection are endoscopists (37), presumably by handling endoscopes contaminated with gastric secretions and/or saliva from patients with *H. pylori*.

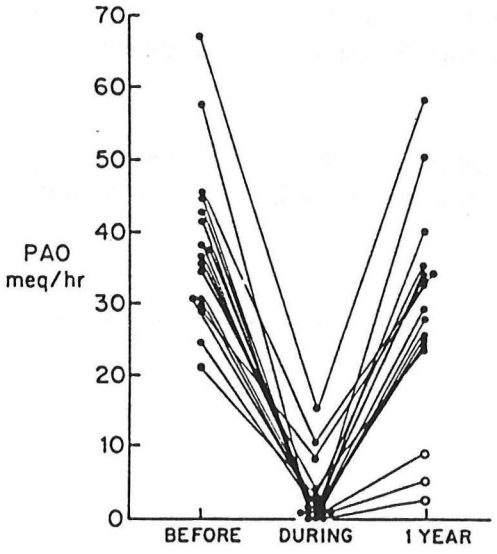
There is no apparent increase in rates of infection in immunocompromised subjects (38) or patients taking drugs which suppress gastric acidity. Patients with Zollinger-Ellison Syndrome or atrophic gastritis with achlorhydria each have low rates of infection (when determined by tissue biopsy), a seeming contradiction. Evidence suggests that *H. pylori* has difficulty surviving at either extreme of gastric pH (39). Furthermore, atrophic mucosa may have large areas of metaplastic intestinal epithelium, tissue which is not suitable for colonization by *H. pylori*.

ACUTE INFECTION WITH *H. pylori*

Acute infection with *H. pylori* has been observed contemporaneously in three individuals. In two cases, investigators themselves knowingly ingested a culture of *H. pylori* after endoscopic biopsy had confirmed absence of pre-existing infection (40-42). Both subjects developed a severe neutrophilic gastritis and, in one, gastric pH was above 7.0 from day 8 to day 39 after ingestion. The third individual, a clinical investigator working with gastric juice, developed an illness characterized by epigastric pain and nausea. On the fifth day of illness an endoscopic biopsy disclosed a neutrophilic gastritis and culture grew *H. pylori* (43). Although this individual did not have a baseline endoscopic exam, sero-conversion occurred between day 14 and 74. Gastric pH was above 7.0 on days 14 and 37, but fell to 2.0 on day 74.

The neutrophilic gastritis with transient hypochlorhydria observed in two of these subjects is reminiscent of cases noted in several research laboratories during the 1970's and 1980's (44,45). In our laboratories at Parkland and the Dallas VA, between October, 1976 and June, 1977, 17/37 healthy volunteers participating in acid secretory studies and one patient with Zollinger-Ellison syndrome became rapidly and profoundly hypochlorhydric (Figure 3).

Figure 3. Peak acid output in 17 subjects with acute gastritis with hypochlorhydria. From Reference 44.



Nine of the 17 volunteers noted a 1-4 day illness consisting of mild to moderate epigastric pain accompanied by nausea in four and vomiting in two. Biopsies of the gastric body in 12 subjects revealed severe neutrophilic gastritis in all 12. Short term follow-up showed lessening of the severity of gastritis and return of acid secretion to near baseline levels in 14/17 within a mean of four months (Figure 3). Despite an intensive search for an etiologic agent, none was found in our subjects. Between July 1977 and December 1981, 16 additional cases occurred, and, in 1987, one further isolated case was noted, bringing to 35 the total number of such cases occurring in our laboratory.

We have since gone back and reviewed the original biopsy material for the presence of *H. pylori*. Additionally, samples of stored serum from before, during, and after hypochlorhydria have been tested in the laboratories of Drs. John Walsh and Martin Blaser for IgG and IgM antibodies to *H. pylori* (46). Data from these studies (Table 6) strongly suggest that, in retrospect, our cases of acute gastritis with hypochlorhydria were temporally related to acute infection with *H. pylori*. It is of interest that biopsies in five subjects showed intense gastritis but no evidence of *H. pylori*. In four of these, convalescent biopsies were positive for *H. pylori* (n=3) or seroconversion to IgG antibodies occurred (n=1). It may well be that intensity of the inflammatory reaction and/or the hypochlorhydria (39) led to suppression of the organisms. As inflammation down-regulated and acid secretion returned, the milieu may have become more conducive to proliferation of the infection.

Table 6. Evidence to suggest that acute gastritis with hypochlorhydria was caused by acute infection with *H. pylori*.

In each case where serum was available before illness, an assay for IgG antibodies to *H. pylori* was negative
H. pylori were found on the initial gastric biopsy during illness in 7 of 12 cases
Assay for IgM antibodies during and shortly after illness was positive in 16 of 17 cases

VIRULENCE FACTORS

A number of candidate virulence factors have been suggested to permit *H. pylori* to escape the bactericidal properties of gastric acid, colonize gastric epithelium, damage epithelial cells, and induce an inflammatory reaction and immune response (47). With the possible exception of immunocompromised hosts, invasion of human epithelial cells by *H. pylori* has not been documented.

MOTILITY

Studies in gnotobiotic piglets have demonstrated that less motile strains of *H. pylori* are unable to colonize the stomach (48). This may be related to the need for the organism to penetrate the mucus layer to avoid the acid milieu of the gastric lumen.

UREASE

Urease also appears necessary for *H. pylori* to successfully colonize gastric epithelium. It was recently reported that a mutant strain of *H. pylori* without urease was unable to colonize gnotobiotic piglets (49). At pH $\leq$ 3.5 , wild type *H. pylori* survives well in phosphate buffered saline as long as urea is present, but a mutant strain will not (39). Theoretically, the breakdown of urea to ammonia raises the pH around the organism and protects it (39,50). At pH $\geq$ 3.5, the generation of ammonia by the wild-type strain leads to pH levels too high for *H. pylori* to survive, while the urease-negative mutant survives well (39). Thus, the generation of ammonia from urea by urease is a two-edged sword. At low pH it is protective; at high pH it is lethal. Urease may also enhance bacterial adherence (51).

Apart from its role in the survival of *H. pylori* , urease is cytotoxic, primarily through production of ammonia, and may be at least partially responsible for the epithelial cell damage associated with the organism (52,53).

ADHERENCE

It is now clear that *H. pylori* possesses lectins that bind to glycolipids and/or glycoproteins on gastric epithelial cells. An N-acetylneuraminyllactose-binding fibrillar hemagglutinin has been described for *H. pylori* as has a specific gastric glycerolipid receptor on gastric mucosal cells (54,55). Tight attachment of this fibrillar adhesin to the carbohydrate on the mucosal cell results in the formation of an attachment pedestal which in turn leads to actin polymerization and epithelial cell disruption (56). Failure of adherence, while having no effect on the inflammatory response, results in less epithelial cell injury (57). Differences in the availability of specific receptors has been suggested as one means to explain genetic differences in susceptibility to infection with *H. pylori* (58).

MEDIATORS OF INFLAMMATION

H. pylori elaborates proteins with chemotactic properties to recruit monocytes and neutrophils to the lamina propria (59) and both lipopolysaccharide (LPS)-dependent and LPS-independent soluble surface proteins which activate these inflammatory cells (60).

Activation of inflammatory cells results in release of other inflammatory cytokines (e.g., interleukins, TNF-alpha) as well as reactive oxygen metabolites (61).

CYTOTOXINS

A vacuolating cytotoxin elaborated by *H. pylori* has been found by several laboratories (62-64). This putative toxin is associated with a 120-128 kDa protein (CagA) which is encoded by the *cag*A gene. Some investigators have found this protein more frequently in *H. pylori*-infected patients with duodenal ulcer than in other subjects infected with the organism. Other investigators find no such difference.

NATURAL HISTORY OF *H. pylori* INFECTION

ASYMPTOMATIC CHRONIC SUPERFICIAL GASTRITIS

The majority of individuals infected with *H. pylori* have asymptomatic chronic superficial gastritis. Dr. William Harford, my colleague at the VAMC, has tracked down 28 of our 35 subjects with acute gastritis with hypochlorhydria (AGH) a mean and median of 12 years after their acute infection. The patient with Zollinger-Ellison syndrome died of metastatic gastrinoma, another subject committed suicide, and five could not be located. At the time of follow-up, questionnaires and sera were obtained from all 28 and EGD with biopsy was done in 22. A paired control subject matched for age, gender, race, and profession was recruited for each of the study subjects. A summary of the results of this study is shown in Table 7.

Table 7. Twelve year follow-up of acute gastritis patients compared to matched controls. From reference 46 and W.V. Harford, M.D., personal communication

	AGH Subjects	Control Subjects	P value
Mean Age (Yr)	40	40	NS
Hp Gastritis	18/22[a] (83%)	8/22 (36%)	<0.01
IgG Hp Ab	22/28 (79%)	9/28 (32%)	<0.001
Symptoms	4/28 (14%)	2/28 (7%)	NS

[a]1 other subject has Hp-negative fundic gastritis

Two points merit emphasis. First, approximately 80% of our AGH subjects have continued evidence of *H. pylori* infection by biopsy and/or serology, a significantly greater prevalence than the control group. Where both biopsy and serology were available, there was perfect agreement. Of the six subjects without *H. pylori* antibodies at follow-up (four of whom were also *H. pylori* negative on biopsy), one had no studies performed between the acute episode of hypochlorhydria and follow-up 11 years later, two had experienced transient seroconversion, and three never had evidence of infection. One of these subjects was found by endoscopic biopsy to have gastritis limited to the body of the stomach, but no evidence of *H. pylori*. The cause of her gastritis remains under investigation. Second, only four subjects (one of whom had developed a duodenal ulcer) reported symptoms of dyspepsia more frequently than once a week, not significantly different than the controls.

Antral biopsies of individuals infected with *H. pylori* show focal epithelial cell damage as well as an inflammatory infiltrate in the lamina propria (5). This infiltrate consists of some polymorphonuclear leukocytes, eosinophils, and mononuclear cells. The latter include B and T lymphocytes [frequently in the form of lymphoid follicles (65)], monocytes, and plasma cells. Biopsies from the body of the stomach also usually demonstrate gastritis but may at times be normal (5). The histologic abnormalities seen with *H. pylori* infection are associated with reduced surface hydrophobicity of gastric mucosa, a phenomenon associated with impaired mucosal defense (66). Biopsies from individuals not infected with *H. pylori* have occasional PMNs and aggregates of lymphocytes, but never lymphoid follicles (65).

Acid Secretion

Mean BAO and PAO before AGH and now in the 22 AGH subjects who consented to follow-up acid secretory studies are compared to their matched control subjects in Table 8.

Table 8. Acid secretion in AGH subjects before illness in AGH subjects and at follow-up in AGH subjects and matched controls.

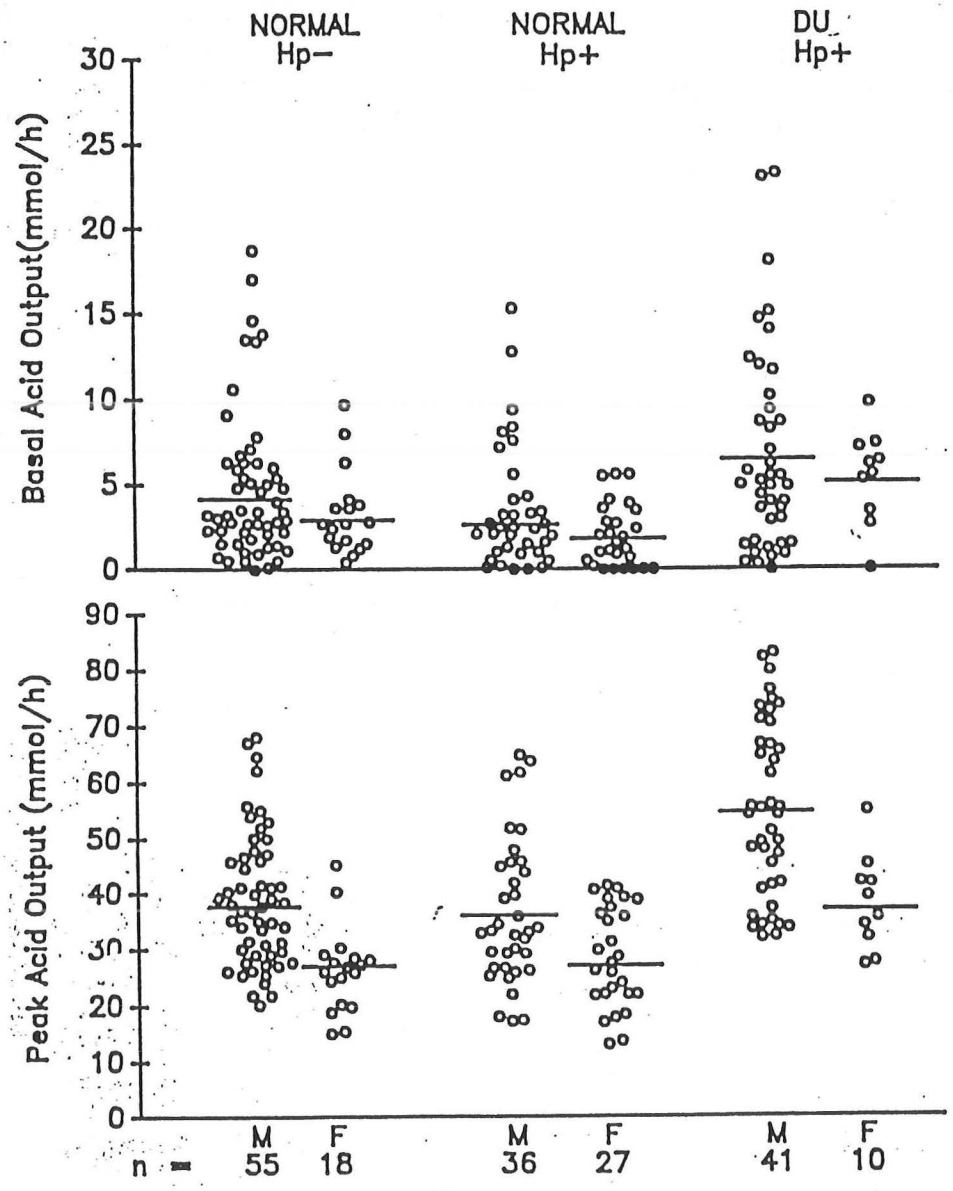
	AGH Subjects		Control Subjects
	Before Illness	Now	Now
BAO (mmol/h)	3.3±0.6	2.2±0.6	5.9±1.2*
PAO (mmol/h)	36±2.2	32.1±3.7**	38.9±2.9

* $p<0.02$ compared to AGH subjects now

** two subjects achlorhydric

We have recently extended our acid secretory studies in a large number of healthy subjects with and without serologic evidence of *H. pylori* infection and have compared them to patients with duodenal ulcer (67). Infected subjects have slightly, but significantly lower basal, but not peak or meal-stimulated, acid outputs when compared to uninfected subjects (Figure 4). The reason(s) for the lower basal acid output is/are not clear but may involve a protein which inhibits acid secretion somewhere late in the enzymatic cascade of the parietal cell (68,69). Patients with duodenal ulcer who are infected with *H. pylori* have high basal and peak acid outputs (Figure 4). The explanation for the discrepancy in acid secretion between healthy subjects and duodenal ulcer patients, each infected with *H. pylori*, is not known.

Figure 4. Basal and peak acid outputs (mmol/h) in normal subjects with and without *H. pylori* infection and in duodenal ulcer patients as a function of gender. Mean values are represented by the horizontal bars.



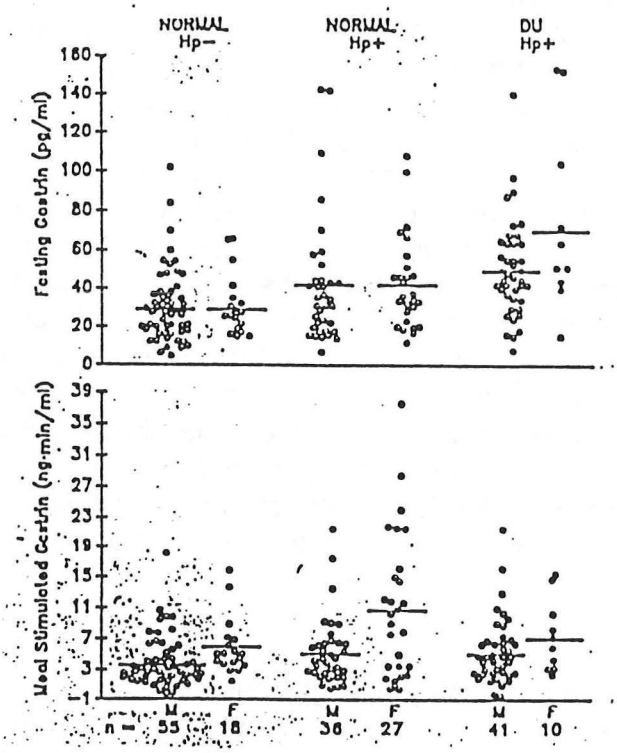
Pepsinogen Secretion

With acute superficial gastritis, serum levels of both PGI and PGII rise (70,71). Since it is made by mucous cells throughout the stomach, not just the oxyntic cells, PGII rises proportionately more than PGI. Thus, the serum PGI/PGII ratio falls. As atrophy occurs and progresses, the levels of PGI fall substantially, while PGII levels fall only modestly. Thus, the serum PGI/PGII ratio continues to fall with increasing degrees of atrophy.

Serum Gastrin

H. pylori positive healthy subjects and duodenal ulcer patients exhibit fasting and meal-stimulated serum gastrin levels significantly higher than uninfected healthy subjects (67) (Figure 5).

Figure 5. Fasting gastrin (pg/ml) and meal-stimulated gastrin (ng.min/ml) in normal men and women with and without *H. pylori* infection and men and women with duodenal ulcer. Mean values are represented by the horizontal bars.



The mechanism of *H. pylori*-associated hypergastrinemia is not clearly understood. Some investigators have reported a deficiency in tissue levels of somatostatin, a natural inhibitor of gastrin release, perhaps due to decreased numbers of D cells (72-74). Other investigators disagree regarding the number of D cells (75). Whether *H. pylori* associated hypergastrinemia has any clinical relevance is unknown.

ATROPHIC GASTRITIS

In some individuals, chronic superficial gastritis has a tendency over time to progress to atrophic gastritis (76). Such progression leads to three patterns of atrophic gastritis: body predominant (Type A), antral predominant (Type B), both body and antrum (Type AB). In one study, the relative proportions of these patterns was 31%, 45%, and 24% (77). As the degree of atrophy progresses, the tissue presence of *H. pylori* decreases. This may be due to hypochlorhydria creating an uninviting milieu for the organism, or to the development of intestinal metaplasia, tissue which cannot support *H. pylori*. While prevalence of *H. pylori* by tissue staining is low, the prevalence of antibodies is very high. This suggests prior infection with *H. pylori*. The mechanism by which atrophy occurs is unclear, and, is probably multi-factorial. However, one recent study found *H. pylori* antibodies which cross-reacted with gastric autoantigens (78).

Type A (body predominant) atrophic gastritis may be associated with pernicious anemia. Such patients have antibodies directed against the proton pump and pepsinogen (79). In these patients, loss of secretory function begins with acid, followed by pepsinogen, and, finally, intrinsic factor (80). This suggests that pernicious anemia is a marker for the most severe, end-stage, form of Type A atrophic gastritis. There has been a long-held belief that the pathogenesis of atrophic gastritis associated with pernicious anemia is "autoimmune", since there are antibodies to the secretory elements. Furthermore, the prevalence of *H. pylori* is low by both tissue staining and serum antibodies, suggesting not even a remote infection with the organism. This concept is now being challenged, with several lines of reasoning put forth. First, antibodies to secretory elements appear to develop after the atrophic process begins. To quote Berstad and Berstad, the "intracellular structures of the gastric mucosa may, as a result of cell damage, be exposed to immunocompetent cells, which do not recognize these structure as 'self'" (81). Second, a recent study of first-degree family members of PA patients found the expected high prevalence of body atrophic gastritis and parietal cell antibodies, but an unexpectedly high prevalence of serum antibodies to *H. pylori* (82). It is plausible, therefore, that in a subset of patients (genetically determined?), *H. pylori* gastritis progresses to a severe form of atrophic gastritis associated with "autoimmunity" and the development of pernicious anemia. The absence of serum antibodies to *H. pylori* in such patients, when compared to patients without pernicious anemia, may simply reflect the greater severity and longer duration of the atrophic process. Said another way, since it takes a long time after *H. pylori* leaves the stomach for serum antibody titers to fall, only patients at the very end of the spectrum (i.e., pernicious anemia) will be without antibodies.

PEPTIC ULCER DISEASE

Two lines of evidence support *H. pylori* gastritis as a major factor in the etiology of peptic ulcer disease, especially duodenal ulcer. First, the relative risk of developing a peptic ulcer is significantly higher among individuals with *H. pylori* gastritis than among those without (83-86). A recently-published paper described a case control study based on a cohort of Japanese-American men who had serum samples stored from 1967-1970 (83). Members of the cohort who developed a peptic ulcer at some time during the ensuing 20 years were 3.4 times more likely to have been serologically positive for *H. pylori* at the beginning of the observation period than those who did not. In an 18 year cohort study, 15% of individuals with serum antibodies to *H. pylori* developed a duodenal ulcer compared to 3% of subjects without antibodies (84). A second cohort study followed patients with or without gastritis for a ten year period of time (85). A peptic ulcer developed in 11% of those with gastritis compared to 1% of those without. Finally,

investigators in Italy recently endoscoped 359 asymptomatic blood donors and found that duodenal and gastric ulcers were present only in those who were infected with *H. pylori* (86) (Table 9).

Table 9. Prevalence of gastric and duodenal ulcer in asymptomatic blood donors. From reference 86.

	H. Pylori Pos. (N=298)	H. Pylori Neg. (n=61)
Duodenal Ulcer	16.8%	0%
Gastric Ulcer	6.7%	0%

Second, numerous studies have shown that recurrence of duodenal or gastric ulcer is markedly decreased following successful eradication of *H. pylori* (87-91) (see page 22). The observed recurrence rates in patients whose ulcers were healed by conventional antisecretory therapy alone ranged from 60 to 100 per cent in these studies, while ulcers recurred in only 0 to 15 per cent of patients in whom *H. pylori* had been successfully eradicated by antimicrobial therapy.

Despite the fact that *H. pylori* is a necessary factor in the etiology of peptic ulcer in most patients, it is far from sufficient. Indeed, only a small proportion, perhaps up to 15%, of infected individuals will develop an ulcer. The reasons why *H. pylori* infection leads to peptic ulcer in some patients, but not in most, remain poorly defined. Factors which, when superimposed upon a background of *H. pylori*, may predispose to ulceration include the use of non-steroidal anti-inflammatory drugs, smoking, and stress. It has also been suggested that the organisms found in patients with ulcer are more likely to possess the cagA gene, which encodes for a cytotoxin, than are the organisms found in patients with gastritis but no ulcer (92). Others dispute this claim (93).

Another possibility is that subjects with *H. pylori* infection who have increased levels of gastric acid secretion will be more likely to develop duodenal ulcer. Indeed, mean basal and peak acid outputs (and serum gastrin levels) are higher in duodenal ulcer patients than in infected (or noninfected) healthy subjects (Figures 4 and 5) (67). Why patients with duodenal ulcer have higher mean acid secretory levels than infected subjects without ulcer, or whether *H. pylori* is even playing a role, is not known.In any regard, only a subset of individual patients with peptic ulcer have acid secretory values above the upper limits of normal.

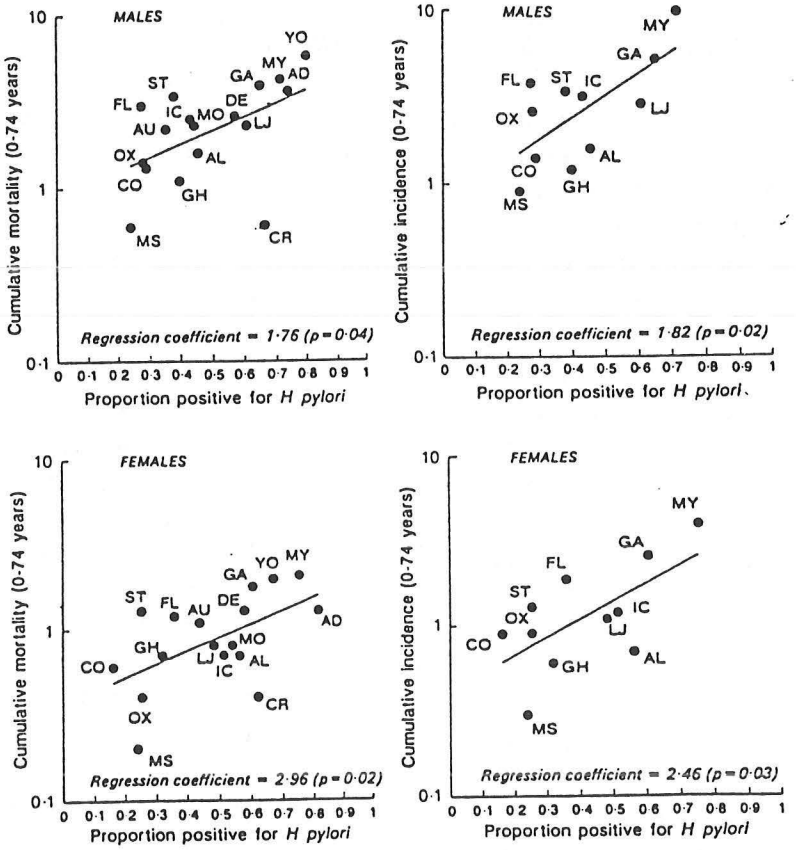
A final consideration is suggested by work from Jon Isenberg's lab at UC San Diego, where detailed studies of duodenal bicarbonate secretion have been ongoing for several years. Patients with chronic duodenal ulcer (all of whom are *H. pylori* positive) have impaired secretion of duodenal bicarbonate in response to acid infusion. Recent work suggests that non-ulcer patients with *H. pylori* infection do not have this abnormality (94). Furthermore, eradication of *H. pylori* in a small sample of duodenal ulcer patients

resulted in normalization of duodenal bicarbonate secretion (94). These data support the concept that there is a subset of individuals in whom, for unknown reasons, *H. pylori* infection impairs duodenal bicarbonate secretion and that it is among these individuals that duodenal ulcers develop.

GASTRIC ADENOCARCINOMA

It has long been known that the sequence of mucosal histologic events leading to the intestinal type of gastric adenocarcinoma is as follows: chronic superficial gastritis - atrophic gastritis - intestinal metaplasia - dysplasia - adenocarcinoma. It is now known that *H. pylori* is the cause of chronic superficial gastritis which in some patients progresses to atrophic gastritis. There is an epidemiologic association between *H. pylori* and adenocarcinoma of the gastric body and antrum, but not the cardia (95-98) (Figure 6, Table 10).

Figure 6. Correlation between lifetime mortality from and incidence of (%) gastric cancer and prevalence of *H. pylori* seropositivity in different populations of the world. From reference 97.



Incidence and mortality rates from gastric cancer by *H pylori* seropositivity (mean of 25–34 years and 55–64 years).

In models in which males and females were combined, regression coefficients were 1·79 (p = 0·002) for mortality and 2·68 (p = 0·001) for incidence.

Centre codes: AL, Algiers; GH, Ghent; CO, Copenhagen; AU, Augsburg; DE, Deggendorf; MO, Mosbach; CR, Crete; IC, Iceland; FL, Florence; MY, Miyagi; YO, Yokote; AD, Adamowka; GA, Gaia; LJ, Ljubljana; OX, Oxford; ST, Stoke; MS, Minneapolis-St Paul.

Table 10. Seroprevalence of *H. pylori* 6-14 years before diagnosis in patients with gastric neoplasms and matched controls (nested case control studies from references 95-97).

	Parsonnet	Nomura	Forman
Cohort Size (N)	128,992	5,908	22,000
Follow-Up (Y)	14.2	13	6
Cancer (N)	246	137	29
Selected as Cases	109	109	29
% H.p.+:Cases	84%	94%	69%
Controls	61%	76%	47%
Odds Ratio:			
All AdenoCa	3.6	6.0	2.8
Intest Type	3.1	4.5	-
Diffuse Type	8.0	-	-

Because most of the patients with gastric cancer and their matched controls were from older age groups, the absolute differences in *H. pylori* prevalence were not striking. Now that the general prevalence of *H. pylori* is decreasing, it would be expected that the difference between patients with and without gastric cancer would widen. Support for this concept is found in a recent study from Japan of young patients with gastric cancer in whom the prevalence of *H. pylori* was 88.4% compared to only 26.9% in young, age-matched controls (99).

As important as *H. pylori* infection appears to be, other factors must also play a role. Only a small proportion of patients progress to atrophic gastritis, much less intestinal metaplasia, dysplasia, and cancer. As with peptic ulcer, *H. pylori* gastritis is in most individuals a necessary, but far from sufficient, factor in the pathogenesis of gastric adenocarcinoma. One area of research concerns the observation that patients with *H. pylori* gastritis have reduced levels of gastric juice ascorbic acid which return towards normal after eradication (43,100).

The diffuse type of gastric cancer is also associated with *H. pylori* (Table 10), but not with atrophic gastritis (101). Sipponen biopsied non-cancerous tissue in the antrum of patients with the diffuse type of gastric cancer and found chronic superficial gastritis in 52% and atrophic gastritis in only 27% (101). Patients with the intestinal type had 26% superficial gastritis and 61% atrophic gastritis. It has recently been suggested that diffuse type of cancer evolves from ECL-cell hyperplasia (102). Since gastrin is trophic for ECL cells, perhaps hypergastrinemia is the link between *H. pylori* and the diffuse type of gastric adenocarcinoma.

GASTRIC LYMPHOMA

Primary gastric lymphoma, virtually all of which are B-cell tumors, is the most common form of extranodal lymphoma (103). The low grade type arises from, and has histologic features which resemble, mucosa-associated lymphoid tissue (MALT). These include the presence of centrocytelike cells, characteristic lymphoepithelial lesions, and lymphoid follicles (104,105). Such features are found in low-grade lymphomas at other extranodal sites (e.g., salivary gland, thyroid) comprising what is now known as MALT lymphomas. Low-grade gastric MALT lymphomas were previously called "pseudo-lymphomas" and, because they often co-existed with peptic ulcers, were believed to be a reaction to the ulcerative process.

The paradox of a lymphoma developing in an organ which is normally devoid of lymphocytes began to be solved with the recognition that *H. pylori* produces chronic gastritis with a large lymphocytic component, including lymphoid follicles. Virtually every patient with gastric lymphoma is infected with *H. pylori* (106) and there is a correlation between the prevalence of *H. pylori* in a population and the incidence of gastric lymphoma (107).

Parsonnet has recently shown in a nested case-control study that the risk of gastric lymphoma is higher in infected than non-infected persons and that infection was present prior to a diagnosis of lymphoma (108). Over 200,000 persons had serum collected and stored after which they were followed for the development of cancer. Thirty-three cases of gastric non-Hodgkins lymphoma occurred a median of 14 years after serum collection and in 31 patients a nongastric lymphoma developed a median of six years following serum collection. Each gastric lymphoma patient was matched to four controls while each non-gastric lymphoma was matched to two controls in the cohort. The results of the study are shown in Table 11.

Table 11. Odds ratio for for association of *H. pylori* infection with gastric non-Hodgkins lymphoma and non-gastric non-Hodgkins lymphoma. From reference 108.

Type of Lymphoma	No. of Patients	H.pylori Infection Patients	Controls	Matched Odds Ratio
Gastric	33	85%	55%	6.3 (2.0-19.9)*
Non-Gastric	31	65%	59%	1.2 (0.5-3.0)

*95% Confidence Interval

More direct evidence for the role of *H. pylori* in gastric lymphoma comes from a study in which cells from patients with various types of lymphoma were cultured in the presence of *H. pylori* (109). Neoplastic B cells and non-neoplastic T cells proliferated, accompanied by increases in IL-2 receptor expression. Examination of the supernatant showed increased levels of IL-2 and tumor immunoglobulin. These responses were abolished by the removal of T cells from the suspension and were not seen in cells from high-grade gastric MALT lymphomas or low-grade B-cell MALT lymphomas from other sites. Thus, specific activation and proliferation of B-cells from low-grade gastric MALT lymphomas occurs upon the release of cytokines from T cells which are themselves specifically activated by *H. pylori* (109). This responsiveness is not seen in non-gastric lymphoma cells or when low-grade gastric MALT lymphoma is transformed to a high-grade lymphoma (110).

In her study, Parsonnet made the important observation that only 3 of the 33 gastric lymphomas were classified as low-grade MALT lymphomas (108). From this observation, and from the cell-biology study described above, three points emerge. First, low-grade gastric MALT lymphomas are diagnosed infrequently. Second, the association of *H. pylori* with high grade lymphomas suggests there may be a continuum from low-grade to high-grade lymphoma. Third, the lack of responsiveness of high grade lymphomas to *H. pylori* suggests that cure of gastric lymphoma by eradication of *H. pylori* will occur only when the tumor is still in the low-grade state.

FUNCTIONAL DYSPEPSIA

The syndrome of epigastric distress, especially related to meals, in patients with no evidence of peptic ulcer, reflux esophagitis, pancreatitis or gallbladder disease has been termed non-ulcer, or more recently, functional dyspepsia. There was a burst of enthusiasm that symptoms in such patients might be related to *H. pylori* gastritis. However, most studies suggest that, when broad groups of patients with dyspepsia are evaluated, the prevalence of *H. pylori* is no greater in patients with functional dyspepsia than age-matched controls (111). While one group has reported a slightly greater prevalence of *H. pylori* in patients with ulcer-like dyspepsia when compared to controls (112), another group has challenged this observation (113).

EFFECTS OF ERADICATION OF *H. pylori*

GASTRITIS

In virtually every instance where there is clear evidence of eradication of the organism , there is accompanying resolution of the neutrophilic component of gastritis and normalization of the surface epithelium (114-116). The lymphocytic infiltrate resolves much more slowly. In one careful morphologic study, lymphocytes (and eosinophils) had not returned to normal after one year, and lymphoid follicles were present throughout the stomach (116).

SERUM ANTIBODY LEVELS

Following successful eradication of *H. pylori* there is a decline over time in IgG antibody titers (8,117,118). In one series, a 20% or greater decline in titer at 6-12 months was 88% sensitive in predicting eradication (8,117). However, in this same series, antibody titers fell into the "normal" range in only a third of the patients. Because the use of serology to confirm eradication requires the testing of paired sera using quantitative measurements rather than a simple positive or negative, and because it cannot be performed sooner than 6-12 months following treatment, it will have limited usefulness in clinical practice.

GASTRIC FUNCTION

Following successful eradication of *H. pylori* there is a fall in fasting, meal-stimulated, and GRP-stimulated gastrin (119-121). The time necessary for a fall in gastrin exceeds that needed to clear the organism, suggesting that it is the inflammation, rather than the organism itself, which is responsible (122). Most, but not all studies, report an increase in somatostatin-producing D cells after eradication (123,124). Basal acid output has been reported to fall in two small, uncontrolled studies of duodenal ulcer patients after eradication, although not to "normal" levels (119,120). Pepsinogen I levels also fall, but peak acid output is unchanged (119,125).

DUODENAL ULCER

The first double-blind study was recently published in which patients with acute duodenal ulcer were treated with either ranitidine alone (300 mg hs) for 6 to 10 weeks or ranitidine plus amoxicillin and metronidazole for the first 12 days (91). Not only was ulcer healing significantly faster with the combination regimen (Table 12), but 12 month endoscopic ulcer recurrence was significantly reduced (Figure 7). An ulcer recurrence was noted in 1/41 *H. pylori* negative patients who completed one year follow-up compared to 45/50 patients who remained *H. pylori* positive. Of the 40 *H. pylori* negative patients who were ulcer-free at 12 months, 39 were followed for another year. No recurrences were noted (126).

One of the issues which would seem self-evident, but which has not been well addressed, deals with the effect of eradication of *H. pylori* on symptoms. The criterion for ulcer recurrence in all published studies is an endoscopic ulcer, but none of the published trials have noted the number of patients who return with symptoms but do not have an ulcer at endoscopy. One study has reported a 7 year follow-up of duodenal ulcer patients after eradication of *H. pylori* (127). Proven DU relapse occurred in 3/37 (8%) of *H. pylori* negative patients compared to 9/26 (35%) of *H. pylori* positive patients ($p<0.01$). However, if they looked at symptom relapse, whether a DU was found or not, the results were 8/37 (22%) in the *H. pylori* negative group compared to 11/26 (42%) of the *H. pylori* positive group (p=ns). Could it be that eradication of *H. pylori* prevents recurrence of the

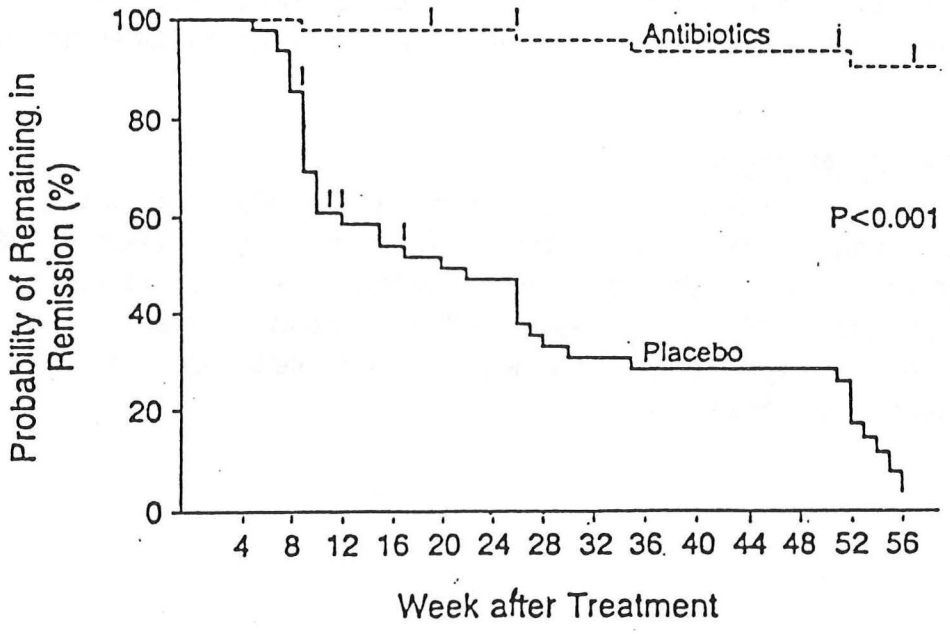
ulcer crater, but has less impact on the symptoms that trouble the patient? Said another way, what has been accomplished if patients still need to take antacids or H_2 receptor antagonists after eradication of *H. pylori*?

Table 12. Treatment of duodenal ulcer with ranitidine and antibiotics: a double-blind trial. From Reference 91.

		6 wk		10 wk
Group	N	Hp Erad	Ulcer Heal	Ulcer Heal
Ranitidine[a] + Placebo	52	1 (2%)	39 (75%)	49 (94%)
Ranitidine[a] + Antibiotics[b]	52	46 (89%)	48 (92%)	51 (98%)
		p<0.001	p=0.01	

[a] 300 mg hs x 6-10 wk
[b] Amox 750 mg tid x 12 da
MNZ 500 mg tid x 12 da

Figure 7. Probability of remaining in remission from duodenal ulcer during one year after healing the ranitidine plus antibiotics or placebo. From Reference 91.



GASTRIC ULCER

One trial with small numbers of patients reported a 1 year recurrence rate of 13% in gastric ulcer patients whose ulcer was healed with rantidine plus H. pyloricidal therapy compared to 74% recurrence in patients healed with ranitidine alone (90). Further studies are needed in patients with gastric ulcer.

BLEEDING ULCER

Controlled trials to document that the subgroup of patients with bleeding ulcers will have long-term results similar to those seen with non-bleeding ulcers are not yet available, although preliminary reports of two small series with follow-up periods averaging 9 and 20 months noted no recurrent bleeding after *H. pylori* eradication (93,94). Larger randomized controlled trials are required.

FUNCTIONAL DYSPEPSIA

A number of studies were conducted to see if eradication of the organism would improve symptoms (130-136). Several of these studies reported success. Unfortunately, every one of these early studies is fatally-flawed and their results are uninterpretable (137). Symptoms were not measured long-term, follow-up diagnostic tests were performed too early to ensure that eradication had occurred, and the regimens used are now known to be poorly effective against *H. pylori*. Recent studies in which more effective regimens have been used, and in which eradication has been properly assessed, suggest that symptoms are improved in patients regardless of whether or not *H. pylori* has been eradicated (138-140). Even these studies evaluate short-term symptom response only (137). Well-designed studies, with large numbers of patients followed for a minimum of one year after treatment, are not available. While it is possible that there is a subset of patients whose symptoms might improve with eradication of *H. pylori*, antimicrobial therapy in patients with functional dyspepsia should be avoided until studies prove this point.

GASTRIC ADENOCARCINOMA

It has been suggested that eradication of *H. pylori* might dramatically reduce the incidence of gastric adenocarcinoma, the second most common cause of death from cancer world-wide. However, the lack of proof that this would actually happen, as well as the costs and logistical difficulties of treating millions of people with antimicrobial therapy, especially in developing countries where the re-infection rate is high, realistically preclude such thoughts at the present time.

GASTRIC LYMPHOMA

Based on their observations that low-grade B-cell MALT lymphomas are immunologically responsive *in vitro* to *H. pylori*, Isaacson's group treated six such patients with *H. pyloricidal* therapy (141). In 5, biopsies repeated up to 15 months after therapy showed no evidence of lymphoma.

TREATMENT OF *H. pylori*

DIAGNOSTIC STRATEGY

The selection of the appropriate test in a given patient depends upon the clinical situation. For patients in whom an EGD is clinically indicated to document the diagnosis of a peptic ulcer, it is reasonable at the same time to obtain mucosal biopsies for histology or a rapid urease test. The rapid urease test is the least expensive of the biopsy tests and should be performed first; extra biopsies may be taken, held, and sent later for histologic examination if the slide test is negative.

For patients in whom it is not indicated for other clinical reasons, EGD should rarely be perfomed solely to diagnose infection with *H. pylori*. Serologic tests or, when available, urea breath tests may be used.

A stong case can be made for not performing any test to diagnose *H. pylori* in patients with duodenal ulcer who have no history of NSAID use and no signs or symptoms of a hypersecretory state. In this situation, the prevalence of *H. pylori* is so high that a diagnostic test adds little except cost. However, in patients taking NSAIDs or in patients with gastric ulcer, the prevalence of *H. pylori* is lower and a diagnostic test is informative.

DEFINITION OF ERADICATION

It has become clear that failure to detect *H. pylori* immediately after a course of antimicrobial therapy does not mean that the organism is truly eradicated. In many instances the organism has only been suppressed, with follow-up studies performed several weeks later readily disclosing its presence. Somehow, *H. pylori* finds "sanctuary sites" which temporarily preclude its detection, but from which it ultimately emerges to regain its foothold once again. Thus, eradication is now defined as absence of the organism by tests performed no sooner than 4 weeks after cessation of therapy.

ERADICATION OF *H. pylori*

H. pylori infection is difficult to cure, requiring concurrent administration of more than one drug (142-144). Effectiveness *in vivo* is predicted poorly by *in vitro* sensitivity and is complicated by the need to test agents in combination. *H. pylori* is inherently resistant to only a few antimicrobial agents (i.e.,vancomycin, nalidixic acid, trimethoprim,

and sulfonamides) but readily becomes resistant to metronidazole, and, to a lesser extent, clarithromycin, when either agent is given as monotherapy (114,145). It does not appear to become resistant to some luminally active agents including bismuth, tetracycline, and amoxicillin. Antibiotics vary considerably in their clearance into gastric juice (146). Luminal pH influences the potency of some drugs to eradicate *H. pylori in vivo* (147), and raising the gastric pH from 3.5 to 5.5 increases the effectiveness of amoxicillin and erythromycin by more than 10-fold. This increased activity at higher pH is one explanation for the effectiveness of regimens that combine potent inhibitors of gastric acid secretion with an antimicrobial.

INDIVIDUAL AGENTS CURRENTLY USED IN REGIMENS TO ERADICATE *H. pylori*

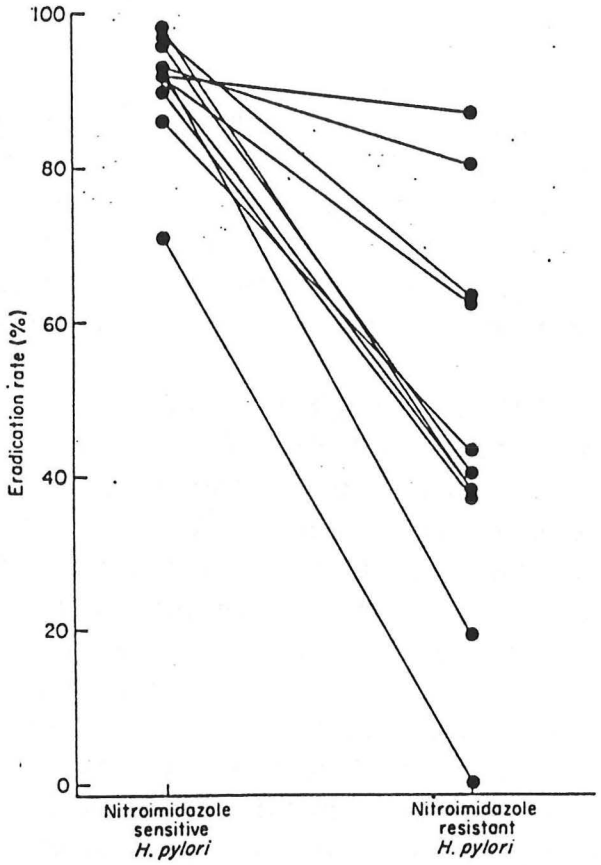
Amoxicillin: *H. pylori* is very sensitive *in vitro* to this antibiotic, which has topical or intraluminal activity, is stable in acid enviroments but most active at neutral pH, and does not accumulate in gastric secretions during intravenous administration (146). Bacterial resistance to amoxicillin is rare.

Tetracycline: *H. pylori* is also very sensitive to this antibiotic, appears to act luminally or topically, and is active at low pH. No bacterial resistance to tetracycline has been reported.

Metronidazole: This antibiotic is a mainstay of "triple therapy". *H. pylori* ordinarily is highly sensitive to metronidazole. Unlike amoxicillin and tetracycline, metronidazole is actively secreted into gastric juice and saliva, can act systemically, with an *in vivo* half life of 8 to 12 hours, and its activity is relatively independent of pH. Mutant organisms with defective nitroreductase activity are resistant to metronidazole (145). Resistance is acquired frequently when metronidazole is administered as a single agent and in areas of the world where it is used frequently for other reasons, rates of resistance are very high (148). Resistance develops less often when metronidazole is given with bismuth or a second antimicrobial agent (149). Resistance to metronidazole is associated with marked reduction in eradication rates when regimens include this antimicrobial (143,150, 151) (Figure 8). Tinidazole is a related nitroimidazole for which most of the same conclusions can be drawn.

Clarithromycin: This antibiotic is a macrolide that blocks protein synthesis. It has an antibacterial spectrum similar to erythromycin but is more acid stable, better absorbed, and has higher activity against *H. pylori*. As with metronidazole, *H. pylori* can become resistant to clarithromycin when the latter is given as a single agent (114). Side effects include taste perversion in a majority of patients.

Figure 8. Effect of nitroimidazole resistance on the eradication rate of *Helicobacter pylori*. From Reference 143.



Bismuth: Bismuth compounds are topical antimicrobial agents that act directly upon bacterial cell walls to disrupt their integrity by accumulating in periplasmic space and along membranes. Bismuth lyses *H. pylori* organisms near the gastric surface and may act in several other ways including prevention of adhesion to gastric epithelium or by inhibition of urease, phospholipase and proteolytic activity. Bismuth compounds are widely used in combination with antibiotics for *H. pylori* eradication because they act by different mechanisms and have complementary activity. In the United States, bismuth is available only as bismuth subsalicylate (PeptoBismol^{R}). Many studies with bismuth elsewhere in the world have utilized colloidal bismuth subcitrate. Equivalence of activity of these two preparations has not been tested critically, but bismuth subsalicylate has proven active in "triple therapy" regimens (90). Antibacterial concentrations of bismuth are achieved in antral mucus only for about 2 h after dosing.

Side effects of bismuth may include central nervous system toxicity at high doses (152). A plasma concentration of 50-100 μg/l is considered borderline for toxicity. The black stool produced by bismuth compounds complicates blinding of antimicrobial regimens that utilize this agent.

H,K ATPase (Proton Pump) Inhibitors: Omeprazole and lansoprazole produce prolonged inhibition of gastric acid secretion. Although these drugs have some direct activity against *H. pylori in vitro* (153), they are rapidly inactivated at acid pH and, therefore, are administered in enteric coated forms that are liberated in the small intestine. Almost no active compound enters the gastric lumen from the circulation. When used alone *in vivo*, they suppress, but do not eradicate, *H. pylori* (154, 155). When used as part of combination therapy to eradicate *H. pylori*, their major activity is related to increasing intragastric pH which creates a less inviting milieu for *H. pylori* and optimizes the effects of antibacterial agents. The decreased gastric juice volume that results from these drugs may also increase intragastric concentrations of antibacterial agents.

THERAPEUTIC REGIMENS TO ERADICATE *H. pylori*

A treatment can be considered useful if it reliably results in more than 80% eradication of *H. pylori* and produces no important clinical or biochemical side effects (144). A review of the literature reveals numerous factors which make it difficult to state with scientific assurance which regimen(s) is/are best to eradicate *H. pylori*. These problems are the best reason that no regimen has yet been approved by the U.S. Food and Drug Administraion for the treatment of *H. pylori*. First, most studies of therapeutic regimens have small sample sizes, are uncontrolled, unrandomized, and unblinded, and fail to use intention-to-treat analysis. Second, among regimens, the dosage and timing of drugs and the duration of their administration vary greatly, making direct comparisons difficult. Third, resistance of *H. pylori* to metronidazole and clarithromycin has a major impact on the success of regimens containing these antimicrobials. Fourth, factors unrelated to the drug regimens themselves may influence eradication rates. Compliance is one such factor (156). It has been estimated that failure to take at least 80 per cent of prescribed doses of antibacterial agents leads to decreased efficacy (156,157). This is a serious problem in regimens that involve taking three or four drugs given three or four times a day, especially when troublesome side effects occur in 20-30 per cent of subjects. Older age, smoking, and a greater severity of mucosal inflammation have also been reported to result in lower eradication rates (157,158). Despite these problems, trends regarding effective antimicrobial therapy are emerging and must serve until the results of ongoing, properly designed, large-scale studies are available.

Regimens to eradicate *H. pylori* may be classified as single, double, or triple antimicrobial therapy with or without a concomitant anti-secretory agent (Table 13).

Table 13. Therapeutic regimens most likely to result in eradication rates of 80% or higher

Antimicrobials	Alone	Plus Antisecretory Agent
Single Therapy	Not Effective	Amoxicillin (2 gm/day) + Omeprazole (20 mg bid)
		Clarithromycin (500 mg tid) + Omeprazole (40 mg daily)
Double Therapy	Not Effective	Amoxicillin (750 mg tid) + Metronidazole (500 mg tid)[a] + Ranitidine (300 mg hs)
Triple Therapy	Bismuth[b] + Metronidazole (500 mg tid) + Amoxicillin (500 mg tid) **or** Tetracycline (500 mg qid)	Bismuth[b] + Metronidazole (250 mg tid)[a] + Tetracycline (500 mg qid) + Raniditine (300 mg hs)

[a] Preliminary reports suggest Clarithromycin, 500 mg tid, can be substituted for metronidazole in this regimen

[b] Bismuth in the United States is avilable only as bismuth subsalicylate (Pepto-Bismol) and should be given in a dose of 2 tablets qid. Colloidal Bismuth Subcitrate (DeNol) is available in other countries and is usually given in a dose of 120 mg qid.

Single Therapy

No antimicrobial agent given alone is adequate to eradicate *H. pylori* (142,143). Bismuth or amoxicillin when given alone eradicate *H. pylori* in just over 20% of patients. *H. pylori* rapidly becomes resistant to metronidazole or the quinolones when they are given alone, resulting in very poor rates of eradication. The best results with an antimicrobial given alone have been reported with clarithromycin, where in one study a 54% (44% intention to treat) eradication rate was achieved with a high dose of 2 gm daily given for 14 days (114).

Results appear to be better when the single antimicrobials amoxicillin or clarithromycin are given with the H,K ATPase inhibitor, omeprazole. The combination of amoxicillin and omeprazole has been best studied, but the eradication rates vary widely from study to study (143,144). Characteristics of the regimens with the best results include the use of at least 2 gm per day of amoxicillin, administration of omeprazole in divided doses totalling at least 40 mg per day, initiation of amoxicillin and omeprazole therapy at the same time, and treatment for at least 14 days (143). Under these conditions, the combination of amoxicillin and omeprazole leads to eradication in approximately 80% of patients (143,159,160). There is some evidence that very high doses of omeprazole (e.g., 120 mg per day) may give slightly higher rates of eradication (159), but such claims must be confirmed by other studies, especially given the high costs of the regimen. Side effects, primarily diarrhea, occur in 6%-10% of patients, requiring cessation of therapy in 2%-5% (143,159,160).

The combination of clarithromycin and omeprazole has been less thoroughly evaluated, but early reports suggest that a single, daily 40 mg dose of omeprazole combined with clarithromycin, 500 mg tid, eradicates *H. pylori* in about 80% of patients (162,163). Almost two-thirds of patients taking clarithromycin experience a metallic taste, but only 3%-5% do not complete therapy.

A new compound, ranitidine bismuth citrate, is comparable to a single antimicrobial plus an anti-secretory agent. Early studies report poor rates of eradication when given alone (164).

Double Therapy

Various combinations of metronidazole/tinidazole, amoxicillin, and bismuth have been used as double therapy, with eradication rates generally between 50 and 60% (142). However, when two antimicrobials are combined with an anti-secretory agent, the results are substantially better. For example, the combination of metronidazole (500 mg) and amoxicillin (750 mg) three times daily with ranitidine (300 mg hs) for 12 days has produced eradication in 89 per cent of infected patients (91). Although only one study, it was a well-designed, randomized, double-blind trial with over 50 patients in each arm. About 15% of patients taking the combination regimen developed diarrhea, but only one patient (2%) stopped the study. Another single report of fewer patients found comparable

patient (2%) stopped the study. Another single report of fewer patients found comparable results when clarithromycin (500 mg tid) was substituted for metronidazole (165).

The combination of ranitidine bismuth citrate plus amoxicillin or clarithromycin may achieve 80-90% eradication rates, but such results are very preliminary (166).

Triple Therapy

Triple therapy with bismuth, metronidazole, and either tetracycline or amoxicillin produces very good eradication rates, especially with organisms sensitive to metronidazole (142-144). Treatment for 14 days is approximately equivalent to 28 days, but there is about a five per cent reduction if treatment is given only for seven days. Analysis of several studies in which treatment was given for 14 days reveals a median eradication rate of 92% with the tetracycline combinations and 84% with the amoxicillin combinations (144). Although a few studies find little effect of metronidazole resistance on eradication rates, most studies find substantially lower rates of eradication in subjects whose *H. pylori* is resistant to metronidazole (143,144). In the United States, a regimen of bismuth, metronidazole, tetracycline, and ranitidine for 14 days eradicated *H. pylori* in 89% of 62 patients (90,167). Substitution of clarithromycin for metronidazole may give similar results (168).

These triple therapy regimens produce significant side effects. Over 30% of patients will report some side effect, primarily nausea, sore mouth, taste disturbance, diarrhea, and candida infections (143). Treatment must be stopped in about 5% of patients (143).

A recent study compared triple therapy with tetracycline plus ranitidine to omeprazole and amoxicillin in patients with active duodenal ulcers (Table 14) (169). Anti-*H. pylori* therapy was given for 14 days followed by 4 weeks of ranitidine alone. While the eradication rates of *H. pylori* were comparable, ulcer healing was slightly better and side effects less frequent with omeprazole/amoxicillin.

FOLLOW-UP OF PATIENTS AFTER ERADICATION THERAPY

Since serologic studies are unsuitable and breath tests are unavailable, the only means currently available to confirm the success of eradication therapy is endoscopic gastric mucosal biopsy, a prohibitively expensive test. Therefore, most patients should have no follow-up test performed, but should be followed using relapse of clinical symptoms as an indication for further diagnostic evaluation. The only exception to this recommendation is for patients who have had a complication of their ulcer, such as bleeding. Some physicians in this situation might wish to discontinue long-term maintenance anti-secretory therapy if eradication of *H. pylori* could be confirmed. While we discourage this practice, it should be done only if endoscopic gastric mucosal biopsy discloses no evidence of continuing *H. pylori* infection.

Table 14. Comparison of triple therapy with tetracycline plus ranitidine to omeprazole plus amoxicillin in active duodenal ulcer. From reference 169.

	Omep/Amox[a]	Triple Therapy[b]
No.	19	19
H.p Erad.	79%	84%
Ulcer Healing	100%	79%
Side Effects	16%	63%

[a] Omeprazole 20 mg bid/Amoxicillin 500 mg qid
[b] Bismuth subsalicylate, metronidazole 400 tid, tetracycline 500 mg tid, ranitidine 300 mg hs

APPROACH TO TREATMENT FAILURE

If at some point it is determined that treatment failed to eradicate *H. pylori*, another course of therapy may be indicated. The best approaches to treament failure have not been determined, but if metronidazole or clarithromycin were used as part of initial therapy, the organism may now be resistant and that agent should not be re-used. It is believed that resistance does not develop to amoxicillin, bismuth or tetracycline.

REINFECTION

Long-term data on recurrence of *H. pylori* infection after successful eradication is sparse, but available data suggest that in Western countries it is as low as 0.4%/year over a 2-4 year period (170) and as high as 13% after the first year (143). In the United States, one study of 118 patients followed a mean of 56 weeks reported a reinfection rate of 3.4% (171).

TREATMENT STRATEGY

Peptic Ulcer Disease

The panel from a recent NIH consensus conference (report attached) recommended that all patients with duodenal or gastric ulcer who are infected with *H. pylori* should be treated with antimicrobial therapy, even with the first ulcer (172). I agree that

most patients with recurrent peptic ulcer disease and who are *H. pylori* positive should be treated. Such individuals might otherwise be candidates for long-term maintenance therapy with H_2 receptor antagonists or surgery, courses of action which are expensive and, with the latter, associated with some morbidity and mortality. A course of antimicrobial therapy directed against *H. pylori* is a reasonable, relatively safe, and inexpensive alternative.

There are, however, several groups of ulcer patients whom I would not treat for *H. pylori*. These include patients with inactive disease for several years off maintenance therapy, patients with Zollinger-Ellison Syndrome, and patients who are old and infirm who might not tolerate the antimicrobial regimens. Patients in whom the development of an uncomplicated ulcer was temporally related to the initiation of NSAIDs may also not require therapy. Finally, the role of eradication of *H. pylori* as a means of preventing further bleeding in patients who have bled once from a peptic ulcer is unsettled. As noted above, only two, small non-randomized trials have been published. While it is reasonable to consider *H. pylori* a risk factor for further bleeding and, therefore, to attempt eradication, I would not consider it sufficient therapy at this time. Until solid trials show that eradication of *H. pylori* works, I would continue treating patients with long-term H_2 receptor antagonists as well.

Gastric Lymphoma

Although data remain scanty, it appears reasonable to treat patients with low-grade B-cell MALT lymphomas in the hope that more radical therapy may be avoided. Patients with high grade lymphomas should, theoretically, not respond to treatment for *H. pylori*. Data to support or refute this supposition are not available.

Other Diseases

Until confirmatory data are available, patients with functional dyspepsia who are infected with *H. pylori* should be treated only as a last resort. There are also no data to support seeking and eradicating *H. pylori* in asymptomatic patients in the hope of preventing any of the diseases associated with its presence.

REFERENCES

1. Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273-74 (letter).

2. Solnick JV, O'Rourke J, Lee A, Paster BJ, Dewhirst FE, Tompkins FS. An uncultured gastric spiral organism is a newly identified Helicobacter in humans. J Infect Dis 1993;168:379-85.

3. Brown KE, Peura, DA. Diagnosis of *Helicobacter pylori* infection. Gastro Clinics N Am. 1993;22:105-15.

4. Graham DY, Evans DG, Evans DJ. Better, accurate, more rapid and convenient physician-office serologic test for detection of *H. pylori* infection. Gastroenterology 1994;106:A83 (Abst).

5. Peterson WL, Lee E, Feldman M. Relationship between Campylobacter pylori and gastritis in healthy humans after administration of placebo or indomethacin. Gastroenterology 1988;95:1185-97.

6. Evans DJ Jr., Evans DG, Graham DY, Klein PD. A sensitive and specific serologic test for detection of campylobacter pylori infection. Gastroenterology 1989;96:1004-8.

7. Talley N, Newell DG, Ormand JE, Carpenter HA, et. al. Serodiagnosis of Helicobacter pylori: comparison of enzyme-linked immunosorbent assays. J Clin Microbiol 1991;29:1635-39.

8. Cutler A, Schubert A, Schubert T. Role of *Helicobacter pylori* serology in evaluating treatment success. Dig Dis Sci 1993;38:2262-66.

9. Graham DY, Evans DJ, ALpert LC, et al. Campylobacter pylori detected noninvasively by the ^{13}C-urea breath test. Lancet 1987;29:1174-77.

10. Podolsky I, Lee E, Cohen R, Peterson WL. Prevalence of C. pylori (CP) in healthy subjects and patients with peptic diseases. Gastroenterolgy 1989;96:A394 (abstract).

11. Dooley CP, Cohen H, Fitzgibbons PL, et al. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. N Engl J Med. 1989;321:1562-66.

12. Graham DY, Malaty HM, Evans DG, Evans DJ Jr., Klein PD, Adam, E. Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Gastroenterology 1991;100:1495-1501.

13. Megraud F. Epidemiology of *H. pylori* infection. Gastroenterol Clin N Am 1993;22:73-88.

14. Malaty HM, Evans DG, Evans DJ Jr., Graham D. *Helicobacter pylori* in hispanics: comparison with blacks and whites of similar age and socioeconomic class. Gastroenterology 1992; 103:813-16.

15. Parsonnet J, Blaser MJ, Perez-Perez GI, Hargrett-Bean N, Tauxe RV. Symptoms and risk factors of *Helicobacter pylori* infection in a cohort of epidemiologists. Gastroenterology 1992;102:41-46.

16. Kuipers EJ, Pena AS, van Kamp G, et al. Seroconversion for Helicobacter pylori. Lancet 1993;342:328-31.

17. Cullen DJE, Collins BJ, Christiansen KJ, et al. When is infection acquired? Gut 1993;34:1681-82.

18. Sitas F, Forman D, Yarnell JWG, et al. *Helicobacter pylori* infection rates in relation to age and social class in a population of Welsh men. Gut 1991;32:25-28.

19. Mendall MA, Goggin PM, Molineaux N, et al. Childhood living conditions and *Helicobacter pylori* seropositivity in adult life. Lancet 1992;339:896-97.

20. Whitaker CJ, Dubiel AJ, Galpin OP. Social and geographical risk factors in Helicobacter pylori infection. Epidemiol Infect 1993;111:63-70.

21. Malaty HM, Graham DY. Effect of childhood socioeconomic status on prevalence of Helicobacter pylori infection. Gastroenterology 1993;104:A139 (Abstract).

22. Forman D, for the EUROGAST Study Group. Epidemiology of, and risk factors for, *Helicobacter pylori* infection among 3194 asymptomatic subjects om 17n populations. Gut 1993;34:1672-76.

23. Haruma K, Kawaguchi H, Kohmoto K, et al. Reduced incidence of Helicobacter pylori infection in Japan during the last 10 years. Gastroenterology 1994; 106:A91 (Abst).

24. Blecker U, Lanciers S, Mehta DI, Hauser B, Lebenthal E, Vandenplas Y. The prevalence of *Helicobacter pylori* positivity in asymptomatic children of different ethnic background living in the same country. Gastroenterology 1994;106:A54 (Abst).

25. Malaty HM, Engstrand L, Pedersen NL, Graham DY. *Helicobacter pylori* infection: Genetic and environmental influences. A study of twins, Ann Intern Med 1994;120:982-6.

26. Vincent P, Gottrand F, Pernes P, et al. High prevalence of *Helicobacter pylori* infection in cohabiting children. Epidemiology of a cluster, with special emphasis on molecular typing. Gut 1994;35:313-16.

27. Drumm B, Perez-Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of Helicobacter pylori infection. N Engl J Med 1990;322:359-63.

28. Malaty HM, Graham DY, Evans DG, Adams E, Evans DJ. Transmission of *Helicobacter pylori* infection. Studies in families of healthy individuals. Scand J Gastroenterology 1991;26:927-32.

29. Bamford KB, Bicjley J, Collins JSA, et al. *Helicobacter pylori*: comparison of DNA fingerprints provides evidence of intrafamilial infection. Gut 1993;34:1348-50.

30. Perez-Perez GI, Witkin SS, Decker MD, Blaser MJ. Seroprevalence of *Helicobacter pylori* infection in couples. J CLin Microbiol 1991;29:642-44.

31. Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of *Helicobacter pylori* from human faeces. Lancet 1992;340:1194-95.

32. Kelly SM, Cummings JH, MacFarlane GT, Grimes V, MacFarlane S, Gibson GR. Occurrence of Helicobacter pylori in fecal specimens from patients with dyspepsia in the UK. Gastroenterology 1993;104:A116 (Abstract).

33. Fox JG, Blanco MC, Yan L, et al. Role of gastric pH in isolation of Helicobacter mustelae from the feces of ferrets. Gastroenterology 1993;104:86-92.

34. Krajden S, Fuksa M, Anderson J, et al. Examination of human stomach biopsies, saliva and dental plaque for Campylobacter pylori. J Clin Microbiol 1989;27:1397-8.

35. Shames B, Krajden S, Fuksa M, Babida C, Penner JL. Evidence for the occurrence of the same strain of Campylobacter pylori in the stomach and dental plaque. J Clin Microbiol 1989;27:2849-50.

36. Nguyen, AH, Engstrand L, Genta RM, Graham DY, El-Zaatari FAK. Detection of *Helicobacter pylori* in dental plaque by reverse transcription-polymerase chain reaction. J Clin Microbiol 1993;31:783-87.

37. Mitchell HM, Lee A, Carrick J. Increased incidence of Campylobacter pylori infection in gastroenterologists: further evidence to support person-to-person transmission of C. pylori. Scand J Gastroenterol 1989;24:396-400.

38. Edwards PD, Carrick J, Turner J, et al. *Helicobacter pylori*-associated gastritis is rare in AIDS: antibiotic effect or a consequence of immunodeficiency? Am J Gastro 1991;86:1761-4.

39. Clyne M, Drumm B. *Helicobacter pylori* requires an acidic environment to survive in the presence of urea. Gastroenterology 1993;106:A666 (Abst).

40. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric campylobacter. Med J Aust 1985;142:436-39.

41. Morris A, Nicholson G. Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastro 1987;82:192-99.

42. Morris AJ, Ali MR, Nicholson GI, Perez-Perez GI, Blaser MJ. Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. Ann Intern Med 1991;114:662-3.

43. Sobala GM, Crabtree JE, Dixon MF, et al. Acute *Helicobacter pylori* infection: clinical features, local and systemic immune response, gastric mucosal histology, and gastric juice ascorbic acid concentrations. Gut 1991;32:1415-18.

44. Ramsey EJ, Carey KV, Peterson WL, et al. Epidemic gastritis with hypochlorhydria. Gastroenterology 1979;76:1449-57.

45. Gledhill T, Leicester RJ, Addis B, et al. Epidemic hypochorhydria. Brit Med J 1985; 290:1383-86.

46. Harford W, Barnett C, Lee E, et al. Epidemic gastritis with hypochlorhydria: 10 year follow up. Gastroenterology 1991;100:A79 (Abst).

47. Blaser MJ. Hypotheses on the pathogenesis and natural history of Helicobacter pylori-induced inflammation. Gastroenterology 1992; 102:720-27.

48. Eaton KA, Morgan DR, Krakowka S. Campylobacter pylori virulence factors in gnotobiotic piglets. Infect Immun 1989;57:1119-25.

49. Eaton KA, Morgan DR, Brooks C, Krakowka S. Essential role of urease in the pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. Infec Immun 1989;59:2470-75.

50. Marshall BJ, Barrett LJ, Prakash C, McCallum RW, Guerrant RL. Urea protects Helicobacter (Campylobacter) pylori from the bactericidal effect of acid. Gastroenterology 1990;99:697-702.

51. Parsons CL, Stauffer C, Mulholland CS, Griffith DP. Effect of ammonium on bacterial adherence to bladder transitional epithelium. J Urol 1984;132:365-66.

52. Smoot DT, Resau JH, Naab T, et al. Bobley HLT, Chippendale GR, Lewison JF, Resau JH. Helicobacter pylori urease activity is toxic in human gastric epithelial cells. Infect Immun 1990;58:1992-94.

53. Xu J, Goodwin CS, Cooper M, Robinson J. Intracellular vacuolization caused by the urease of *Helicobacter pylori*. J Infect Dis 1990;161:1302-4.

54. Evans DG, Evans DJ, Moulds JJ, Graham DY. N-acetylneuraminyllactose-binding fibrillar hemagglutinin of Campylobacter pylori: a putative colonization factor antigen. Infect Immun 1988;56:2896-2906.

55. Lingwood CA, Pellizzari A, Law H, Sherman P, Drumm B. Gastric glycerolipid as a receptor for Campylobacter pylori. Lancet 1989;2:238-41.

56. Smoot DT, Mobley HLT, Gilliam T, Phelps P, Resau JH. Pedestal formation of *Helicobacter (Campylobacter) pylori* with gastric epithelial cells in vitro may require actin polymerization. Gastroenterology 1990;98:A127 (abstract).

57. Hessey SJ, Spencer J, Wyatt JI, et al. Bacterial adhesion and disease activity in Helicobacter associated chronic gastritis. Gut 1990;31:134-8.

58. Boren T, Falk P, Roth KA, Larson G, Nomark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 1993;262:1892-5.

59. Craig PM, Karnes WE, Territo MC, Walsh JH. Helicobacter pylori secretes a chemotactic factor for monocytes and neutrophils. Gut 1992;33:1020-23.

60. Mai UEH, Perez-Perez GI, Wahl LM, et al. Soluble surface proteins from *Helicobacter pylori* activate monocytes/macrophages by lipopolysaccharide-independent mechanism. J Clin Inv 1991;87:894-900.

61. Mooney C, Keenan J, Munster D, et al. Neutrophil activation by *Helicobacter pylori*. Gut 1991;32:853-7.

62. Leunk RD, Ferguson MA, Morgan DR, Low DE, Simar AE. Antibody to cytotoxin infection by Helicobacter pylori. J Clin Microbiol 1990;28:1181-84.

63. Hupertz V, Czinn S. Demonstration of a cytoxin from *Campylobacter pylori*. Eur J Clin Micribiol Infect Dis 1988;7:576-8.

64. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from Helicobacter pylori. J Biol Chem 1992;267:10570-5.

65. Genta RM, Hamner HW, Graham DY. Gastric lymphoid follicles in *Helicobacter pylori* infection: frequency, distribution, and response to triple therapy. Hum Pathol 1993;24:577-83.

66. Goggin PM, Marrero JM, Spychal RT, et al. Surface hydrophobicity of gastric mucosa in *Helicobacter pylori* infection: effect of clearance and eradication. Gastroenterology 1992;103:1486-90.

67. Peterson W, Barnett C, Evans DJ et al. Acid secretion and serum gastrin in normal subjects and patients with duodenal ulcer:the role of *Helicobacter pylori* Am J Gastroenterol 1993;88:2038-43.

68. Cave DR, Vargas M. Effect of a Campylobacter pylori protein on acid secretion by parietal cells. Lancet 1989;2:187-89.

69. Taha AS, Fraser WD, Kelly RW, Gemmell CG, Lee FD, Russell RI. Inhibition of human gastric cyclic AMP production by *Helicobacter pylori* protein - possible involvement of mucosal prostaglandin E_2. Aliment Pharmacol Ther 1991;5:379-89.

70. Asaka M, Kimura T, Kudo M, et al. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology 1992;102:760-66.

71. Mossi S, Meyer-Wyss B, Renner EL, Merki HS, Gamboni G, Berlinger C. Influence of *Helicobacter pylori*, sex, and age on serum gastrin and pepsinogen concentrations in subjects without symptoms and patients with duodenal ulcer. Gut 1993;34:752-6.

72. Murthy UK, Linscheer R, Cho C. The hypergastrinemia in Helicobacter pylori (HP)-gastritis is due to a decrease in antral D cell density and D:G cell ratio. Gastroenterology 1992;102:A130 (abstract).

73. Kaneko H, Nakada K, Mitsuma T, Uchida K, Furusawa A, et al. Helicobacter pylori infection induces a decrease in immunoreactive-somatostatin concentrations of human stomach. Dig Dis Sci 1992;37:409-16.

74. Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. Lancet 1992;340:930-32.

75. Graham DY, Lew G, Lechago J. Antral G-cell and D-cell numbers in *Helicobacter pylori* infection: Effect of *H. pylori* eradication. Gastroenterology 1993;104:1655-60.

76. Ihamaki T, Kekki M, Sipponen P, Siurala M. The sequela and course of chronic gastritis dring a 30- to 34-year bioptic follow-up study. Scand J Gastroenterol 1985;20:485-91.

77. Karnes WE, Samloff IM, Siurala M, et al. Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. Gastroenterology 1991;101:167-74.

78. Negrini R, Lisato L, Zanelli I, et al. *Helicobacter pylori* infection induces antibodies cross-reacting wiht human gastric mucosa. Gastroenterology 1991;101:437-45.

79. Mardh S, Song Y-H. Characterization of antigenic structures in auto-immune atrophic gastritis with pernicious anemia... Acta Physiol Scand 1989; 136: 581-7.

80. Poliner IJ, Spiro HM. Independent secretion of acid, pepsin, and 'intrinsic factor' by the human stomach. Gastroenterology 1958;34:106-109.

81. Berstad K, Berstad A. *Helicobacter pylori* infection in peptic ulcer disease. Scand J Gastroenterol 1993;28:561-67.

82. Varis O, Valle J, Siurala M. Is *Helicobacter pylori* involved in the pathogenesis of the gastritis characteristic of pernicious anemia? Scand J Gastroenterol 1993;28: 705-8.

83. Nomura A, Stemmerman GN, Chyou P-H, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. Ann Intern Med 1994;120:977-81.

84. Cullen DJE, Collins BJ, Christiansen KJ, Epis J, Warren JR, Cullen KJ. Long term risk of peptic ulcer disease in people with helicobacter pylori infection - a community based study. Gastroenterology 1993;104:A60 (Abstract).

85. Sipponen P, Varis K, Fraki O, Korri U-M, Seppala K, Siurala M. Cumulative 10-year risk of symptomatic duodenal and gastric ulcer in patients with or without chronic gastritis. Scand J Gastroenterol 1990;25:966-73.

86. Vaira D, Miglioli M, Menegatti M. Endoscopic findings in *Helicobacter pylori* seropositive and seronegative asymptomatic blood donors. Gastroenterology 1994;106:A202 (Abst).

87. Coghlan JG, Humphries H, Dooley C, et al. Campylobacter pylori and recurrence of duodenal ulcers - A 12-month follow-up study. Lancet 1987;2:1109-11.

88. Marshall BJ, Goodwin CS, Warren JR, et al. Prospective double-blind trial of duodenal ulcer relapse after eradication of Campylobacter pylori. Lancet 1988;2:1437-42.

89. Rauws EAJ, Tytgat GNJ. Cure of duodenal ulcer associated with eradication of Helicobacter pylori. Lancet 335;1990:1233-35.

90. Graham, DY, Lew, GM, Klein PD, Evans DG, Evans DJ, Saeed ZA, Malaty HM. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer: a randomized, controlled study. Annals of Internal Medicine 1992;116:705-8.

91. Hentschel E, Brandstatter G, Dragosics B, et. al. Effect of ranitidine and amoxicillin plus metronidazole on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N Engl J Med 1993;328:308-12.

92. Cover TL, Glupczynski Y, Lage AP, Tummuru MKR, Blaser MJ. The 120-128kDa cytotoxin-associated protein (CagA) as a marker for ulcerogenic *Helicobacter pylori* strains. Gastroenterology 1994;106:A65 (Abst).

93. Go MF. The putative virulence gene cagA is equally prevalent in *H. pylori* isolates obtained from individuals with duodenal ulcer or aymptomatic gastritis. Gastroenterology 1994;106:A82 (Abst).

94. Rapier RC, Dreilinger AD, Nyberg LM, Koss MA, Hogan DL, Isenberg JI. *Helicobacter pylori* diminishes proximal duodenal mucosal bicarbonate secretion in patients with duodenal ulcer disease. Gastroenterology 1994;106:A164 (Abst).

95. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127-31.

96. Nomura A, Stemmermann GN, Chyou P, Kato I, et al. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132-6.

97. Forman D and the Eurogast Study Group. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet 1993;341:1359-62.

98. Hansson L-E, Engstrand L, Nyren O et al. *Helicobacter pylori* infection: Independent risk indicator of gastric adenocarcinoma. Gastroenterology 1993;105: 1098-1103.

99. Kohmoto K, Haruma K, Kawaguchi H, et al. Helicobacter pylori infection in young Japanese patients is a major risk factor for gastric carcinoma. Gastroenterology 1994;106:A109 (Abst).

100. Sobala GM, Schorah CJ, Shires S, et al. Effect of eradication of *Helicobacter pylori* on gastric juice ascorbic acid concentrations. Gut 1993;34:1038-41.

101. Sipponen P, Riihela M, Hyvarinen H, Seppala K. Chronic nonatrophic ('superficial') gastritis increases the risk of gastric carcinoma. Scand J Gastroenterol 1994;29:336-40.

102. Waldum HL, Haugen OA, Isaksen C, Mecsei R, Sandvik AK. Enterochromaffin-like tumour cells in the diffuse but not the intestinal type of gastric carcinomas. Scand J Gastroenterol 1991;26(Suppl180):165-9.

103. Isaacson PG. Gastric lymphoma and *Helicobacter pylori*. N Engl J Med 1994;330:1310-11.

104. Cogliatti SB, Schmid U, Schumacher U, et al. Primary B-cell gastric lymphoma: a clinicopathological study of 145 patients. Gastroenterology 1991;101:1159-70.

105. Papadaki LP, Wotherspoon AC, Isaacson PG. The lymphoepithelial lesion of gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALT): an ultrastructural study. Histopathology 1992;21:415-21.

106. Wotherspoon AC, Ortiz-Hidalgo C, Falzom MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991;338:1175-6.

107. Dogiioni C, Wotherspoon AC, Moschini A, de Boni M, Isaacson PG. High incidence of primary gastric lymphoma in northeastern Italy. Lancet 1992;339:834-5.

108. Parsonnet J, Hansen S, Rodriquez BS, et al. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994;330:1267-71.

109. Hussell T, Isaacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to *Helicobacter pylori*. Lancet 1993;342:571-4.

110. Isaacson PG. Gastric lymphoma and *Helicobacter pylori*. N Engl J Med 1994;330:1310-11.

111. Talley NJ. The role of *Helicobacter pylori* in nonulcer dyspepsia. Gastroenterol Clin N Am 1993;22:153-67.

112. Tucci A, Corinaldesi R, Stanghellini V, et al. *Helicobacter pylori* infection and gastric function in patients with chronic idiopathic dyspepsia. Gastroenterology 1992;103:768-74.

113. Holtmann G, Goebell H, Holtmann M, Talley NJ. Dyspepsia in healthy blood donors. pattern of symptoms and association with *Helicobacter pylori*. Dig Dis Sci 1994;39:1090-8.

114. Peterson WL, Graham DY, Marshall B et al. Clarithromycin as monotherapy for eradication of *Helicobacter pylorie*. A randomized. double-blind trial. Am J Gastro 1993;88:1860-64.

115. Valle J, Seppala K, Sipponen P, Kosunen T. Disappearance of gastritis after eradication of *Helicobacter pylori*. Scand J Gastroenterol 1991;26:1057-65.

116. Genta RM, Lew GM, Graham DY. Changes in the gastric mucosa following eradication of *Helicobacter pylori*. Mod Pathol 1993;6:281-9.

117. Cutler A, Schubert T. Long-term follow up of *H. pylori* serology following successful eradication. Gastroenterology 1993;104:A61 (Abst).

118. Kosunen TU, Seppala K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titers after eradication of *Helicobacter pylori*. Lancet 1992;339:893-5.

119. Moss SF, Calam J. Acid secretion and sensitivity to gastrin in patients with duodenal ulcer:effect of eradication of *Helicobacter pylori*. Gut 1993;34:888-92.

120. El-Omar E, Penman I, Dorrian CA, Ardill JES, McColl KEL. Eradicating *Helicobacter pylori* infection lowers gastrin meditated acid secretion by two thirds in patients with duodenal ulcer. Gut 1993;34:1060-65.

121. Graham DY, Opekun A, Lew G et al. Ablation of exaggerated meal-stimulated gastrin release in duodenal ulcer patients after clearance of *Helicobacter pylori* infection. Am J Gastroenterol 1990;85:394-98.

122. Graham DY, Go MF, Lew G, Genta RM, Rehfeld JF. *Helicobacter pylori* infection and exaggerated gastrin release: Effects of inflammation and progastrin processing. Scand J Gastroenterol 1993;28:690-94.

123. Moss SF, Legon S, Bishop AE, Polak JM, Calam J. Effect of *Helicobacter pylori* on gastric somatostatin in duodenal ulcer disease. Lancet 1992;340:930-2.

124. Graham DY, Lew GM, Lechago J. Antral G-cell and D-cell numbers in *Helicobacter pylori* infection: effect of *H. pylori* eradication. Gastroenterology 1993;104:1655-60.

125. Chittajallu RS, Dorrian CA, Ardill JES, McColl KEL. Effect of *Helicobacter pylori* on serum pepsinogen I and plasma gastrin in duodenal ulcer patients. Scand J Gastroenterol 1992;27:20-4.

126. Schutze K, Hentschel E, Brandstatter G. More on the eradication of *Helicobacter pylori* and the recurrence of duodenal ulcer. N Engl J Med 1993;329:1356 (letter).

127. Forbes GM, Glaser ME, Digby JEC, et al. Duodenal ulcer treated with *Helicobacter pylori* eradication: seven year follow-up. Lancet 1994;343:258-60.

128. Graham DY, Hepps KS, Ramirez FC, Lew GM, Saeed ZA. Treatment of Helicobacter pylori reduces the rate of rebleeding in peptic ulcer disease. Scand J Gastroenterol 1993;28:939-42.

129. Labenz J, Borsch G. Role of *Helicobacter pylori* eradication in the prevention of peptic ulcer bleeding relapse. Digestion 1994;55:19-23.

130. Morgan D, Kraft W, Bender M, Pearson A. Nitrofurans in the treatment of gastritis associated with Campylobacter pylori. Gastro. 1988;95:1178-84.

131. Glupczynski Y, Burette A, Labbe M, Deprez C, De Reuck M, Deltenre M. Campylobacter pylori-associated gastritis: A double-blind placebo-controlled trial with amoxycillin. Am. J. Gastro. 1988;83:365-72.

132. Rokkas T, Pursey C, Uzoechina E, et al. Non-ulcer dyspepsia and short term De-Nol therapy: a placebo controlled trial with particular reference to the role of Campylobacter pylori. Gut 1988;29:1386-91.

133. Lambert JR, Dunn K, Borromeo M, Korman MG, Hansky J. Campylobacter pylori - A role in non-ulcer dyspepsia? Scan. J. Gastroenterol 1989;24(Suppl 160):7-13.

134. Loffeld R, Potters H, Stobberingh E, Flendrig J, Van Spreeuwel J, Arends J. C. pylori associated gastritis in patients with non-ulcer dyspepsia: a double blind placebo controlled trial with colloidal bismuth subcitrate. Gut 1989;30:1206-12.

135. Kang JY, Tay HH, Wee A, Guan R, Math MV, Yap I. Effect of colloidal bismuth subcitrate on symptoms and gastric histology in non-ulcer dyspepsia. A double blind placebo controlled study. Gut 1990;31:476-80.

136. Marshall BJ, Valenzuela JE, McCallum RW, et al. Bismuth subsalicylate suppression of *Helicobacter pylori* in nonulcer dyspepsia: a double-blind placebo-controlled trial. Dig Dis Sci 1993;38:1674-80.

137. Talley NJ. A critique of therapeutic trials in *Helicobacter pylori* - positive functional dyspepsia. Gastroenterology 1994;106:1174-83.

138. Patchett S, Beattie S, Leen E, et al. *Helicobacter pylori* and non-ulcer dyspepsia. Gastroenterology 1992;103:340-7.

139. Elta G, Barnett J, Scheiman J, et al. The effect of treatment for *Helicobacter pylori* on non-ulcer dyspepsia symptoms. Gastroenterology 1992;102:A63 (abstract).

140. Holcombe C, Thom C, Kaluba J, Lucas SB. *Helicobacter pylori* clearance in the treatment of non-ulcer dyspepsia. Aliment Pharmacol Ther 1992;6:119-23.

141. Wotherspoon AC, Doglioni C, Diss TC et al. Regression of primary low-grade B-cell gastric lymphoma on mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 1993;342:575-77.

142. Chiba N, Rao BV, Rademaker JW, Hunt RH. Meta-analysis of the efficacy of antibiotic therapy in eradicating H. pyori. Am J. Gastro.1992;87:1716-27.

143. Penston JG. Review article: *Helicobacter pylori* eradication-understandable caution but no excuse for inertia. Aliment Pharmacol Ther 1994;8:369-90.

144. Tytgat GNJ. Review article: treatments that impact favourably upon the eradication of *Helicobacter pylori* and ulcer recurrence. Aliment Pharmacol Ther 1994;8:359-68.

145. Cederbrant G, Kahlmeter G, Ljungh A. Proposed mechanism for metronidazole resistance in *Helicobacter pylori*. J Antimicrob Chemoth 1992;29:115-20.

146. Veldhuyzen van Zanten SJO, Goldie J, Hollingworth J, Siletti C, Richardson H, Hunt RH. Secretion of intravenously administered antibiotics in gastric juice: implications for management of *Helicobacter pylori*. J Clin Pathol 1992;45:225-7.

147. Grayson ML, Eliopoulos GM, Ferraro MJ, Moellering RC. Effect of varying pH on the susceptibility of Campylobacter pylori to antimicrobial agents. Eur J Clin Microbiol Infect Dis 1989;8:888-9.

148. Glupczynski Y. Results of a multicenter European survey in 1991 of metronidazole resistance in Helicobacter pylori. Eur J Clin Microbiol Infect Dis 1992;11:777-81.

149. Goodwin CS, Marshall BJ, Blincow ED, Wilson DH, Blackburn S, Phillips M. Prevention of nitroimidazole resistance in Campylobacter pylori by coadministration of colloidal bismuth subcitrate: clinical and in vitro studies. J Clin Pathol 1988;41: 207-10.

150. Bell GD, Powell K, Burridge SM, et al. Experience with 'triple' anti-*Helicobacter pylori* eradication therapy: side effects and the importance of testing the pre-treatment bacterial isolate for metronidazole resistance. Aliment Pharmacol Ther 1992;6:427-35.

151. Rautelin H, Seppala K, Renkonen OV, Vaino U, Kosunen TU. Role of metronidazole resistance in therapy of *Helicobacter pylori* infections. Anitmicrob Agents Chemother 1992;36:163-6.

152. Gorbach SL. Bismuth therapy in gastrointestinal diseases. Gastroenterology 1990; 99:863-75.

153. McGowan CC, Cover TL, Blaser MJ. The proton pump inhibitor, omeprazole, inhibits survival of *Helicobacter pylori* at low pH by a urease-independent mechanism. Gastroenterology 1994;106:A135 (Abst).

154. Daw MA, Deegan P, Leen E, O'Morain C. The effect of omeprazole on *Helicobacter pylori* and associated gastritis. Aliment Pharmacol Ther 1991;5:435-9.

155. Weil J, Bell GD, Powell K, et al. Omeprazole and *Helicobacter pylori*: temporary suppression rather than true eradication. Aliment Pharmacol Ther 1991;5:309-13.

156. Graham DY, Lew GM, Malaty HM, et al. Factors influencing the eradication of *Helicobacter pylori* with triple therapy. Gastroenterology 1992;102:493-6.

157. Cutler AF, Schubert TT. Patient factors affecting *Helicobacter pylori* eradication with triple therapy. Am J Gastroenterol 1993;88:505-9.

158. Labenz J, Leverkus F, Madeya S, Peitz U, Borsch G. Omeprazole plus amxicillin for cure of *H. pylori* infection: Factors influencing the treatment success. Gastroenterology 1994;106:A114 (Abst).

159. Labenz J, Gyenes E, Ruhl GH, Borsch G. Omeprazole plus amoxicillin:efficacy of various treatment regimens to eradicate *Helicobacter pylori*. Am J Gastroenterol 1993;88:491-5.

160. Labenz J, Ruhl, GH, Bertrams J, Borsch G. Medium- or high-dose omeprazole plus amoxicillin eradicates *Helicobacter pylori* in gastric ulcer disease. Am J Gastroenterol 1994;89:726-30.

161. Mannes GA, Bayerdorffer E, Hele C, Ruckdeschel G, Stolte M. An increasing dose of omeprazole combined with amoxicillin increases the eradication rate of Helicobacter pylori. Gastroenterology 1993;104:A140 (Abst).

162. Logan RPH, Gummett PA, Schaufelberger HD, et al. Eradication of *Helicobacter pylori* with clarithromycin and omeprazole. Gut 1994;35:323-326.

163. Logan RPH, Bardhan KD, Celestin LR, et al. Healing of DU and eradication of *H. pylori* by clarithromycin and omeprazole. Gastroenterology 1994;106:A126 (Abst).

164. Bardhan KD, Dekkers CPM, Nowak A, et al. GR122311X (ranitidine bismuth citrate): a new drug for the treatment of duodenal ulcer. Gastroenterology 1994;106:A46 (Abst).

165. Al-Assi MT, Genta RM, Karttunen TJ, Graham DY. Clarithromycin-amoxycillin therapy for *Helicobacter pylori* infection. Aliment Pharmacol Ther 1994;8:In press.

166. Wyeth JW, Pounder RE, DeKoster E, et al. GR122311X (ranitidine bismuth citrate) with antibiotics for the eradication of *Helicobacter pylori*. Gastroenterology 1994; 106:A212 (Abst).

167. Graham DY, Lew GM, Evans DG, Evans DJ, Klein PD. Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. Ann Intern Med 1991; 115:266-9.

168. Graham DY, Ramirez FC, Lew GM et al. Tetracycline, clarithromycin, bismuth: a new effective triple therapy for *Helicobacter pylori* eradication. Gastroenterology 1993;104:A90 (Abst).

169. Labenz J, Gyenes E, Ruhl GH, Borsch G. Amoxicillin plus omeprazole versus triple therapy for eradication of *Helicobacter pylori* in duodenal ulcer disease: a prospective, randomized and controlled trial. Gut 1993;34:1167-70.

170. Borody TJ, Andrews P, Mancuso N et al. *Helicobacter pylori* reinfection rate, in patients with cured duodenal ulcer. Am J Gastroenterol 1994;89:529-32.

171. Cutler AF, Schubert TT. Long-term *Helicobacter pylori* recurrence after successful eradication with triple therapy. Am J Gastroenterol 1993;88:1359-61.

172. NIH Consensus Development Panel. *Helicobacter pylori* in peptic ulcer disease. J Am Med Assoc 1994;272:65-9.