THE SEROEPIDEMIOLOGY OF HELICOBACTER PYLORI AND ITS RELATIONSHIP TO CHRONIC ATROPHIC GASTRITIS

Dissertation submitted for the degree of D. Phil

University of Oxford

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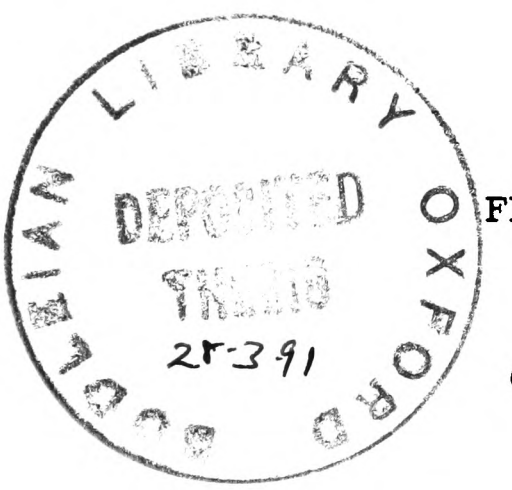

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ABSTRACT

There were three main aims to this thesis:

1. to test whether infection with Helicobacter pylori was related specifically to chronic atrophic gastritis (CAG), a precursor of gastric cancer; and to measure the specificity and sensitivity of Helicobacter pylori antibody markers for detecting H.pylori infection of the gastric mucosa; and CAG;

2. to measure the distribution of H.pylori in relation to sociodemographic, dietary and lifestyle characteristics in non-clinical populations from Caerphilly (Wales), Italy, China, and Kenya;

3. to examine the relationship between H.pylori and gastric cancer.

H.pylori was strongly associated with CAG and H.pylori antibody level was a sensitive and specific marker of both infection (84.8%, 92.7%) and CAG (71.4%, 90.9%).

In Caerphilly and Italy, the prevalence of H.pylori increased steeply to 40-50% in those aged 45-54 years and levelled off in older age groups. In China, over 50% of those aged 35-44 were infected. In Kenya, almost all people studied between 18-30 years were infected with H.pylori.

In Caerphilly, H.pylori infection was significantly higher in poorer socioeconomic groups, whilst in Italy, socioeconomic differences with regard to infection were in the same direction but not as pronounced. In Caerphilly, H.pylori infection was related to the number of the subject's siblings but not to the number of children or adults sharing the house of the subject.

In Caerphilly, those infected with H.pylori consumed less vitamin C and more alcohol than those not infected. Those infected in Italy consumed more vitamin C but similar amounts of alcohol than those not infected. In China, smoking was inversely related to H.pylori infection.

In China, a geographic correlation of +0.34 ($2p = 0.02$) was found between the prevalence of H.pylori infection (measured in 46 counties) and gastric cancer mortality. There was also a positive (but not significant) correlation with peptic ulcer mortality. No other type of cancer showed a significant association with H.pylori.

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CHAPTER 1

CHRONIC ATROPHIC GASTRITIS AND HELICOBACTER PYLORI

INTRODUCTION

The broad aims of this thesis were to clarify the relationship between *Helicobacter pylori* and chronic atrophic gastritis (CAG) and to describe the relationship between *H.pylori*, sociodemographic and lifestyle factors, CAG and gastric cancer. In order to answer these questions a series of five interrelated studies were carried out in Oxford, Caerphilly (Wales), Italy, China and Kenya. These are described in Chapters 3 to 7.

This chapter is divided into 3 sections. Section 1 deals with chronic atrophic gastritis, section 2 with *Helicobacter pylori* and section 3 with the aims of the thesis.

The chapter begins (section 1.1) by comparing the different histological and phenotypic classification schemes of gastritis, and then focusses on Strickland and Mackay's (1973) type B CAG as the lesion of interest.

The evidence that CAG is related to gastric cancer is reviewed in section 1.2 by examining studies comparing the prevalence of CAG and its distribution by age in areas of differing gastric cancer rates. This section also looks at some of the case-control (1.2.4) and follow-up (1.2.5) studies that were conducted in order to quantify the risk between CAG and gastric cancer.

Section 1.3 reviews the literature on the relationship

between CAG and the proenzyme pepsinogen. One of the major limitations of studying the epidemiology of CAG has been the absence of any specific clinical symptoms and hence the reliance on gastric biopsies for the diagnosis of this disease. To circumvent the need for invasive gastroscopic procedures, it has been suggested that the level of pepsinogen which enters the circulatory system may be used as a diagnostic marker of CAG. This section describes the physiological background to this relationship, as well as the studies that have tested the relationship between serum or plasma pepsinogen levels and gastric mucosal histology.

Section 1.4 reviews what is currently known about the aetiology of the relationship between CAG and gastric cancer. This includes the role of chemical irritants, diet and social conditions. The aetiology of CAG is multifactorial but remains poorly understood. A model of gastric carcinogenesis, linking CAG to gastric cancer has been proposed and updated since 1975 (Correa et al 1975, Correa 1988).

Helicobacter pylori a spiral shaped bacteria (defined in Section 2.1), has been shown to cause chronic active gastritis but it is unclear whether it also plays an important role in the aetiology of CAG.

Section 2.2 is devoted to reviewing the evidence of the association between H.pylori and gastric mucosal histology, especially chronic active gastritis and CAG. These studies

include endoscopic studies, studies of voluntary or accidental ingestion of the organism and treatment studies.

Because of the strong association between H.pylori and gastritis, section 2.3 describes how an Enzyme Linked Immunosorbent assay for antibodies against H.pylori can be useful in identifying subjects with gastritis, and especially CAG.

Section 2.4 looks at the current role ascribed to H.pylori in the gastric cancer model and section 2.5 describes non-clinical seroprevalence studies of H.pylori throughout the world.

Section 3 gives descriptions of the individual studies and detailed hypotheses of the aims of the thesis.

SECTION 1

CHRONIC ATROPHIC GASTRITIS

1.1 DEFINITIONS

1.1.1 Histological definition

Chronic gastritis encompasses any diffuse inflammation of the gastric mucosa. It does not have any distinctive symptoms and for this reason its diagnosis depends on the histological examination of gastric biopsy material. There are several classification systems currently being used and there is no universal agreement as to which is the better. One of the most popular has been that of Whitehead (1973).

The main features of Whitehead's classification have been the degree of infiltration of the lamina propria by inflammatory cells and the degree of atrophy of the glandular epithelium. In chronic superficial gastritis, the inflammation is limited to the level of the gastric pits, whereas in CAG the inflammation is seen throughout the thickness of the mucosa. Polymorphonuclear leucocytes can be present or absent in both types of gastritis, their presence being an indication of gastric activity. CAG can be accompanied by two types of metaplasia, pyloric or intestinal. In pyloric metaplasia the gastric epithelium is replaced by cells commonly found in the pylorus. In intestinal metaplasia (IM) the gastric epithelium is replaced by cells resembling those of the intestinal epithelium (Whitehead 1973). There are two types of IM, complete and incomplete, indicating the presence or absence of intestinal absorptive cells (Filipe and Jass 1986).

Table 1

Phenotypes of gastritis

Author	Year	Main site affected			Other
		Fundus	Antrum & Fundus	Antrum	
Strickland & Mackay	1973	Type A	{-----Type B----}		
Glass & Pitchumoni	1975	Type A	{-----Type B----}		Type AB+ (with PCA*) Type AB- (without PCA)
Correa	1980	Autoimmune	Environmental	Hypersecretory	
Correa	1988	"	Multifocal	"	
Kekki et al	1988	Type A	Type AB	Type B	Superficial gastritis
Dixon	1989	Type A	{--Type B (Bacterial)--} {--Type C (Chemical)---}		
Wyatt & Dixon	1988	Type A	{--Type B&C as above---}		Reflux gastritis (post operative)

* PCA = Parietal cell antibodies

Other classification systems are also used, for example that of Rao et al (1975), who graded the degree of gastritis on the basis of chronic inflammation, rather than the degree on atrophy. Kekki and co-workers (1987), from Finland, use the degree of the loss of glands, irrespective of the presence or absence of inflammation or intestinal metaplasia. Stolte et al (1989) and Steininger et al (1989), use the density of lymphocytes and plasma cells in the tunica propria as an index of severity of gastritis. Miki et al (1987) use a Congo Red test to assess the degree of absence of acid decreasing glands as an indirect measure of the severity of atrophic gastritis.

1.1.2 Phenotypes of gastritis

There are several topological classifications of gastritis, as shown in Table 1, based largely on whether the antrum, fundus or both parts of the stomach are affected. These phenotypes can only be distinguished by assessing biopsies from both the antrum and fundus.

1.1.2.1 Type B (Strickland and Mackay), AB (Kekki et al), or Correa's Multifocal/Environmental gastritis

This type of gastritis is the most prevalent form and will be the focus of this review. It has been termed 'type B' by Strickland and Mackay (1973) and 'type AB' by Kekki et al (1987), and 'multifocal gastritis' by Correa (1988). Glass and Pitchumoni (1975) subdivided this form of gastritis into those with and without parietal cell antibodies.

1.1.2.3 Type B (Kekki et al) or Correa's Hypersecretory gastritis

This type of gastritis affects the antrum and is associated with duodenal ulceration. It has been termed 'hypersecretory' chronic gastritis by Correa (1980) and 'type B' (sic) chronic gastritis by Kekki et al (1987). About 6% of the Finnish population is reported to be affected (Kekki et al 1987). Strickland and Mackay (1973) do not distinguish between antral predominant and diffuse gastritis.

1.1.2.4 Other forms of gastritis

There are numerous other types of gastritis. These include reflux (Wyatt and Dixon 1989) post-gastrectomy gastritis (Correa 1988), 'Type C', suggested by Dixon (1989) and Wyatt and Dixon (1989) to have a Chemical aetiology, and other rare forms such as infectious, varioliform, lymphocytic, and eosinophilic gastritis, as reviewed elsewhere (Whitehead 1973, Paull and Yardley, 1989).

One of the major limitations of these systems has been that they have evolved independently of one another without interchange of histological material to assess the internal or external comparability of these classification schemes. These schemes (histological and phenotypic) used by different workers, imply that comparisons of studies on CAG must be treated with some caution, as they may not be comparable with one another. The emphasis of this review will be on the diffuse type of CAG termed 'environmental'

'multifocal' by Correa (1980, 1988, respectively), or 'type B' by Strickland and Mackay (1973).

1.2 RELATIONSHIP BETWEEN CAG AND GASTRIC CANCER

1.2.1 Background

Despite the absence of specific clinical symptoms, CAG is an important lesion because it is thought to predispose to gastric cancer of the intestinal type (Taylor 1987). Despite falling incidence rates, especially in developed countries, (Howson et al 1986), gastric cancer was still just the most common cancer worldwide in 1980 (Parkin et al 1988). Evidence that CAG is considered to be a major pathological precursor of gastric cancer comes from descriptive, case-control and follow-up studies. This section describes some of the most important studies relating CAG to gastric cancer and on the few studies that have attempted to examine the aetiology of CAG.

Because a gastric biopsy is needed to diagnose CAG, studies have been restricted mainly to patients attending endoscopy clinics, usually with upper abdominal symptoms. However, clinic based studies may present a picture of CAG which may be different from that in the general population. There have been a few studies that involved endoscopy of non-clinical populations, and these are described below. In order to circumvent the need for gastric biopsies, the level of serum pepsinogen has been proposed as a less invasive marker of CAG. This relationship between CAG and serum pepsinogen, as well as epidemiological studies using serum pepsinogen are also discussed.

Table 2
Prevalence of chronic atrophic gastritis in different populations

Authors	Year	Country	Population type	N	Prevalence of CAG* (%)	Gastric Ca. mortality /100,000
Siurala et al	1968	Finland	Rural, random	142	28**	36.9#
Ihamäki et al	1978	Finland	Rural, random	431	20B 29A	
Villako et al	1976	USSR, Estonia	Rural, random	155	20B 29A	55.8##
"	1981	" "	Urban, random	226	37B 34A	
Correa et al	1970	Colombia	Narino, Agricultural workers	286	56.3	150.0
	1976		Narino low g.c. risk pop	168	33.3	40.0
Haenszel et al	1976		Cali, Narino migrants	45	37.8	68.0
Cuello et al	1976		Cali, poor s.e.c.	57	26.3	23.0
			Cartagena, patients unrelated to gastric symptoms	30	13.3	6.0
Stemmermann 1978		Japan	Screening program	52	90.4	95.3
		Hawaii	Japanese population	59	47.5	45.9
Imai et al 1971		Japan	Autopsies	260	58.5	M=68.6, F=35.3
		USA	"	350	18	M=9.4, F=4.7
Cheli et al 1980		Hungary	Endoscopy patients	100	24	42.9
		Italy	" "	100	11	29.7
Zhang et al 1984		China	Random, High g.c. risk	19,445	4.02	30.9-113.2
		"	Low g.c. risk	21,379	0.54	7.5-12.0

* Chronic atrophic gastritis may have been classified using different criteria, thus prevalence rates between studies are not necessarily comparable.
** A = Based on antral biopsies, B = Based on body, fundal biopsies. # Doll et al 1976. ##Napalkov et al 1983.

1.2.2 Population-based studies

There were five sets of studies that have compared the distribution of CAG in populations with different gastric cancer mortality rates (Table 2).

The first set of studies was undertaken by a USSR-Finnish team (see Villako and Siurala (1981), Siurala et al (1985) for summary) which conducted four population-based endoscopic surveys. Two were in Finland (Siurala et al 1968; Ihamaki et al 1979) in which the annual gastric cancer mortality rate for men in 1966-70 was 36.9 per 100,000 (Doll et al, 1976). Two were in Estonia (USSR), where the comparable annual gastric cancer mortality rate for the region for 1966-1970 was 55.8/100,000 (Napalkov et al 1983). There were no striking differences in the overall prevalence of CAG in the Finnish/Estonian studies, with rates for CAG based on biopsies of the gastric body ranging between 20 to 37% in Estonia and between 17 to 28% in Finland. There was, however, an increased prevalence of severe CAG in Estonia (6% in both studies) in comparison with Finland (3% and 2%).

The second set of studies was conducted in Colombia. A number of population-based endoscopic surveys of CAG and IM were conducted in regions of widely varying gastric cancer mortality (ranging from 6/100,000 to 150/100,000 per year) (Haenszel et al 1976, Cuello et al 1976, Correa et al 1976). The prevalence of CAG and IM among populations from high gastric cancer risk areas who migrated to areas of lower gastric cancer risk was also studied (Correa et al 1970).

Agricultural workers from the Narino district who had the highest gastric cancer mortality rates (150/100,000) had the highest prevalence rates for CAG (56.3%). By contrast, natives of Cartagena, where the gastric cancer mortality rate was low (6/100,000), had a lower prevalence rate for CAG (13.3%). Residents of Cali, a moderate gastric cancer risk area (40/100,000), had an intermediate rate (26.3%). The prevalence of CAG in migrant agricultural workers from Narino who moved to Cali was 37.8%, that is higher than the prevalence in Cali but lower than the prevalence in Narino.

A third set of endoscopic studies compared CAG among Japanese natives from Akita and Japanese living in Hawaii. These were carried out by Stemmerman et al (1978). These two populations had gastric cancer mortality rates for men of 95.3 and 45.9 per 100,000 respectively (Stemmerman et al 1978). CAG (with IM) among Japanese men in Akita prefecture was substantially higher (90.4%) than in Japanese Hawaiians (47.5%). Similar differences were found for women (Stemmerman et al 1987).

A fourth study compared autopsy specimens for 260 Japanese in Japan and 350 US Americans by Imai et al (1971). Gastric cancer mortality per 100,000 amongst Japanese was 68.6 for men and 35.3 for women. For Americans, gastric cancer mortality was 9.4 per 100,000 for men and 4.7 per 100,000 for women. A greater prevalence of CAG was found at autopsy in Japanese (58.5%) than in US Americans (18.0%) (Imai et al 1971).

The fifth study comprised a large cross-sectional study on the aetiology of gastric cancer in 10 Chinese counties, where 40,824 subjects were recruited from farming and factory brigades in areas of varying prevalence of gastric cancer (Zhang et al 1984). Areas of high gastric cancer risk (with mortality rates ranging from 31-113/100,000) had eight times the prevalence of CAG (4% vs 0.5%) than areas of low gastric cancer risk (with mortality rates ranging from 7.5-12/100,000). The correlation coefficient (r) between prevalence of CAG and mortality due to gastric cancer in 10 districts was 0.65 ($p < 0.05$).

In addition to these population-based studies, there was a similar relationship between CAG and gastric cancer in patients attending endoscopy clinics in Hungary and Italy. The annual mortality from gastric cancer is higher in Hungary (49/100,000) than in Italy (29.7/100,000). Hungarian patients had a greater prevalence of CAG and IM (24%) than comparable Italian patients (11%) (Cheli et al 1980).

The studies comparing the Finnish and Estonian populations did not show large differences in the prevalence of CAG comparable with the differences in gastric cancer mortality, apart from the differences in severe CAG. In Japan, Hawaii, USA, Colombia and China the rates for CAG did reflect the gastric cancer mortality of the population, probably because of the larger contrasts in gastric cancer mortality between and within these populations. In

addition, migrants from high-risk areas moving to a low-risk area (within Colombia and from Japan to Hawaii) had intermediate CAG and gastric cancer rates. This implies that environmental conditions might have played an important role in the aetiology of both CAG and gastric cancer.

1.2.3 CAG and age

Both clinic-based and population-based studies showed that the prevalence of CAG increased with age. In an early clinic-based study of 1,000 consecutive biopsies in Australia, Joske et al (1955) found the frequency of CAG increased from 11% in the age group 0 to 20 years to 61% in those over 60 years old. In the Finnish and Estonian population-based studies referred to above, the prevalence of CAG was less than 10% in those under 30 years and over 40% in those over 65 years old.

There are some interesting observations of the prevalence of CAG in young people in populations with high and low gastric cancer mortality rates. Agricultural workers in the Narino district of Colombia had a high gastric cancer mortality rate and 45% of young agricultural workers (aged less than 29 years) in the same district had CAG (Correa et al 1976). In contrast, the same age group of Narino non-agricultural workers, who had a lower gastric cancer mortality rate, had a lower prevalence of CAG (14.6%) (Correa et al 1976). Populations of intermediate gastric cancer risk, Narino migrants and residents of Cali, had intermediate rates of gastritis in those aged less than 29

years: 30.0% and 23.9%. In the lowest gastric cancer risk area of Cartagena the prevalence of CAG-IM amongst those less than 29 years was 10.0% (Correa et al 1976).

Although not directly comparable, Colombian rates for CAG were substantially higher than the Finnish rates especially at young ages. Only at the age of 60 or more did the prevalence of CAG in the Finnish population samples attain the level of the Columbian high risk population at 20-29 years old. Evidently CAG had a younger age at onset in populations of high gastric cancer incidence than low incidence. This implies that the age of onset of CAG may have been more important in gastric cancer than differences in prevalence at old age. The effect of environmental mutagens over a longer time period on a gastritic stomach may nave been more important than their effect on a normal mucosa (Correa et al 1986).

1.2.4 Case-control studies

The presence of CAG in groups with and without gastric cancer was assessed by comparing autopsy material of individuals at post mortem or by comparing biopsy material from patients attending endoscopy clinics, or from population surveys.

There were numerous studies dating as far back as the late 1800's which used post mortem material comparing the histological appearance of the gastric mucosa in people with and without gastric cancer. A common observation around the

turn of the century was that the frequency of gastritis in stomachs with gastric cancer was higher than in stomachs without (e.g. Fenwick 1870, Konjetzny 1913). Konjetzny (1913) argued that chronic gastritis constituted a precancerous condition. Although most studies were concordant with this finding, there were dissenting views. Guiss and Steward (1943) for example, found no differences in chronic gastritis between those with and without gastric cancer. Some of these early studies may have been misleading because unless fixed with formalin immediately after death, autolysis of the stomach may have yielded unreliable results (Taylor 1987).

Several endoscopic studies have examined the relationship between CAG, IM and gastric cancer but only two Finnish studies, which used community based controls, will be reviewed. The presence of CAG and IM in stomach biopsies was assessed by Sipponen et al (1983) from 257 gastric cancer cases and 358 age-matched population controls. The prevalence of antral CAG & IM was significantly higher (51%) in the gastric mucosa of gastric cancer patients than of controls (27%) and particularly so in patients with antral tumours of the intestinal type (68%). In another study, Sipponen et al (1985) assessed the risk of gastric cancer in patients with different grades of gastritis in 131 patients and 371 population based controls. Those with severe CAG were 18.1 times more likely, and those with severe body CAG were 4.6 times more likely, to develop gastric cancer than

people with a normal mucosa or superficial gastritis. There was also a positive correlation between the severity of CAG and the risk of developing the disease.

There has been some recent interest in the sub-type of IM and the risk of gastric cancer. This was investigated in a Portuguese case-control study of 1,041 patients attending an endoscopy clinic for upper gastrointestinal symptoms (Silva and Filipe 1986) Forty-one of the 110 patients (37.2%) with gastric cancer had incomplete IM, but none of those with a benign condition had incomplete IM.

The major limitation of these case-control studies is that the order of events is unknown; that is, gastric cancer could have caused the CAG or IM rather than the other way round. It was only with the long term follow-up of subjects that the temporal relationship between CAG and gastric cancer was established.

1.2.5 Cohort studies

There were only two studies in which more than 100 subjects with and without CAG were followed up for a long period of time. Cheli et al (1973) followed 114 subjects for 11-18 years after biopsies were taken. Gastric cancer was recorded as the cause of death in 9 of 65 subjects with 'atrophic' or 'pre atrophic gastritis' and in none of those with a normal mucosa or superficial gastritis.

Ihamäki et al (1978) followed 261 subjects with a normal

mucosa or superficial gastritis and 116 with CAG in body biopsies for 27 years. During this period, one gastric cancer was seen amongst those with either normal or superficial gastritis, in contrast to 11 cases in those with CAG. The CAG group also had a higher prevalence of IM (89%) than the control group (36%). Fifty-eight percent of subjects with a normal mucosa developed superficial gastritis and 14% developed CAG. In another analysis of the same population, Siurala (1985) showed after 27 years that the increase in CAG from body biopsies was at a constant 1.5% per year up to geriatric age.

These follow-up studies have answered three important points in relation to CAG and gastric cancer: firstly, a normal mucosa precedes CAG and CAG preceded gastric cancer; secondly, this transition took place over 2-3 decades; and thirdly, as many as 10-15% of those with severe CAG may have developed gastric cancer over a 20-30 year period.

1.3 SEROLOGICAL MARKERS OF CAG

As mentioned above, one of the main limiting factors in the epidemiology of CAG has been its poor association with specific symptoms and the necessity of a gastric biopsy to determine the status of the gastric mucosa. In order to circumvent the need for taking gastric biopsies, the level of pepsinogen has been proposed as a serological marker of CAG.

Pepsinogen, as well as being secreted into the gastric

Table 3

Sensitivity and specificity of selected studies using pepsinogen as a marker to diagnose type B CAG and gastric cancer.

Author	Year	N	Population	Disease endpoint	Prevalence (%)	Assay endpoint	Sens (%)	Spec (%)
Miki et al	1987	121	Endoscopy	Advanced CAG	32.2	PGA/C < 2.5	87.2	84.1
Westerveld et al	1987	341	Endoscopy	Gastric ca. & Precurs	45.8	PGA < 25 µg/ml & PGA/C <1.5	46.0	96.4
Borsch et al	1989	273	Endoscopy	Severe CAG Type A	53.9	PGA < 71.6 µg/ml	100.0	86.0
Miki et al	1989	425	Endoscopy	Gastric Ca.	47.6	PGA/C < 2	87.5	82.5

PGA = Serum pepsinogen A levels
PGC = Serum pepsinogen C levels

Table 4

Population studies relating pepsinogen levels with gastric cancer or precursors

Author	Year	Country	Population	Percent with: IM	Percent with: low PGA	Gastric cancer mortality per 100,000
Stemmermann et al	1987	Japan	Japanese	75.6	16.1	95.3
Stemmermann et al	1978	Hawaii	Japanese	43.7	9.3	45.9
			Caucasians	--	2.3	16.9
Burr et al	1988	U.K.	Bath		7.7	94 (SMR)
			Caerphilly		12.6	111(")
			Non manual		11.9	--
			Manual		15.4	--

SMR = Standardised mortality rate, adjusted for social class.

lumen, appears in the serum in two immunologically distinct forms, pepsinogen A (PGA or PGI) and pepsinogen C (PGC or PGII) (Defize and Meuwissen 1987). PGA originates only from the chief and mucus neck cells of the fundic region of the stomach, but PGC originates from the gland cells throughout the stomach and the proximal duodenum (Defize and Meuwissen 1987). With increasing gastric atrophy affecting the fundic region PGA levels tend to fall. PGA is highly specific for CAG but not very sensitive (Westerveld et al 1987). This is because those with atrophy not yet reaching the fundus will still have high PGA levels and will therefore be misclassified as normal. In contrast, PGC levels do not fall as sharply as PGA levels, because gastric metaplasia in the proximal duodenum (which accompanies intestinal metaplasia and atrophy in the stomach) compensates for some of the loss of PGC-producing glands in the stomach (Defize and Meuwissen 1987).

By using a combination of PGA and C levels, Westerveld et al (1987) obtained a specificity of 96% for diagnosing CAG, although the sensitivity was only 46%. Miki et al (1987) using the ratio of PGA:PGC, reported a sensitivity and specificity of 87% and 84% respectively in detecting CAG as measured by the Congo Red test described in section 1.1.1. These studies are summarised in Table 3.

Despite the poor sensitivity of PGA and PGC levels in detecting the presence of CAG, they have been used in a few epidemiological and aetiological studies, relating CAG to

dietary and social factors and gastric cancer (Table 4).

When comparing Japanese and Caucasians living in Hawaii, Stemmermann et al (1978) found the Japanese had a greater prevalence of low PGA levels (9.3%) than Caucasians, in keeping with the gastric cancer differences between the two populations. In a recent cohort study of 8,006 men of Japanese descent living in Hawaii, Stemmermann et al (1987) compared the PGA and PGC levels of 88 cases of gastric cancer to 250 controls. Patients with gastric cancer of the intestinal type were 9 times more likely to have had a low PGA level (< 20 μg/ml) and 10 times more likely to have had a low PGA/PGC ratio (< 2.0) than controls.

Studies using serum pepsinogen showed results that were consistent with what was already known about CAG and gastric cancer. However, the low sensitivity of PGA or PGA/C as a marker for CAG found by some workers may limit the usefulness of these markers in epidemiological studies.

1.4 AETIOLOGY OF CAG

1.4.1 Model for gastric carcinogenesis

Evidence from studies such as those described above led Correa and co-workers (Correa et al 1975, Correa 1983; 1988) to propose and refine an aetiological model for gastric cancer. Correa suggested that gastric cancer of the intestinal type was an end result of cellular changes or mutations which began in early life. These changes were multicausal but resulted in inflammation, (chronic

gastritis), atrophy (CAG) and abnormal or loss of cellular differentiation (IM and dysplasia, respectively).

Correa suggested that irritants such as alcohol, aspirin, salt, coarse grains and, most recently, the bacterial organism Helicobacter pylori were responsible for altering the microenvironment of the stomach and assisting in the production of mucosal atrophy Correa 1988).

Atrophy is the key event linking CAG to gastric cancer, being present in autoimmune 'type A' and environmental CAG but absent in hypersecretory chronic gastritis (Correa 1980). Atrophy could also be caused by inadequate cellular repair due to poor nutritional status, for example diets low in vitamin C and beta carotene (Correa 1988).

The achlorhydria which accompanies CAG, due to atrophy of acid-secreting cells, creates an environment which is favourable for bacterial proliferation. Certain bacterial species which are able to colonise the stomach possess nitrate reductase enzymes which can reduce dietary nitrate to nitrite. For example, subjects with CAG formed nitrite up to 10 times more efficiently than those without (Eisenbrand et al 1984). In a study from China, a higher proportion of those with CAG had nitrate-reducing bacteria in relation to those with superficial gastritis and a normal mucosa, these proportions being 36%, 24% and 12%, respectively (Zhang et al 1984). Nitrite is highly reactive and may combine with nitrogen compounds to produce N-

nitroso-compounds (NOC's), many of which are known to be mutagens and potent carcinogens (Correa 1983). Different dietary factors could also inhibit or catalyse the nitrosation reaction (Correa 1988). The interplay of these dietary and other agents may cause the progression of CAG to intestinal metaplasia and subsequently the transition to dysplasia and gastric cancer.

1.4.2 Risk and protective factors

Despite the importance of CAG in the epidemiology of gastric cancer, only a few studies have focussed specifically on its aetiology (Table 5). Whereas some have been based on nutritional and biochemical surveys of populations with and without CAG, others were inferred from studies of gastric cancer and animal experiments. This section examines the relevance of chemical irritants like salt and aspirin, dietary factors, and N-Nitroso-compounds in the aetiology of CAG. The next section deals with the epidemiology of *Helicobacter pylori* which has recently been shown to be playing a prominent role in the aetiology of CAG.

1.4.3 Chemical irritants and mutagens

1.4.3.1 Salt

In the Colombian high risk region for CAG (Narino) described earlier, the custom of salting meat for preservation was practiced by 52% of the population. By contrast, only 18% reported this custom in the other areas (Haenszel *et al* 1976). To corroborate this, the urinary

sodium and sodium:potassium ratio was found to be higher in villages with high rates of CAG than in villages with low rates (Correa et al 1983, Montes et al 1985).

People in areas of high gastric cancer mortality (and presumably with a higher prevalence of CAG) consumed more salt than people in areas of low gastric cancer mortality. This geographical correlation was also shown in worldwide comparisons of salt consumption and gastric cancer and cerebrovascular disease mortality, which suggested as the common aetiological factor (Joosens and Geboers 1986). Similarly, salt consumption and gastric cancer mortality was shown to be positively correlated within individual countries, for example China (Lu and Quin, 1987), and in case control studies of diet and gastric cancer (Hu et al 1988).

There were however some studies which did not show a correlation between dietary salt and gastric cancer. Whelton and Goldblatt (1981) found a concordance between gastric cancer and cerebrovascular disease mortality, but they also found a concordance between gastric cancer and other causes of death unrelated to salt consumption, such as pancreatic and lung cancers. Their results suggested that salt, gastric cancer and cerebrovascular disease were not causally linked.

Kono et al (1983) compared the mortality of cerebrovascular disease and gastric cancer in areas of

differing salt consumption in Japan and found a correlation between salt and cerebrovascular disease but not with gastric cancer. The association between foods pickled in salt and gastric cancer was more consistent (Howson et al 1986) which suggested that a combination of salt and other agents may both damage the mucosa and initiate carcinogenesis. Salt appeared to have damaged the stomach mucosa of laboratory animals (Kodama et al 1984) and also seemed to potentiate the action of N-nitroso-carcinogens in rats (Takamashi et al 1984, Correa 1988).

1.4.3.2 Aspirin

Evidence that aspirin is an irritant of the gastric mucosa came from patients on long term aspirin therapy and from animal studies. Rheumatic patients on long term aspirin therapy had a greater frequency of erythema (76%) than patients not on aspirin therapy. Only 4% of the latter had gastric erythema (Silvoso et al 1979). In a large follow-up study of 6.25 years of 22,781 subjects, those using aspirin on a daily basis had elevated but non-significant risks for admission due to gastrointestinal bleeding, and no elevated risks due to gastric cancer (Paganini-Hill et al 1989). The authors commented that these observations may have underestimated any association because people with stomach complaints may have avoided using analgesics.

In a randomised controlled trial of aspirin in the prevention of cardiovascular mortality, a significantly

greater proportion of those taking aspirin reported gastric bleeding when compared with those taking the placebo (Steering Committee of the Physicians' Health Study Research Group, 1989). Aspirin also seemed to potentiate the carcinogenic action of N-nitroso-compounds in rats (Takamashi et al 1984, Tsung Hsien et al 1983).

1.4.3.3 Pepsinogen

Chronic or acute superficial gastritis, especially in the fundus, are causes of high PGA and PGC secretion into the gastric lumen (Samloff 1989, Defize and Meuwissen, 1987). In the presence of acid, pepsinogens are converted to pepsins which hydrolyse any available substrate, including gastric mucin. The role of pepsin has been reviewed as a possible mechanism of peptic ulceration (Samloff 1989), but it is not known whether the action of increased pepsin in the stomach due to chronic superficial gastritis predisposes to autolysis of the gastric mucosal glands and subsequent gastric atrophy.

1.4.3.4 N-nitroso-compounds (NOCs)

The production of endogenously synthesised NOCs in the achlorhydric stomach is an integral part of Correa's model of gastric carcinogenesis (Correa 1988). Although the significance of NOCs to gastric and other cancers remains to be proven, (Forman 1987) it is still generally accepted that if NOCs have a role to play, it will be as a consequence of CAG rather than in the establishment of this condition. Thus although nitrate exposure (Cuello et al 1976) and

exposure to an extremely mutagenic NOC in the Fava bean (Correa 1988) has been found at an increased level in high risk Colombian populations, this probably has no bearing on the development of CAG in these populations. In contrast, Stemmermann and Mower (1981) suggested that NOC may be a direct cause of CAG based on work in which CAG metaplasia and gastric cancer was established in rats after exposure to specific NOCs. The relevance of such animal experiments to the human situation in which NOC is endogenously synthesised has still to be established.

1.4.3.5 Mycotoxins

Mycotoxins, especially aflatoxins, have been reported to be strong hepatocarcinogens in both human and animal studies (Wild et al 1990) but there has been very little written about the role of mycotoxins in relation to CAG and gastric cancer. In a population based study in China (Zhang et al 1984), a significantly ($p < 0.05$) higher proportion (28%) of the population living in a high gastric cancer risk area had Aspergillus versicolor (a producer of sterigmatomycin, which is quite similar to aflatoxin) present in gastric juice aspirates in comparison with those living in low gastric cancer areas (16%). Also, those with CAG in the high risk area had a higher prevalence of A.versicolor when compared to those with CAG in the low gastric cancer risk areas, 31% and 16%, respectively, but this difference was not significant ($p > 0.05$). Besides this population study, there have been reports on gastric carcinogenicity of aflatoxins in rats, especially if used in conjunction with

Table 5 Studies of diet and CAG

Author	Year	Population	Disease	Risk factors	Protective factors
Haenszel et al	1976	Colombia	CAG & IM	Moras*, corn, salting of meat	Lettuce
Correa et al	1983	Colombia	CAG & IM	Fava beans, ?salt, low K+	Fresh fruit, vegetables vitamin A, ?beta carotene
Haenszel et al	1985	Colombia	CAG & IM	Salt	beta carotene+, vitamin E+
Fontham et al	1986	U.S.A.	CAG & IM	Milk#, bread# smoking	Fruit, vegetables, vitamin C
Burr et al	1987	U.K.	Low PGA	low social class	Fruit, vegetables plasma ascorbate

* Local blackberries
# See text (Section 1.4.4.)
+ Protective for gastric dysplasia, not CAG.

N-Nitroso-compounds (Newberne and Connor 1980).

1.4.4 Dietary risk factors

Consumption of corn and acidic blackberries (Moras) have been associated with areas with a higher prevalence of CAG in Colombian populations (Haenszel et al 1976). Elevated risks for smoking, milk and bread were found amongst patients attending an endoscopy clinic in South Louisiana (Fontham et al 1986). The authors argued however that milk and bread was consumed to relieve upper abdominal discomfort in the latter study.

1.4.5 Dietary protective factors

In Colombia, an increased consumption of lettuce was associated with regions of low prevalence rates of CAG (Haenszel et al 1976). In another nutrition survey of four villages in the Colombian Narino district, the frequency of consumption of fresh fruit and vegetables was inversely related to the prevalence of CAG & IM (Correa et al 1983). The same association with fruit and vegetables was also found amongst people attending an endoscopy clinic in South Louisiana (Fontham et al 1986). Consumption of vitamins A and C (commonly found in fresh fruit and vegetables), has also been inversely associated with CAG (Correa et al 1983, Fontham et al 1986). Vitamin E and beta carotene have been found to be inversely associated with gastric dysplasia, but not with CAG (Haenszel et al 1985). Consumption of vitamin A and C (or a component of fresh fruit and vegetables) may be protective against CAG, or retard its development.

Vitamin E and beta-carotene may be protective against dysplastic lesions, after the development of CAG (Correa 1988, Haenszel et al 1985).

In the only aetiological study using pepsinogens as a marker of CAG, Burr et al (1987) compared serum PGA levels in men from Bath (UK) (where the gastric cancer mortality rate was low) to men from Caerphilly (where the gastric cancer mortality rate was high). A greater proportion of low PGA levels were found in Caerphilly (14.5%) than in Bath (7.7%). Men in manual occupations had lower ascorbate levels, consumed less fruit and had lower PGA levels than men in non manual jobs in both towns.

1.5 SUMMARY

The epidemiological studies described above, and the studies on animal models have all contributed to the hypothesis that CAG is a major pathological precursor of gastric cancer. Gastritis seemsto advance with age, and is more prevalent and occurred at an earlier age in populations with higher gastric cancer mortality. One of the major limiting factors on the studies on CAG has been the lack of uniformity on the topological and histological classification of gastritis, making comparisons difficult.

Dietary factors may have played an important role in the age of onset of gastritis and in the rate of progression of gastritis to CAG, metaplasia and dysplasia. A consistent finding has been that vitamin C and fresh fruit and vegetables play a protective

role in the development of CAG, and gastric cancer.

A major recent addition to the model of gastric carcinogenesis has been that of H.pylori as an important cause of chronic active and other forms of gastritis. The evidence for the association between H.pylori and mucosal histopathology, in particular CAG, is described in the next section.

SECTION 2

HELICOBACTER PYLORI

INTRODUCTION

Reports of the presence of spiral bacteria in the human gastric mucosa can be traced as far back as 1874, when Bottcher observed them in stomach tissues. Kreinitz (1906) described seeing spiral bacteria in gastric contents of patients with ulcerative cancer, and Luger and Neuberger (1921) found bacteria absent in a healthy stomach but present in diseased stomachs. There was a resurgence of interest in spiral gastric bacteria after Steer and Colin-Jones described their presence in 1975 (mistakenly identified as Pseudomonas aeruginosa) in association with gastric ulceration (Steer and Colin Jones, 1975). However, Marshall and Warren (1984) were the first to identify these as Campylobacter pyloridis and since the publication of this first observation its name has been changed to Campylobacter pylori. More recently it has been reclassified in its own genus, Helicobacter (Goodwin et al 1989). This chapter will review the literature on the epidemiology of H.pylori in relation to gastritis, and more specifically, chronic atrophic gastritis.

2.1 IDENTIFICATION OF H.PYLORI

Helicobacter pylori is a spiral shaped, gram negative bacteria about 0.5 μm wide and 3.5 μm long. It is specifically found attached to the surface of gastric mucosal cells, beneath the mucus layer and is usually associated with the intercellular junctions of the mucosal epithelium (Hazell et al 1986). H.pylori is also found deep in the gastric epithelial pits

(Hazell et al 1986) but does not invade the surface of the mucosa. This bacteria has been identified outside the stomach, but only in tissues exhibiting a gastric morphology, for example Barrett's oesophagus, (Talley et al 1988) and gastric metaplasia in the duodenum (Wyatt et al 1987).

The methods currently used for the detection of H.pylori in individuals include culturing of the organism, visual observation from mucosal biopsies or the detection of circulatory antibodies. In addition, H.pylori produces a large amount of urease and this property has been useful in the diagnosis of the organism, by using isotopically labelled ^{13}C or ^{14}C urea, and measuring the residual labelled carbon in exhaled CO_2 (urea breath tests) Graham et al 1987, Bell et al 1985). Biopsy material containing H.pylori will hydrolyse urea and cause a change in pH and a colour change of a pH indicator solution. This property was first described by McNulty and Wise (1985) and is now available as a commercial kit (CLO test).

2.1.1 Immune response

Subjects with histological or microbiological proven H.pylori infection of the gastric mucosa produce a specific localised and a systemic immunological response (Jones et al 1987, Newell et al 1988, Perez-Perez et al 1988). H.pylori organisms attached to the gastric mucosa are coated with immunoglobulin but the antibodies do not eliminate the organisms nor prevent the re-establishment of infection after clearance (Newell and Stacey 1989). This implies that H.pylori has the means to protect itself from the host response (Newell and Stacey 1989, Morgan and

Table 6
Sensitivity and specificity of serological tests
in diagnosing H.pylori infection

Author	Year	Preparation	sens(%)	spec(%)
Perez-Perez et al	1988	Whole cell	93.1	94.4
Newell et al	1988	Acid extract	92.1	88.0
Dent et al	1988	Urease	100	79.0
Evans et al	1989	High MW cell proteins	98.7	97.8
Fox et al	1989	Whole cell	89.0	75.0
Bolton et al	1989	Whole cell	100	73.0
Mitchell et al	1988	Whole cell	100	94.0

Leunk 1989). H.pylori also elicits a systemic antibody response, predominantly of the IgG and IgA classes but IgM immunoglobulins are rarely detected (Newell and Stacey 1989). The IgG response is significantly raised and remains constant throughout infection but amongst the IgG class, IgG_3 immunoglobulins (indicative of an acute infection) are not detected (Newell and Stacey 1989). IgG seroconversion occurred 22-33 days after infection in one volunteer study (Morris and Nicholson 1987)

H.pylori-associated proteins of molecular weight of 14-16, 20-22, 31, 54, 56, 57-63 110-120 kilodaltons have most frequently been detected in the blood of H.pylori infected subjects, but there has been no single protein that reacted consistently in all subjects, suggesting a complex and variable response to H.pylori infection (Newell and Stacey 1989). An H.pylori flagellar protein of 54 and 56 kilodaltons was not specific to H.pylori and appeared to cross-react with some Campylobacter species (Newell and Stacey 1989).

Despite the heterogeneity of antibody response to H.pylori, serological techniques for the diagnosis of H.pylori infection by the identification of IgG and IgA antibodies are now widely available, yielding sensitivities and specificities exceeding 80% (table 6). These techniques have included the use of whole cells (Jones et al 1986) whole cell sonicates, (Booth et al 1986, Perez-Perez et al 1988) acid extractable preparations (Newell et al 1988), and urease preparations (Dent et al 1988) as antigen preparations for immunological tests (usually Enzyme Linked Immunosorbent Assays (Newell and Rathbone 1989)).

When compared to cell culture as a gold standard, the specificity and sensitivity of surface antigen, acid extract and urease preparations in detecting H.pylori was measured in 117 subjects (Bolton and Hutchingson 1989). Although the authors concluded that the urease test performed better than the other tests, there was broad agreement between the three preparations, the sample size being too small to detect a significant difference between the three methods. This indicates that serological antibody tests could be a useful tool for the identification of current and past H.pylori infection (Newell and Rathbone 1989).

2.2 ASSOCIATION BETWEEN H.PYLORI AND GASTRITIS

Several lines of investigation have led to the current hypothesis that H.pylori is a cause of gastritis, and especially chronic active gastritis. These have included clinic-based studies, studies of voluntary or accidental ingestion of H.pylori, and studies of antibacterial treatment of H.pylori. One of the major limitations in many of these studies has been the absence of uniformity in the classification of mucosal histology, despite reference to the most popular classification scheme of Whitehead (1973). For example, some workers include superficial gastritis and CAG into one category of 'gastritis', whereas others classify as 'gastritis' only those lesions which displayed intestinal metaplasia. In other examples, the degree of severity of 'chronic gastritis' has been judged in relation to the density of polymorphonuclear leucocytes, (that is the degree of activity), whereas in other studies, severity has been judged in relation to the degree of atrophy.

Because H.pylori has been regarded as a major causative agent of 'gastritis' in general, the term 'Campylobacter pylori or Helicobacter pylori-associated gastritis' has also been used in the literature. In some of these studies it is not always made clear whether a mucosal biopsy has been classified independently of the presence or absence of H.pylori organisms. If mucosal histopathology is not classified independently of the presence or absence of H.pylori, then sections with gastritis but without H.pylori could be misclassified as normal. Similarly normal tissue but with H.pylori organisms present may be misclassified as 'gastritis'. Such misclassification would therefore bias any association between H.pylori and gastric mucosal histology.

Much of the work on H.pylori has been in relation to duodenal and gastric ulceration and the presence of polymorphonuclear leucocytes (the presence of activity) as a response to infection. It is only in a few studies that CAG has been looked at as a lesion of interest.

The aim of this section is to review the literature on the relationship between H.pylori and specific subtypes of gastritis, particularly chronic active and chronic atrophic gastritis. However, because of the lack of adherence to well established histological nomenclature, and the use of different criteria to classify mucosal histopathology, caution must be exercised when comparing some of these studies.

2.2.1 H.pylori and mucosal histopathology

Since the first reports of the association between H.pylori

and 'gastritis' (Marshall and Warren 1984) there has been an abundance of papers confirming this association in clinical studies. The relationship between H.pylori and gastritis of all forms has been the subject of a number of reviews (Pearson (1986), Blaser (1987, 1990) Dooley and Cohen (1988), Yeomans (1989), Dixon (1989) Graham and Klein (1988)).

A common approach in these reviews has been to pool the results of several studies. For example, in one of the earlier reviews, Blaser (1987) showed H.pylori as being present in 6-51% of subjects without 'gastritis' and in 38-100% in those with 'gastritis'. Such an approach does not take into consideration the magnitude of an association between H.pylori and mucosal histopathology and does not allow comparison between histological subgroups of gastritis.

Another way of summarising the literature on H.pylori is to use the odds ratio as a measure of association between mucosal histopathology and H.pylori (Armitage and Berry 1987). An odds ratio (and corresponding confidence intervals) describes the ratio of the odds of H.pylori infection in those with a normal mucosa in relation to the odds of infection in those with various grades of gastritis (for example, superficial, chronic atrophic, etc.). If a number of studies have been conducted using roughly the same methodology, a pooled odds ratio could be used to summarise the magnitude of the association of H.pylori in relation to mucosal histopathology by lesion of interest in these studies (Demets, 1987).

In order to measure the association between H.pylori and gastric mucosal histopathology, odds ratios (and corresponding upper and lower 95% confidence intervals) were calculated from each study of H.pylori status and gastritis since 1984. In order to reduce the effect of random variation, Figure 1 only lists endoscopic surveys of subjects which had an original sample size of over 50 subjects. However, after those with ulcers were excluded, the sample size of some studies was reduced to under 50 subjects.

Figure 1 shows the odds of having H.pylori in relation to the degree of gastritis (against a baseline of normal mucosa), as classified by each author in subjects with non-ulcer dyspepsia.

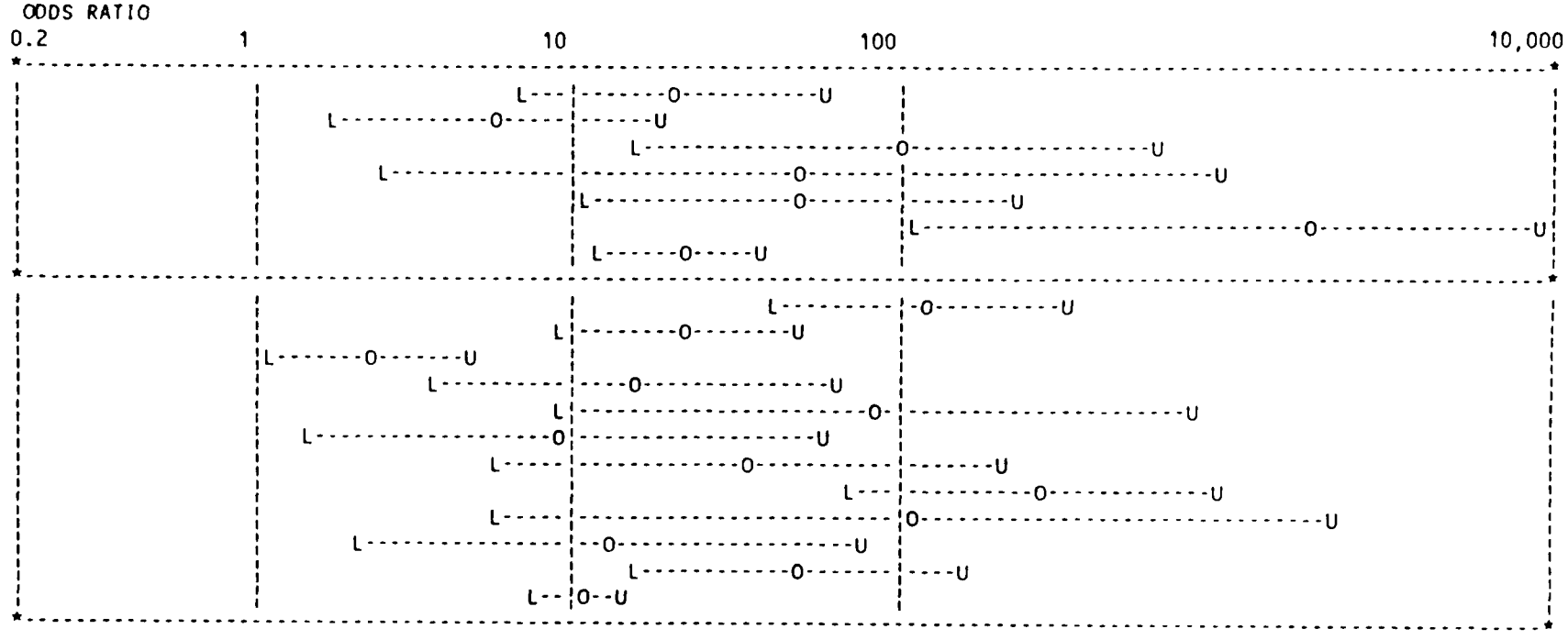
Most studies reported results from antral biopsies, body biopsies or both. Results from body biopsies only have been excluded from Figure 1. Because culture of H.pylori, histology or the urea breath test have been considered as the gold standards against which antibody tests are compared, and for the sake of brevity, this analysis excluded the results which used only H.pylori antibody tests. However, inclusion of studies which used antibody tests for H.pylori positivity did not have a significant effect on the magnitude of the associations reported in Figure 1.

Clearly, this type of pooled analysis may serve as a useful adjunct for reviewing the literature, but it is not a substitute for well designed studies looking at the association between H.pylori and mucosal histology and in particular, CAG and gastric

FIGURE 1

RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
BEDOSSA	1987	FRA2	110	SG	20.0
BEDOSSA	1987	FRA1	110	SG	5.6
JONES	1984	U.K.	50	SG	98.0
MANGANARO	1988	ITAL	83	SG	48.4
SIURALA	1988	FINL	157	SG	48.5
STOLTE	1989	GERM	6300	SG	1826*
POOLED ODDS RATIO				SG	20.9
ANDERSEN	1987	DENM	153	CSG	118
BOOTH	1986	U.K.	220	CSG	20.9
JASKIEWICZ	1989	SAFR	178	CSG	2.3
JIANG	1987	CHIN	209	CSG	15.0
JONES	1986	U.K.	64	CSG	84.0
PRICE	1985	U.K.	51	CSG	9.1
PRICE	1988	U.K.	50	CSG	34.8
SIPPONEN	1988	FINL	194	CSG	260
SIPPONEN	1988	FINL	57	CSG	110
SIPPONEN	1988	FINL	39	CSG	12.6
SIURALA	1988	FINL	179	CSG	48.6
POOLED ODDS RATIO				CSG	10.3



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AG=ACTIVE GASTRITIS, CG=CHRONIC GASTRITIS, CAG=CHRONIC ATROPHIC GASTRITIS
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

* NOT INCLUDED IN POOLED ODDS RATIO CALCULATION
# p > 0.05 (NOT SIGNIFICANT)

FIGURE 1

RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
BEDOSSA	1987	FRA2	110	SG	20.0
BEDOSSA	1987	FRA1	110	SG	5.6
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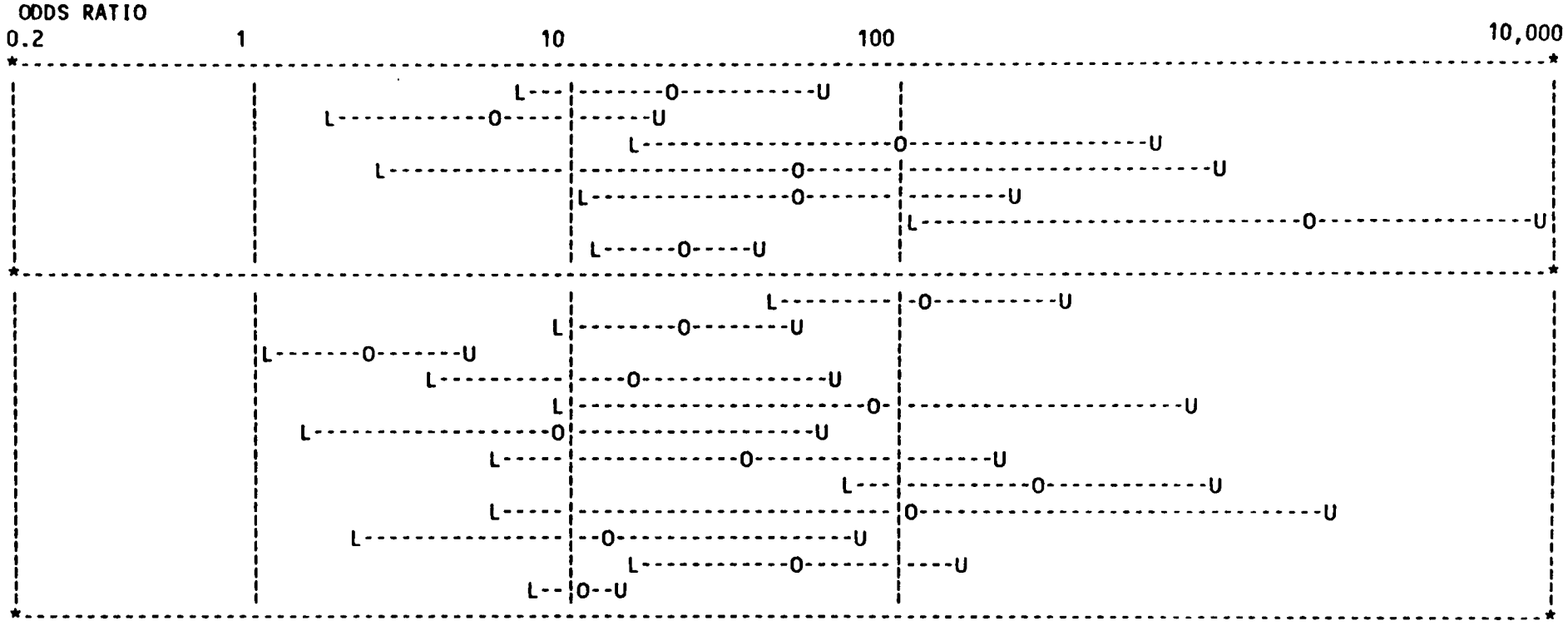
MANGANARO	1988	ITAL	83	SG	48.4
SIURALA	1988	FINL	157	SG	48.5
STOLTE	1989	GERM	6300	SG	1826*
POOLED ODDS RATIO				SG	20.9
ANDERSEN	1987	DENM	153	CSG	118
BOOTH	1986	U.K.	220	CSG	20.9
JASKIEWICZ	1989	SAFR	178	CSG	2.3
JIANG	1987	CHIN	209	CSG	15.0
JONES	1986	U.K.	64	CSG	84.0
PRICE	1985	U.K.	51	CSG	9.1
PRICE	1988	U.K.	50	CSG	34.8
SIPPONEN	1988	FINL	194	CSG	260
SIPPONEN	1988	FINL	57	CSG	110
SIPPONEN	1988	FINL	39	CSG	12.6
SIURALA	1988	FINL	179	CSG	48.6
POOLED ODDS RATIO				CSG	10.3



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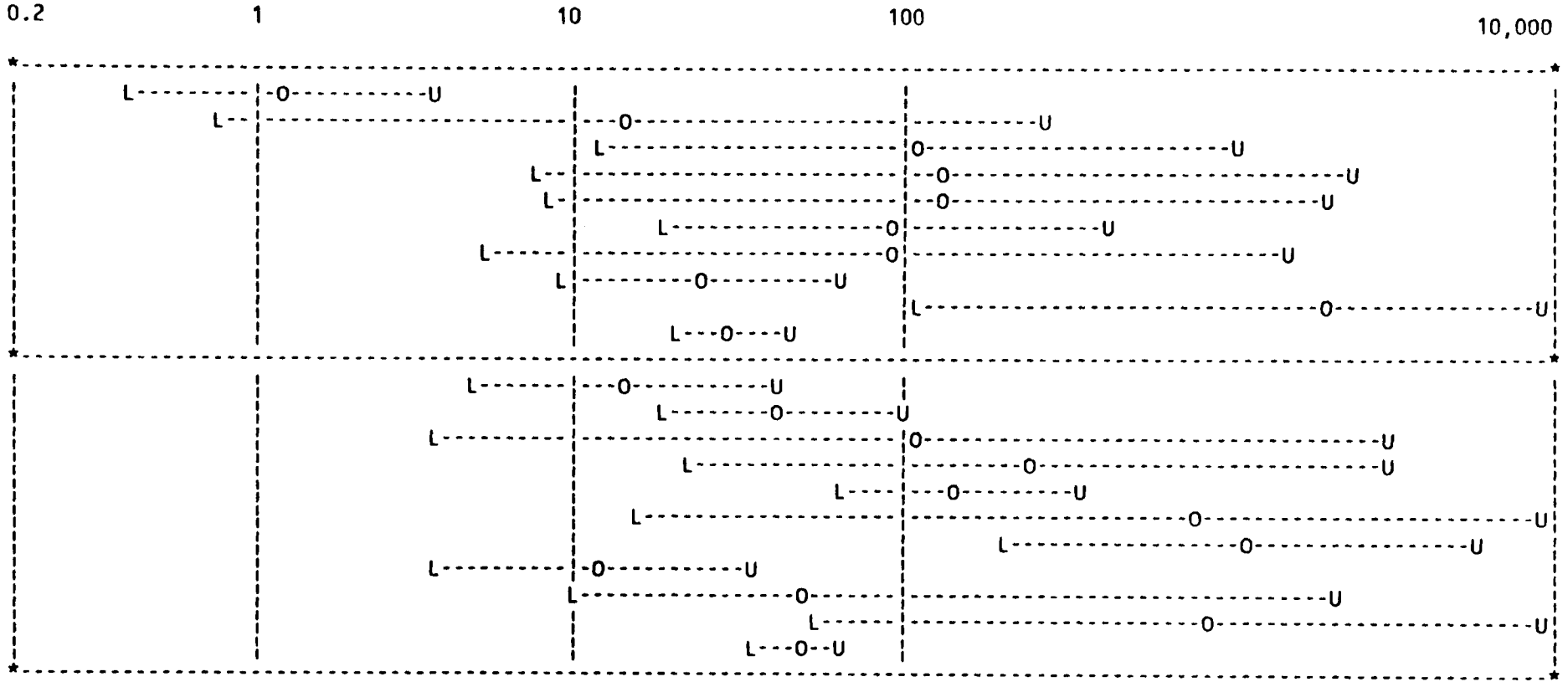
* NOT INCLUDED IN POOLED ODDS RATIO CALCULATION
# p > 0.05 (NOT SIGNIFICANT)

Fig 1 continued

RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	CAGTY	ODDS RATIO
ANDERSEN	1988	DENM	72	G	1.2#
FENDRI	1988	TUNI	104	G	14.3#
LANGENBERG	1984	NETH	50	G	109
MCNULTY	1984	U.K.	80	G	132
MITCHELL	1988	AUST	189	G	128
MORRIS	1988	NZEL	54	G	88.7
MUSGROVE	1988	U.K.	97	G	89.0
VON WULFFEN	1988	GERM	206	G	23.7
WYATT	1987	U.K.	254	G	1917
POOLED ODDS RATIO				G	29.0
COELHO	1989	U.K.	96	AG	14.2
DENT	1988	U.K.	202	AG	42.1
JONES	1984	U.K.	50	AG	106
MARSHALL	1984	AUST	69	AG	252
MILLER	1988	SAFR	224	AG	150
RAUWS	1988	NETH	34	AG	825
RAUWS	1988	NETH	240	AG	1132
ROUVROY	1987	RWAN	184	AG	11.1
SORENSEN	1988	NORW	61	AG	47.3
STEININGER	1989	GERM	784	AG	901
POOLED ODDS RATIO				AG	46.9



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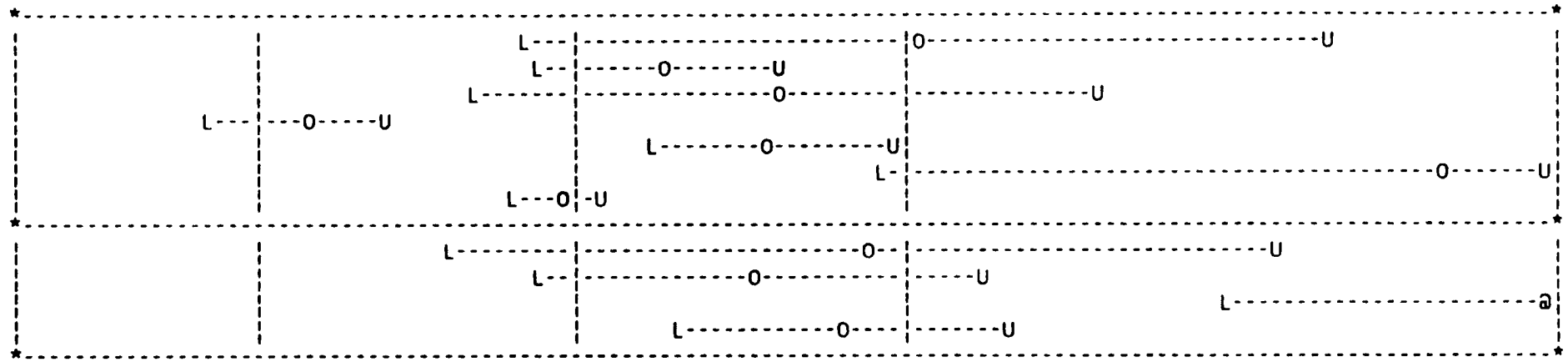
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

p > 0.05 (NOT SIGNIFICANT)

Fig 1 continued

RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
BLOMBERG	1988	SWED	112	CG	114
BOOTH	1986	U.K.	220	CG	17.8
MARSHALL	1984	AUST	69	CG	42.0
ROUVROY	1987	RWAN	184	CG	1.4#
STANESCU	1989	GERM	207	CG	38.4
VAIRA	1988	U.K.	66	CG	4485
POOLED ODDS RATIO				CG	8.7
FENG	1988	CHIN	310	CAGA	74.1
JIANG	1987	CHIN	209	CAGA	35.2
STOLTE	1989	GERM	6300	CAGA	1E4*
POOLED ODDS RATIO				CAGA	62.8



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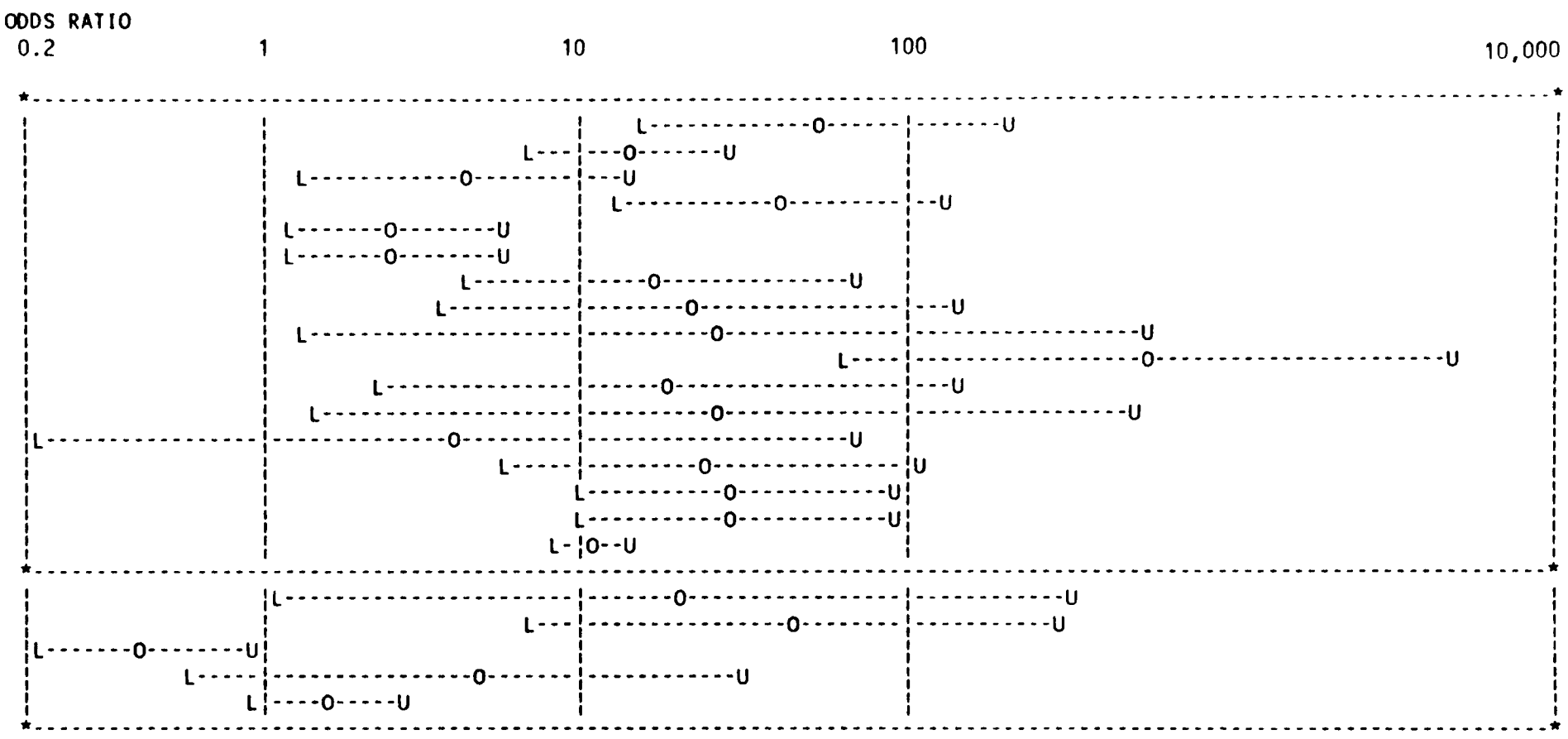
AG=ACTIVE GASTRITIS, CG=CHRONIC GASTRITIS, CAG=CHRONIC ATROPHIC GASTRITIS
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

* NOT INCLUDED IN POOLED ODDS RATIO CALCULATION
# p > 0.05 (NOT SIGNIFICANT)

Fig 1 continued

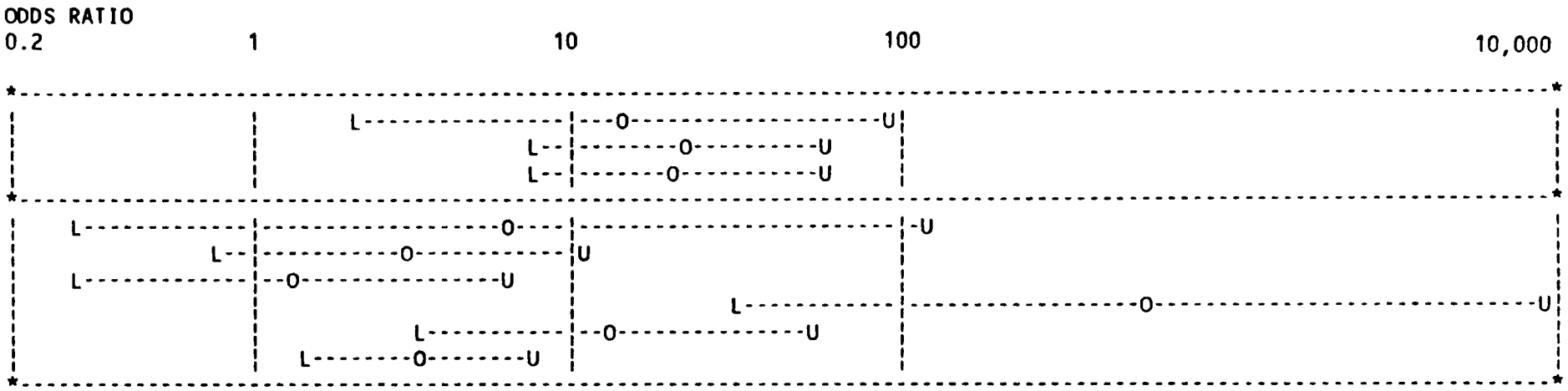
RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
ANDERSEN	1987	DENM	153	CAG	55.9
BOOTH	1986	U.K.	220	CAG	14.4
COELHO	1989	U.K.	96	CAG	4.3
DOOLEY	1988	USA	113	CAG	41.4
FOX	1989	USA	169	CAG	2.6
JASKIEWICZ	1989	SAFR	178	CAG	2.6
JIANG	1987	CHIN	209	CAG	17.3
JONES	1986	U.K.	64	CAG	22.5
MANGANARO	1988	ITAL	83	CAG	27.0
MARTIN	1988	USA	112	CAG	559
PRICE	1985	U.K.	51	CAG	17.6
SIPPONEN	1988	FINL	57	CAG	27.1
SIPPONEN	1988	FINL	57	CAG	4.0#
SIPPONEN	1988	FINL	194	CAG	24.7
SIURALA	1988	FINL	157	CAG	29.1
SIURALA	1988	FINL	179	CAG	29.1
POOLED ODDS RATIO				CAG	10.2
FENG	1988	CHIN	310	CAGQ	19.2
MILLER	1988	SAFR	224	CAGQ	45.0
ROUVROY	1987	RWAN	184	CAGQ	0.4#
SORENSEN	1988	NORW	61	CAGQ	4.5#
POOLED ODDS RATIO				CAGQ	1.6#



G=GASTRITIS, SG=SUPERFICIAL GASTRITIS, CSG=CHRONIC SUPERFICIAL GASTRITIS
AG=ACTIVE GASTRITIS, CG=CHRONIC GASTRITIS, CAG=CHRONIC ATROPHIC GASTRITIS
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
MILLER	1988	SAFR	224	IM	13.5
SIURALA	1988	FINL	142	IM	21.4
POOLED ODDS RATIO				IM	20.4
FENG	1988	CHIN	310	CA.	6.1#
JASKIEWICZ	1989	SAFR	178	CA.	2.9#
JIANG	1987	CHIN	179	CA.	1.3#
STEININGER	1989	GERM	784	CA.	542*
VON WULLFEN	1988	GERM	206	CA.	13.0
POOLED ODDS RATIO				CA.	3.2



G=GASTRITIS, SG=SUPERFICIAL GASTRITIS, CSG=CHRONIC SUPERFICIAL GASTRITIS
AG=ACTIVE GASTRITIS, CG=CHRONIC GASTRITIS, CAG=CHRONIC ATROPHIC GASTRITIS
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

* NOT INCLUDED IN POOLED ODDS RATIO CALCULATION
# p > 0.05 (NOT SIGNIFICANT)

cancer.

2.2.1.1 H.pylori and 'gastritis'

There is now good evidence that H.pylori is associated with almost all forms of gastritis, but the pooled analysis of the odds ratio showed the strongest association being with Strickland and Mackay's criteria (1973) of type B chronic active gastritis (O.R. = 62.8, $p<0.05$) and also with active gastritis which was neither chronic nor atrophic (O.R.= 51.5, $p < 0.05$). The pooled odds ratio was 8.7 ($p<0.05$) for chronic gastritis and 10.2 ($p<0.05$) for CAG. An association was also found for CSG (10.3, $p<0.05$).

Studies on quiescent gastritis (that is, gastritis but without polymorphonuclear leucocytes) showed some contradictory results; two studies showed a positive association (Feng and Yang 1988, Sorensen et al 1988), the latter study being not statistically significant, and the third study (Rouvroy et al 1987) showed a negative but non-significant association. These three contradictory studies resulted in an overall pooled odds ratio of the association between H.pylori and quiescent gastritis of 1.6, ($p>0.05$).

2.2.1.2 H.pylori and CAG

Evidence of an association between H.pylori and CAG has been shown in population-based studies, (Siurala et al 1988, Sipponen et al 1988), healthy volunteers (Dooley et al 1988) outpatient populations (Sipponen et al 1988) and non-ulcer dyspepsia patients attending endoscopy clinics (e.g. Jones et al 1986,

Andersen et al 1987, Fox et al 1989, Jiang et al 1987) (Figure 1). The pooled odds ratio between H.pylori and CAG was 10.2 ($p < 0.05$). This was lower than the association between H.pylori and active gastritis (O.R. for active 'gastritis' was 46.9 and for active CAG, 62.8). One reason for the lowered odds ratio in relation to CAG may have been because CAG is sometimes associated with IM, which H.pylori does not colonise. This introduces the potential for missing H.pylori in CAG positive biopsies. Another reason, as suggested by Fox et al 1989, is that CAG may be a multifactorial disease (as described above) with H.pylori playing a role in selected individuals.

2.2.1.3 H.pylori and Intestinal Metaplasia

H.pylori has not been found to be associated with intestinal metaplasia (IM). In a population-based study by Sipponen et al (1988), there was no significant difference in prevalence of H.pylori relation to the severity of IM. This poor association between H.pylori and IM was corroborated in histological studies which failed to detect H.pylori organisms in intestinalised gastric epithelial cells (Siurala et al 1988).

2.2.1.4 H.pylori and gastric cancer

There was a positive and significant (pooled O.R. = 3.2; $p < 0.05$) association between H.pylori and gastric cancer, but this was only so when the proportion of H.pylori positive cases in those with gastric cancer was compared to those with a normal mucosa. In the case of gastric cancer (and IM), it was not possible to tell whether this association was due to H.pylori being present in CAG which usually accompanies gastric cancer

(and IM), or due to H.pylori being present in tissue affected with gastric cancer. Some studies did not specify whether H.pylori was detected in biopsies taken from the cancerous lesion or from the area surrounding the cancer which is invariably CAG.

Because of the close relationship between H.pylori chronic active gastritis and CAG, several people have speculated about the relevance of H.pylori in relation to gastric cancer (Graham and Klein (1987), Correa and Ruiz (1989), Parsonnet (1989), Smallwood (1989)). This pooled analysis provided some evidence of a relationship between H.pylori and gastric cancer but it must be remembered that people attending endoscopy clinics because of dyspepsia are a selected group, not representative of the population at large. It could be argued that H.pylori may have been a confounder of other unmeasured factors causing dyspepsia and ultimately gastric cancer. No studies have tested this relationship between H.pylori and gastric cancer in a non-clinical setting.

2.2.1.5 Type A gastritis and H.pylori

In order to rule out the possibility that H.pylori is an opportunistic coloniser of an already gastritic stomach, the prevalence of H.pylori was assessed in patients with Strickland and Mackay's (1973) 'type A' gastritis (O'Connor et al 1984, Flejou et al 1989, Siurala et al 1988, Steininger 1989). In all these studies, there was no relationship between H.pylori and type A gastritis, suggesting that H.pylori was unlikely to have occurred as a secondary coloniser.

Table 7

Studies involving ingestion of Helicobacter pylori

Ramsey et al	1979	Infected nasogastric tube or pH electrode used for acid secretion studies caused hypochlorohydria and 'gastritis' in 17/37 volunteers. Agent unknown.
Gledhill et al	1985	Infected tube for removal of gastric juice caused epidemic hypochlorohydria.
Marshall et al	1985	Found Helicobacter-like organisms in biopsies of patients from Ramsey et al.
Marshall et al	1985	H.pylori colonises mucosa of healthy volunteer causing polymorphonuclear leucocyte infiltration (chronic active gastritis) on 10th day.
Morris & Nicholson	1987	Colonisation by H.pylori successful in volunteer after 800 mg cimetidine causing polymorphonuclear leucocyte infiltration on 11th day.
Peterson et al	1987	Rise in serum H.pylori antibody levels in those infected by pH electrode (Ramsey et al, 1979).

2.2.2 Ingestion studies

The most convincing evidence that H.pylori actually causes a pathological lesion came from studies where there was either experimental or accidental infection of H.pylori, proving at least one of Koch's postulates of a causal relationship between a bacterial agent and disease. These studies are listed chronologically in Table 7.

Two subjects with a previously normal gastric mucosa voluntarily ingested the organism (Marshall et al 1985, Morris and Nicholson 1987). Both developed chronic active gastritis, but in the latter study, only after the prior ingestion of 800 mg Cimetidine. An organism was also implicated as a cause of epidemic hypochlorohydria in gastric juice pH studies. The infection was thought to have been carried by an infected nasogastric tube (Ramsey et al 1979, Peterson et al 1987), or by an infected pH electrode (Gledhill et al 1985). Biopsies taken from subjects in the study by Ramsey and co-workers were found later to be H.pylori-like organisms (Marshall et al 1985).

2.2.3 Treatment studies

Evidence of the relationship between H.pylori and chronic active gastritis also came from treatment studies which recorded the state of the gastric mucosa before and after drug treatment against H.pylori. For example, Rauws et al (1988) and Glupczynski et al (1988) found that the stomach mucosa became normal in patients where H.pylori had disappeared after drug treatment of about four weeks directed against the organism. H.pylori soon reappeared after cessation of treatment however.

Table 8

Sensitivity and specificity of H.pylori serology in diagnosing gastritis

Authors	year	Endpoint	sens(%)	spec(%)
Newell et al	1988	antral gastritis	95.6	83.0
von Wulffen et al	1988	antral gastritis	81.0	44.1
Dent et al	1988	antral gastritis	86.6	86.7
Perez-Perez et al	1988	antral gastritis	67.8	>90.0
		active	93.3	--
		chronic	47.4	--
Fox et al	1989	CAG+IM	96.0	67.0

In the study by Rauws et al (1988) for example, 45% of those treated with amoxycillin and colloidal bismuth showed an initial reduction immediately after treatment, but after one month of follow-up, only 18% of patients remained free of infection.

2.3 H.PYLORI SEROLOGY AND CAG

H.pylori serology could be of potential use in detecting those with CAG, but no studies have measured the sensitivity and specificity of H.pylori antibodies in detecting this lesion. The association between the presence of H.pylori organisms and CAG was lower than that for active gastritis, implying that H.pylori antibodies would be of limited use in detecting CAG when compared to other forms of gastritis.

Table 8 shows the studies which reported the specificity and sensitivity of H.pylori antibodies in detecting various forms of gastritis. Newell et al (1988) developed an antibody assay with a sensitivity of 95.6% and a specificity of 83.0% in identifying those with gastritis. Two studies have looked at the relationship between H.pylori seropositivity and chronic gastritis. The first, (Perez-perez et al 1988, found a sensitivity and specificity of 47.4% and >90% respectively for the diagnosis of chronic gastritis (not necessarily atrophic), and the second (Fox et al 1989) a sensitivity and specificity of 96% and 67% respectively in diagnosing CAG with IM. The latter study included IM which is an additional marker of high risk of gastric cancer, but excluded all those with CAG which are IM negative, a subgroup still at high risk of gastric cancer. This underlines the need to elucidate the relationship between

Table 9
Prevalence of H.pylori in studies with more than 100 participants

Author	Year	Country, population	Sampling method	N	Age range	Seroprevalence %
Jones et al	1986	UK	Patients without g.i. symptoms, volunteers, screening clinics	607	6-90	20.6
Morris et al	1986	N. Zealand	Blood donors, syphilis survey sera, occupational groups	483	15-70	36.2
Siurala et al	1988	Finland	Random, population	179	Adult	A=49.0 B=53.0
Dwyer et al	1989	Australia White	Lab staff, blood donors	154	10-59	14.6
		Aborigine, N. Australia	Syphilis, Hepatitis B surveys	274	10-59	0.7
		S. Australia	Not stated	285	10-59	50.0
		Vietnamese	Immigration health centres	190	20-59	18.0
Marshall et al	1989	USA Virginia	Blood donors: White Black	510	20-65	12.0 36.0
Fitzgibbons et al	1989	USA	Healthy volunteers	116	19-91	A=31.0

...continued

Table 9
Prevalence of H.pylori in studies with more than 100 participants

Author	Year	Country, population	Sampling method	N	Age range	Seropre-valence %
Megraud et al	1989	Ivory Coast	Cluster sample	374	0-10	55.2
					Adult	>80.0
		Algeria	Blood donors	277	0-10	45.2
						>80.0
Dooley	1988 1989	USA	Paid volunteers	113	18-91	32.0
Perez-Perez et al	1988	USA	Volunteers, healthy controls, medical staff, students	347	0-39	<20.0
					40+	>50.0
Wyatt et al	1988	UK, Leeds	Blood donors	247	<30	19.0
					50+	50.0
Kosunen	1989	Finland	Blood donors	500	18-25	10.0
					56-65	60.0

A = Based on antral biopsies
B = Based on fundal biopsies

Table 10
Studies of H.pylori in populations from Africa

Author	Year	Country	N	Population	Mean age	H.L.O.(%)*	H.pylori Ab's (%)+
Rouvroy	1987	Rwanda-Burundi	173	Endoscopy	34	75	N/T
Wyatt	1987	Ghana	39	Endoscopy	41	100	N/T
Lachlan	1988	Kenya	40	Asymptomatic health workers	22	74.4	100
Glupczynski	1989	Zaire	197	Endoscopy	adults	95	N/T
Holcombe	1989	Nigeria	57	Endoscopy	34	87	N/T
Olive	1989	Reunion	40	Blood bank	30	N/T	63
Fendri	1988	Tunisia	104	Endoscopy	adults	70.2	N/T

* H.L.O. = H.pylori-like organisms identified at histology or culture

+ Ab's = IgG antibody prevalence.

H.pylori serology and CAG. What is still to be established is whether a combined test for serum pepsinogen and H.pylori antibodies will be a more accurate test than the use of either test alone.

2.4 H.PYLORI AND THE MODEL FOR GASTRIC CARCINOGENESIS

Correa (1988) recently added H.pylori to the gastric cancer model as an aetiological factor in chronic antral active gastritis. Correa (1988) and Correa and Ruiz (1989) argued that H.pylori could be designated a promoter of gastric cancer, by being associated with cell proliferation. The irritation and excessive replication of epithelial cells caused by H.pylori may result in the promotion of carcinogenesis, especially in the presence of genotoxic or mutagenic agents. One recent animal study has shown that rats fed on 0.01% ammonia (which is produced in large amounts by H.pylori) developed gastric atrophy after 4 weeks (Tsujii et al 1990). On the basis of population-based studies Siurala et al (1988) and Kekki et al (1987) argued that H.pylori may be an important trigger for the onset of chronic superficial gastritis but not for its further progression.

2.5 GEOGRAPHICAL DISTRIBUTION OF H.PYLORI INFECTION

H.pylori has been identified in every population studied. This is illustrated by the diverse countries of origin of the studies reported in Figure 1 and by a number of non-clinical studies from different geographic areas (Table 9) examining the prevalence of H.pylori in selected populations. Table 9 shows the seroprevalence of H.pylori in recent non-clinical studies of sample size exceeding 100 subjects. These population studies

have been recently reviewed (Dwyer 1989, Parsonnet 1989), but typically they have included symptom-free health worker staff volunteers (Barthel et al 1988, Burnett et al 1988), responders to local newspaper advert campaigns (Dooley et al 1988, 1989), blood donor and other volunteer groups (Graham et al 1988, Fitzgibbons et al 1988, Marshall et al 1989 Jones et al 1986), occupational groups (Morris et al 1986), and children (Klein et al 1989). Only one study from Finland examined the prevalence of Helicobacter-like organisms from gastric biopsies of randomly selected individuals (Siurala et al 1988).

Bearing in mind differences in sample selection and the type of serological assay, prevalence was reported to be 0.7% amongst northern Australian aborigines but over 80% in adults from the Ivory Coast and Algeria (Megraud et al 1989). Seroprevalence rates in European adult populations ranged from 20 to 60%. There were also considerable worldwide variations in relation to age, with populations from Africa and South America having higher rates amongst young adults than Western populations. For example, in a study from Kenya, 85% of those endoscoped showed H.pylori-like organisms identified histologically (Lachlan et al 1988). In the same study, 13/14 healthy volunteers of average age of 22 had H.pylori organisms present on mucosal biopsies (Lachlan et al 1987). In a study from Ghana, 97% of those endoscoped showed the presence of H.pylori antibodies (Wyatt et al 1987), and the organism was cultured in 75% of 173 patients attending an endoscopy clinic in Rwanda-Burundi (Rouvroy et al 1987). Similar reports of high prevalence rates of H.pylori in endoscopic populations also came from Zaire (95%, Glupczynski et

al 1989), and Nigeria (87%, Holcombe et al 1989) as shown in Table 10.

H.pylori appeared to be more prevalent amongst poorer socioeconomic groups. This has been shown in studies from Colombia (Guttierez et al 1988), Peru (Klein et al 1989) and USA (Dehesa et al 1989). The question that needs to be asked is whether these populations from African and South American countries are at an increased risk of CAG and subsequently gastric cancer. This is difficult to answer because gastric cancer rates are only sporadically recorded in a few African countries, and cancer rates are generally not recorded in relation to social class in South America.

Contrary to expectation, evidence suggests that gastric cancer mortality in Africa is low, with the highest recorded annual rates in 1976 being in Rwanda-Burundi of 23/100,000 (P. Cook-Mozzafari and D.Burkitt, unpublished data), and 20/100,000 in Mali in 1986 (Bayo et al 1990). One explanation could be that, in keeping with a multifactorial model of gastric cancer, H.pylori infection in African populations may not progress any further because of an absence of promoting factors. However there have been no attempts to study the relationship between H.pylori CAG and gastric cancer in an African country.

It has to be borne in mind that clinical studies provide no information as to the prevalence of H.pylori in the population as a whole. It is also difficult to compare rates between clinical studies because of probable differences in referral rates in

different countries. Studies of non randomly selected population groups may also have been unrepresentative of the true picture in a population. For example blood donors generally need to be 'fit' and free of medication. In addition, the different age structures of the populations studied make it very difficult to compare studies with each other. This underlines the need to undertake studies in randomly selected populations.

2.6 SUMMARY

H.pylori is a pathogenic bacterium which plays an important role in the aetiology of gastritis, especially chronic active antral gastritis. H.pylori may also be related to chronic atrophic gastritis (CAG) which is an established pathological precursor of gastric cancer. It has also been established that H.pylori serology can diagnose gastritis with a sensitivity and specificity exceeding 80%. What is not yet known is whether H.pylori serology can be used to specifically diagnose CAG.

As is the case of CAG, the prevalence of H.pylori increased with age and was higher in poorer countries and amongst poorer socioeconomic groups. However, previous studies of the prevalence of H.pylori have been carried out on selected populations such as blood donors and healthy volunteers. Such studies may have misrepresented the true distribution of H.pylori in the population. Except for a few studies exploring the relationship between diet and CAG, there is very little known of dietary and other sociobehavioural factors associated with H.pylori infection. There has been some speculation as to the relationship between H.pylori infection and gastric cancer, but

no study has formally tested this association.

SECTION 3

AIMS OF THE THESIS

The aims of the thesis are as follows:

1. To test the hypothesis that infection with the bacterium Helicobacter pylori is related specifically to the development of chronic atrophic gastritis;

2. To measure the sensitivity and specificity of assaying serum or plasma anti-H.pylori IgG antibodies in diagnosing both H.pylori infection, and the presence of chronic atrophic gastritis; and to also test the effect of including an assay for serum pepsinogen on the sensitivity and specificity of diagnosing chronic atrophic gastritis.

3. To measure the distribution of H.pylori antibody levels in relation to age and social class in unselected populations;

4. To measure the distribution of H.pylori infection in relation to other lifestyle and sociodemographic indices of social class, such as household density, occupation, smoking, alcohol consumption and a range of dietary indices (in particular Vitamin C and beta carotene intakes).

5. To examine the relationship between seroprevalence of H.pylori antibody levels, pepsinogen levels and gastric cancer mortality;

6. To test whether H.pylori is associated with epidemic dyspepsia

and gastric cancer in Kenya.

In order to achieve these aims, five interrelated studies were undertaken in Oxford, Caerphilly, Italy, China and Kenya. Briefly, the Oxford study deals with the relationship between H.pylori and mucosal histopathology, the Caerphilly and Italian studies measure the distribution of H.pylori infection in relation to social class and dietary and behavioral determinants of social class. The Chinese study looks at the relationship between H.pylori and gastric cancer and the Kenyan study looks at the relationship between H.pylori, aflatoxins, CAG, dyspepsia and gastric cancer.

Standardised laboratory techniques, were used to analyse the blood or urine collected in these studies. The laboratory methods used are described in Appendix 1.

Study 1. H.pylori and mucosal histopathology (Chapter 2)

Chapter 2 describes a study conducted in 1987 of 95 subjects attending an endoscopy clinic in Oxford. The aims of this study were to:

1) Test the hypothesis that H.pylori is associated with chronic atrophic gastritis;
2) Test the hypothesis that H.pylori IgG antibodies can diagnose the presence of the organism on the gastric mucosa;
3) Test the hypothesis that H.pylori IgG antibodies can be

used as a diagnostic test for chronic atrophic gastritis; and also test the effect of including an assay for pepsinogen on the sensitivity and specificity of diagnosing CAG.

Study 2 Prevalence of H.pylori in Caerphilly (Chapter 3)

The Caerphilly study was originally set up in 1978 to examine dietary and other determinants of heart disease in 797 men. Another smaller study of 343 older men from Caerphilly was set up in 1983 to examine the relationship between diet and CAG. Plasma, sociodemographic, behavioral and dietary information was made available from 749 men in both studies to determine:

1) the seroprevalence of H.pylori IgG antibodies in relation to age and social class;
2) whether H.pylori is related to other social indicators of class, such as father's occupation, household density, number of siblings and ownership of a refrigerator;
3) whether H.pylori is related to dietary and behavioral indices of social class, such as smoking, alcohol consumption and intake of vitamin C, beta-carotene and other nutrients.

Study 3: Prevalence of H.pylori in Italy (Chapter 4)

The Italian study comprised randomly selected population controls of a multicentre case-control study of gastric cancer. These were age matched to cases of gastric cancer. Plasma, sociodemographic, dietary and behavioral information was made available for 959 of these controls (558 male, 401 female) to examine:

1) the distribution of H.pylori in relation to age and social class;
2) the relationship between H.pylori and other social and behavioral indicators such as smoking and alcohol consumption and the intake of 17 food items, but specifically fresh fruit and vegetables, and dairy products;
3) the distribution of H.pylori in relation to other social indices such as urban or rural residence, occupation, number of siblings and ownership of a refrigerator.
4) the geographical distribution of H.pylori in relation to residence in regions of high or low gastric cancer mortality;
5) the relationship of H.pylori to a reported history of peptic ulcer and gastritis, and a family history of gastric cancer;

Study 4: H.pylori and gastric cancer in China (Chapter 5)

China has enormous geographical variations in mortality due to different causes, including gastric cancer. Because of this variation in mortality rates, a large geographic correlation study was carried out in 1982, in which 286 dietary, biochemical and sociodemographic characteristics of about 6,400 adult men and women living in 65 rural counties were correlated to 78 causes of death. In order to measure the association between H.pylori and gastric cancer, plasma from a subset of 1,882 men from 46 counties (with a 25-fold variation in gastric cancer mortality) was made available in order to correlate:

1) the seroprevalence of H.pylori with mortality from gastric cancer;
2) the seroprevalence of H.pylori with mortality from other

diseases including peptic ulcer disease;

3) the seroprevalence of H.pylori to 286 other factors, including 110 blood components, 26 urinary components, 33 dietary components analysed from food samples and 68 socio-behavioral and demographic characteristics.

Study 5: H.pylori and dyspepsia in Kenya (Chapter 6)

The aim of this study was to assess whether H.pylori was responsible for an epidemic of dyspepsia in a rural Kenyan hospital. In addition, an increase in the proportion of gastric cancer amongst younger people in this hospital has been anecdotally reported. It was hypothesised that the increase in dyspepsia and possibly of gastric cancer could be explained by exposure to H.pylori. It was also thought that a change in storage conditions over the recent past may have led to an increase in dietary aflatoxin levels and this may have also been responsible for the dyspepsia epidemic. Chapter 7 describes a study of 25 young adults attending a hospital in rural Kenya because of dyspepsia, and compares them with 40 volunteers. All subjects provided gastric biopsies for the determination of CAG, a serum sample for the determination of H.pylori antibody levels and albumin-bound aflatoxin adduct levels, and a urine sample for the determination of urinary aflatoxin levels. Serum was also collected from an additional sample of 96 children aged 1-19 years old attending the hospital outpatient clinic. This serum was used to determine H.pylori antibody levels. Hospital records of gastric cancer cases were also available from 1965 to 1988.

The aims of the study were to test:

1) whether there has been a increase in gastric cancer in this region;
2) the association between H.pylori, CAG, and dyspepsia;
3) the relevance of aflatoxins to the development of dyspepsia.

The concluding Chapter 7 critically evaluates this information in relation to the aetiology of CAG and indirectly, gastric cancer. It also reviews the public health implications of this organism and poses some questions for further study.

CHAPTER 2

HELICOBACTER PYLORI AND SERUM PEPSINOGEN A AND C: SEARCHING FOR SEROLOGICAL MARKERS OF CHRONIC ATROPHIC GASTRITIS

1 INTRODUCTION

'Type B' (Strickland and MacKay 1973) chronic atrophic gastritis (CAG) is defined as a diffuse inflammatory process of unknown aetiology involving the stomach mucosa and is thought to predispose to gastric cancer of the intestinal type (Taylor 1987). A model of gastric cancer has been proposed (Correa et al 1975, Correa 1983, 1988) in which has been suggested that CAG precedes gastric cancer by 2-3 decades. By focussing on CAG, it may therefore be possible to gain a better understanding of some of the processes involved in the epidemiology of gastric cancer before the appearance of clinical symptoms.

As shown in Chapter 1, relatively little research has been carried out on the aetiology of CAG. This is partly because CAG often does not produce distinctive symptoms (Taylor 1987, Villako et al 1984) and its diagnosis depends on the histological examination of gastric biopsy material (Whitehead 1973). Most of our knowledge about CAG is, therefore, largely based on patient groups presenting symptomatically to gastroenterology clinics. Large variations in admission rates in relation to duodenal ulcer have been reported in the literature (Joensson and Silverburg 1982). The pattern of referral for less specific symptoms such as non ulcer dyspepsia would be expected to be more variable and for this reason patients attending endoscopy clinics may be unrepresentative of all those with CAG. It has been suggested that certain serum markers such as the level of the proenzyme pepsinogen and the presence of

H.pylori antibodies may be of value in indirectly establishing the presence of gastritis, including CAG, circumventing the need for endoscopic procedures (See Chapter 1). The use of serology to classify subjects into those with and those without CAG would enable the design of more extensive studies of CAG than has hitherto been possible.

This study was designed to:

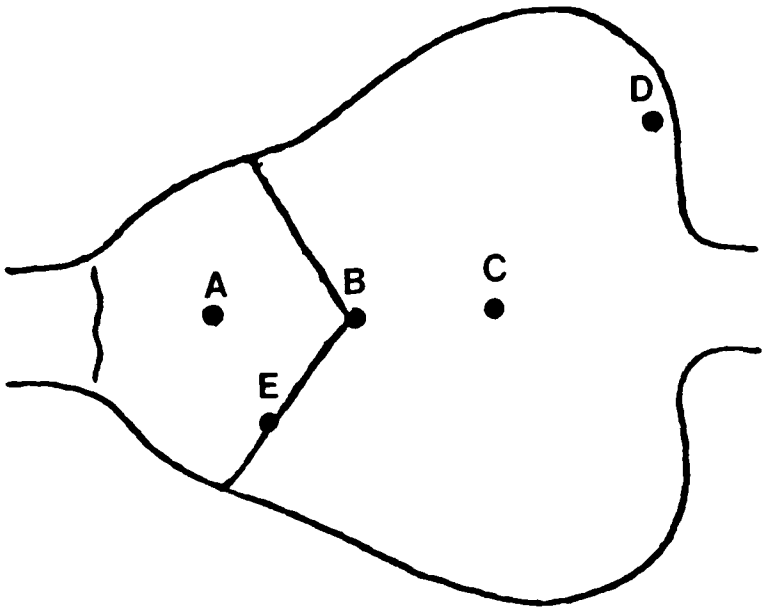
1) test the hypothesis that the presence of H. pylori IgG antibodies is an accurate diagnostic test for the presence of H. pylori organisms on the gastric mucosa;

2) test the hypothesis that H. pylori infection is associated with chronic atrophic gastritis;

3) test the hypothesis that the presence of H. pylori IgG antibodies could be used as a diagnostic test for the presence of histologically defined chronic atrophic gastritis; and also to test the effect of including an assay for serum pepsinogen on the sensitivity and specificity of diagnosing CAG.

Figure 1

Biopsy protocol



A: Antrum (Lesser Curve)
B: Incisura
C: High Lesser Curve
D: Fundus
E: Posterior Wall (at Incisura)

Inclusions: All cases 20-69 years.

Exclusions: Gastric cancer, previous gastric surgery, liver and kidney disease, kidney transplant patients, oesophageal varices, haemorrhagic diathesis, emergency endoscopy, gastrointestinal bleeding.

2 METHODS

2.1 Subjects

Systematic gastric biopsies were taken from 95 consecutive patients, aged 20-69 years, attending a routine endoscopy clinic. Information gathered from each subject included age, sex and endoscopic findings. Patients were excluded from the series only if they were outside the age range or had one of the following conditions: gastric or oesophageal cancer, previous gastric surgery, oesophageal varices, liver or kidney disease, or a haemorrhagic diathesis. Five biopsies per patient were taken according to a predetermined protocol from the antrum, incisura (lesser curve), high lesser curve, fundus and from the posterior wall at the incisura (Figure 1). Biopsies were immediately fixed in formol saline solution. Just before endoscopy a 10 ml blood sample was obtained. This was allowed to clot and serum was separated, aliquoted and frozen to -20°C on the same day.

2.2 Histology

A Warthin Starry stain (Bancroft and Stevens 1982) was used to identify *Helicobacter* - like organisms (HLO) in each biopsy site. Other sections were stained using haematoxylin and eosin (Bancroft and Stevens 1982) for the classification of the gastric mucosa. Biopsies were classified by site (antrum, body) and severity of gastritis into normal (N), chronic superficial gastritis (CSG) and CAG (mild-CAG1, moderate-CAG2) using Whitehead's (1973) classification. The presence of polymorphonuclear leucocytes and intestinal

metaplasia (IM) was also noted. The presence of Helicobacter - like organisms and the grading of gastritis was assessed separately by 2 different histopathologists.

In order to assess the relationship between H.pylori and mucosal histopathology subjects were grouped into 7 categories according to the part of the stomach affected (antrum, body) and the severity of the lesions (N, CSG, CAG1 and CAG2). Thus the first group (N/N) contained those who had a normal mucosa in both antrum and body, the second group (N,CSG/N,CSG) contained those with CSG in either antrum or body biopsies (or both) and no CAG. The third (CAG1/N,CSG) and fourth (CAG2/N,CSG) groups contained, respectively, those with mild or moderate antral CAG but a normal histology or CSG in the body. The fifth group (N,CSG/CAG1,CAG2) comprised those who had mild or moderate fundal CAG but only CSG or a normal histology in the antrum. The sixth group (CAG1/CAG1) contained all those with mild CAG in both antrum and body and the last group (CAG1,CAG2/CAG1,CAG2) contained those who have CAG in both the antrum and body and some moderate CAG at one or both sites. A single subject with severe antral CAG was grouped with those with moderate CAG.

In order to assess the sensitivity and specificity of H.pylori and serum pepsinogen in detecting the presence or absence of chronic atrophic gastritis, an alternative grouping system was used in which subjects were divided into those with (groups 3-7 above) and without (groups 1-2 above)

chronic atrophic gastritis. For some analyses the CAG group was further subdivided into those with and without evidence of IM.

2.3 Reliability of classification of chronic atrophic gastritis

In order to assess the variability of the histological assessment of chronic atrophic gastritis between different observers, all 5 biopsies from a random sample of 17 subjects were classified independently by another histopathologist.

2.4 Serology

Serum PGA and C levels were measured with an enzyme linked immunosorbent assay (ELISA) using purified PGA and PGC antigens (Westerveld *et al* 1987), described in Appendix 1.

The presence of *H. pylori* IgG serum antibodies was measured with an ELISA procedure using an acid extractable antigen from the surface of *H. pylori* cells (NCTC 11638) (Newell *et al* 1988, 1989), described in Appendix 1.

2.4.1 Criteria for positivity

Subjects with PGA concentrations lower than 25 μg/ml or a PGA/C ratio less than 1.5 were considered to have 'low' pepsinogen levels. These cutoff points were found to optimise discrimination in previous studies using an identical assay system (Westerveld *et al* 1987). A *H.pylori*

IgG antibody concentration greater than 10.0 μg/ml was considered positive. Again, this cutoff point was used on the basis of previous research using the same assay (Newell et al 1989).

2.5 Statistical tests

All statistical procedures were carried out using the SAS-PC (1985) statistical package. The Chi-squared test was used to test for the association between the variables in question (Armitage and Berry 1987). The Chi-squared test for trend was used to test for linear trend between extent or severity of gastritis and presence of H. pylori antibody, presence of HLO and low PG levels. The groups comprising increasing extent of CAG were 1 and 2 (none), 3 and 4 (limited to antrum), and 6 and 7 (antrum and body). The groups representing severity of CAG were 1 and (none), 3 and 6 (mild CAG), and 4 and 7 (moderate CAG). Those having only fundal CAG were excluded from the trend calculations.

The ELISA procedure for the measurement of H. pylori antibody recorded a maximum value of 90 μg/ml for high antibody titres. Thus the frequency distribution of H. pylori antibodies was non-normal. PGA and PGA/C levels were also skewed and for this reason the Wilcoxon Sign Rank test, a distribution - free technique (Armitage and Berry 1987) was used to compare the levels of these markers between different grades of gastritis.

2.5.1 Agreement on classification of chronic atrophic gastritis

Agreement between the two histopathologists was statistically assessed using the kappa statistic (Fleiss 1981). The kappa statistic is an improvement over 'percentage agreement' between two readers because it is independent of prevalence of the disorder, and adjusts for agreement on the basis of chance alone.

2.5.2 Sensitivity, specificity and predictive values

The sensitivity, specificity and the positive and negative predictive values (PV+, PV- respectively) of single or combined markers was calculated using standard formulas (Galen and Gambino 1975). In order to obtain the optimum (best sensitivity and specificity) cutoff of *H. pylori* antibody levels in diagnosing *H. pylori* infection and the optimum cutoff for detecting chronic atrophic gastritis, a Receiver Operating Characteristic (ROC) curve (Erdereich and Lee 1981) was obtained by plotting the sensitivity against the false positive rate of detection of *H. pylori* organisms (and CAG in those without ulcers) for increasing *H. pylori* antibody levels (1, 2, 5, 10, 50 and 90 μg/ml). ROC curves were also drawn for increasing levels of serum PGA (10, 20, 25, 40, 60 and 80 μg/ml) and increasing levels of the ratio PGA/C (1, 1.5, 2.5, 3 and 4).

3 RESULTS

3.1 Completeness of the information

Eight out of 95 subjects endoscoped (8.4%) had inadequate biopsies from either antrum (n=2) or body (n=6) sites and were, therefore, excluded. Fifty-seven (60.0%) of the 87 remaining subjects were men (mean age = 47.6 years, SD = 13.5) and 38 (40.0%) were women (mean age = 48.2 years, SD = 14.2).

Of the 87 subjects, 26 had ulcers present. Because pepsinogen levels tend to be elevated in subjects with ulcers (Westerveld et al 1987, Samloff 1989), some analyses using PGA and PGC levels made a distinction between those with and without ulcers.

3.2 Grading of gastritis (Table 1)

Of the 87 subjects with adequate biopsies, 26 (29.9%) had a normal histology in both antrum and body at all five sites while 13 subjects (14.9%) had superficial gastritis in either the antrum or body. Forty-eight subjects (55.2%) had CAG (mild or moderate) at any site, 26 (29.9%) had antral CAG only, 3 (3.5%) had body CAG only and 19 (21.8%) had CAG at both sites. All three subjects with body CAG also had an ulcer.

Two subjects (7.7%) of those with a normal mucosa had polymorphonuclear leucocytes present. In contrast, 46.2% of those with CSG and 80-100% of those with antral CAG had polymorphonuclear leucocytes present. The proportion of

Table 1

Gastric histological findings in patients attending endoscopy (all subjects) (n=87)*

Gastritis Group* Antrum/body	N (%)	N (%) with Polys	N (%) with I.M.
1 N/N	26 (29.9)	2 (7.7)	0 (0)
2 N,CSG/N,CSG	13 (14.9)	6 (46.2)	0 (0)
3 CAG1/N,CSG	15 (17.2)	12 (80.0)	6 (40.0)
4 CAG2/N,CSG	11 (12.6)	11 (100.0)	2 (18.2)
5 N,SG/CAG1,2	3 (3.5)	2 (66.7)	0 (0)
6 CAG1/CAG1	9 (10.4)	8 (88.9)	2 (22.2)
7 CAG1,2/CAG1,2	10 (11.5)	10 (100.0)	5 (50.0)
Total	87 (100.0)	51 (58.6)	15 (17.2)
X^2 for trend (1 d.f.):			
by extent+		35.9#	14.1#
by severity++		47.6#	12.9#

Polys = Polymorphonuclear leucocytes
I.M. = Intestinal Metaplasia
* 8 subjects not classifiable (insufficient biopsies)
+ Score 1 & 2, 3 & 4, 6 & 7
++ Score 1, 2, 3 & 6, 4 & 7
# $p<0.0001$

Table 2

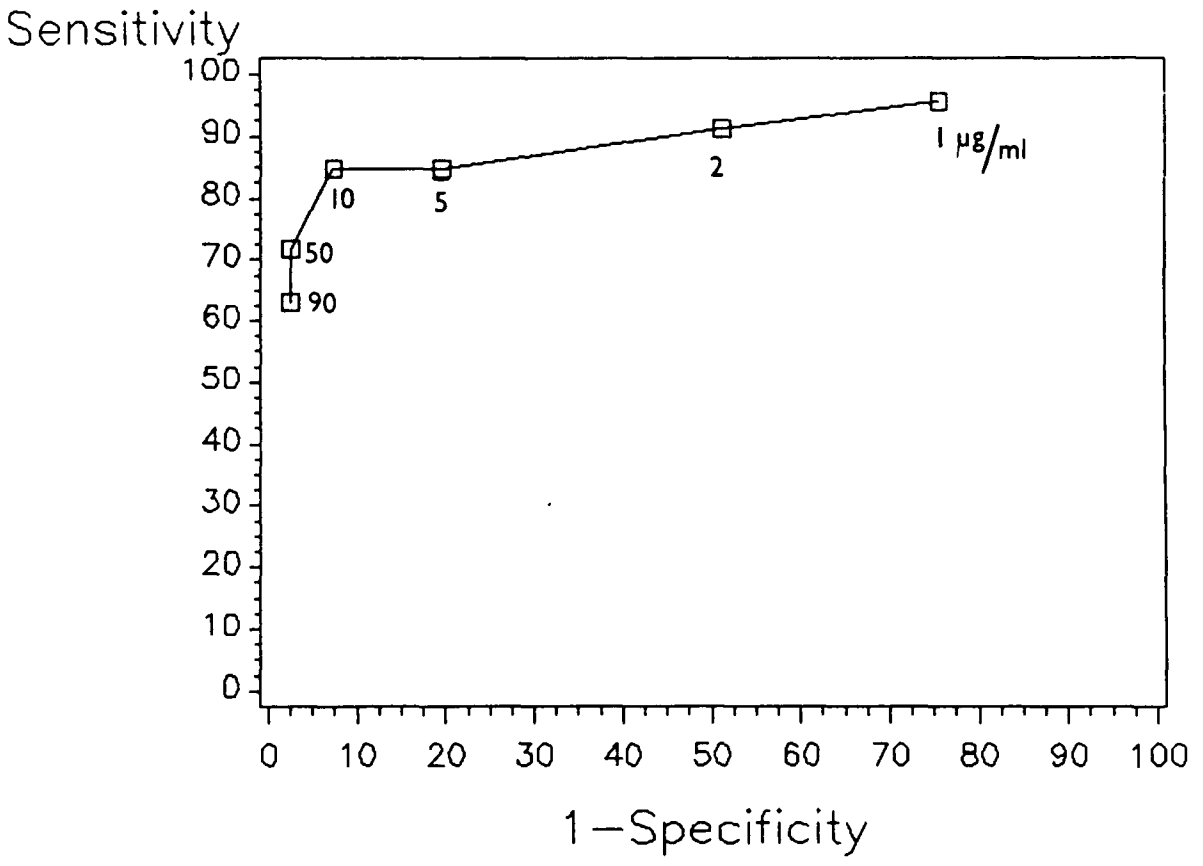
Relationship between H.pylori and mucosal histology (all subjects)

Mucosal Histology (Antrum/body)	N	Number and (%) of positives for: HLOs*	HP >10 ug/ml**	Median IgG levels ug/ml
1 N/N	26	3 (11.5)	2 (7.7)	1.4
2 CSG	13	4 (30.8)	3 (32.1)	2.4
3 CAG1/N,CSG	15	10 (66.7)	9 (60.0)	74.8
4 CAG2/N,CSG	11	10 (90.1)	10 (90.1)	90.0
5 N,SG/CAG1,2	3	2 (66.7)	3 (100.0)	51.3
6 CAG1/CAG1	9	8 (88.9)	6 (66.7)	67.6
7 CAG1,2/CAG1,2	10	9 (90.0)	9 (90.0)	90.0
Overall CAG	48	39 (81.3)	37 (77.1)	
Total	87	46 (52.9)	42 (48.3)	
X^2 for trend (1 d.f.):				
by extent+		31.2#	27.7#	
by severity++		35.4#	36.3#	

Mucosal Histology	N	HLOs*	HP >10 ug/ml**	Median IgG levels ug/ml
N,SG	45	7 (15.6)	7 (15.6)	1.5
CAG+IM-	34	26 (76.5)	23 (67.7)	55.1
CAG+IM+	16	13 (81.3)	14 (87.5)	90.0
All CAG	50	39 (78.0)	37 (74.0)	90.0
Total	95	46	44	
X^2 for trend (1 d.f.):		30.5#	31.9#	

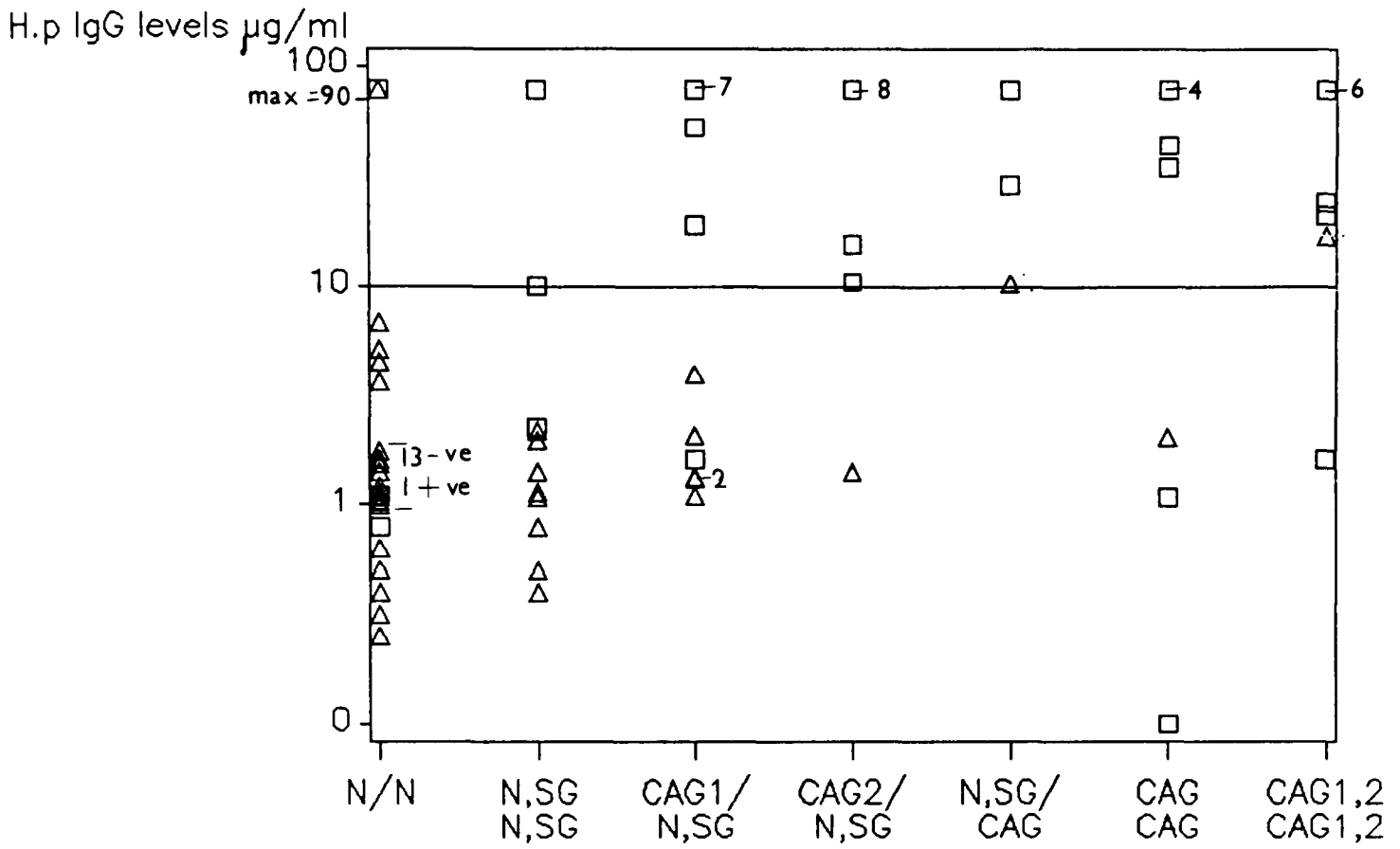
* HLO = Helicobacter like organisms
** HP = H.pylori antibody levels
+,++ See table 1
# $p<0.0001$

Figure 2
Receiver Operating Characteristic
H.pylori IgG antibody levels diagnosing infection



Number of cases = 87

Figure 3
H.PYLORI IgG ANTIBODY LEVELS AND STOMACH MUCOSAL HISTOLOGY



All Subjects, N = 87

those with polymorphonuclear leucocytes was strongly related to both the extent of CAG (X^2 trend = 35.9; 1 df; $p < 0.0001$) and the severity of CAG (X^2 trend = 47.6; 1 df; $p < 0.0001$). Two out of the three cases with body CAG had polymorphonuclear leucocytes present.

The proportion of those with IM was also strongly related to CAG. None of those with a normal mucosa or CSG had IM. In contrast, 15 out of 48 with CAG (31.3%) had IM. IM was related to the extent of CAG (X^2 trend = 14.1; 1df; $p < 0.0001$) as well as to the severity of CAG (X^2 trend = 12.9; 1df; $p < 0.0001$).

3.2.1 Interobserver agreement on classification of chronic atrophic gastritis

There was good agreement between the two histopathologists with 15 out of the 17 (88%) randomly selected subjects classified by both histopathologists into either N/CSG or chronic atrophic gastritis. The kappa statistic, that is the level of agreement above that expected by chance, was 0.76 ($2p < 0.00001$).

3.3 Helicobacter pylori IgG antibodies and Helicobacter-like organisms (Table 2 and Figures 2 and 3)

Figure 2 shows the receiver operating characteristic curve of detection of HLO infection at different cutoff points of H.pylori antibody concentration. It appears from the plot that a cutoff of 10 μg/ml gave the best sensitivity (84.8%) and specificity (92.7%) in detecting HLO. In other

words, 39 out of 46 subjects with HLO had H.pylori antibody levels above 10 μg/ml and 3 out of 41 subjects without HLO had antibody levels over 10 μg/ml (X^2 = 52.1; 1 d.f.; p < 0.0001).

3.4 Helicobacter - like microorganisms and mucosal histology (Figure 3 and Table 2)

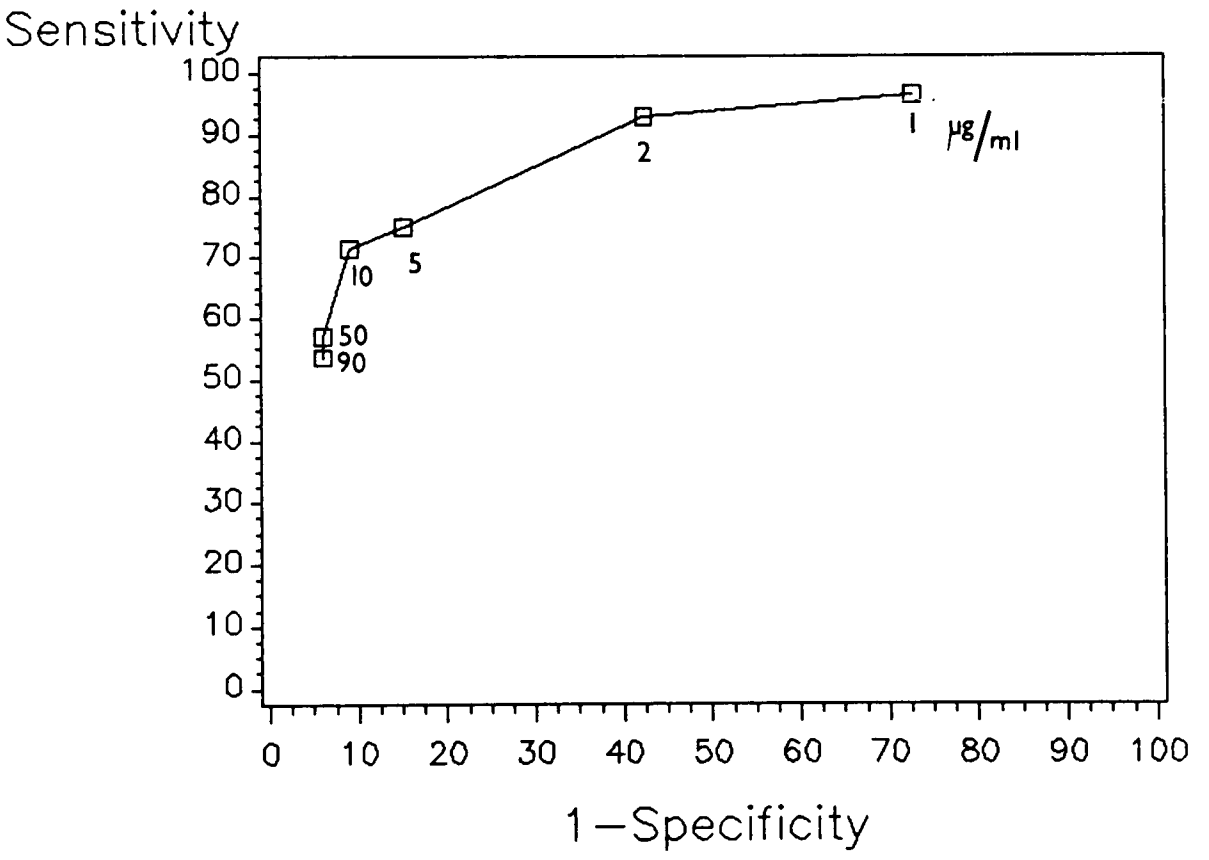
Because of the close association (high sensitivity and specificity) between H.pylori antibody levels and HLO the distribution of H.pylori antibodies in relation to gastritis was similar to that shown with H.pylori histology. Forty-six out of 87 subjects (52.9%) were found on histological examination to have HLO at any one site. H. pylori infection appeared widespread throughout the antrum and body, with only three cases out of the 46 having HLO in only antral and not in body biopsies. Two out of the three subjects with only fundal CAG had HLO. Presence of these organisms was strongly related to the extent of CAG (X^2 trend = 31.2; 1 df; p < 0.0001) as well as to the severity of CAG (X^2 = 35.4; 1 df; p < 0.0001). This association held irrespective of the presence or absence of those with peptic ulcers. There was also a strong association between the presence of polymorphonuclear leucocytes (in any biopsy site) and the presence of HLO (X^2 = 69.4; 1 df; p < 0.0001).

There was an increasing trend in the proportion showing the presence of HLO in relation to IM. The proportion with these organisms was 18.0% in those with a normal histology

Figure 4

Receiver Operating Characteristic

H.pylori IgG antibody levels diagnosing CAG



Subjects without ulcers, N = 61

or CSG; 78.9% in those with CAG but without IM and 86.7% in those with CAG and IM (X^2 trend = 30.5; 1 df; $p < 0.0001$).

H.pylori IgG antibody levels ranged from 0.0 μg/ml to a maximum level of 90.0 μg/ml. There was a significant difference (Wilcoxon Rank Sum z = 6.5; p = 0.0001) in antibody levels between the 46 subjects with HLO (median level = 90.0 μg/ml) and the 49 without HLO (median level = 1.8 μg/ml).

3.5 Helicobacter pylori antibody levels and mucosal histopathology (Figure 4 and table 2)

There was a statistically significant increasing trend of prevalence of H. pylori antibodies in relation to both the extent of CAG (X^2 trend = 27.7; 1 df; $p < 0.0001$) and the severity of CAG (X^2 trend = 36.3; 1 df; $p < 0.0001$). All three subjects with body CAG had elevated H. pylori antibody levels. There was also an increasing trend in the proportion showing a positive antibody response in relation to IM (X^2 trend = 35.4; 1 df; $p < 0.0001$). The proportion with H. pylori antibodies was 16.6% in those with a normal histology or CSG, 67.7% in those with CAG but without IM and 87.5% in those with CAG and IM (Table 2).

If the data is looked at quantitatively, there was a positive trend between median antibody levels and mucosal histology (Table 2). Those with a normal mucosa or with CSG had median antibody levels of 1.4 μg/ml and 2.4 μg/ml respectively. Those with antral CAG had much higher median

Table 3
Subjects with low pepsinogen levels according to mucosal histology (n=85)

Mucosal histology (antrum/body)	Number and (%) positives for: All subjects			Subjects without ulcers		
	N	Low PGA#	Low PGA/C#	N	Low PGA	Low PGA/C
		N (%)	N (%)		N (%)	N (%)
1 N/N	26	10 (38.5)	3 (11.5)	22	9 (40.9)	1 (4.5)
2 N,CSG/N,CSG	13	2 (15.4)	1 (7.7)	11	1 (9.1)	1 (9.1)
3 CAG1/N,CSG	15	2 (13.3)	3 (20.0)	7	2 (28.6)	1 (14.3)
4 CAG2/N,CSG	11	0 --	2 (18.2)	8	0 --	1 (12.5)
5 N,SG/CAG1,2*	2	0 --	0 --	0	0 --	0 --
6 CAG1/CAG1*	8	3 (33.3)	3 (33.3)	6	2 (33.3)	2 (33.3)
7 CAG1,2/CAG1,2	10	2 (20.0)	3 (30.0)	7	2 (28.6)	3 (42.9)
Total	85	19 (22.4)	15 (17.7)	61	16 (26.2)	9 (14.8)
X^2 for trend (1 d.f.):						
by extent+		0.52;p=0.05	8.8;p=0.002		0.1;p=0.8	5.1;p=0.02
by severity++		4.5;p=0.03	2.1;p=0.2		2.4;p=0.1	2.5;p=0.1
N,SG	39	12 (30.8)	4 (10.3)	33	10 (30.3)	2 (6.1)
CAG+IM-	31	3 (9.68)	6 (19.4)	18	2 (11.11)	2 (11.1)
CAG+IM+	15	4 (26.7)	5 (33.3)	10	4 (40.0)	5 (50.0)
All CAG	46	7 (14.6)	11 (23.9)	28	7 (25.0)	7 (25.0)
Total	85	19 (22.4)	15 (17.7)	61	16 (26.2)	9 (14.8)
X^2 for trend (1 d.f.):		0.8;p=0.4	5.1;p=0.02		0.0;p=1.0	6.9;p=0.008

* Serum unavailable for PG measurement on 2 subjects
\# Low PGA = Pepsinogen A concentration <25 ug/ml
PGC = Pepsinogen C concentration
Low PGA/C = PGA:PGC ratio <1.5
+,++ See table 1

Table 4

Median pepsinogen levels and mucosal histology (n=85*)

Mucosal histology (antrum/body)	Subjects with ulcers			Subjects without ulcers			All subjects		
	N	PGA (ug/ml)	PGA/C	N	PGA (ug/ml)	PGA/C	N	PGA (ug/ml)	PGA/C
1 N/N	4	56.4	4.5	22	28.0	2.5	26	29.6	2.7
2 N,SG/N,CSG	2	33.6	2.2	11	70.4	4.0	13	59.2	3.2
3 CAG1/N,CSG	8	47.2	2.8	7	44.0	2.3	15	44.0	2.7
4 CAG2/N,CSG	3	75.2	3.4	8	41.6	2.5	11	43.2	2.7
5 N,SG/CAG1,2		48.8	2.5		--	--	2	48.8	2.4
6 CAG1/CAG1	2	25.6	1.8	6	32.4	1.7	8	28.8	1.7
7 CAG1,2/CAG1,2	3	50.0	2.3	7	67.2	2.2	10	58.6	2.2
N,CSG	6	45.6	3.3	33	35.2	2.8	39	36.0	2.8
CAG+IM-	13	43.2	2.1	18	43.6	2.6	31	43.2	2.2
CAG+IM+	5	59.2	3.4	10	34.4+	1.3#~	15	38.4	2.3
All CAG	18	46.6	2.5	28	42.4	2.2	46	42.4	2.3
Total	24	44.0	2.7	61	40.0	2.5	85	40.8	2.6

* 1 subject with PGC = 0 ug/ml was excluded from statistical calculations

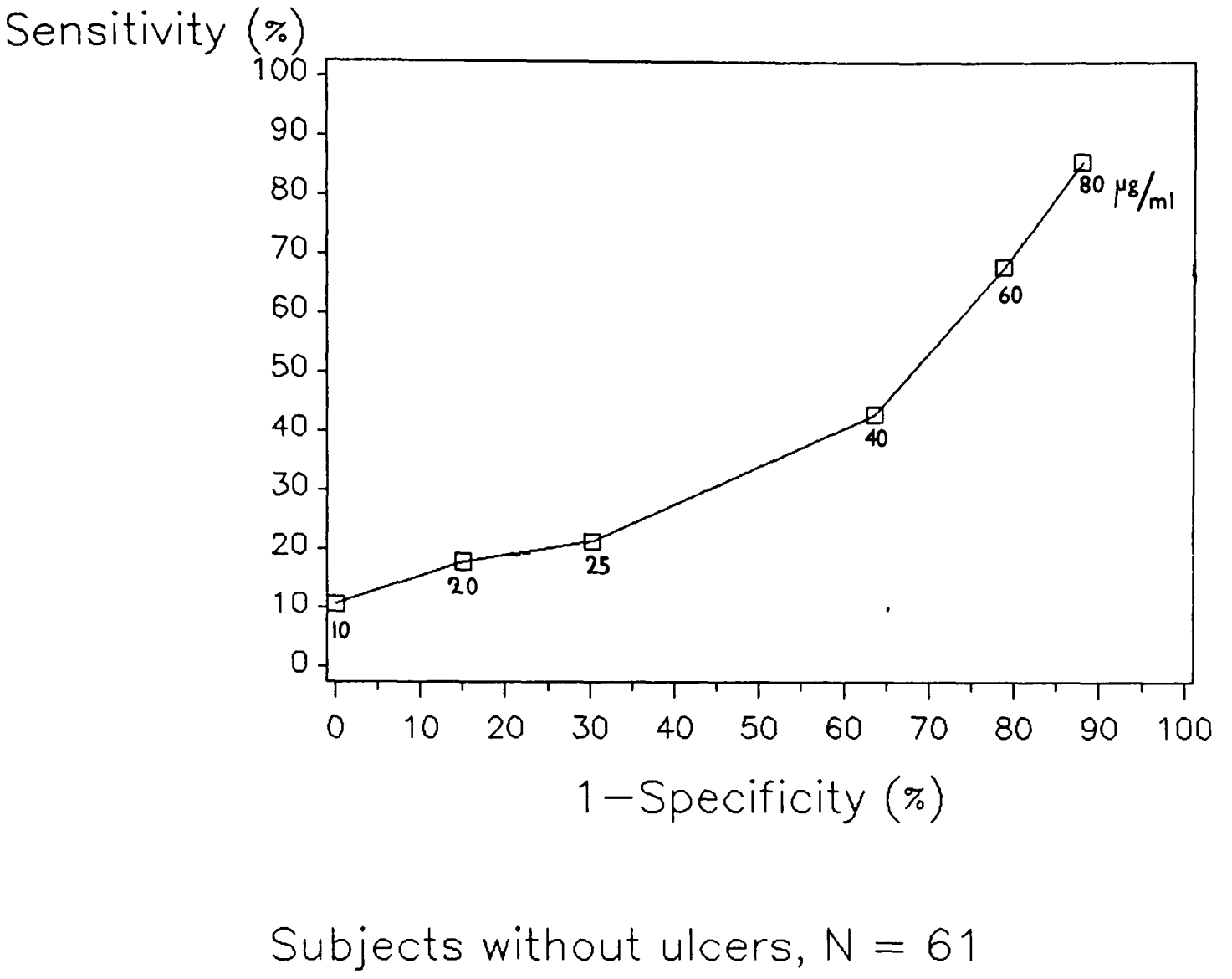
Wilcoxon sign rank test

Differences in PGA and PGA/C levels between ulcer and non-ulcer groups

+ $p<0.1$ # $p<0.05$

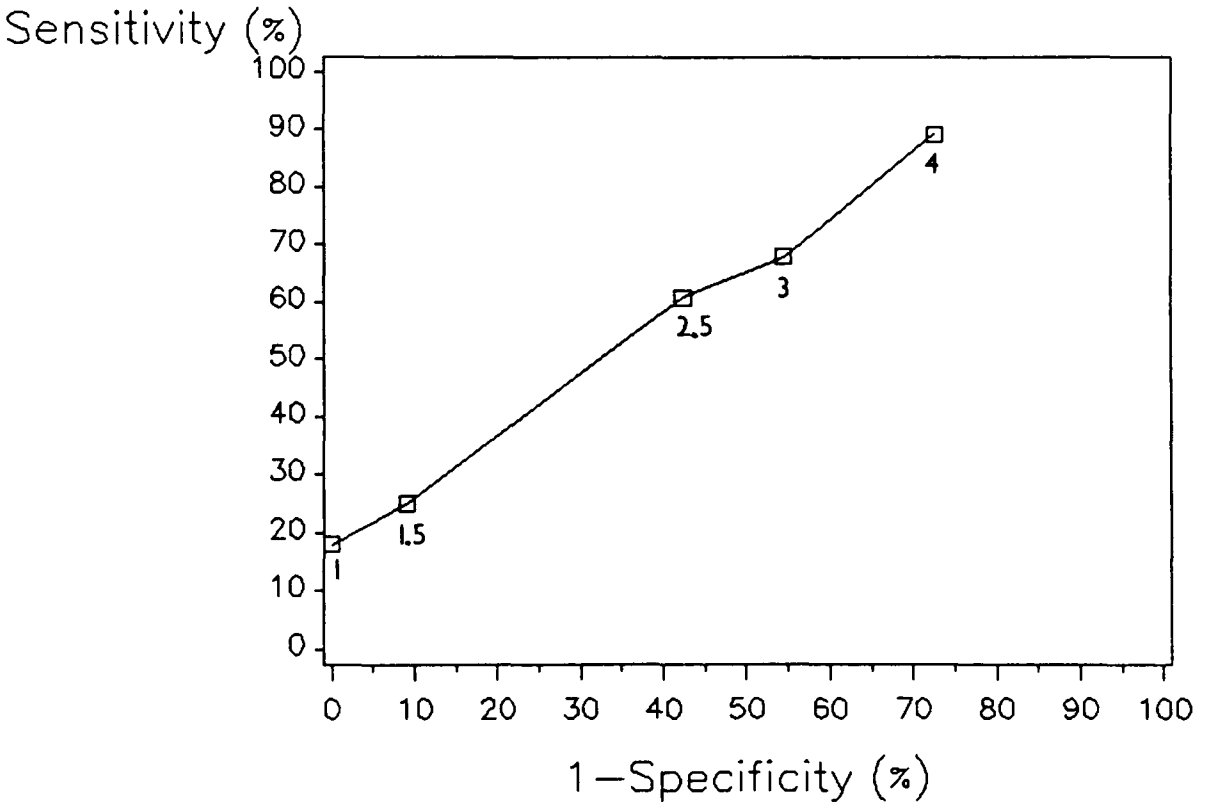
~ IM-versus IM- $p<0.05$ (non-ulcer group)

Figure 5
Receiver Operating Characteristic
PGA level diagnosing CAG



Subjects without ulcers, N = 61

Figure 6
Receiver Operating Characteristic
PGA/PGC ratio diagnosing CAG



Subjects without ulcers, N = 61

antibody levels of 74.8 μg/ml in those with mild antral CAG, and 90.0 μg/ml in those with moderate antral CAG. Those with mild CAG in both antrum and body had median antibody levels of 67.6 μg/ml; and those with moderate CAG in antrum or body had median antibody levels of 90.0 μg/ml.

Figure 4 shows the relationship between sensitivity and 1-specificity (false positive fraction) of detecting CAG at different H.pylori antibody cutoff levels in those without ulcers. An antibody level of 10 μg/ml gave the best sensitivity (71.4%) and specificity (90.9%) in detecting CAG.

3.6 Pepsinogen levels and mucosal histology (Tables 3 & 4 and Figures 5 & 6)

Serum for PGA and PGC determinations was available for 85 subjects from whom adequate biopsies were taken. The overall proportion of those with a low PGA level (< 25 μg/ml) or low PGA/C ratio (< 1.5) was respectively 22.4% and 17.7% (Table 3).

In comparison with those of H. pylori antibodies, the sensitivity of PG for detecting CAG was relatively poor. Of 46 subjects with some degree of CAG only 7 (14.6%) and 11 (23.9%) were identified as such using PGA alone or PGA/PGC respectively. This compares with 37 out of 48 (77.1%) being correctly identified using the H. pylori antibody assay. Specificity is also low for the PGA assay with 27 out of 39 subjects (69.2%) without CAG having a "normal" PGA level

(above 25 μg/ml). However, 35 of these subjects (89.7%) had "normal" PGA/PGC ratios (above 1.5) while 34 (87.2%) were negative for H. pylori antibodies. Restricting the analysis to those 61 subjects without ulcers improved both sensitivity and specificity of PGA (25.0% and 69.7%, respectively) and the sensitivity and specificity of the PGA/PGC ratio (25% and 93.9% respectively). For the latter there was also a significant trend in relation to extent of CAG (X^2 trend = 5.1; 1 df; p = 0.02) and presence of IM (X^2 trend = 6.9; 1 df; p = 0.008). Figures 5 and 6 show the receiver operating characteristic curves of serum PGA levels and the ratio of serum PGA/C in detecting CAG.

Median PGA and PGA/C levels in relation to presence or absence of ulcers and mucosal histology are shown in Table 4. These were slightly higher in the ulcer group (44.0 μg/ml, 2.7 respectively) than in the non-ulcer group (40.0 μg/ml, 2.5 respectively), in both cases the difference was not significant. There was a significant difference in PGA/C levels between those with and without ulcers amongst those with IM, these being 3.4 and 1.3 respectively ($p < 0.05$). PGA levels in those with and without ulcers were 59.2 and 34.4 μg/ml respectively ($p < 0.1$).

In the non-ulcer group, median PGA levels were similar in those with a normal histology or CSG to those with IM (35.2 μg/ml, 34.4 μg/ml respectively). However, those with IM had significantly lower PGA/C levels than those without IM (1.3 versus 2.6; $p < 0.05$).

Table 5

Serum H.pylori antibody levels and PGA/C levels in 61 subjects without ulcers

Gastritis score (antrum/body)	HP*	N	N, (%) PGA/C <1.5	Median levels PGA/C
1 N/N	+	1	0 (0.0)	2.2
	-	21	1 (4.8)	2.6
2 N,CSG/N,CSG	+	2	0 (0.0)	4.5
	-	9	1 (11.1)	3.2
3 CAG1/N,CSG	+	3	1 (33.3)	2.3
	-	4	0 (0.0)	2.4
4 CAG2/N,CSG	+	7	1 (14.3)	2.4
	-	1	0 (0.0)	4.9
5 N,CSG/CAG1,2	+	0	-- --	
	-	0	-- --	
6 CAG1/CAG1	+	4	1 (25.0)	2.5
	-	2	1 (50.0)	0.9
7 CAG1,2/CAG1,2	+	6	3 (50.0)	1.5
	-	1	0 (0.0)	2.7
I.M.				
Normal/CSG	+	3	0 (20.0)	4.0
	-	30	2 (6.7)	2.7
CAG+ IM-	+	11	1 (9.1)	2.5
	-	7	1 (14.3)	2.7
CAG+ IM+	+	9	5 (55.6)	1.0
	-	1	0 (0.0)	1.6
All CAG	+	20	6 (30.0)	2.3
	-	8	1 (12.5)	2.2
Total	+	23	6 (26.1)	2.3
	-	38	9 (9.1)	2.7
		61	9 (14.8)	2.5

* HP = H.pylori antibody +ve (>10 ug/ml)

3.7 PG levels and H. pylori (Table 5 and Figure 2)

A more detailed analysis of the interrelationship between serum H. pylori antibody levels and the PGA/PGC ratio was carried out on the patients without ulcers. This is shown in table 5 and figure 6. In this group there were 33 subjects with a normal mucosa or CSG only and of these, 3 had a positive antibody level for H. pylori. However none of these three subjects had a low PGA/PGC level. Thus, by accepting as "normal" subjects with neither H. pylori antibodies nor a low PGA/PGC ratios - all the patients with a normal mucosa or CSG were correctly classified, i.e. there were no false positives and specificity was 100%. Of the 28 subjects with CAG in the non ulcer group, 20 were positive for H.pylori antibodies but only 6 had both H.pylori antibodies and a low PGA/C ratio and the sensitivity of this combined marker was 21.4%. Five out of the 10 subjects with IM were positive to both H.pylori antibodies and low PCA/C ratio, resulting in an increased sensitivity of 50.0% and no appreciable loss in specificity, 98.0%.

The sensitivity and specificity of using a PGA level of below 25 μg/ml in detecting CAG was 21.4% and 100.0%, respectively. The sensitivity and specificity of PGA alone in detecting IM was 40.0% and 76.5%. Out of the 28 subjects with CAG in the non ulcer group, only 3 had both H.pylori antibodies and a low PGA level. Thus the sensitivity for a combined H.pylori and PGA marker was reduced from 21.4% to 10.7%. Only 3 out of the 10 subjects with IM were positive to both H.pylori antibodies and low PGA level, resulting in

Table 6

Diagnostic power of various serological tests (subjects without ulcers)

	Histology N/CSG		CAG					
	Test + ve	- ve	+ ve	- ve	SPEC	SENS	PV+*	PV-*
HP IgG >10 ug/ml	3	30	20	8	90.9	71.4	87.0	79.0
PGA/C <1.5	2	31	7	21	93.9	25.0	77.8	59.6
HP & PGA/C	0	33	6	22	100.0	21.4	100.0	60.0
HP & PGA	0	33	6	22	100.0	21.4	100.0	60.0
PGA <25 ug/ml	0	33	3	25	100.0	10.7	100.0	56.9

	Histology Intestinal Metaplasia - ve		+ ve					
	Test + ve	- ve	+ ve	- ve	SPEC	SENS	PV+*	PV-*
HP IgG >10 ug/ml	14	37	9	1	72.6	90.0	39.1	97.4
PGA/C <1.5	4	47	5	5	92.2	50.0	55.6	90.4
HP & PGA/C	1	50	5	5	98.0	50.0	83.3	90.9
HP & PGA	0	51	3	7	100.0	30.0	100.0	87.9
PGA <25 ug/ml	12	39	4	6	76.5	40.0	25.0	86.7

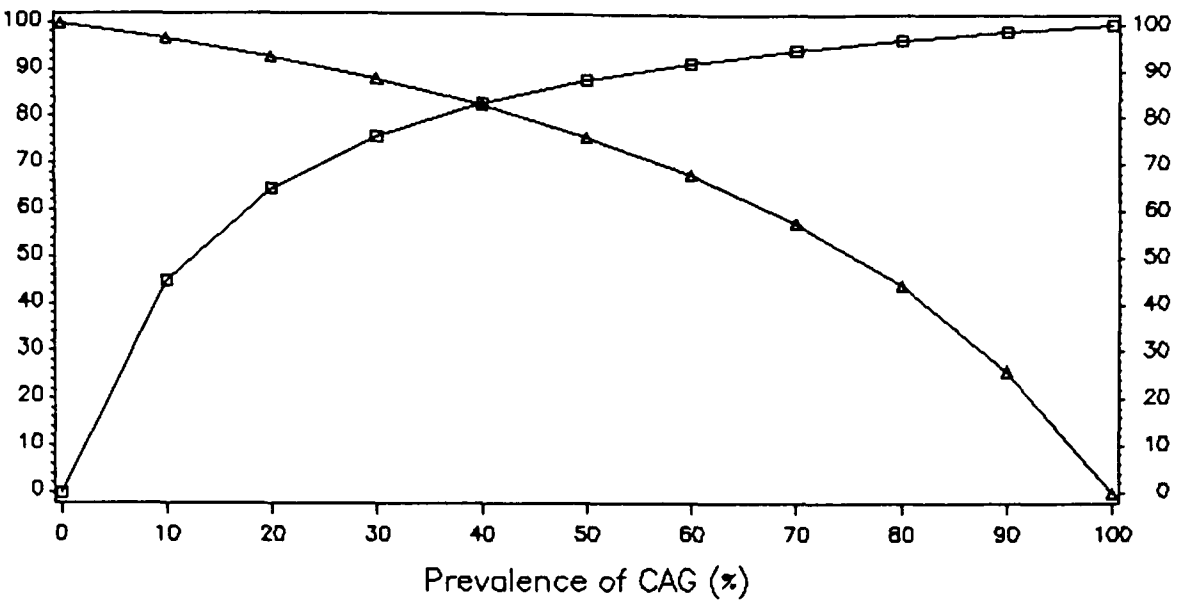
* PV+ and PV- calculated for prevalence rate at endoscopy. For PV+ and PV- at different rates see Fig 3.

FIGURE 7

RELIABILITY OF H.PYLORI AND PEPSINOGENS AS MARKERS OF CAG AND IM

PV+ AND PV− VALUES FOR H.PYLORI DIAGNOSING CAG*

Subjects without ulcers

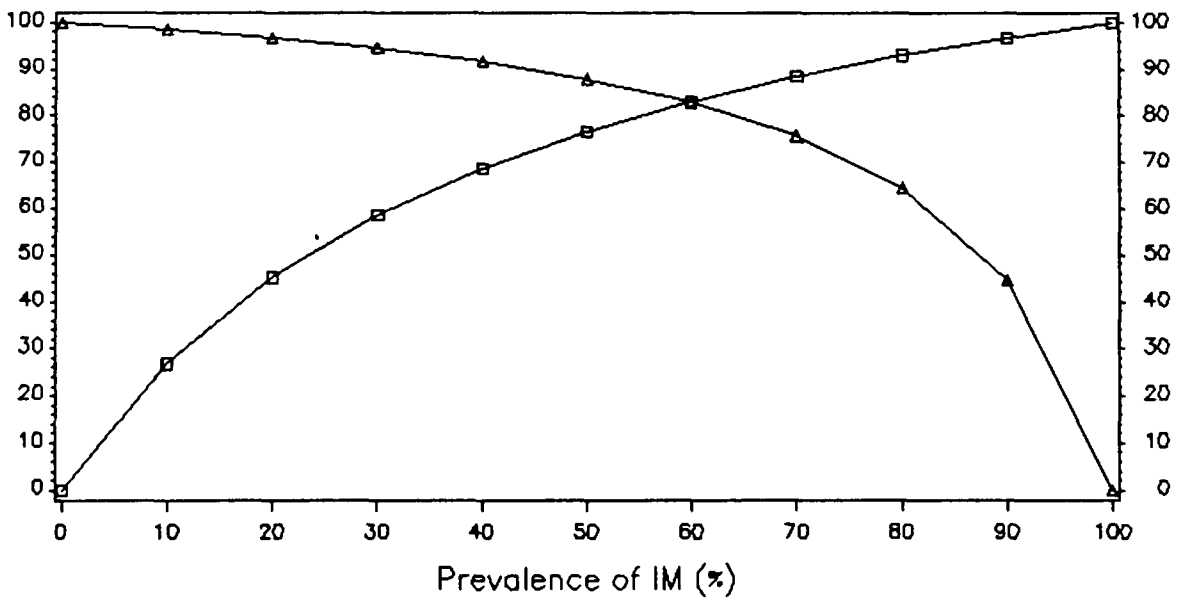


PV+ = SQUARE, PV− = TRIANGLE

*SENS = 71.4%, SPEC = 90.9%

PV+ AND PV− VALUES FOR H.PYLORI DIAGNOSING IM*

Subjects without ulcers

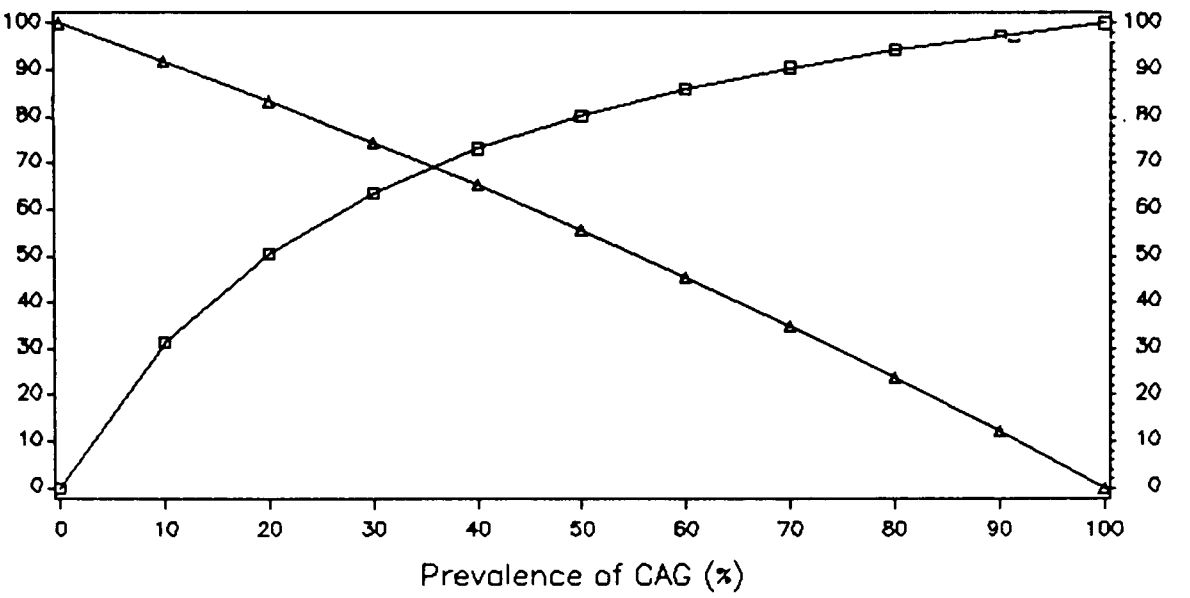


PV+ = SQUARE, PV− = TRIANGLE

*SENS = 90.0%, SPEC = 72.6%

PV+ AND PV− VALUES FOR PGA/C DIAGNOSING CAG*

Subjects without ulcers

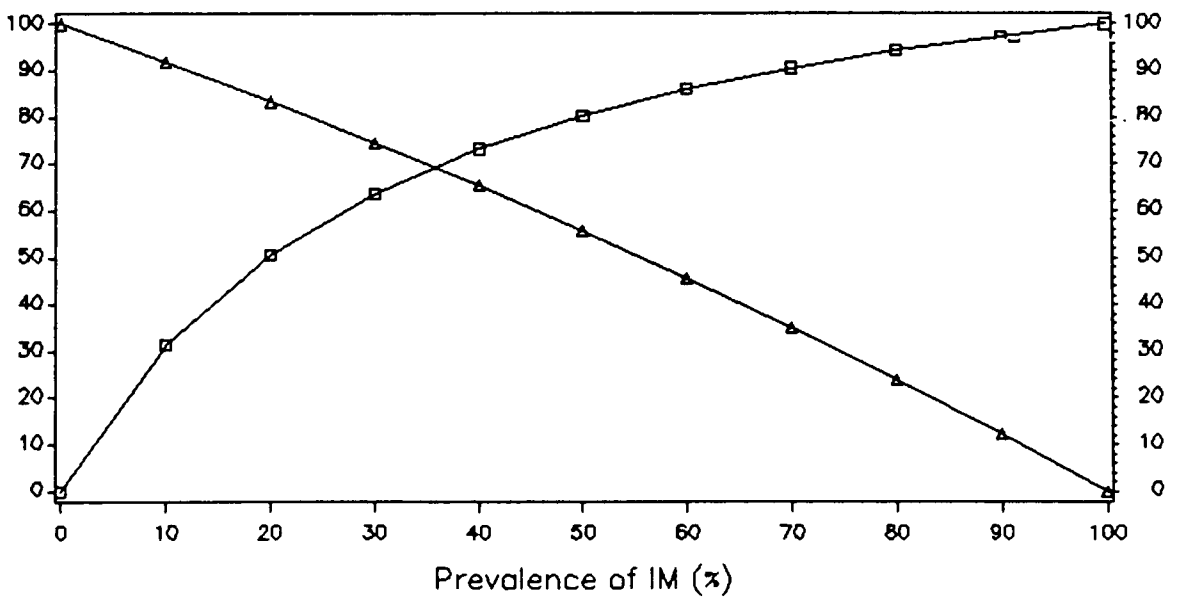


PV+ = SQUARE, PV− = TRIANGLE

*SENS = 25.0%, SPEC = 93.9%

PV+ AND PV− VALUES FOR PGA/C DIAGNOSING IM*

Subjects without ulcers

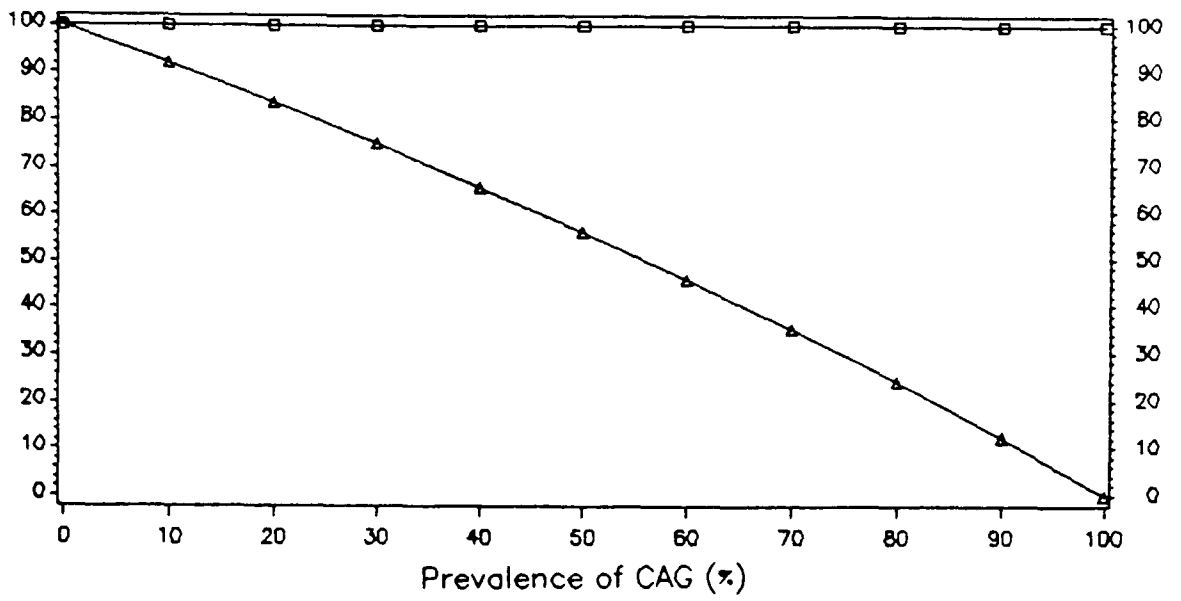


PV+ = SQUARE, PV− = TRIANGLE

*SENS = 50.0%, SPEC = 92.2%

PV+ AND PV− VALUES FOR PGA/C & H.PYLORI DIAGNOSING CAG*

Subjects without ulcers

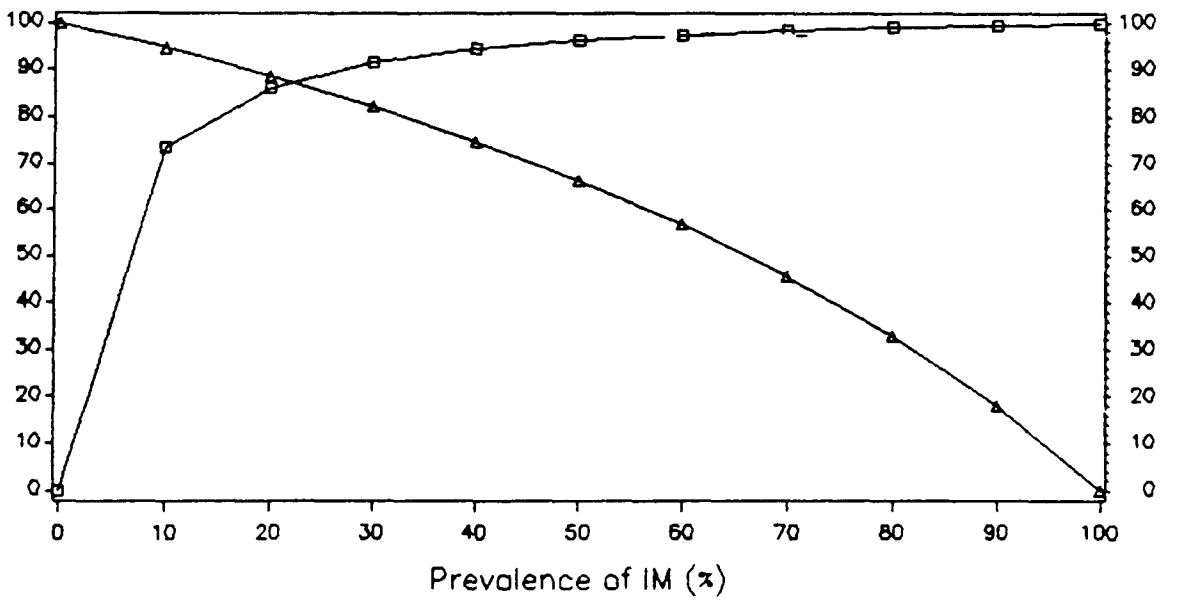


PV+ = SQUARE, PV− = TRIANGLE

*SENS = 21.4%, SPEC = 100.0%

PV+ AND PV− VALUES FOR PGA/C & H.PYLORI DIAGNOSING IM*

Subjects without ulcers



PV+ = SQUARE, PV− = TRIANGLE

*SENS = 50.0%, SPEC = 98.0%

a sensitivity of 30.0%. All of those without IM were both H.pylori and PGA negative, resulting in a specificity of a combined H.pylori and PGA marker of 100.0%

3.8 Reliability of serum markers in diagnosing CAG and IM (Table 6)

Table 6 shows the sensitivity, specificity, positive and negative predictive values (PV+ and PV-) of H. pylori, the PGA level and the PGA/C ratio used singly, or combined, in diagnosing either CAG or IM in those without ulcers. To take account of the possibility of the prevalence of CAG and IM being different from that seen in non-clinical populations, the PV+ and PV- of these tests were calculated for prevalence rates ranging from 0-100% and are shown in Figure 6.

3.8.1 Single tests

The presence of H. pylori antibodies (above and below 10 μg/ml) was more sensitive than the level of PGA (above and below 25 μg/ml) or PGA/C (above and below 1.5) in diagnosing CAG (71%, 10.7% and 25.0% respectively) and IM (90.0%, 40.0 and 50.0% respectively). However, H. pylori levels were less specific than the PGA and the PGA/C levels in diagnosing CAG (90.9% vs 100.0% and 93.9%, respectively) and IM (72.6% vs 76.5% and 92.2%, respectively). The effect of prevalence of disease (CAG or IM) on the predictive value of these markers is seen in Figure 7.

Because of the higher sensitivity of H. pylori antibodies

when compared with PGA/C, the PV- of H. pylori fell less sharply than the PV- of PGA/C with increased prevalence of CAG (or IM).

3.8.2 Combined tests

The combination of H. pylori and PGA/C yields a sensitivity of 21.4% and an improved specificity of 100% in diagnosing CAG and a sensitivity of 50.0% and an improved specificity of 98.0% in diagnosing IM. A specificity of the combined H. pylori and PGA/C marker of 100% had a dramatic effect on the PV+ value (Figure 7), in that it remains constant at 100.0% irrespective of the prevalence of CAG. A specificity of 98.0% in diagnosing IM has improved the PV+ value but only at low prevalence rates of IM. In both instances (diagnosis of CAG or IM) the PV- decreased with increasing prevalence of disease.

4 DISCUSSION

General comments

This study was designed to test whether H.pylori antibodies can accurately diagnose the presence of Helicobacter-like organisms. A second aim was to test the association between H.pylori and chronic atrophic gastritis (CAG) and a third aim was to test whether an H.pylori antibody test and/or a test for pepsinogen, can diagnose the presence of CAG. An underlying concern about the literature on H.pylori and mucosal histopathology has been the use of the term 'gastritis' without reference to the degree of activity or atrophy (See Chapter 1). Particular attention was paid therefore to the classification of gastritis and to assess the repeatability of diagnosis of CAG between histopathologists.

4.1 Histological classification of biopsy material

The relationship between CAG, polys and IM was similar to that reported elsewhere (Whitehead 1973); polymorphonuclear leucocytes being generally absent in those with a normal mucosa and present in a proportion of those with CSG and CAG. IM was absent in those with a normal mucosa and CSG and only present in patches in some of those with CAG.

All those with CAG, with the exception of three subjects, had Strickland and MacKay's (1973) 'type B' gastritis affecting the antrum and in most cases, both antrum and fundus. Two of the subjects had CAG in the fundus and CSG in the antrum and one subject apparently had CAG in the

fundus with a normal antrum (possibly 'type A' gastritis). All three had ulcers and H. pylori infections, the latter being an atypical feature of 'type A' CAG (Flejou et al 1989, O'Connor et al 1984).

4.1.1 Level of agreement between histopathologists

According to Fleiss (1981) kappa levels above 0.75 (in this case, $k = 0.76$, $2p < 0.00001$) represented 'excellent agreement' beyond that expected by chance alone. This level of agreement between two histopathologists was encouraging in that it gives added confidence that the lesion of interest (CAG) was in most cases correctly identified.

4.2 Relationship between H. pylori antibody levels and HLO infection

The 'gold standard' of the presence of H.pylori infection ought to have been bacterial culture and identification of the organism, because other spiral organisms may be confused, on visual observation, with H.pylori. However, the strong association between presence of HLO on the mucosa and high serum H. pylori antibody levels was seen in other studies (Newell and Rathbone 1989), and provided indirect but good evidence that the gram-negative curved bacteria seen on the mucosa were indeed H. pylori.

There is always likely to be some misclassification between histology and serology for a number of reasons. First, there is a lag period between newly acquired infection and development of an immune response. In a

deliberate infection of a volunteer, H. pylori IgG antibodies were formed 4 weeks after ingestion of the organism (Morris and Nicholson 1987). This could have accounted for some of the seven false negative serology results found in this study. Second, other organisms resembling H. pylori may have been present on the mucosa, especially in subjects with achlorhydria, might have been misread as HLO false (histology) positive but serology negative (Newell and Rathbone 1989). Thirdly, the three subjects with false positive serology results might have occurred as a result of a temporary clearance of H. pylori from the mucosa, or a reduction in bacterial numbers in subjects with extensive intestinal metaplasia (in fact, one of the three false positives had IM). This may have given the impression of a clear mucosa but there would have been a persistent antibody response. Fourthly, even though five biopsies were taken per subject, patches of early H. pylori infection may have been missed. If the infection was sufficient to elicit an antibody response, this could have resulted in a 'false' positive result. Two of the antigens used in the H. pylori preparation for this study were common with some Campylobacter species (Newell et al 1988). The antigen preparation had a small degree of cross-reaction with other enteric pathogens such as C. jejuni or C. coli. However, this was unlikely to have accounted for the three false positive results because of the high specificity of the antigenic preparation to H. pylori (Newell et al 1988). An issue affecting both false negative and positive results was the choice of cutoff for positivity. The present cutoff

of 10 μg/ml gave the best sensitivity and specificity between those who were infected and those who were not (Figure 2). A lower cutoff would have resulted in a higher sensitivity, but at the expense of a reduced specificity. For example, using a lower cutoff point of 2 μg/ml, the sensitivity and specificity was, respectively, 91.3% and 48.8%.

4.3 Relationship between H. pylori and chronic atrophic gastritis

The relationship between H. pylori and active gastritis shown in this study was in agreement with current knowledge on the pathogenicity of this organism (Blaser 1987, Dooley and Cohen 1988).

H. pylori infection (as determined by histology or antibody levels) was strongly related to both the extent and the severity of CAG and also with the presence of IM. Despite the strong association which has been demonstrated between H. pylori and CAG of all forms, it is not yet clear whether H. pylori is a sufficient cause of CAG and IM. This is because there were still about 20% of subjects with CAG who did not have any H. pylori infection. This implies that CAG is a multifactorial lesion, not entirely dependent on H. pylori infection. In a Finnish population, H. pylori was histologically found to be associated with CSG but neither with severe CAG nor with the presence of IM (Siurala et al 1988). One of the reasons for these discordant findings may have been the use of different classification systems in the

evaluation of CAG. It might also be possible that once active gastritis has been established, other factors caused the transition to CAG and IM.

4.4 Helicobacter pylori antibody levels as a diagnostic test for chronic atrophic gastritis

Because of the persistence of infection and elevated H. pylori antibody levels regardless of the extent or severity of CAG, or the presence of polymorphonuclear leucocytes, these antibodies have not been found to be as sensitive and specific marker for diagnosing CAG (and IM) as were found in other studies.

The H. pylori antibody test used in this study yielded a sensitivity and specificity of 71.4% and 90.9% respectively for diagnosing CAG in subjects without ulcers. Newell et al (1988) reported a sensitivity of 95.6% and a specificity of 83% for diagnosing 'gastritis' from antral biopsies. Similarly, using antral biopsies, Perez-Perez et al (1988) reported a sensitivity of 67.8% and a specificity of over 90% for diagnosing 'gastritis'. Loffeld et al (1989) reported a sensitivity and specificity of 85.4% and 100% respectively for diagnosing 'H. pylori associated gastritis'. By lowering the cutoff point, their sensitivity was 100% at the expense of a reduced specificity of 72.7%. Musgrove et al (1988) found H. pylori antibodies to be 100% sensitive and 79% specific in detecting 'gastritis' in antral and fundal biopsies.

One of the drawbacks of studies relating H. pylori serology to the status of the gastric mucosa, has been the tendency to classify 'gastritis' irrespective of the degree of atrophy. A second drawback was that in some studies it was not made clear whether the classification of gastritis was based on the degree of depletion of the mucosal glands, the presence of H. pylori on the mucosa or both. These discrepancies in classification of gastritis could have accounted for some of the differences in the value of H. pylori for diagnosing CAG between the different studies. This study differs from the others in that an attempt was made to evaluate the sensitivity and specificity of H.pylori and PG levels in detecting specifically CAG because it is an important precursor of gastric cancer.

4.5 Pepsinogen levels as diagnostic markers of CAG

This study confirmed the complex relationship between pepsinogen levels and gastric disorders, as reviewed elsewhere (Defize and Meuwissen 1987, Samloff 1989). Subjects with duodenal ulcer had higher median PGA and PGA/C levels than those without, consistent with findings of others (Defize and Meuwissen 1987, Westerveld et al 1987). This is probably due to inflammation of the mucosa as described by Samloff (1989). In a study amongst children, Oderda et al (1989) found PGA levels to be elevated when associated with the presence of H. pylori. After treating the children for H. pylori, PGA levels were reduced, suggesting raised PGA could be used as a marker of H. pylori associated (or active) gastritis. PGA levels were highest

in those with CSG as found by Westerveld et al (1987). This evidence may add an interesting dimension to the role of PGA and H. pylori, in that it may be possible to differentiate between active and inactive CAG by comparing PGA levels.

Whereas high PGA levels may be associated with active H. pylori associated gastritis, PGA levels are markedly reduced once gastric atrophy affects the fundal region. This has been illustrated in studies of PG levels in relatives of patients with pernicious anaemia (Samloff et al 1982, Varis et al 1979). Such groups had a high proportion of 'type A' CAG affecting the fundus and sparing the antrum (Defize and Meuwissen 1987) and showed a clear relationship between PG levels and fundal CAG. Using a reduction in PGA as a marker, Varis et al (1979) reported a sensitivity of 91% and a specificity of 97% in diagnosing severe CAG. In another study amongst relatives of PA patients, Samloff et al (1982) found the sensitivity and specificity of PGA was 59.4% and 43.4%, respectively for diagnosing severe CAG. Using the PGA/C ratio, the same authors found an improved sensitivity and specificity of 84.4% and 69.8% respectively for diagnosing severe fundal CAG.

In 'type B' CAG, the lesion most often found among patients attending upper endoscopy clinics and in the general population, the fundal mucosa is only affected in late stages of atrophy (Defize and Meuwissen 1987, Samloff 1989). PGA is produced by the fundal glands and for this reason PGA levels are insensitive to changes restricted to

the antrum leading to a poorer sensitivity in detecting antral CAG. No losses in the specificity of PGA would be expected because those with a normal mucosa will still have high PGA levels and thus will be correctly classified as normal. Westerveld et al (1987) used both antral and fundal biopsies to classify the gastric mucosa and, with PGA alone and found a sensitivity of 50.4% and specificity of 92.7% in diagnosing 'CAG, IM and gastric cancer'. PGC is more sensitive to changes in the antrum because the net level of this proenzyme increased slightly, possibly due to gastric metaplasia in the proximal duodenum (Samloff 1989). This made the PGA/C ratio a more sensitive marker of CAG than the PGA level, especially if CAG mainly affected the antrum. For example, using PGA/C, Westerveld et al (1987) reported a sensitivity and specificity of 66.4% and 83.0% respectively in diagnosing subjects with 'CAG, IM and gastric cancer'. Using PGA, the sensitivity and specificity was 21.4% and 69.7% respectively for diagnosing CAG and 45.5% and 77.2% for diagnosing IM. In this study the sensitivity and specificity of PGA/C was 25% and 90.9% respectively in diagnosing CAG and 54.5% and 89.4% in diagnosing IM. These data are, however, not strictly comparable to those of Westerveld et al (1987) because there was only one case with severe CAG and gastric cancer cases were not included in this study.

The potential of pepsinogen as a marker of severe CAG has already been utilised in clinical and epidemiological studies of CAG, IM and gastric cancer. Stermmermann et al

(1978) found Japanese subjects having a greater prevalence of low PGA than Caucasians, in keeping with the contrasting rates of IM and gastric cancer between these two populations. In case-control studies of PG and gastric cancer, patients with stomach cancer were 9 times more likely to have had low PGA levels (Nomura et al 1980, Stemmermann et al 1987) and 10 times more likely to have had low PCA/C levels (Stemmermann et al 1987). Because of the relationship between PG, CAG and gastric cancer, Miki and co-workers (Miki et al 1989) have suggested PGA and PGA/C levels be studied as a potential screening tool in Japan for the identification of early gastric cancer. In a study by Burr et al (1987) twice as many men in Caerphilly had low PGA levels when compared to those in Bath which had the lower gastric cancer rate. Low PGA levels were also associated with a lower social class status, low plasma ascorbate levels and a low consumption of fruit.

4.5.1 Combined results

In this study, the major advantage of using both serum PG and H. pylori antibody levels to detect the presence of CAG or IM was that almost all of those subjects without CAG or IM were correctly identified as normal, that is the specificity of this test was raised to over 90%. This was despite a reduction in sensitivity from 71% to 21% (i.e. an increase in the number of cases with CAG being missed). A high specificity had a substantial effect on the PV+ and PV- of these tests. At a high prevalence of CAG, the positive predictive value increases but at the expense of the PV- (Figure 7).

5 SUMMARY

This study has shown H.pylori to be related to both chronic active and chronic atrophic gastritis. 10 μg/ml was the best cutoff for detecting the presence of both H.pylori - like organisms and chronic atrophic gastritis. Using PGA or PGA/C as an additional marker of chronic atrophic gastritis resulted in improved specificity but in a loss of sensitivity. An increased specificity improved the PV+ of these tests (that is the percentage of test results which were truly positive). However, the low sensitivity of these tests implies that if they are used to screen asymptomatic populations, many people with abnormal histologies will be classed as negative and therefore missed.

These results must be treated with caution for the following reasons. First, the results would only apply to populations without ulcers. It was felt necessary to exclude ulcer cases from analysis of sensitivity and specificity because peptic ulceration increases PGA levels. When those with ulcers were included in this study, the sensitivities and specificities are reduced at the expense of the PV+ value. The exclusion of the ulcer cases reduced the sample size of the study, which had the effect of increasing random variation. The prevalence of peptic ulcer in the general population would be expected to be lower than that found amongst those referred for endoscopies. and also be expected to vary both between and within populations (Langman and Logan 1986). It would therefore be difficult

to ascertain the precise effect of these factors in a screening program or in a population based study. These reservations underline the need to reconfirm these results in other settings, to assess the robustness of these serological markers in diagnosing CAG, especially if they are to be used as screening tools for early gastric cancer.

However, pepsinogens have been used in epidemiological studies on CAG as described earlier, and have shown results consistent with studies in which gastric biopsies have been taken. One of the limitations of studying precursors to gastric cancer such as CAG has been the reliance on expensive endoscopic procedures for confirmatory diagnosis. As a consequence, there have been only a few studies on the aetiology of precursors of gastric cancer. Despite their inaccuracy in populations with a high prevalence of CAG, these serum markers used singly or in combination could be extremely versatile tools to examine populations for the presence or absence of CAG by circumventing endoscopic procedures, in order to ascertain dietary and other environmental factors related to CAG.

CHAPTER 3

HELICOBACTER PYLORI INFECTION RATES IN MEN FROM CAERPHILLY

1 INTRODUCTION

There have been several studies which have attempted to determine the true seroprevalence of H.pylori amongst non-clinical populations. These studies are reviewed in Chapter 1 and have recently been reviewed by Dwyer et al (1989) and Parsonnett (1989). Typically, they have included symptom-free health worker staff volunteers (Barthel et al 1988, Burnett et al 1988, Dooley et al 1988, Graham et al 1988), responders to local newspaper advertisements (Fitzgibbons et al 1988), blood donor and other healthy volunteer groups (Jones et al 1986, Morris et al 1986, Marshall et al 1989, Wyatt et al 1988, occupational groups (Vaira 1988, Morris et al 1986) and children (Klein et al 1988). Clinical studies or studies of selected groups (for example blood donors) may be unrepresentative of the true picture in the population. Only one report from Finland examining the prevalence of H. pylori-like organisms in biopsies, rather than the seroprevalence of H. pylori IgG antibodies, has studied randomly selected individuals (Siurala et al 1988).

This chapter describes a seroprevalence study of H. pylori in a randomly selected population from Caerphilly in South Wales. Its aims were:

1. to describe the seroprevalence of H. pylori in relation to age and social class;
2. to test whether H. pylori is related to other social indices of social class such as father's occupation and household density;

3. to test whether <u>H. pylori</u> infection is associated with dietary and behavioral indices of social class such as smoking, alcohol consumption, and intake of vitamin C, total carotene and other nutrients.

2 SUBJECTS AND METHODS

2.1 Subjects

Two randomly selected population based studies, conducted in Caerphilly, South Wales in 1979 and in 1983, provided plasma samples and questionnaire information. The first study, designed to look at heart disease risk factors, comprised 797 men, an 8% random sample of the 9,659 men aged 30-69 years on the electoral register (Caerphilly and Speedwell Collaborative Group (CSCG) 1984). In this study, an initial letter was sent to a random sample of all men and responders were included if they were between 30-69 years. The second study, part of a geographic correlation study comprised 343 men aged 65-75 years, a 12% random sample of the estimated 2,789 total population in this age group, selected from medical practitioner records (Burr et al 1987). Twenty-five men were included in both studies and, in 11 cases where plasma was available from both studies, the results for 1983 (Burr et al 1987) were excluded.

2.2 Plasma samples

Plasma samples for each subject were stored, in each of the two studies, at -20°C and had been thawed twice prior to analysis. H. pylori IgG antibody concentrations were measured with a reliable and standardised enzyme-linked immunosorbent assay (ELISA) procedure described in Appendix 1 (Newell et al 1989, Steer et al 1987). An ELISA optical density reading of greater than 0.7, which corresponded to an IgG antibody titre of 10 μg/ml was considered as positive evidence of H. pylori infection. This cut-off showed the

optimal sensitivity and specificity in detecting H. pylori infection on the basis of culture from gastric biopsy material, the rapid urease test (Newell et al 1989) or histopathology (Newell et al 1989, Chapter 2).

2.3 Questionnaire information

Both studies required completion of a questionnaire, which included information on age, occupation and smoking status. Categorisation of social class was based on the current or most recent occupation of each subject based on the standard UK divisions into six major groups: I, II, IIIN, IIIM, IV and V (OPCS 1980).

In addition to the information on age, social class and smoking habits, a structured self-completed questionnaire, was used in the CSCG study (1984) to obtain information on place of birth (Caerphilly, elsewhere), household size (number of children <17 years in the household and number of siblings), occupation of the subject's father when the subject was 10 years old, alcohol consumption and dietary information. Dietary information was based on the reported frequency of consumption of 54 separate food items, over the year prior to the interview (Yarnell et al, 1983). The questionnaire was validated against seven-day dietary records (Yarnell et al 1983).

2.4 Categorisation of information

The first part of the analysis was to test for trends in prevalence of H.pylori in relation to social class and age

(using 9 five-year age groups: 30-34, 35-39 ... 70-74) and smoking habits (non-, ex-, current smoker) from subjects in both studies for whom plasma, age and social class information was available. Because the combination of nine age and six social class groups resulted in specific age-class groups with no subjects, for statistical analysis, social class categories were combined as follows: I & II, IIIN & M, and IV & V.

The second part of the analysis was restricted to those subjects drawn from the CSCG study (1984) for whom father's occupation (when the subject was 10 years old), household density, dietary and alcohol consumption information was available.

The social class of the subject's father at the age of 10 was also categorised, as above, into three class categories. Because of the smaller sample size, the analysis in relation to father's occupation used five age groupings (30-34, 35-44, 45-54, 55-64 65-69 years).

Household density was determined on the basis of number of children (aged <17 years) and adults (>=17 years) eating in the subject's household at the time of the interview. An additional question requested information on the number of the subject's siblings.

The nutrient composition of each of 53 food items was elicited from the dietary frequency questionnaires, and was

based on the amount of each nutrient contained in a standard portion based on UK food tables (Paul and Southgate 1978). A computer program, developed by the Medical Research Council (Wales), was used to calculate nutrient levels based on information from frequency of consumption. Alcohol intake was calculated using the same approach as that used for food consumption. Energy intake was calculated as follows: 3.75X(Sugar+Starch) + 4X(Protein) + 9X(Fat) + 5.53X(Alcohol%).

Plasma ascorbate levels were also available for those aged 65-74 years who participated in the study by Burr et al (1987).

As a validation exercise, the relationship between food frequency questionnaire and information from seven-day dietary records was measured in a population sample of about 119 men in Caerphilly (Yarnell et al 1983). Correlation coefficients between seven-day dietary and frequency questionnaire methods were as follows: alcohol: 0.75, cereal fibre: 0.41, total fibre: 0.37, total protein: 0.41, sugar: 0.45, carbohydrate: 0.27, fat: 0.31, saturated fat 0.31, vitamin C: 0.36, and total energy: 0.30. All correlations were statistically significant ($2p < 0.001$), but except for alcohol, all other nutrients showed significant differences when comparing paired mean values (Yarnell et al 1983).

2.5 Statistical methods

Prevalence rates for age and social class categories were

indirectly standardised for each other. The significance of linear trends of infection, by age or social class (adjusted for each other) and statistical interaction between age and social class were assessed using a goodness of fit Chi-squared statistic in a logistic regression model (Armitage and Berry 1989). The Generalised Linear Interactive Modelling statistical package (GLIM) was used for this analysis (Healy, 1988).

The Mantel and Haenszel Chi-squared test for linear trend (Armitage and Berry 1987), adjusted for age (five groups 30-34, 35-44, 45-54, 55-64, 65+) and social class (three groups) was used to test the statistical significance of the relationship between prevalence of H.pylori and household density and smoking habits (Non smoker, ex-smoker, current smoker, 1-14, 15-24, 25+ cigarettes / day) (PROC FREQ, SAS 1985).

Mean nutrient intake levels were calculated in relation to the presence or absence of H.pylori. Analysis of covariance (Armitage and Berry 1987) using a multiple regression procedure (PROC GLM, SAS 1985, Lee 1987) was used to calculate mean nutrient levels in relation to H.pylori infection, adjusted for age (four groups 30-34, 35-44, 45-54, 55+) and social class (three groups).

Plasma ascorbate levels were originally measured in individuals in the study by Burr et al (1987). Analysis of covariance (PROC GLM SAS, 1985) was also used to calculate

mean plasma ascorbate levels in relation to H.pylori infection, adjusted for age (two groups, 65-69, 70-75) and social class (three groups) in subjects from the study by Burr et al (1987).

3 RESULTS

3.1 Completeness of information

Out of the 797 men selected, 711 (89.2%) participated in the CSCG study (1984). For 124 subjects there was no plasma left over for this analysis and an additional 24 subjects had no social class information available. Thus the total number of subjects available for this study was 711 - (124 + 24) = 563, 70.6% of the original sample. In the study by Burr et al (1987), 246 out of 343 men participated (71.7%) and plasma samples were available for 197, 57.4% of the original sample. Omission of the 11 men for whom plasma samples were available in both the 1979 and 1983 studies effectively reduced the number of subjects in the latter study from 197 to 186.

By combining the two studies, plasma and information on both age and social class was available for (563 + 186) 749 subjects which represents 67% of the original sample of (797 + 343 - 25) 1,115. There was no difference in the social class distribution (X^2 = 4.6; 2df; p = 0.5) or the age distribution (X^2 = 8.1; 8df; p = 0.4) between those who had plasma available and those who did not.

Dietary information and information on alcohol consumption, father's occupation and household density was only available from the 563 men aged 30-69 years participating in the first study (CSCG, 1984).

Table 1

Age specific prevalence rates for **Helicobacter pylori** IgG antibodies amongst 749 Caerphilly males

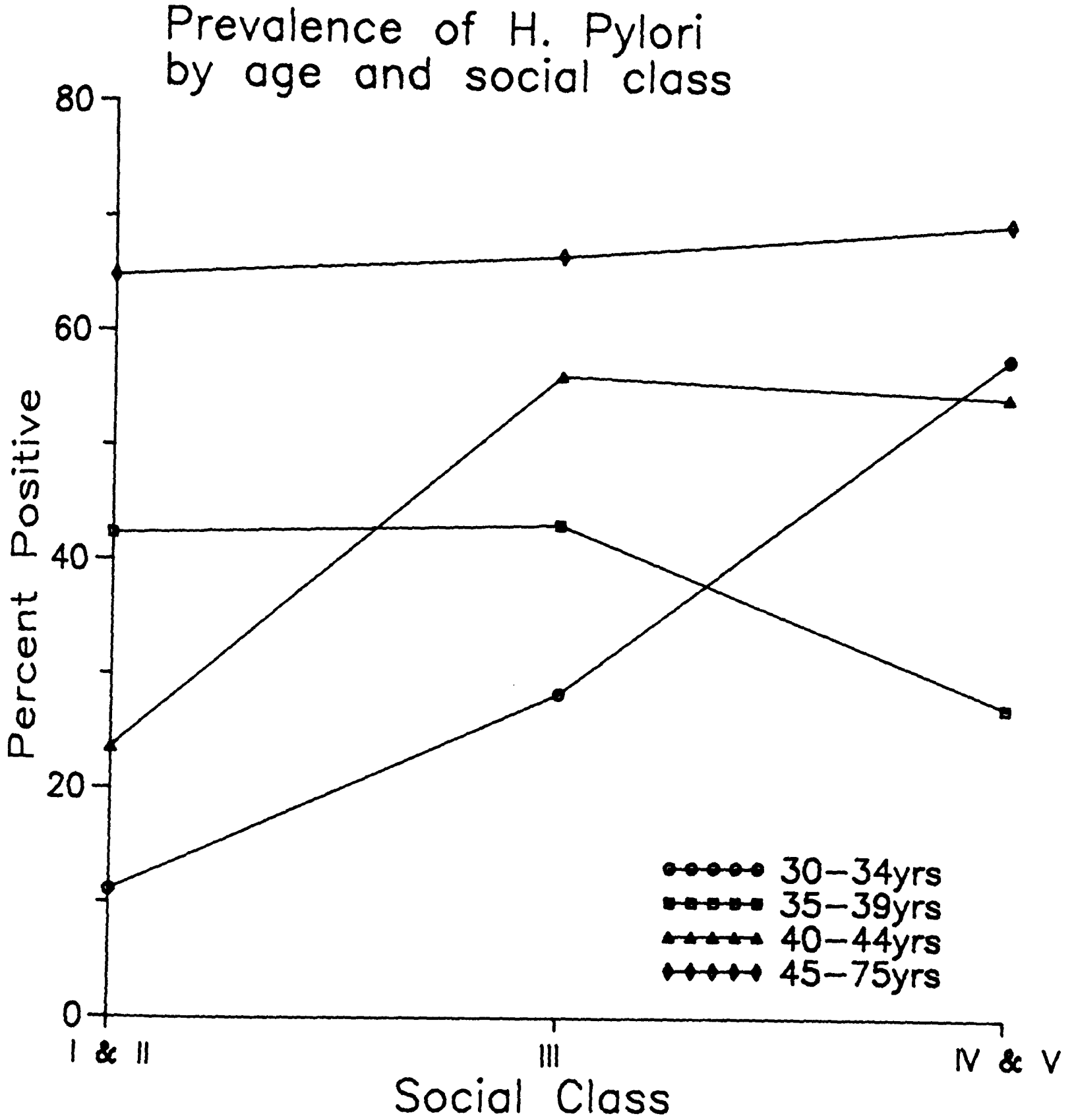
Age group	No.	No. Positive for **H.pylori**	Crude (%)	Crude (95% C.I.)	Adjusted for social class (%)	Adjusted for social class (95% C.I.)	Median O.D.
30-34	106	31	29.3	(23.1-35.4)	29.8	(23.5-36.0)	0.39
35-39	81	33	40.7	(33.1-48.3)	42.1	(34.3-49.7)	0.58
40-44	87	43	49.4	(41.9-56.9)	48.8	(41.3-56.3)	0.68
45-49	62	45	72.6	(64.7-80.5)	74.5	(66.5-82.0)	0.91
50-54	84	56	66.7	(59.5-73.9)	66.2	(58.9-73.3)	0.82
55-59	68	44	64.7	(56.6-72.8)	63.4	(55.2-71.6)	1.00
60-64	42	29	69.1	(59.1-79.0)	69.2	(59.2-79.1)	0.90
65-69	131	79	60.3	(54.3-66.3)	59.4	(53.4-64.4)	0.81
70-75	88	66	75.0	(68.5-81.5)	73.9	(67.3-80.5)	0.93
Total	749	426	56.9	(54.4-59.4)			0.78
X^2 trend (1 d.f.)			46.7		42.3		
p value			<0.0001		<0.0001		

Table 2

Prevalence rate for **H.pylori** IgG antibodies in relation to social class

Social class grouping	No.	No. Positive for **H.pylori**	Crude (%)	Crude (95% C.I.)	Adjusted for age (%)	Adjusted for age (95% C.I.)
I	30	10	33.3	(21.3-45.4)	36.3	(24.0-48.6)
II	128	65	50.8	(44.6-51.0)	52.0	(45.8-58.2)
IIIN	70	35	50.0	(41.6-58.4)	51.4	(43.0-59.8)
IIIM	350	207	59.1	(55.5-62.8)	58.7	(55.0-62.3)
IV	125	82	65.6	(59.7-71.6)	65.2	(59.2-71.1)
V	46	27	58.7	(48.5-68.9)	54.5	(44.2-64.8)
Total	749	426	56.9	(54.4-59.4)		
X^2 trend (1 d.f.)			8.8		6.3	
p=			0.003		0.01	

Figure 1.


Prevalence of H. Pylori
by age and social class
Percent Positive
80
60
40
20
0
I & II
III
IV & V
Social Class
30–34yrs
35–39yrs
40–44yrs
45–75yrs

3.2 Prevalence of H. pylori in relation to age

Table 1 shows the crude and social class adjusted prevalence rates of H. pylori antibody for the age categories 30-34, 35-39, ... 70-75 years. Approximately 30% of the population aged 30-34 years (29.8% adjusted for social class), had a positive antibody titre for H. pylori. This prevalence increased steeply with age until 45-49 years, after which it remained constant, at about 60% or greater. A test for linear trend with age (adjusted for social class) was statistically significant (X^2 trend = 42.3, 1df, $p < 0.0001$). Comparison of median optical density (OD) levels by age (and social class) showed a similar pattern to the results using a dichotomous categorisation of H. pylori infection. Median OD levels were lowest in the youngest age group (30-34 years) and increased with age, levelling off in those older than 45 years.

3.3 Prevalence of H.pylori in relation to social class

Table 2 shows the distribution of H. pylori infection in relation to social class. The age-standardised rates for H. pylori prevalence were lowest in social class categories I and II (49.2%), intermediate in categories IIIN & M (57.5%) and highest in categories IV & V (62.2%). A test for linear trend of increasing prevalence rates with decreasing social class was statistically significant after adjusting for age ($X^2 = 6.3$; 1df; $p = 0.01$). The median OD level for those in social class IV and V was higher (0.84) when compared to that for social class I and II (0.68), while those in social

class IIIM and N showed an intermediate level (0.77).

3.3.1 Age and social class interaction

Prevalence rates by social class on an age-specific basis are shown in Figure 1. Because there were no differences in age groups above 45 years and for the sake of simplicity, men aged 45-75 years are shown as a single age category. There were large differences within the youngest age group (30-34 years), the rate in social class categories IV and V (57.9%) being more than double that of categories IIIN & M (28.3%), and about five times higher than that in categories I & II (11.1%). In those aged 35-39 years and 40-44 years, social class differences were present but not with any particular trend. Prevalence in those aged over 45 years was very similar in all class categories.

When an interaction term (for age and social class) was added to the model examining the age-adjusted trend in social class of the subject, there was no significant improvement in fit (X^2 = 11.43; 8df; p = 0.2). However, having examined the data, a model was fitted in which those aged 45 years and over were combined into a single age category. In this post hoc analysis, addition of the interaction term resulted in a significant improvement in fit (X^2 = 11.04; 3df; p = 0.01). This implies that the distribution of H.pylori by social class in this study was largely due to the distribution of H.pylori in those aged less than 45 years.

Table 3

Prevalence of Helicobacter pylori infection in relation to father's social class

Age of subject	Father's social class							
	I&II		IIIM&N		IV&V		Total	
	N	%	N	%	N	%	N	%
30-34	10	(20.0)	36	(16.7)	56	(39.3)	102	(29.4)
35-44	15	(26.7)	39	(43.6)	108	(47.2)	162	(44.4)
45-54	14	(71.4)	32	(62.5)	87	(71.3)	133	(69.2)
55-64	15	(46.7)	20	(45.0)	101	(66.3)	136	(61.0)
Total	54	(42.6)	127	(40.9)	352	(57.4)	533 *	

* 30 subjects, no information.
Test for linear trend in relation to father's social class:

Unadjusted: $X^2_{Trend} = 4.9$; 1 df; $p = 0.03$

Adjusted for age (4 groups): $X^2_{Trend} = 8.2$; 1 df; $p = 0.004$.

Table 4

Was father ever unemployed at age of 10?	N	% Infected
No	336	48.8
Yes	123	61.0
	459	
Missing =	104	
X^2 =	5.3	p=0.02
X^2 (adjusted)* =	0.0	p=0.9

* Adjusted for age (5 groups) only

(Adjusted for age + social class $X^2 = 0.03$; $p = 0.9$)

3.3.2 Father's social class

Table 3 shows a trend of increasing prevalence rates with decreasing social class of the subject's father. A Chi-squared test for linear trend was significant after adjustment for age (X^2 trend = 8.2; 1df; p = 0.004). Differences in prevalence rates in relation to father's social class were also marked in those under 35 years, the rate for those in social class IV & V being almost double (40.4%) that of those whose fathers were in social class I & II (18.2%). The differences in prevalence rates in relation to the social class of the father amongst those aged 35 and older were not as marked as the differences amongst those aged 35 years or less, these being 47.8, 50.0 and 61.0% for social class I & II, IIIM & N and IV & V, respectively.

Information on whether the subject's father was ever unemployed when the subject was 10 years old was available from 459 out of 563 subjects (81%). Table 4 shows the prevalence of H.pylori infection in relation to fathers' employment status. Those with an unemployed father when the subject was aged 10 had significantly higher infection rates (61.0%) when compared to those whose father was employed (48.8%), (X^2 = 5.3; 1 df; p = 0.02), but this difference was not found to be significant after adjustment for age (p = 0.9) or age and social class combined (p = 0.9)

3.4 Place of birth

Prevalence of H.pylori amongst those born outside Caerphilly was significantly lower (44.8%) when compared

Table 5

Helicobacter pylori infection rates in relation to total number of children (aged <17 years) in household

Total number of children in household	N	% Infected			
		Crude	95% CI	Adjusted for age	95% CI
0-1	367	57.2	(53.6-60.8)	52.9	(49.2-56.5)
2-3	164	44.5	(39.1-50.0)	53.6	(48.1-59.0)
4+	32	46.9	(34.5-59.2)	50.8	(38.4-63.2)
X^2 trend =	7.8	p=0.02			
X^2 trend = (adjusted 1*)	0.5	p=0.5			
X^2 trend = (adjusted 2*)	0.05	p=0.8			

*1 Adjusted for age (5 groups)

*2 Adjusted for age (5 groups) and social class (3 groups)

Table 6

Helicobacter pylori infection rates in relation to number of adults in household

Total number of adults in household (17+yrs)	N	% Infected			
		Crude	95% CI	Adjusted for age	95% CI
1-2	361	48.5	(44.8-52.1)	51.9	(48.2-55.6)
3	117	59.8	(53.5-66.2)	53.8	(47.3-60.2)
4+	85	62.4	(55.0-69.7)	55.4	(47.9-63.0)
	563				
X^2 trend =		8.14	p=0.02		
X^2 trend = (adjusted 1*)		0.5	p=0.5		
X^2 trend = (adjusted 2*)		0.4	p=5		

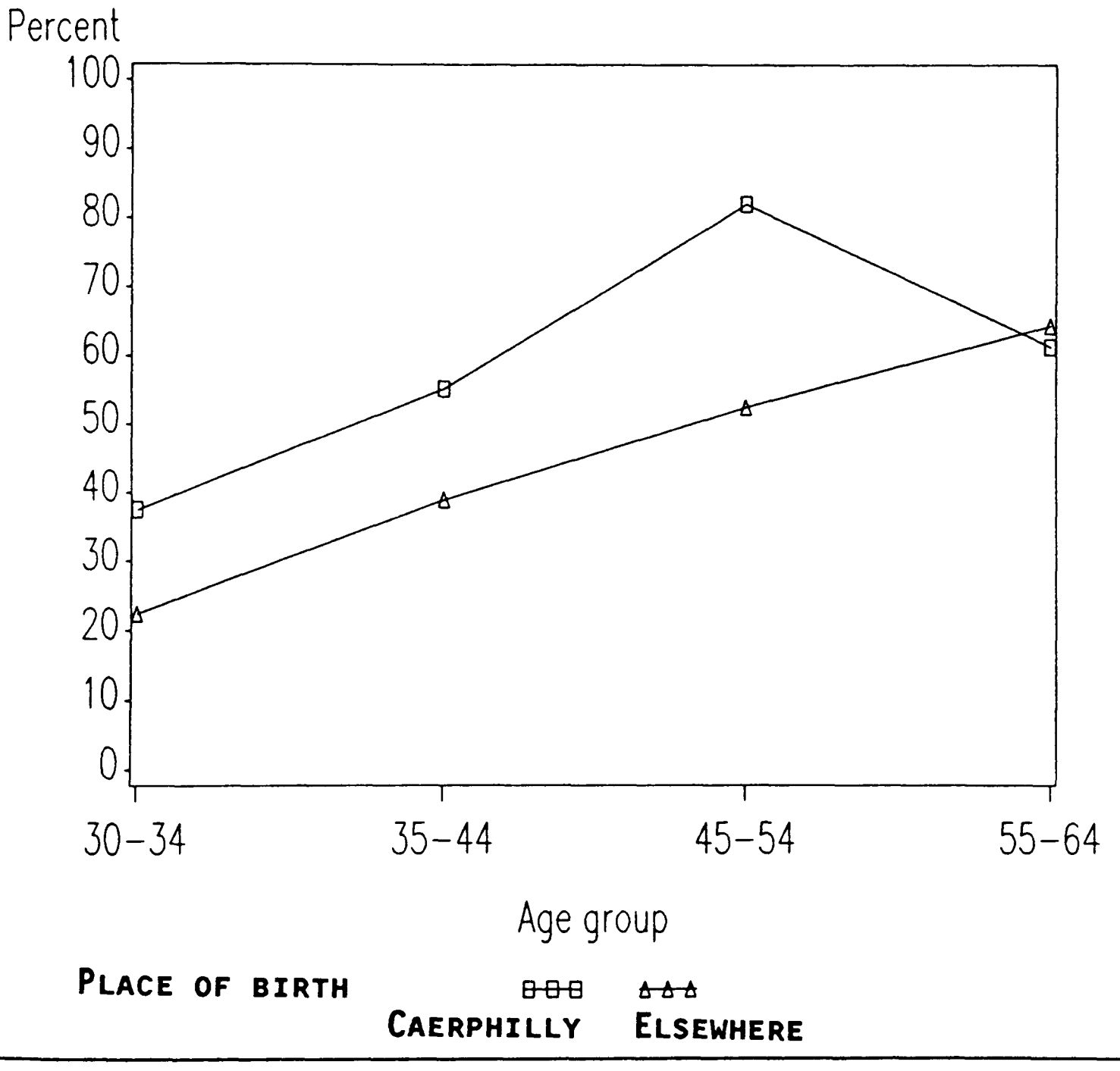
*1 Adjusted for age (5 groups)

*2 Adjusted for age (5 groups) and social class (3 groups)

Figure 2

PREVALENCE OF H.PYLORI

IN CAERPHILLY MEN BY PLACE OF BIRTH



with those born in Caerphilly (62.1%) (X^2 = 4.8; 1 df; p = 0.02). Figure 2 shows the age-specific prevalence of infection in those born in and out of Caerphilly. With the exception of those aged 55 and over, age specific prevalence rates were higher in those born in Caerphilly when compared to those born elsewhere, but this difference in prevalence of H.pylori did not reach statistical significance after adjustment for age and social class (X^2 = 1; 1df; p =0.9).

3.5 Household density

3.5.1 Number of children in household:

Table 5 shows those with one or no children having the highest infection rates (57.2%) when compared to those having 2-3 children (44.5%) or more (46.9%) A test for trend in relation to number of children is significant (X^2_{Trend} = 7.8; 1 df; p = 0.02), but this trend was not significant after adjusting for age only (X^2_{Trend} = 0.5; 1 df; p = 0.5) or age and social class (X^2_{Trend} = 0.05; 1 df; p =0.8).

3.5.2 Number of adults in household

Table 6 shows those with two or fewer adults (aged 17+ years) having the lowest infection rates (48.5%), those with three adults showing intermediate rates (59.8%) and those households with four or more adults having the highest H.pylori infection rates (62.4%). This trend in relation to number of adults was significant (p = 0.02) but the significance disappears after adjustment for age only (X^2_{Trend} = 0.5; 1 df; p = 0.5) or age and social class (X^2_{Trend} = 0.3; 1 df; p = 0.6).

Table 7

Helicobacter pylori infection rate in relation to number of siblings (subject excluded)

Number of siblings	N	% Infected			
		Crude	95% CI	Adjusted for age	95% CI
0,1	150	39.3	(33.8-44.9)	43.4	(37.8-49.1)
2,3	193	52.3	(47.3-57.3)	52.3	(47.3-57.3)
4+	220	62.7	(58.2-67.3)	59.0	(54.3-63.6)
	563				
X^2 trend =		19.6		p=0.0001	
X^2 trend = (adjusted 1*)		9.6		p=0.002	
x^2 trend = (adjusted 2*)		6.69		p=0.01	

*1 Adjusted for age (5 groups)

*2 Adjusted for age (5 groups) and social class (3 groups)

Table 8

Relationship between **Helicobacter pylori** and dietary and alcohol intake, adjusted for age and social class

	Units per day	Mean levels					
		With **H.pylori**		Without **H.pylori**			
		Unadj	Adj	Unadj	Adj	p-value	r*
Vitamin C	mg	50.5	51.2	56.3	55.4	0.03	0.38
Fat	g	101.9	100.8	99.7	101.0	0.92	0.21
Sugar	g	95.2	98.8	99.0	95.4	0.32	0.35
Starch	g	144.9	146.1	147.1	147.1	0.95	0.41
Cereal fibre	g	7.0	7.1	7.3	7.2	0.68	0.53
Vegetable fibre	g	8.5	8.7	9.1	9.0	0.25	0.35
Protein	g	73.3	74.0	73.8	73.0	0.55	0.33
Alcohol	g	25.8	26.6	22.0	21.2	0.05	0.65
Energy	KCal	2288.7	2288.9	2265.7	2265.4	0.68	0.34
Total Carotene	ug	2845.0	2858.9	2965.4	2950.2	0.64	

* r = correlation coefficient between weighted 7 day dietary survey and food frequency questionnaire based on 640 cases in a related study from Caerphilly (Fehilly et al 1984)

Table 9

Consumption of vitamin C (mg/day) in Caerphilly men, by age and social class

Class	Age group				
	30-34	35-44	45-54	55-64	Total
I&II	58.5	56.3	59.3	60.8	58.5
IIIM&N	59.3	54.5	51.6	48.9	53.0
IV&V	51.0	47.2	42.2	40.5	47.7
	57.6	53.6	51.7	51.2	53.2

3.5.3 Number of siblings

Table 7 shows those subjects with 0-2 siblings having the lowest H.pylori infection rates (39.3%), those with 3-4 siblings having intermediate rates (52.3%) and those subjects with five or more siblings having the highest infection rates (62.7%). This trend in relation to number of siblings was highly significant before (X^2_{Trend} = 19.5; 1 df; p = 0.0001) or after adjustment for age (X^2_{Trend} = 9.6; 1 df; p = 0.002) or after adjustment for age and social class (X^2_{Trend} = 6.7; 1 df; p = 0.005).

3.6 Dietary factors

Table 8 shows the relationship between mean and adjusted (for age and social class) nutrient levels of nine nutrients, and energy and alcohol consumption in relation to H.pylori infection.

There was an increasing trend of consumption of vitamin C with decreasing social class (p < 0.0001) and age (p < 0.01) as shown in table 9. Intake of vitamin C was significantly lower in those who were infected when compared to those who were not (51.2 vs 55.4 mg/day), and this difference was significant even after adjustment for age and social class (p = 0.03). Adjusting for energy intake made virtually no difference to the vitamin C intake values, these being 51.3 and 55.4 μg/day, in those with and without H.pylori infection, respectively.

Amongst those aged 65-75, there were no differences in

Table 10

Helicobacter pylori infection rates (%) in relation to smoking habits in Caerphilly men

Smoking status	N	% **H.pylori** positive			
		Crude	95% CI	Adjusted for age	95% CI
Never	132	50.8	(44.7-56.9)	56.4	(50.3-62.4)
Ex-smoker	204	62.8	(58.0-67.5)	58.4	(53.6-63.3)
Pipe/cigar smoker	77	59.7	(51.9-67.6)	57.1	(49.2-65.0)
1-14 cigs/day	113	62.0	(55.6-68.3)	58.3	(51.8-64.8)
15-24 cigs/day	117	58.1	(51.7-64.5)	58.6	(52.2-65.0)
25+ cigs/day	103	42.7	(35.9-49.5)	48.2	(41.3-55.0)
Total	747 (3 missing)				

X^2 Trend (Never, 1-, 15-, 25+, cigs per day) = 0.08; 1df; p = 0.8

X^2 Trend (Never, 1-, 15-, 25+, adjusted for age and social class) = 0.3; p = 0.6

plasma ascorbate concentrations in relation to the presence or absence of H.pylori infection; mean adjusted levels being 0.29 and 0.30 μg/100ml plasma, respectively (p = 0.9).

Those with H.pylori infection consumed slightly lower levels of total carotene than those without H.pylori infection, (2858.9 vs 2950.2 μg per day, respectively) but this difference was not statistically significant (p = 0.64).

There were no significant differences in the levels of fat, sugar, starch, cereal fibre, vegetable fibre and protein consumed between those who were infected with H.pylori and those who were not.

3.7 Alcohol consumption

Table 6 also shows the level of alcohol consumption in relation to H.pylori infection. After adjusting for age and social class, those with H. pylori infection consumed more alcohol than those without, consumption levels being, respectively, 26.6 g vs 21.2 g per day (p < 0.05).

3.8 Smoking

Non-smokers had a lower prevalence rate than the other groups combined (ex- and current smokers), 50.8% vs 58.0%, but this difference was not significant (p = 0.13). Ex-smokers and pipe/cigar smokers had elevated rates of 62.8 and 59.7%, respectively. There was no evidence of a trend in relation to frequency of tobacco use (never, 1-14, 15-24,

25+ cigarettes per day) before (X^2_{Trend} = 0.08; 1 df; p = 0.8) or after adjustment for age and social class (X^2 trend = 0.3; 1df; p = 0.6), Table 10.

4 DISCUSSION

4.1 Completeness of information

Although based on plasma collected for other purposes, this is the first study in which the prevalence of H. pylori antibodies has been measured in a large population selected at random. Full data were available for only 67% of those selected but the refusal rate was only 16% (183/1,115) as the other 17% of the original sample of 1,115 represents those for whom no plasma (159 subjects) or social class (24 subjects) information was available. The similarity in age and social class distribution between those who did and those who did not have plasma available, the high participation rate and the random sampling technique means that the data were relatively free from the biases associated with volunteer and other selected groups.

4.2 Quality of the plasma

All the plasma samples were subjected to two freeze-thaw cycles prior to our investigation. There has been no evidence as yet suggesting that this detrimentally affects the performance of the H.pylori ELISA but in any case, any damage caused by these cycles would have affected the whole population and would not be biased towards any sub-group.

4.3 Age

Overall 56.9% (95% CI 54.4-59.4) of the 749 men in Caerphilly showed positive evidence of infection with H. pylori and, as in all other studies, there was a pronounced association with age. Prevalence figures were higher than

those from other studies of non-clinical populations (see Table 10 in Chapter 1) except for a study from Algeria and the Ivory Coast in which 80-90% of adults were positive (Megraud et al 1989).

Prevalence increased sharply between 30 and 45 years and over the age of 45 years the proportion infected was 67.1% (95% CI 64.1-70.2). In two other British studies, from Manchester (Jones et al 1986) and Leeds (Wyatt et al 1988), the prevalence of infection above the age of 50 years (by when there should be relatively little effect of age) was 45.9% (95% CI 40.3-51.5) and 50% (95% CI 38.9-61.1) respectively compared with the figure in this study of 66.3% (95% CI 63.1-69.6). In a Finnish study of healthy blood donors the prevalence of infection in those over 50 years was reported to be over 50% (Kosunen et al 1989).

It is difficult to make comparisons between studies without making allowance for the different analytical techniques, the age composition of other study populations and the selection effects of using non-random samples. Some of the differences between studies were likely to be a result of methodological differences (for example different assays and selection procedures), but it is also possible that infection rates in Caerphilly were genuinely elevated.

There are no data to assess whether the prevalence of chronic atrophic gastritis in Caerphilly was correspondingly elevated but it may be noted that the SMR for gastric cancer

in the period 1968-78 was 138 (England and Wales = 100) and this part of Wales has traditionally been associated with high gastric cancer mortality (Burr *et al* 1987, Craven *et al* 1979, Gardner *et al* 1984). In the same period, male mortality rates from gastric and duodenal ulcer in this area of Wales were at (SMR = 90-109) and below (SMR = 75-89) the national average (Gardner *et al* 1984), but mortality rates are unlikely to reflect the true incidence of peptic ulcer at this period of time.

4.4 Social class

The data show an inverse relationship between prevalence of *H. pylori* infection and social class; that is, infection rates being highest in the lowest social class categories. Examination of Figure 1 indicates that this trend with social class is predominated by those in two of the three youngest five-year age groups. A test for statistical interaction between age and social class was not, however, significant without the *post hoc* combination of those aged 45-75 years into a single category. As this latter analysis was conducted after examining the data, the interaction requires confirmation in other independent studies. Also, even after the combination of social classes, the numbers in each age-class category become quite small.

At this stage, therefore, undue emphasis should not be placed on the social class effect being specific to young men. If confirmed, however, it would indicate that the higher social class groups are potentially protected from or

are less susceptible to infection at a young age. It is not possible to say whether young men in the high social class groups will remain free of infection as they become older (that is a cohort effect) or whether, by the age of fifty, they will have the same prevalence of infection as those in lower class groups. Also, other reports from South America (Klein et al 1989, Guittierez et al 1988), Africa (Megraud 1989, Lachlan et al 1988) and USA (Dehesa et al 1989), have shown a similar high prevalence of H. pylori antibodies amongst poor population groups particularly at young ages.

England and Wales data for the last 2-3 decades show an inverse relationship between both gastric and duodenal ulcer prevalence and social class. In general terms, peptic ulcer is about twice as common in those of low social class (Langman and Logan 1986, OPCS 1978, OPCS 1986). Low social class has also been a strong determinant of gastric cancer in England and Wales for over 50 years (Logan 1982) and this may be in part related to the high prevalence of H. pylori infection and associated gastritis in such men in early life. Correa et al 1976 have shown in Colombia that the prevalence of antral atrophic gastritis at young ages is a major factor distinguishing populations at high risk of gastric cancer from those at low risk. If H. pylori associated gastritis progresses into atrophic gastritis then these results are in keeping with the findings in Colombia.

4.5 Place of birth

Although the difference in prevalence between those born

in and out of Caerphilly was not significant, it is of interest that being born outside Caerphilly had lower age specific rates. This may be in keeping with the geographic differences in gastric cancer rates between Caerphilly and the rest of the country, but the data were too sparse to draw definite conclusions.

4.6 Household density

Household density, as measured by the number of children (<17 years old) and adults (17+ years old) in a household did not seem to play any role in infection rates. Household density is usually related to both age and social class and therefore both these factors could act as confounders of household density. There was a increasing prevalence of infection in relation to the number of the subject's siblings, and this relationship remained significant after adjustment for age and social class. This implied that household density at the time when the subject was living with his siblings, seems to have a greater effect on H.pylori infection rates than the current number of persons living in the household, and this effect is independent of social class. If confirmed in other studies, the relationship between number of siblings and infection points to the effects of age and social class being a cohort phenomenon rather than one of gradual acquisition of infection with age. Drumm et al (1990) found that a greater proportion of parents of infected children had H.pylori antibodies when compared to non-infected controls. Also, siblings of infected children were more likely to be

infected with H.pylori than siblings of controls. This intrafamilial clustering of H.pylori in families described by Drumm et al (1990) is in keeping with the data presented here on the relationship between H.pylori and number of siblings. Data from physicians in the 1970's exhibited similar prevalence rates to age matched physicians in the 1980's (unpublished data, Parsonett 1989), and on this basis, Parsonett (1989) concluded that the pattern of H.pylori infection was attributed to age rather than a cohort effect. However, this is still in keeping with a cohort phenomenon, infection rates in physicians (presumably belonging to social class I) remaining the same over time.

The prevalence of H.pylori has been reported to be higher in mentally retarded inmates (Berkowitz and Lee 1987, Reiff et al 1989), in orphanage children and in endoscopy clinic staff (Reiff et al 1989), and among families of children infected with H.pylori (Mitchell et al 1987). Besides the evidence of higher prevalence rates amongst endoscopy staff pointing to contaminated endoscopes being the mode of transmission, people living in close contact are at a higher risk of transmission of H.pylori, possibly via a faecal-oral or oral-oral route, via a direct salivary, droplet infection or due to some other common source. In this study, the increased prevalence of H.pylori infection in relation to number of the subject's siblings (but not in relation to number of children currently living in the subject's household) is in keeping with infection occuring at an early age.

In a study of factors influencing gastric cancer in the UK, overcrowding at a young age was found to be the most important risk factor for subsequent gastric cancer by Barker *et al* (1990), who concluded that this pointed to an infective agent influencing the subsequent risk of gastric cancer. This is the first time that an association between *H. pylori* and household size has been reported. If confirmed in other studies, *H. pylori* transmission in large households may be an important risk factor for gastric cancer in later life.

4.7 Dietary factors

4.7.1 Vitamin C

The strong inverse relationship between vitamin C consumption and social class in this population was described before by Fehily *et al* (1984) and compares well with weighted dietary records (Fehily *et al* 1984). In fact, in the UK as a whole vitamin C consumption has been inversely related to both social class and to household density (Ministry of Agriculture, Fisheries and Food 1978, 1988).

In this study, an association was found between *H.pylori* infection and vitamin C levels after adjustment for social class and age, in those aged 30-64 years. The data on plasma ascorbate concentrations in those aged 65-75 showed no differences in relation to infection.

Rather than being of aetiological importance to *H.pylori*

infection, low consumption of vitamin C was probably associated with a combination of lifestyle factors related to social class and household size, both shown to be risk factors for H.pylori in this study.

In a recent study of an endoscopic population Sobaler et al (1989) found lower plasma ascorbate levels in those with CAG when compared to those with a normal mucosa and suggested that those with severe chronic atrophic gastritis consume less vitamin C than those with normal stomachs.

The relationship between vitamin C intake, chronic atrophic gastritis and gastric cancer is of particular interest because vitamin C is an important inhibitor of nitrosation, preventing the formation of potentially carcinogenic N-nitroso compounds in persons who already have severe chronic atrophic gastritis (Correa 1988, Correa and Ruiz 1989, Chapter 1).

Vitamin C, or the consumption of fresh fruit and vegetables has been shown to be specifically inversely associated with chronic atrophic gastritis (Fontham et al 1986, Haenszel et al 1976, Correa et al 1983, Burr et al 1987, see Chapter 1) and with gastric cancer (Buiatti et al 1989, La Veccia et al 1987, Burr and Halliday 1989, You et al 1988, Correa et al 1985, Kono et al 1988). If H.pylori infection progresses to CAG, and vitamin C is protective against gastric cancer, then overcrowded households (which are at a higher risk of acquiring H.pylori) consuming less

vitamin C might be at an increased risk of gastric cancer.

4.7.2 Total carotene

Although there was no evidence of an association between carotene and H.pylori infection, beta carotene was implicated as being protective for gastric cancer in subjects with gastric dysplasia, a more severe gastric lesion than chronic atrophic gastritis, (Correa et al 1983, Haenszel et al 1976), and in relation to gastric cancer (Wald et al, 1988).

4.8 Smoking

Smoking did not seem to be associated with H. pylori infection. This is in contrast to an endoscopy study of subjects with upper abdominal symptoms which found that more H. pylori culture positive patients were smokers in comparison to H. pylori negative patients (Andersen et al 1988). In a study by Martin et al (1989), smoking among H.pylori positive subjects appears to increase the risk of peptic ulceration, and presumably the likelihood of undergoing endoscopy and clearly, the relationship between smoking and H.pylori infection (and chronic atrophic gastritis) needs to confirmed in other population based studies.

4.9 Alcohol

Alcohol consumption has been a known irritant of the gastric mucosa (Correa et al 1983, Correa 1988) and a cause of acute gastritis (Whitehead et al 1973), and pangastritis among alcoholics (Bouchier et al 1984). Alcohol consumption

be an important factor in addition to H.pylori infection in the aetiology of chronic atrophic gastritis.

5 SUMMARY

These results have shown that the prevalence of H. pylori infection can be very high, even in Western countries. Infection rates showed significant differences, between age and social class, especially in those aged under 45 years. The strong relationship between number of siblings and infection points to the effects of age and social class being a cohort phenomenon rather than one of gradual acquisition of infection with age.

The lower vitamin C consumption level amongst those infected with H.pylori after age and social class adjustment is of interest because of its relevance to chronic atrophic gastritis and to the gastric cancer model. Similarly, the relationship between gastric irritation due to alcohol consumption and H.pylori infection may be important in the aetiology of H.pylori-associated gastritis.

The relationship between H. pylori infection and socioeconomic, dietary, and lifestyle characteristics needs to be confirmed in other studies, especially in areas of varying peptic ulcer and gastric cancer incidence.

CHAPTER 4

HELICOBACTER PYLORI INFECTION IN RELATION TO GEOGRAPHICAL, SOCIODEMOGRAPHIC AND DIETARY FACTORS IN ITALY

1 INTRODUCTION

Chapter 1 described the background to and the need for randomly selected population based epidemiological studies of *H.pylori*. Chapter 3 described a study of *H.pylori* infection rates in relation to sociodemographic, behavioral and dietary variables in a random sample of Caerphilly men. This Chapter attempts to reconfirm some of the findings in the study from Caerphilly by looking at the distribution of *H.pylori* infection in another setting. It describes a seroprevalence survey of *H.pylori*, based on 1,159 randomly selected population controls of a case-control study of diet and gastric cancer in Italy, conducted in 1985-7 (Buiatti *et al* 1989a,b). Questionnaire information and plasma samples were made available from this study in order to examine:

1. the distribution of *H.pylori* in relation to age, sex and social class;

2. the geographical distribution of *H.pylori* in relation to residence in regions of high or low gastric cancer mortality and urban or rural residence;

3. the relationship between *H.pylori* and the dietary intake of 17 food items, specifically vitamin C, fresh fruit and vegetables, citrus fruit and dairy products; and lifestyle factors such as smoking and alcohol consumption;

4. the distribution of H.pylori in relation to food storage indices such as ownership of a refrigerator and food storage methods;

5. the relationship of H.pylori to number of siblings, a reported history of peptic ulcer and gastritis and a family history of gastric cancer.

2 SUBJECTS AND METHODS

2.1 Subjects

Seven centres (Forli, Cremona, Imola, Florence, Siena, Genoa and Cagliari) of varying gastric cancer mortality participated in the case-control study of diet and gastric cancer (Buiatti et al 1989a,b). Cases were identified in surgery and gastroenterology departments and endoscopy clinics. All subjects with a histologically confirmed gastric cancer were included in the study. A total of 1,016 gastric cancer cases were recruited from these seven centres.

The 1,235 population controls, which were the subjects of interest in this study, were randomly selected using computerised municipal lists of residents and National Health Service files, matched to gastric cancer cases by 5 year age group, sex and area of residence (Buiatti et al 1989a,b). Each subject was questioned personally by a trained interviewer, using a previously validated structured questionnaire, and provided a blood sample (Buiatti et 1989b). The questionnaire included questions on residential, occupational, medical history, dietary information, alcohol consumption, and smoking habits, and food storage conditions.

2.2 Plasma samples

Plasma samples were collected from 5 of the 7 participating centres (Cagliari, Florence, Forli, Genoa and

Imola). Plasma aliquots were stored after collection at -20°C, and thawed once before being analysed for H.pylori IgG antibodies using the same ELISA procedure to that used in all studies (Chapters 2 to 7), described in Appendix 1. Consistent with the study reported in Chapter 2 (and all other studies reported in this thesis), an optical density reading of 0.7, corresponding to an IgG antibody titre of 10 μg/ml, was considered as positive for H.pylori infection.

2.3 Geographical information

Subjects were grouped according to whether they were resident in an urban or rural area, and by place of birth (northern/southern Italy). Subjects were also grouped by the gastric cancer risk of the area of residence (High = Forli, Imola and Florence; Low = Genoa and Cagliari). Gastric cancer rates (per 100,000) for these centres are shown in Table 1.

2.4 Sociodemographic information

Information on social class (low, middle, high) was based on occupation and educational level. Teachers, clerks, and professionals were classified in the highest social class, skilled artisans and technicians were classified in the middle class category, and manual labourers classified in the lowest class category. Married women were classified according to the occupation of their husband.

2.5 Family and medical history

Subjects were asked whether they had any siblings, a history of peptic ulcer, gastric resection, dyspepsia and chronic gastritis. Another question related to whether there were any gastric cancer cases in the subject's family. Medical history was confirmed by examining the medical records of these subjects.

2.6 Dietary information

Dietary information was based on the usual frequency of consumption of 146 food items over a 12 month period, two years prior to the interview, using a structured and validated food frequency questionnaire (Buiatti *et al* 1989a,b). These 146 items were condensed into 18 food groups as shown in Table 6. Questions on preference for salty foods and alcohol consumption were also asked.

2.7 Food storage

Food storage indicators were sought by asking the age of the subject when a refrigerator was first brought into the home. The habit of storing left over food in the refrigerator was also elicited (always/often, seldom/never).

2.8 Categorisation of variables

Subjects were categorised into four age groups, <45, 45-54, 55-64, and 65+ years. All food intakes were grouped into tertiles of consumption. Social class was divided into three categories, low, middle and high.

2.9 Statistical tests

The significance of linear trends of H.pylori infection by age (4 groups, <45, 45-54, 55-65, 65+) or social class (low, middle, high) (adjusted for each other) and interaction between age and social class were assessed using a goodness of fit Chi-squared model (Armitage and Berry 1989).

In order to test for a statistical interaction between age and social class, age was divided into two categories (<45, 45+ years) the hypothesis being that an interaction between age and social class would be expected only in the younger age group. The Generalised Linear Interactive Modelling System (GLIM) was used for this analysis (Healy, 1988).

The Mantel-Haenszel Chi-squared statistic (Armitage and Berry 1987) was used to test for linear trends in H.pylori infection rates in relation to diet, alcohol consumption, salt preference, number of siblings and number of gastric cancer relatives. A Chi-squared statistic was used to test for differences in prevalence of H.pylori in relation to smoking, ownership of a refrigerator and history of gastrointestinal complaints. Both Chi-squared and Chi-squared trend statistics were also adjusted for age (continuous), sex, place of birth, and social class using a multiple logistic regression procedure (SAS, LOGIST Procedure, 1980). Age, place of birth and social class were found to be the

most significant demographic variables (p = 0.008, 0.19, 0.13 respectively) in a multivariate model of H.pylori containing, age (continuous), sex (m/f), social class (low, middle, high) place of birth (north/south), and the frequency of consumption of the food groups described earlier (tertiles of consumption). The effect of sex was not found to be significant in this model (p = 0.7) but it was nevertheless included as an adjustment variable.

3 RESULTS

3.1 Completeness of information

Table 1 shows the number of subjects selected and the breakdown of the response rates in this study. Out of a total of 1,235 subjects initially selected 1,134 were found eligible for the study and 1,005 (88.6%) agreed to be interviewed as controls for a case-control study on gastric cancer (Buiatti *et al* 1989b). An additional 464 subjects were interviewed at a later date giving a total of 1469 persons interviewed. Information on the number that refused to be interviewed at this later phase of the study was not available.

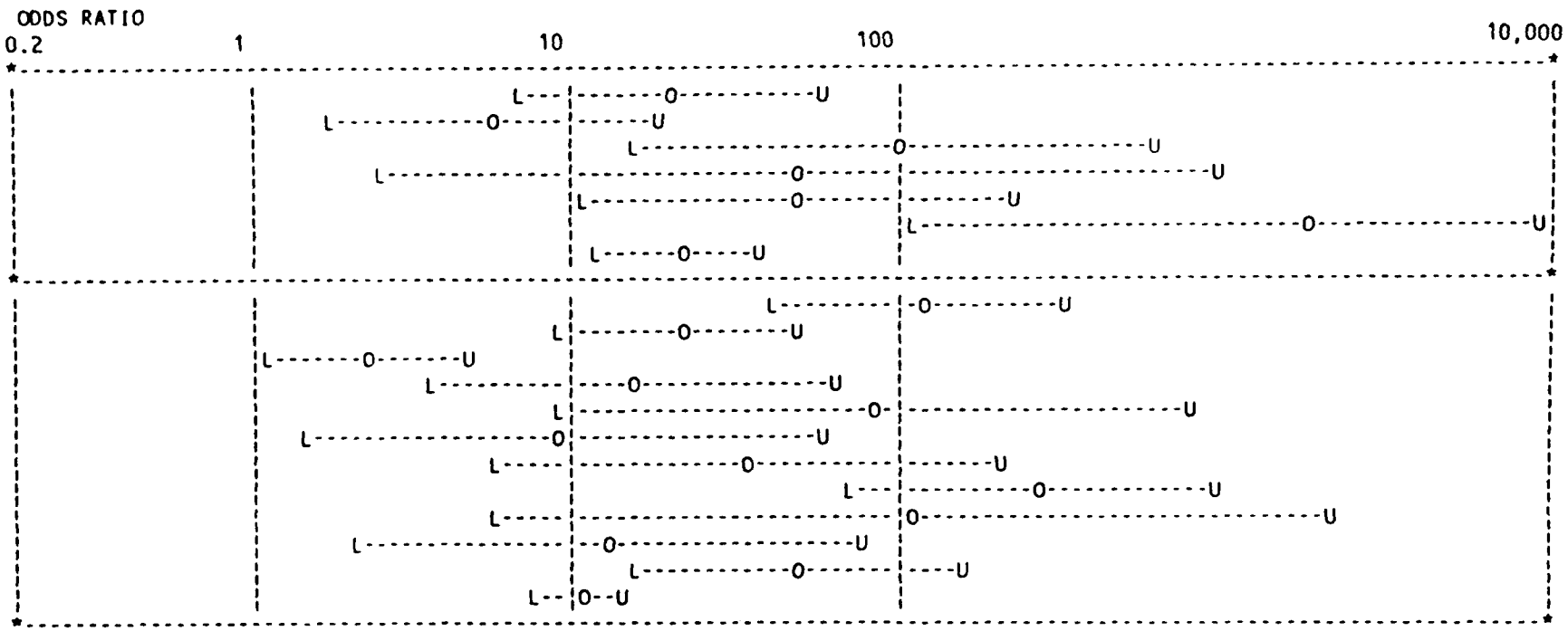
Of the 1,469 persons interviewed, 1056 agreed to provide a blood sample and sufficient blood for the analysis of *H.pylori* antibodies was available from 959 subjects. The closest estimate of the blood collection rate was therefore (100 X 1056/ 1469) 71.9%. The overall blood collection rate was (88.6% X 71.9%) 63.7%.

There were no differences between those who provided a blood sample and those who did not in relation to social class, migratory status, urban or rural residence, smoking status, alcohol drinking status or Quetelet index. A greater proportion of older people (>65 years) (53.3% versus 38.9%) refused to provide a blood sample (X^2 = 32.8; 3 df; p < 0.0001). Response rates to providing blood also varied by centre, ranging from 60.8% in Calgiari to 91.8% in Imola (X^2

FIGURE 1

RELATIONSHIP BETWEEN H.PYLORI AND MUCOSAL HISTOPATHOLOGY

AUTHOR	YEAR	LOCATION	NUMBER	TYPE	ODDS RATIO
BEDOSSA	1987	FRA2	110	SG	20.0
BEDOSSA	1987	FRA1	110	SG	5.6
JONES	1984	U.K.	50	SG	98.0
MANGANARO	1988	ITAL	83	SG	48.4
SIURALA	1988	FINL	157	SG	48.5
STOLTE	1989	GERM	6300	SG	1826*
POOLED ODDS RATIO				SG	20.9
ANDERSEN	1987	DENM	153	CSG	118
BOOTH	1986	U.K.	220	CSG	20.9
JASKIEWICZ	1989	SAFR	178	CSG	2.3
JIANG	1987	CHIN	209	CSG	15.0
JONES	1986	U.K.	64	CSG	84.0
PRICE	1985	U.K.	51	CSG	9.1
PRICE	1988	U.K.	50	CSG	34.8
SIPPONEN	1988	FINL	194	CSG	260
SIPPONEN	1988	FINL	57	CSG	110
SIPPONEN	1988	FINL	39	CSG	12.6
SIURALA	1988	FINL	179	CSG	48.6
POOLED ODDS RATIO				CSG	10.3



G=GASTRITIS, SG=SUPERFICIAL GASTRITIS, CSG=CHRONIC SUPERFICIAL GASTRITIS
AG=ACTIVE GASTRITIS, CG=CHRONIC GASTRITIS, CAG=CHRONIC ATROPHIC GASTRITIS
CAGA=ACTIVE CAG, CAGQ=QUIESCENT CAG, IM=INTESTINAL METAPLASIA, CA=GASTRIC CANCER

* NOT INCLUDED IN POOLED ODDS RATIO CALCULATION
# p > 0.05 (NOT SIGNIFICANT)

Table 1

Gastric cancer rates, sample size and response rates in participating centers

Centre	G.C. rate* M	F	No. subjects identified A	Ineligible B	Initial interview C	Extra subjects interviewed D	Total interviewed E	Number providing blood F	Blood for *H.pylori* analysis G	Response rate (F/E %)
Florence+	56.8	40.9	547	33	440	121	561	384	376	68.5
Forli+	69.8	53.3	281	12	259	171	430	357	311	83.0
Imola+	40.8	29.5	74	3	61	-	61	56	44	91.8
Genoa	26.4	19.7	205	51	137	109	246	155	149	63.0
Cagliari	19.5	16.3	118	2	108	63	171	104	79	60.8
			1235	101	1005	464	1469	1056	959	71.9

* Per 100,000, (Buiatti *et al* 1989).
+ High gastric cancer risk centers

Number eligible (A-B) 1235 - 101 = 1134
Interview success rate (C/(A-B)) = (1005/1134) = 88.6%
Overall blood collection rate = 71.9% X 88.6% = 63.7%

Table 2

Prevalence rates for H.pylori infection, by centre among 959 subjects in Italy

Centre	No. positive for H.pylori	Total	Crude prevalence rate %	(95% C.I.)	Prevalence adjusted for age %	(95% C.I.)
1. Florence	173	376	41.0	(42.4-49.6)	46.2	(42.7-49.9)
2. Forli	135	311	43.4	(39.4-47.3)	44.3	(40.3-48.2)
3. Imola	23	44	52.3	(41.7-62.8)	51.3	(46.1-61.2)
4. Genoa	67	149	45.0	(39.3-50.7)	42.8	(37.1-48.5)
5. Cagliari	44	79	55.7	(47.9-63.5)	55.8	(48.0-63.6)
Total	433	959	45.2	(43.0-47.5)		
High gastric cancer (1,2,3)	331	731	45.2	(42.6-47.8)	45.8	(43.2-48.3)
Low gastric cancer (4,5)	102	228	44.7	(40.1-49.3)	43.3	(38.7-47.9)
X^2 (1 df) =			0.0		0.0	
(h/l g.c.) p =			0.9		0.9	
Urban	336	760	44.2	(41.7-46.7)	44.4	(41.9-46.9)
Rural	97	199	48.7	(43.8-53.2)	48.0	(43.0-53.0)
X^2 (1 df) =			1.3		0.9	
(urban/rural) p =			0.3		0.4	

Table 2

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4. Genoa	67	149	45.0	(39.3-50.7)	42.8	(37.1-48.5)
5. Cagliari	44	79	55.7	(47.9-63.5)	55.8	(48.0-63.6)
Total	433	959	45.2	(43.0-47.5)		
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X^2 (1 df) =			0.0		0.0	
(h/l g.c.) p =			0.9		0.9	
Urban	336	760	44.2	(41.7-46.7)	44.4	(41.9-46.9)
Rural	97	199	48.7	(43.8-53.2)	48.0	(43.0-53.0)
X^2 (1 df) =			1.3		0.9	
(urban/rural) p =			0.3		0.4	

= 65.8; 5 df; $p < 0.0001$).

3.2 Geographical distribution of H.pylori infection (Table 2)

Overall prevalence of H.pylori in the five study centres was 45.2% (95% C.I. = 43.0-47.5%), as shown in Table 3. Prevalence of H.pylori was roughly similar in relation to area of residence, the highest age adjusted rates being in Cagliari, 55.8% (95% C.I. = 48.0-63.6%) and the lowest in Genoa, 42.8% (95% C.I. = 37.1-48.5%). There was no significant difference between age adjusted prevalence rates of H.pylori in relation to gastric cancer mortality rates, these being lower 43.3% (95% C.I. = 38.7-47.9%) in the two centres of low gastric cancer incidence and 45.8% (95% C.I. = 43.2-48.3%) in the three centres of high gastric cancer incidence (High versus low, $X^2 = 0.2$; 1df; $p = 0.2$, adjusted for age, social class, place of birth).

Those originally born in the south of Italy had elevated rates of H.pylori infection (51.7%, 95% C.I. = 44.2-59.2%) when compared to those born in the north (44.5%, 95% C.I. = 42.1-46.9%), but a Chi-square test was not significant after adjustment for age and social class (X^2 (adjusted) = 1.7; 1 d.f.; $p = 0.12$).

Table 3

Age specific prevalence rates for H.pylori infection among 959 subjects in Italy

Age group	No. positive for H.pylori	Total	Crude prevalence rate		Prevalence adjusted for class	
			%	(95% C.I.)	%	(95% C.I.)
<45	45	151	29.8	(24.6-35.0)	31.0	(25.7-36.3)
45-54	96	204	47.1	(42.2-52.0)	47.6	(42.7-52.5)
55-64	109	234	46.5	(41.9-51.1)	46.3	(41.7-52.2)
65+	183	370	49.5	(45.9-53.1)	48.6	(45.0-52.2)
Total	433	959	45.2	(43.0-47.5)		
(X^2_{Trend} (1 df) =			17.6		12.0	
p =			0.0001		0.0005	

Table 4

Prevalence rates for H.pylori infection in relation to social class among 959 subjects in Italy

Social class Category	No. positive for H.pylori	Total	Crude prevalence rate %	(95% C.I.)	Prevalence adjusted for age %	(95% C.I.)
High	55	143	38.5	(32.8-44.2)	41.2	(35.4-47.0)
Middle	101	241	41.9	(37.5-46.4)	42.5	(38.0-47.0)
Low	277	575	48.2	(45.3-51.1)	47.1	(44.2-50.0)
Total	433	959	45.2	(43.0-47.5)		
(X^2_{Trend} (1 df) =			5.64		2.6	
p =			0.02		0.11	

3.3 Prevalence of H.pylori in relation to socio-demographic information

3.3.1 Age

Table 3 shows the prevalence of H.pylori infection rates in relation to age. Those aged 30-45 years had a prevalence rate of 31.0% (adjusted for social class). At older age groups (45+ years), the prevalence of H.pylori infection remained constant, at about 46-49%, and a test for linear trend was statistically significant after adjustment for social class (X^2_{Trend} = 12.0; 1df; p = 0.0005).

3.3.2 Sex

The study population comprised 558 men and 401 women of mean age of 59.5 and 57.8 years, respectively. There were no significant differences in H.pylori infection rates in relation to sex, these being 46.4% (95% C.I. = 43.4-49.4%) and 43.4% (95% C.I. = 39.9-46.9) for males and females, respectively (X^2 = 0.1 ; 1df; p = 0.7).

3.3.3 Social class

Table 4 shows the prevalence of H.pylori infection in relation to social class. Prevalence rates (adjusted for age) were highest (47.1%) in the lowest social class category, intermediate (42.5%) in the middle class category and lowest (41.2%) in the highest social class category. A Chi-square test for trend in infection rates with social class was statistically significant (5.6; 1 df; p = 0.02), but was non-significant after adjustment for age (X^2_{Trend} =

Figure 1
Seroprevalence of H.pylori in Italy

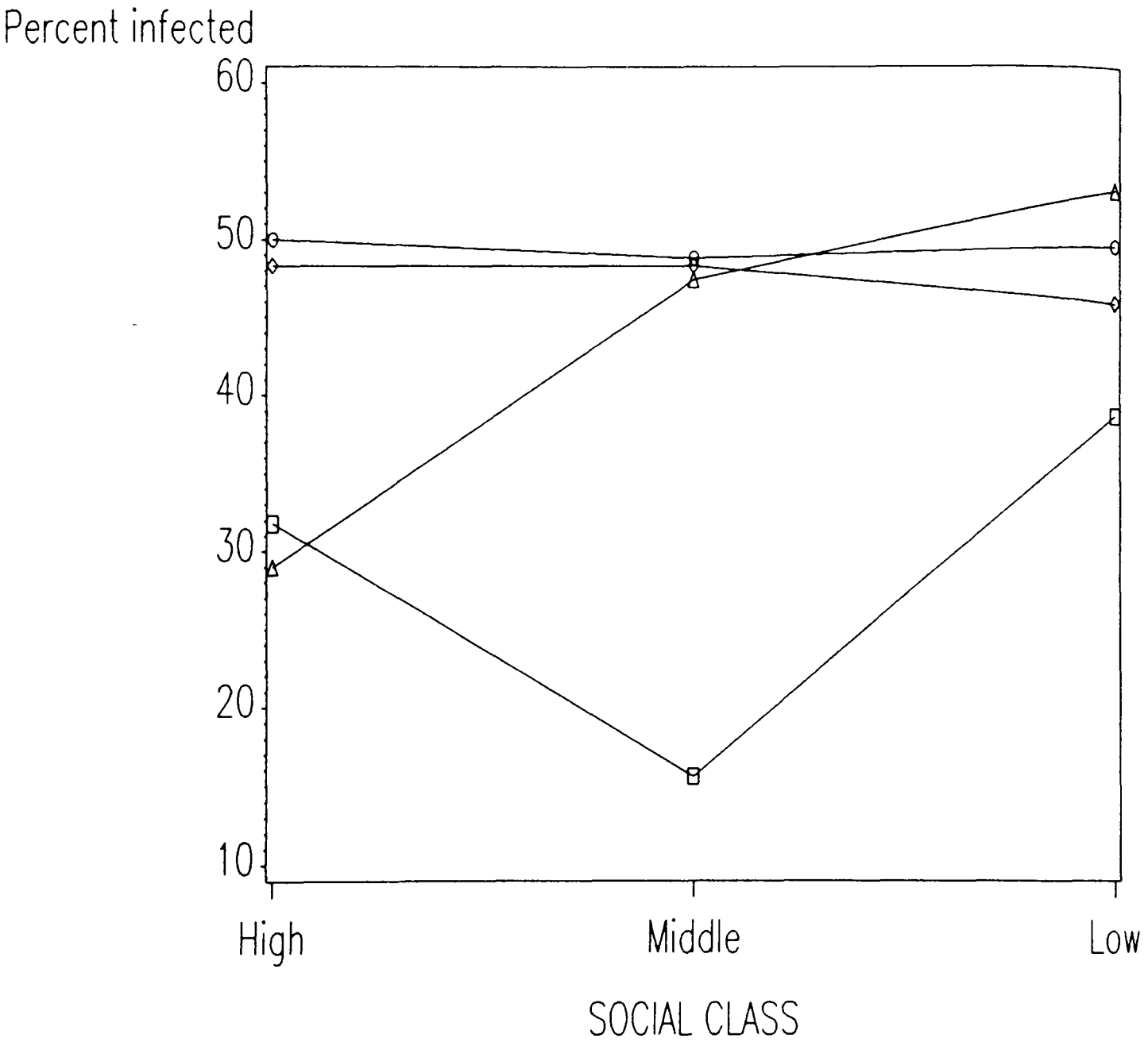


Table 5

H.pylori infection rates in relation to number of siblings in family

Number of siblings	N	% infected
0-1	351	39.9
2-3	282	49.7
4+	326	46.9

X^2_{2Trend} = 3.5; 1 df; p = 0.06 (Unadjusted)
X^2_{Trend} = 2.4; 1 df; p = 0.12 (Adjusted for age (continuous) sex, place of birth, class)

2.6; 1df; p = 0.11).

3.3.4 Age and social class interaction

Figure 1 shows the prevalence rates for H.pylori on an age specific basis. Prevalence rates in those over 54 years were similar and for the sake of simplicity they have been shown in a single category in Figure 1. In those aged 45-54 there appeared to be a trend in relation to social class, with those in the low social class having almost twice the infection rate when compared with those in the high social class (53.2 versus 29.0%), and those in the middle social class category showing intermediate rates (47.4%). In the youngest age group (<45 years) those in the middle social class had the lowest rates (15.6%) followed by those in the highest social class (31.8%) and those in the lowest social class (38.7%).

A statistical model for interaction was fitted in which those older than 45 were grouped into a single category, the a priori hypothesis being that if there was an interaction it would have been evident in those in the youngest age group. However, addition of an interaction term after adjustment for age and social class did not result in a significant improvement in fit (X^2 = 0.12; 3df; p = 0.7).

3.4 Household density

Table 5 shows the prevalence of H.pylori in relation to the number of the subject's siblings. Those with 0-1, 2-3,

Table 6

Relationship between H.pylori and dietary factors

Item	Prevalence of H.pylori infection (%) by tertile of consumption			Test for trend (Unadjusted)		Test for trend (Adjusted*)	
	Low	Med	High	X^2(1 df)	p	X^2(1 df)	p
Bread and pasta	41.2	46.5	47.8	2.9	0.09	1.8	0.2
Trad. Soup	56.7	58.0	49.8	2.9	0.09	1.1	0.3
Meats	45.6	43.9	45.9	0.0	0.9	0.02	0.89
Dried meat	46.6	45.6	43.3	0.7	0.4	0.06	0.8
Dried fish	43.5	42.4	49.5	2.3	0.13	1.73	0.19
Fresh fish	46.9	45.6	43.2	0.9	0.3	0.39	0.5
Dairy products	41.2	49.5	44.7	0.7	0.4	1.12	0.3
Mature cheeses	43.9	46.1	45.2	0.08	0.8	0.24	0.6
Raw vegetables	45.6	41.5	48.3	0.5	0.5	1.14	0.3
Cooked vegetables	42.6	43.4	49.4	2.9	0.09	1.99	0.16
Frozen vegetables	48.9	42.7	46.9	0.03	0.9	0.42	0.5
Legumes	41.3	46.8	47.3	2.3	0.13	2.23	0.14
Spices	46.6	45.8	43.0	0.8	0.4	0.28	0.6
Garlic, onions	44.9	44.1	46.7	0.2	0.7	0.02	0.9
Fresh fruit	43.0	46.7	45.8	0.5	0.5	0.84	0.4
Dried fruit	45.9	45.3	44.3	0.2	0.7	0.09	0.8
Desserts	45.6	44.7	45.2	0.0	0.9	0.05	0.8
Citrus fruit	41.4	47.1	47.0	2.1	0.15	3.35	0.07
Salt taste	43.2	45.7	45.1	0.1	0.8	0.3	0.6
Alcohol	43.9	48.3	43.3	0.0	0.9	0.3	0.6

* Adjusted for age (continuous), sex, place of birth, class.

and 4 or more siblings had prevalence rates of H.pylori of 39.4, 49.7 and 46.9%, respectively. There was a positive trend with increasing number of siblings, (X^2_{Trend} = 3.5; 1 df; p = 0.06) but this trend became non-significant after adjustment for age, sex, place of birth and social class (X^2_{Trend} = 2.4; 1 df; p = 0.1).

3.5 Dietary factors

Table 6 shows the prevalence rates for H.pylori in relation to tertiles of consumption of 18 food groups, to the habit of adding salt at the table (seldom, sometimes, always) and to the subject's salt preference. Also shown in Table 6 are unadjusted and adjusted (for age sex, social class and place of birth) Chi-squared tests for trend in the prevalence of H.pylori infection, in relation to tertiles of consumption. There was a marginally significant positive trend in relation to increasing bread and pasta consumption and a negative trend in relation to consumption of traditional soups (in both instances X^2_{Trend} = 2.9; 1 df; p = 0.09), but the adjusted trends were not significant for either items. There was a positive trend in relation to consumption of dried fish, cooked vegetables and legumes, but in all three instances the trend remained non-significant both before and after adjustment (adjusted p = 0.19, 0.16 and 0.14, respectively). There was a positive trend in relation to consumption of citrus fruit and this was of borderline significance after adjustment (X^2_{Trend} = 3.35; 1df; p = 0.07).

Table 7

Relationship between H.pylori and smoking status

	N	% infected	O.R.
never	372	50.3	1
ex	276	37.8	0.56 (p=0.05)*
current	311	45.7	0.71 (p=0.001)*

*Adjusted for age (continuous), sex, place of birth, class.

Table 8

Relationship between H.pylori and storage of leftover food

Storage of leftover food	N	% infected
Often/always	604	45.7
Seldom/never	355	44.2

X^2T =0.2; 1df; p = 0.7 (Unadjusted)
X^2T =0.7; 1df; p = 0.4 (Adjusted for age (continuous), sex, place of birth, class)

Table 9

Relationship between H.pylori and storage conditions

Age of acquisition of fridge (years)	N	% infected
<32	473	41.4
32-42	259	44.0
42+	227	54.2

X^2T = 9.2; 1df; p = 0.002 Unadjusted;
X^2T = 1.2; 1df; p = 0.27 Adjusted for age (continuous), sex, place of birth, class.

Table 10

Relationship between H.pylori infection and reported medical history

	% infected without disorder	% infected with disorder	N with disorder	X^2T(1df)	p	X^2T(1df)*	p
Gastric resection	45.2	42.9	21	0.1	0.8	0.2	0.7
Peptic ulcer	45.0	50.0	6	0.1	0.8	0.0	1.0
Duodenal ulcer	44.7	50.0	90	0.9	0.3	0.5	0.5
Gastric ulcer	44.8	60.0	20	1.8	0.2	0.8	0.4
Chron. atroph. gastrit.	45.1	66.7	3	0.6	0.5	0.77	0.4
Gastritis n.o.s.	44.5	47.8	180	0.6	0.4	0.6	0.4
Quetelet Index		0.8		0.3		1.0	0.3

* Adjusted for age (continuous), sex, place of birth, class.

3.6 Lifestyle factors (alcohol and smoking)

Alcohol consumption was not related to H.pylori (X^2_{Trend} = 0.3; 1df; p = 0.6), Table 6. Current smokers (Table 7) had significantly lower prevalence rates when compared to those who never smoked (45.7% versus 50.3%, respectively, (X^2 adjusted for age, sex, class and place of birth = 10.8; 1 df; p = 0.001). Ex-smokers had the lowest H.pylori infection rates (37.7%) and the difference between ex-smokers and those who never smoked was statistically significant (X^2 adjusted for age, sex, class and place of birth = 3.8; 1 df; p = 0.05).

3.7 Food storage methods

There was a positive trend of H.pylori infection in relation to age of acquisition of a refrigerator, but this relationship was not statistically significant after adjustment for age, social class, place of birth and sex (X^2_{Trend} = 1.2; 1df; p = 0.27). No association was found in relation to habit of storing leftover foods in the refrigerator (X^2_{Trend} = 0.7; 1df; p = 0.4, table 8 and 9).

3.8 Family and medical history

Table 10 shows the prevalence rates for H.pylori infection in relation to a medical history of peptic ulcer, gastritis and gastric resection. Prevalence rates for H.pylori were elevated in those who reported a history of ulcers (duodenal, gastric and peptic) and chronic gastritis

Table 11

H.pylori infection rates in relation to gastric cancer in family

Number affected	N	% infected
0	856	48.2
1	96	41.7
2+	7	100.0

X^2_{Trend} = 0.7; 1 df; p = 0.4 (Unadjusted)
X^2_{Trend} = 0.01; 1 df; p = 0.9 (Adjusted for age (continuous) sex, place of birth, class)

when compared to those who did not, but none of these differences reached statistical significance. Prevalence rates in those who reported non-specific gastritis and in those who underwent gastric resection were marginally lower than those who did not, but again, none of these differences were statistically significant.

Table 11 shows the prevalence of H.pylori in relation to a family history of gastric cancer, expressed as the number of first degree relatives affected (0, 1, 2+). Ninety-six subjects reported having had a relative with gastric cancer and seven reported having two or more relatives with gastric cancer. Prevalence rates for H.pylori in those 96 subjects with one relative with gastric cancer were 41.7%, slightly lower than those 856 who reported no gastric cancer among first degree relatives (48.2%) but rates were 100% in those seven who reported having two or more relatives with gastric cancer. This trend in relation to gastric cancer in the family was however, not statistically significant.

4 DISCUSSION

4.1 Completeness of information

This is the second study of H.pylori prevalence rates measured in a large randomly selected population. Plasma was available from 63.7% of the total population, but the age distribution between those who did and did not have plasma available for analysis was statistically significant, with older people being less keen to provide a blood sample. However, it is unlikely that refusal to participate in this study was related to H.pylori status. Therefore this bias towards younger people participating is unlikely to have affected the conclusions in this study.

4.2 H.pylori and age

Age was the most important factor influencing the prevalence of H.pylori in this population. Overall, the prevalence was 45.2%. However, the subjects in this study were matched controls of gastric cancer cases. Gastric cancer is a disease of older people and because H.pylori infection is related to age, estimates of the prevalence of H.pylori will reflect the age structure of the sample. In order to correct for this difference in age structure and to allow for comparisons with the study from Caerphilly described in Chapter 3, the age structure of the 'World Standard Population' (Doll and Smith 1982) was used to standardise both the Italian and Caerphilly prevalence rates. This adjustment yielded an age standardised prevalence rate (ASPR) of those aged 30-75 of 41.9%, which

is 17.8% lower than the ASPR for Caerphilly (Chapter 3) of 59.7%. However, because the Italian population was slightly biased towards the younger age groups the prevalence of 41.9% was possibly an underestimate of the true level of infection in this population.

4.3 H.pylori and social class

Although the results were statistically non-significant ($p = 0.11$), those in the lowest social class category had higher prevalence rates of H.pylori than those in the highest class category. These data were in keeping with the differences in relation to social class found in the study from Caerphilly (Chapter 3). The lack of statistical significance in the Italian study could be ascribed to several reasons: First, there could have been methodological differences in ascertaining social class status between the two studies. In the Caerphilly study occupation was derived by reference to occupation alone (OPCS 1981) but in this study, educational status was also taken into consideration in determining social class categories. However this case-control study of gastric cancer and diet (Buiatti et al 1989b) did find a significant inverse association between social class and gastric cancer risk consistent with other studies on gastric cancer in the UK and other countries (Logan 1982, Leon 1989, Howson et al 1986).

Second, the differences in relation to social class in Caerphilly were primarily due to the differences in the

youngest age group (30-35 years). In the Italian study, there were only seven subjects under the age of 35 (compared with 106 in Caerphilly) and it is possible that the data were numerically too sparse to show a relationship with social class (or with any other variable), as was found in the study from Caerphilly. Evidence of early acquisition of infection (relationship between prevalence of infection and number of siblings) was not as clear as in the Caerphilly study, but it may still be possible that age and cohort effects were operating in this population.

4.4 Geographical distribution of H.pylori

If H.pylori associated chronic active gastritis progresses to chronic atrophic gastritis, then one might have expected a correlation between regional H.pylori prevalence rates and gastric cancer rates. There was little variation in the prevalence of H.pylori in relation to gastric cancer risk, or in relation to urban/rural residence. The development of gastric cancer is likely to be related to environmental conditions that existed in the past; thus current prevalence rates of H.pylori may have been an inappropriate indicator of past infection, especially in a country such as Italy which has been undergoing rapid economic changes.

Because of the multifactorial aetiology of gastric cancer, and the relatively low variation in gastric cancer rates between these centres when compared to, for example,

China (Chapter 5) it may be inappropriate to have expected a straightforward correlation between H.pylori prevalence and gastric cancer mortality, especially on the basis of only five data points (one data point per participating centre). In addition the age distribution of this population may have been too old to show any geographical variation in H.pylori prevalence rates.

4.5 Dietary and lifestyle factors

In this study there was a positive association between H.pylori prevalence and consumption of citrus fruits. Citrus fruits in this study comprised the bulk of the vitamin C intake of this population (D. Palli pers comm.) These results were in contrast to the data from Caerphilly, where those with H.pylori consumed less vitamin C than those without. In other studies reviewed in the Introduction (Chapter 1) and the Caerphilly study (Chapter 3), those with CAG consumed less vitamin C than those with normal stomachs. Whereas the pattern of vitamin C consumption in the UK is inversly related to social class (Fehily et al 1984, Ministry of Food and Agriculture, 1988) this did not appear to be so in Italy. In Italy, consumption of vitamin C (derived mainly from citrus fruit) was highest in the poorer and more southerly centres (Cagliari) and lowest in the more affluent northern centres, in keeping with low gastric cancer rates in the south and high gastric cancer rates in the north (Buiatti et al 1990b). These regional or socioeconomic differences in consumption of vitamin C (or

other nutrients) could explain the differences in relation to H.pylori found in different studies.

4.5.1 Lifestyle factors

In this study, ex- and current smokers had significantly lower rates of H.pylori infection, but there was no evidence of a trend in relation to tobacco use (non, ex-, current smokers). However a negative association in relation to having ever smoked was in contrast to the study from Caerphilly where no association was found in relation to smoking (Chapter 3). An explanation of this contrasting finding could be that in Italy smoking was particularly prevalent amongst those aged 30-39 years, and had no obvious trend in relation to social class, as measured by years of education (La Vecchia 1985). It may be that even if these factors were adjusted for in the assessment of trends, cigarette smoking in the young may have been a measure of other lifestyle factors that were inversely associated with H.pylori infection.

4.6 Household density

Although the relationship between H.pylori and number of siblings was not as striking as that found in the Caerphilly study (Chapter 3), there was a positive (but non significant) trend ($p = 0.12$) between number of siblings and H.pylori prevalence rates; those with only one sibling having lower infection rates than those with two or more siblings. If age of acquisition (or period if a cohort

effect) was an important factor in H.pylori prevalence, then the Italian subjects may have been too old to show a positive relationship with household size.

4.7 History of gastroduodenal disorders

Although there were no significant differences in H.pylori infection rates between those who had a history of peptic (not otherwise specified), duodenal, gastric ulcers, chronic atrophic gastritis and unspecified gastritis, those with these complaints had slightly higher prevalence rates of H.pylori than those without. These relatively small differences in prevalence of H.pylori between those with and without gastroduodenal disorders is surprising, considering the prominent role it is thought to play in duodenal (Wyatt 1989, Graham 1989), gastric ulceration (Graham 1989) and chronic atrophic gastritis (Chapter 1). This poor relationship between gastroduodenal disease and H.pylori could, again, have been due to the study population being too old, diluting any association between infection and disease. No attempt was made to confirm the medical history of those not reporting any complaints, and this could have also diluted the putative associations between H.pylori and gastroduodenal disease.

4.8 H.pylori in relation to gastric cancer in family

Although there were no significant trends between number of relatives with gastric cancer and H.pylori infection, all seven subjects with two or more first degree relatives with

gastric cancer had H.pylori infection. This unusual familial clustering of gastric cancer in relation to H.pylori and intestinal metaplasia was noted by Scott et al (1990) who investigated a family in which two out of four siblings had gastric cancer. H.pylori was noted in five, and intestinal metaplasia noted after five years in three out of the eight children (aged 10-26 years) of these affected families, suggesting a genetic predisposition to intestinal metaplasia and gastric cancer. Scott et al (1990) suggested that H.pylori acted as a promoter in the progression from a normal to metaplastic epithelium by inducing a hyperproliferative state in the gastric mucosa. However this family cluster syndrome could also be explained as being due to genetic predisposition to H.pylori infection.

5 SUMMARY

The aims of this study were to determine the distribution of H.pylori infection in relation to sociodemographic, dietary and lifestyle characteristics in a population from Italy.

H.pylori was strongly related to age, but only slightly to social class. Social class in Italy is not routinely measured and even in this study the gradients between the upper and lower social class groups may not be comparable to data from the U K. In addition, the age of the subjects may have been too old to show any heterogeneity in infection rates in relation to social class.

H.pylori infection rates were higher, but not significantly so, in areas of high gastric cancer rates. In addition to the Italian population being too old to show any heterogeneity in H.pylori infection rates, the variation in gastric cancer may have been too small, when compared to a country like China, to show significant differences in infection rates between high and low gastric cancer risk areas.

Associations between H.pylori and dietary and lifestyle characteristics contrary to those shown in the study from Caerphilly have been shown in relation to smoking and vitamin C consumption, probably due to different sociodemographic and lifestyle characteristics of the study

populations.

H.pylori infection rates were higher in those with gastroduodenal complaints when compared to those without, and H.pylori was found in all those with two or more relatives with gastric cancer.

CHAPTER 5

GEOGRAPHIC ASSOCIATION OF HELICOBACTER PYLORI ANTIBODY PREVALENCE AND GASTRIC CANCER MORTALITY IN RURAL CHINA

1 INTRODUCTION

Infection of the human gastric mucosa by the bacterium *Helicobacter pylori* can cause chronic antral gastritis (Dooley and Cohen 1988, Chapter 1). Eradication of *H.pylori* is rarely achieved by normal immunological defence mechanisms (Morgan and Leunk 1989, Newell and Stacey 1989) and in the absence of specific chemotherapy an established infection can act as a persistent inflammatory stimulus to the gastric epithelium. *H.pylori*-induced gastritis may eventually progress to chronic atrophic gastritis (CAG) (Paull and Yardley 1989) a precursor lesion for gastric cancer (Siurala *et al* 1985, Chapter 1). *H.pylori* infection might, therefore, be a cause of human gastric cancer (Paull and Yardley 1989, Correa and Ruiz 1989, Parsonnet 1989) and some preliminary studies in communities at increased risk of gastric cancer have indicated a high prevalence of *H.pylori* infection Correa and Ruiz, 1989, Fox *et al* 1989) especially at young ages (Gutierrez *et al* 1988, Klein *et al* 1989, Dehesa *et al* 1989). In Chapter 1, the pooled odds ratio of the relationship between *H.pylori* and gastric cancer in subjects attending endoscopy clinics was calculated as 3.2, but there has not been a systematic attempt to examine the relationship between *H.pylori* and gastric cancer in non-clinical populations.

This chapter describes a geographic correlation of gastric cancer mortality rates in 46 rural Chinese counties

with H.pylori antibody prevalence rates in a randomly selected sample of the population. The aims of the study were:

1. to correlate the seroprevalence of H.pylori with mortality from gastric cancer;
2. to correlate the seroprevalence of H.pylori with mortality from other diseases, in particular, peptic ulcer disease;
3. to correlate the prevalence of H.pylori to 286 other blood, urinary, dietary and lifestyle factors, and
4. to correlate the levels of plasma pepsinogen to gastric cancer mortality.

2 SUBJECTS AND METHODS

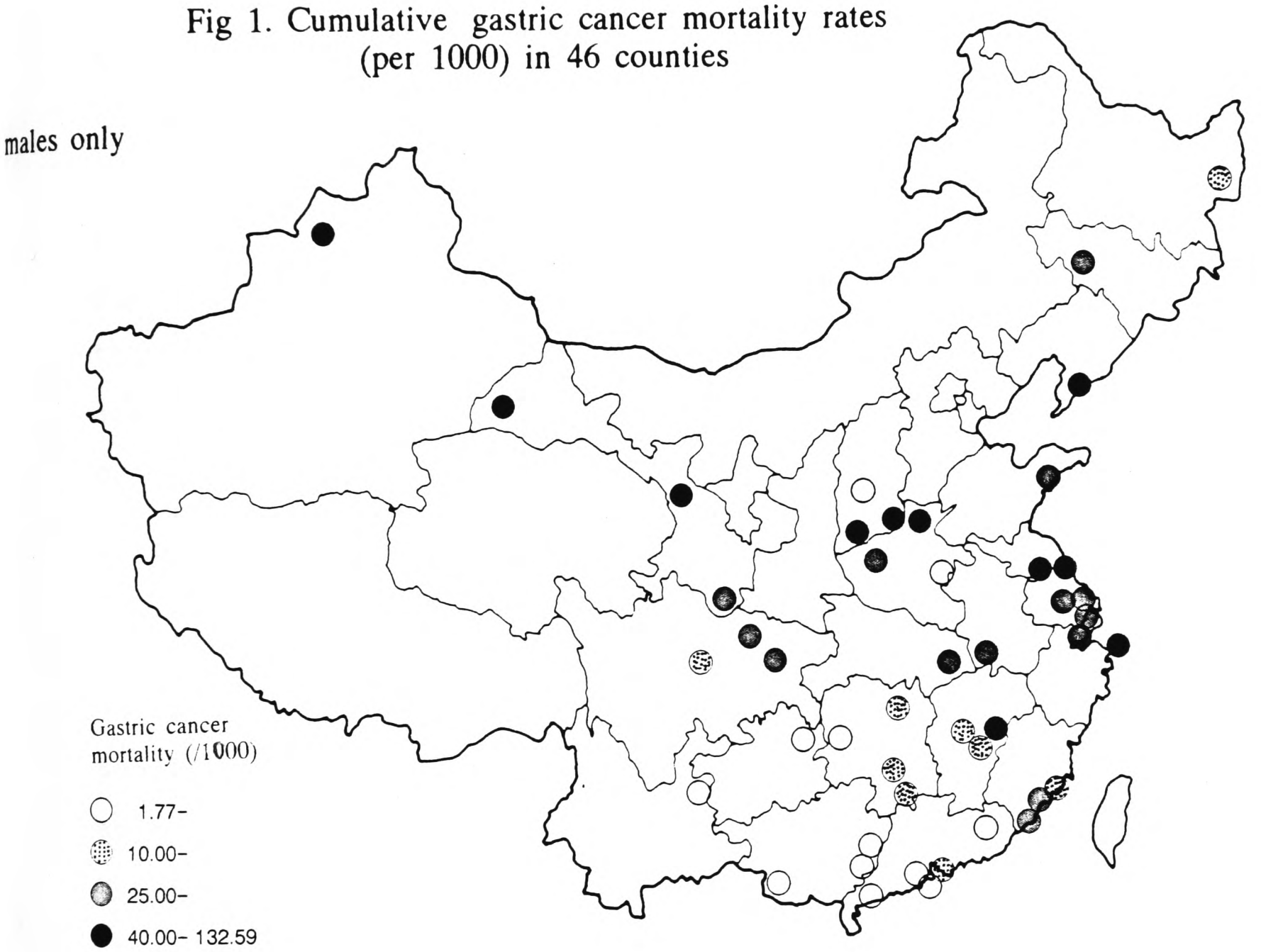
2.1 Subjects

Blood samples were collected as part of a large geographical correlation survey in rural China in the period September to December 1983, the methods of which have been described in detail elsewhere (Chen et al, 1990). Sixty-five out of approximately 2,000 counties throughout China were selected for the survey on the basis of a wide variation between their mortality rates from seven major types of cancer (including gastric cancer) during the period 1973-75 (Li et al 1981). A random sampling procedure was used to identify two villages in different communes within each county and then an age-stratified sample of approximately 25 males and 25 females from each village (total: about 50 males and 50 females per county) were invited to participate. All participants were in the age range 35-64 years and were divided approximately equally into three male and three female ten-year age groups: 35-44, 45-54 and 55-64 years.

2.2 Blood collection

A 10 ml fasting venous blood sample was collected from each participant into a heparinised vacutainer. On the day of collection, plasma fractions were aliquoted and stored at -20°C. After several weeks, the frozen samples were transferred to Beijing where they were stored for some years at -30°C.

Fig 1. Cumulative gastric cancer mortality rates (per 1000) in 46 counties



Variation in:	min	max	diff
Gastric cancer mortality (/1000)	2.8	69.4	25X
Peptic ulcer mortality (/1000)	1.5	29.0	19X

For the present study, individual plasma samples were available from only male participants, and from only 46 of the 65 counties (the samples from females and from the other counties having been depleted), shown in Figure 1. In 44 counties samples were available from both villages in the county while in the other two counties plasma was available from only a single village.

A total of 1882 samples from the 46 counties were air-freighted on dry ice to the UK and were analysed for H.pylori antibody status in mid-1989, that is, slightly less than six years after collection. Prior to thawing out for analysis the samples had undergone only one thaw-freeze cycle in Beijing.

2.3 Laboratory assays

2.3.1 H.pylori IgG antibody tests

Specific anti-H.pylori antibodies for each individual sample were analysed with an enzyme-linked immunosorbant assay (ELISA), using an acid-extractable cell surface H.pylori antigen. The method was the same as the one used in all the other studies (Chapters 2-7, Appendix 1) except that the samples were diluted 1 in 200. An optical density reading of 0.6 or more was used to define a positive antibody response (Newell et al, 1990). This corresponded to a value of 10 μg IgG/ml of plasma (Steer et al 1987).

2.3.2 Pooled blood and urinary samples

For each village, equal aliquots of the individual blood and urine samples were pooled into age (35-44, 45-54, 55-64 years) and sex specific pools for biochemical analysis of 26 urinary and 110 blood constituents shown in Table 1. Thus a 'pooled' level of a particular constituent would reflect the mean village/age/sex specific level for that particular constituent.

2.3.3 Tests for plasma pepsinogen A

Serum or plasma pepsinogen A (PGA) levels have been used as serological markers of chronic atrophic gastritis in other studies, reviewed in Chapter 1. PGA levels are affected at the late stages of gastric atrophy, and therefore, if there were any correlation between PGA and gastric cancer, it would have been expected to be most obvious in the oldest age group.

In order to measure the correlation between pepsinogen levels and gastric cancer mortality, pooled blood samples (Section 2.3.2) rather than individual samples (used to measure H.pylori antibody levels, Section 2.3.1), were made available for the oldest age group (55-64 years) from 63 out of the total of 65 counties. In 46 counties, there was sufficient pooled plasma from both villages in each county, and for the remaining 17 counties, sufficient pooled plasma was only available from onr of the two villages.

Plasma PGA levels in the pooled samples were analysed using an identical ELISA technique (Westerveld et al 1987, Pals et al 1985) described in Appendix 1 and used in Chapter 2.

2.4 Calculation of H.pylori prevalence rates

The percentage of samples positive for H.pylori in each village was used as an estimate of the village prevalence rate and the mean of the two village prevalence rates was used as an estimate of the county prevalence rate. As a test of consistency between villages in the same county, the prevalence rates in each of the two villages were correlated against each other for the 44 counties with samples for both villages. Because the age structure of those selected for study within each county was similar, comparisons between H.pylori prevalence rates in different counties were not confounded by age.

2.5 Calculation of mortality rates

Mortality rates in each of these counties from various diseases had been recorded in a nationwide survey in 1973-5, and all were standardised for age by calculating the cumulative mortality rate per 1,000 subjects by 65 years of age (Chen et al, 1990). A cumulative mortality rate of 40 per 1,000 for a particular disease would, for example, correspond to a probability of about 4% of dying from that disease before 65 years of age, if all other causes of death had been eliminated.

2.6 Statistical tests

This study comprised one aspect of a much larger correlation study comparing the mortality of 78 causes of death to 286 blood, urinary dietary and demographic characteristics in rural China (Chen et al 1990). For the sake of consistency, standard computer programmes were written to calculate correlation coefficients (r and corresponding 2-sided significance tests) between mortality and all the other variables in question (Chen et al 1990). The same correlation coefficients were used to correlate the prevalence of H.pylori between village pairs to the 78 mortality variables and to the 286 blood, urinary lifestyle and demographic characteristics, as shown in Table 1.

Chi-squared trend tests (1 df) were used, to test for linear trends of H.pylori infection in relation to age. All reported significance levels (2p) are based on two-sided significance tests.

3 RESULTS

3.1 Gastric cancer rates

For gastric cancer, the cumulative mortality rates per 1,000 by age 65 for the 46 counties ranged from 3 to 69 (mean = 22, sd = 16).

3.2 H.pylori prevalence rates

Of the 1,882 individual samples analysed, 1,137 (60%) were positive for H.pylori IgG antibodies. Table 2 shows the percentage of positive samples, broken down by five-year age group. There was a significant increase in H.pylori prevalence with age, from 44.2% in those aged 35-39 to 62.5% in those aged 60-64 years (X^2_{Trend} = 9.7; 1 df; p = 0.002).

3.3 Within-village variability

There was a seven-fold variability of H.pylori prevalence of infection between villages, the proportion of individuals positive for H.pylori antibodies ranging from 14% to 100% (mean = 60%, sd = 22.1%). A correlation coefficient (r) for the comparison of the H.pylori prevalence rates between the two villages within each of 44 counties was 0.59 (Figure 2) and was highly significant ($p < 0.001$). This demonstrated that the variation between these counties in H.pylori antibody prevalence rates chiefly represented real geographic variation and not just random scatter. The prevalence of H.pylori antibodies in the 46 counties ranged from 28% to 96% (mean 60%, sd 19.9%), a 3.4-fold variability.

TABLE 1

Pearson's Correlation Coefficients and 2-sided significance levels (* = 0.05, ** = 0.01, *** = 0.001) of plasma Helicobacter pylori antibody prevalence in 45 Chinese Counties and all variables measured in study.

Pearson's r(%)	Variable
27	- mortality ALL CAUSES PER 1000 POPULATION AGED <1 YEAR (1973-5) (NB Slightly exceeds deaths/1000 births)
18	- mortality ALL CAUSES (cumulative rate, 0-14)/1000
20	- mortality ALL CAUSES (cumulative rate, 0-64)/1000
8	- mortality ALL CANCERS (cumulative rate, 0-14)/1000
17	- mortality ALL CANCERS (cumulative rate, 0-64)/1000
18	- mortality ALL NON-CANCER CAUSES (cumulative rate, 0-14)/1000
12	- mortality ALL NON-CANCER CAUSES (cumulative rate, 0-64)/1000
-22	- mortality NASOPHARYNGEAL CANCER (cumulative rate, 0-64)/1000
8	- mortality OESOPHAGEAL CANCER (cumulative rate, 0-64)/1000
34*	- mortality STOMACH CANCER (cumulative rate, 0-64)/1000
16	- mortality LIVER CANCER (cumulative rate, 0-64)/1000
-17	- mortality RECTAL CANCER (cumulative rate, 0-64)/1000
-23	- mortality COLON CANCER (cumulative rate, 0-64)/1000
-16	- mortality COLORECTAL CANCER (cumulative rate, 0-64)/1000
-24	- mortality FEMALE LUNG CANCER (cumulative rate, 0-64)/1000
-20	- mortality MALE LUNG CANCER (cumulative rate, 0-64)/1000
-7	- mortality FEMALE BREAST CANCER (cumulative rate, 0-64)/1000
3	- mortality CERVIX CANCER (cumulative rate, 0-64)/1000
-1	- mortality LEUKAEMIA (cumulative rate, 0-14)/1000
4	- mortality LEUKAEMIA (cumulative rate, 0-64)/1000
19	- mortality BLADDER CANCER (cumulative rate, 0-64)/1000
-23	- mortality PENIS CANCER (cumulative rate, 0-64)/1000
11	- mortality LYMPHOMA (cumulative rate, 0-14)/1000
32	- mortality LYMPHOMA (cumulative rate, 0-64)/1000
24	- mortality CHORIOEPITHELIOMA (cumulative rate, 0-64)/1000
1	- mortality BRAIN CANCER (cumulative rate, 0-14)/1000
4	- mortality BRAIN CANCER (cumulative rate, 0-64)/1000
-5	- mortality PULMONARY TUBERCULOSIS (cumulative rate, 0-14)/1000
10	- mortality PULMONARY TUBERCULOSIS (cumulative rate, 0-64)/1000
-7	- mortality TUBERCULOSIS OTHER THAN PULMONARY (cumulative rate, 0-14)/1000

-7 - mortality TUBERCULOSIS OTHER THAN PULMONARY (cumulative rate, 0-64)/1000
8 - mortality INFECTIOUS DISEASE OTHER THAN TUBERCULOSIS (cumulative rate, 0-14)/1000
2 - mortality INFECTIOUS DISEASE OTHER THAN TUBERCULOSIS (cumulative rate, 0-64)/1000
-23 - mortality SCHISTOSOMIASIS (cumulative rate, 0-64)/1000
-6 - mortality PARASITIC DISEASE OTHER THAN SCHISTOSOMIASIS (cumulative rate, 0-14)/1000
-8 - mortality PARASITIC DISEASE OTHER THAN SCHISTOSOMIASIS (cumulative rate, 0-64)/1000
19 - mortality EXTERNAL CAUSES (cumulative rate, 0-14)/1000
17 - mortality EXTERNAL CAUSES (cumulative rate, 0-64)/1000
19 - mortality DIABETES (cumulative rate, 0-64)/1000
32 - mortality METABOLIC AND ENDOCRINE DISEASE OTHER THAN DIABETES (cumulative rate, 0-14)/1000
29 - mortality METABOLIC AND ENDOCRINE DISEASE OTHER THAN DIABETES (cumulative rate, 0-64)/1000
-1 - mortality DISEASE OF BLOOD AND BLOOD FORMING ORGANS (cumulative rate, 0-14)/1000
11 - mortality DISEASE OF BLOOD AND BLOOD FORMING ORGANS (cumulative rate, 0-64)/1000
-13 - mortality BRAIN AND NEUROLOGICAL DISEASE (cumulative rate, 0-14)/1000
-16 - mortality BRAIN AND NEUROLOGICAL DISEASE (cumulative rate, 0-64)/1000
-15 - mortality MYOCARDIAL INFARCTION AND CORONARY HEART DISEASE (cumulative rate, 0-64)/1000
11 - mortality HYPERTENSIVE HEART DISEASE (cumulative rate, 0-64)/1000
-7 - mortality RHEUMATIC HEART DISEASE (cumulative rate, 0-64)/1000
17 - mortality CONGENITAL HEART DISEASE (cumulative rate, 0-14)/1000
-22 - mortality KESHAN DISEASE (cumulative rate, 0-14)/1000
-30 - mortality KESHAN DISEASE (cumulative rate, 0-64)/1000
33 - mortality OTHER HEART DISEASE (cumulative rate, 0-14)/1000
(other than specified in 0046-0051)
21 - mortality OTHER HEART DISEASE (cumulative rate, 0-64)/1000
(other than specified in 0046-0051)
8 - mortality STROKE (cumulative rate, 0-64)/1000
0 - mortality HYPERTENSIVE HEART DISEASE AND MI/CHD (cumulative rate, 0-64)/1000
6 - mortality STROKE, HYPERTENSIVE HEART DISEASE AND MI/CHD (cumulative rate, 0-64)/1000
-5 - mortality PNEUMONIA (cumulative rate, 0-14)/1000
-3 - mortality PNEUMONIA (cumulative rate, 0-64)/1000
10 - mortality PNEUMOCONIOSIS AND DUST DISEASE (cumulative rate, 0-64)/1000
-6 - mortality CHRONIC BRONCHITIS, EMPHYSEMA AND RESPIRATORY HEART DISEASE (cumulative rate, 0-64)/1000
-3 - mortality OTHER RESPIRATORY DISEASE (cumulative rate, 0-14)/1000
(other than chronic bronchitis, emphysema and respiratory heart disease)
3 - mortality OTHER RESPIRATORY DISEASE (cumulative rate, 0-64)/1000
(other than chronic bronchitis, emphysema and respiratory heart disease)
18 - mortality INTESTINAL OBSTRUCTION (cumulative rate, 0-14)/1000
9 - mortality INTESTINAL OBSTRUCTION (cumulative rate, 0-64)/1000
19 - mortality CIRRHOSIS OF THE LIVER (cumulative rate, 0-64)/1000
11 - mortality PEPTIC ULCER (cumulative rate, 0-64)/1000
0 - mortality DIGESTIVE DISEASE OTHER THAN PEPTIC ULCER (cumulative rate, 0-14)/1000
9 - mortality DIGESTIVE DISEASE OTHER THAN PEPTIC ULCER (cumulative rate, 0-64)/1000

6	- mortality NEPHRITIS (cumulative rate, 0-14)/1000
15	- mortality NEPHRITIS (cumulative rate, 0-64)/1000
-1	- mortality KIDNEY DISEASE OTHER THAN NEPHRITIS (cumulative rate, 0-14)/1000
-2	- mortality KIDNEY DISEASE OTHER THAN NEPHRITIS (cumulative rate, 0-64)/1000
15	- mortality ECLAMPSIA (cumulative rate, 0-64)/1000
-5	- mortality DISEASE OF PREGNANCY AND BIRTH OTHER THAN ECLAMPSIA (cumulative rate, 0-64)/1000
36*	- mortality CONGENITAL DEFECT, EXCLUDING HEART DISEASE (cumulative rate, 0-14)/1000
16	- mortality TETANUS OF THE NEWBORN (cumulative rate, 0-14)/1000
43*	- mortality PREMATURITY (cumulative rate, 0-14)/1000
36*	- mortality OTHER PERINATAL (cumulative rate, 0-14)/1000 (other than congenital defect, tetanus and prematurity)
26	- mortality OTHER KNOWN CAUSES (cumulative rate, 0-14)/1000 (any specified cause that is not included in causes 0008-0078)
15	- mortality OTHER KNOWN CAUSES (cumulative rate, 0-64)/1000 (any specified cause that is not included in causes 0008-0078)
-5	- mortality CAUSE NOT KNOWN (cumulative rate, 0-14)/1000
-7	- mortality CAUSE NOT KNOWN (cumulative rate, 0-64)/1000
-1	- plasma GLUTATHIONE PEROXIDASE (nmoles NADPH oxidized/10ul/min)
-15	- plasma TOTAL CHOLESTEROL (mg/dl)
10	- plasma HIGH DENSITY LIPOPROTEIN CHOLESTEROL (mg/dl)
-20	- plasma NON-HDL CHOLESTEROL (mg/dl)
25	- plasma TRIGLYCERIDE (mg/dl)
-4	- plasma APOLIPOPROTEIN A1 (mg/dl)
-29*	- plasma APOLIPOPROTEIN B (mg/dl)
-20	- plasma LIPID PEROXIDE (nmol/dl)
-25	- plasma ALPHA CAROTENE (ug/dl)
-25	- plasma BETA CAROTENE (ug/dl)
3	- plasma ALPHA TOCOPHEROL (ug/dl)
-11	- plasma GAMMA TOCOPHEROL (ug/dl)
-11	- plasma RETINOL (ug/dl)
-11	- plasma VITAMIN C (ASCORBIC ACID) (mg/dl)
18	- plasma RETINOL BINDING PROTEIN (mg/dl)
-2	- plasma HEPATITIS B ANTI-CORE ANTIBODY (% of individual samples that were positive; non-pooled analysis)
21	- plasma HEPATITIS B SURFACE ANTIGEN (% of individual samples that were positive; non-pooled analysis)
-6	- plasma CANDIDA ALBICANS ANTIBODIES (Absorbance at 1:100 plasma dilution, 1:16000 antigen coat plate)
-7	- plasma EPSTEIN-BARR VIRUS ANTIBODIES (antibody titre, expressed to log base2)
33*	- plasma TYPE 2 HERPES SIMPLEX VIRUS ANTIBODIES (-1.5+log10 antibody titre)
19	- plasma COPPER (mg/dl)
-8	- plasma IRON (mg/dl)
-19	- plasma POTASSIUM (mg/dl)
-7	- plasma MAGNESIUM (mg/dl)
0	- plasma TOTAL PHOSPHORUS (mg/dl)

-25	- plasma INORGANIC PHOSPHORUS (mg/dl)
-3	- plasma SELENIUM (ug/dl)
12	- plasma ZINC (mg/dl)
7	- plasma FERRITIN (ng/ml)
-5	- plasma TOTAL IRON BINDING CAPACITY (ug/dl)
-16	- plasma GLUCOSE (mg/dl)
7	- plasma UREA NITROGEN (mg/dl)
-12	- plasma CREATININE (mg/dl)
17	- plasma UREA NITROGEN STANDARDISED FOR CREATININE (mg/mg creatinine)
16	- plasma URIC ACID (mg/dl)
-10	- plasma PREALBUMIN (mg/dl)
17	- plasma ALBUMIN (g/dl)
26	- plasma TOTAL PROTEIN (g/dl)
32*	- plasma BILIRUBIN (mg/dl)
-40**	- plasma COTININE (ng/ml)
12	- plasma OESTRADIOL (pg/ml) IN WOMEN AGED 35-44 YEARS
-6	- plasma OESTRADIOL (pg/ml) IN WOMEN AGED 45-54 YEARS
-4	- plasma OESTRADIOL (pg/ml) IN WOMEN AGED 55-64 YEARS
10	- plasma OESTRADIOL (pg/ml) IN WOMEN AGED 35-54 YEARS
-8	- plasma SEX HORMONE BINDING GLOBULIN (nmol/l) IN WOMEN AGED 35-44 YEARS
-2	- plasma SEX HORMONE BINDING GLOBULIN (nmol/l) IN WOMEN AGED 45-54 YEARS
7	- plasma SEX HORMONE BINDING GLOBULIN (nmol/l) IN WOMEN AGED 55-64 YEARS
-3	- plasma SEX HORMONE BINDING GLOBULIN (nmol/l) IN WOMEN AGED 35-64 YEARS
7	- plasma PROLACTIN (ng/ml) IN WOMEN AGED 35-44 YEARS
-31*	- plasma PROLACTIN (ng/ml) IN WOMEN AGED 45-54 YEARS
0	- plasma PROLACTIN (ng/ml) IN WOMEN AGED 55-64 YEARS
-10	- plasma PROLACTIN (ng/ml) IN WOMEN AGED 35-64 YEARS
5	- plasma TESTOSTERONE (ng/dl) IN MEN AGED 35-44 YEARS
-4	- plasma TESTOSTERONE (ng/dl) IN MEN AGED 45-54 YEARS
-42**	- plasma TESTOSTERONE (ng/dl) IN MEN AGED 55-64 YEARS
-18	- plasma TESTOSTERONE (ng/dl) IN MEN AGED 35-64 YEARS
-18	- plasma TESTOSTERONE (ng/dl) IN WOMEN AGED 35-44 YEARS
13	- plasma TESTOSTERONE (ng/dl) IN WOMEN AGED 45-54 YEARS
28	- plasma TESTOSTERONE (ng/dl) IN WOMEN AGED 55-64 YEARS
3	- plasma TESTOSTERONE (ng/dl) IN WOMEN AGED 35-64 YEARS
100***	- plasma HELICOBACTER PYLORI IgG ANTIBODY (% of individual samples that were positive; non-pooled analysis)
15	- red blood cell GLUTATHIONE REDUCTASE [activity coefficient (A/C)]
12	- red blood cell CATALASE (units/g haemoglobin)
-31*	- red blood cell SUPER OXIDE DISMUTASE (units/g haemoglobin)
12	- red blood cell HAEMOGLOBIN (g/dl of whole blood) (measured in individual samples)
-24	- red blood cell TOTAL LIPID PALMITIC ACID (% of total fatty acid by weight)
-15	- red blood cell TOTAL LIPID STEARIC ACID (% of total fatty acid by weight)

3	- red blood cell TOTAL LIPID OLEIC ACID (% of total fatty acid by weight)
23	- red blood cell TOTAL LIPID TETRACOSAENOIC ACID (% of total fatty acid by weight) + contamination by co-elution of docosapentaenoic acid (22:5(6))
-12	- red blood cell TOTAL LIPID LINOLEIC ACID (% of total fatty acid by weight)
22	- red blood cell TOTAL LIPID LINOLENIC ACID (% of total fatty acid by weight)
-11	- red blood cell TOTAL LIPID DI-HOMO-GAMMA-LINOLENIC ACID (% of total fatty acid by weight)
11	- red blood cell TOTAL LIPID ARACHIDONIC ACID (% of total fatty acid by weight)
7	- red blood cell TOTAL LIPID EICOSAPENTAENOIC ACID (% of total fatty acid by weight)
-19	- red blood cell TOTAL LIPID DOCOSATETRAENOIC ACID (% of total fatty acid by weight)
-2	- red blood cell TOTAL LIPID DOCOSAPENTAENOIC ACID (% of total fatty acid by weight)
-3	- red blood cell TOTAL LIPID DOCOSAHEXAENOIC ACID (% of total fatty acid by weight)
-25	- red blood cell TOTAL LIPID SATURATES (% of total fatty acid by weight)
-1	- red blood cell TOTAL LIPID n6 POLYUNSATURATES (% of total fatty acid by weight)
3	- red blood cell TOTAL LIPID n3 POLYUNSATURATES (% of total fatty acid by weight)
1	- red blood cell TOTAL LIPID POLYUNSATURATES (% of total fatty acid by weight)
8	- red blood cell TOTAL LIPID POLYUNSATURATES/SATURATES
22	- red blood cell PHOSPHATIDYLCHOLINE MYRISTIC ACID (% of total phosphatidylcholine fatty acids by weight)
-14	- red blood cell PHOSPHATIDYLCHOLINE PALMITIC ACID (% of total phosphatidylcholine fatty acids by weight)
-37*	- red blood cell PHOSPHATIDYLCHOLINE STEARIC ACID (% of total phosphatidylcholine fatty acids by weight)
-14	- red blood cell PHOSPHATIDYLCHOLINE ARACHIDIC ACID (% of total phosphatidylcholine fatty acids by weight)
-10	- red blood cell PHOSPHATIDYLCHOLINE BEHENIC ACID (% of total phosphatidylcholine fatty acids by weight)
-2	- red blood cell PHOSPHATIDYLCHOLINE MYRISTOLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
15	- red blood cell PHOSPHATIDYLCHOLINE PALMITOLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
-17	- red blood cell PHOSPHATIDYLCHOLINE OLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
11	- red blood cell PHOSPHATIDYLCHOLINE GADOLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
14	- red blood cell PHOSPHATIDYLCHOLINE ERUCIC ACID (% of total phosphatidylcholine fatty acids by weight)
6	- red blood cell PHOSPHATIDYLCHOLINE LINOLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
-4	- red blood cell PHOSPHATIDYLCHOLINE GAMMA-LINOLEIC ACID (% of total phosphatidylcholine fatty acids by weight)
-23	- red blood cell PHOSPHATIDYLCHOLINE EICOSADIENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
3	- red blood cell PHOSPHATIDYLCHOLINE DI-HOMO-GAMMA-LINOLENIC ACID (% of total phosphatidylcholine fatty acids by
17	- red blood cell PHOSPHATIDYLCHOLINE ARACHIDONIC ACID (% of total phosphatidylcholine fatty acids by weight)
2	- red blood cell PHOSPHATIDYLCHOLINE DOCOSATETRAENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
11	- red blood cell PHOSPHATIDYLCHOLINE DOCOSAPENTAENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
9	- red blood cell PHOSPHATIDYLCHOLINE LINOLENIC ACID (% of total phosphatidylcholine fatty acids by weight)
19	- red blood cell PHOSPHATIDYLCHOLINE EICOSAPENTAENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
29	- red blood cell PHOSPHATIDYLCHOLINE DOCOSAPENTAENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
21	- red blood cell PHOSPHATIDYLCHOLINE DOCOSAHEXAENOIC ACID (% of total phosphatidylcholine fatty acids by weight)
-30	- red blood cell PHOSPHATIDYLCHOLINE SATURATES (% of total phosphatidylcholine fatty acids by weight)
-9	- red blood cell PHOSPHATIDYLCHOLINE n9 MONOUNSATURATES (% of total phosphatidylcholine fatty acids by weight)
11	- red blood cell PHOSPHATIDYLCHOLINE n6 POLYUNSATURATES (% of total phosphatidylcholine fatty acids by weight)
25	- red blood cell PHOSPHATIDYLCHOLINE n3 POLYUNSATURATES (% of total phosphatidylcholine fatty acids by weight)
24	- red blood cell PHOSPHATIDYLCHOLINE POLYUNSATURATES (% of total phosphatidylcholine fatty acids by weight)

24	- red blood cell PHOSPHATIDYLCHOLINE POLYUNSATURATES/SATURATES
-12	- urine VITAMIN C EXCESS (ASCORBIC ACID) (mg excreted in first 4 hours after 500mg oral load) (measured in individual samples)
6	- urine VITAMIN B2 EXCESS (RIBOFLAVIN) (mg excreted in first 4 hours after 5mg oral load) (measured in individual samples)
-29	- urine OROTIDINE EXCRETED IN 4 HOURS (umol/kg body weight)
1	- urine CHLORIDE EXCRETED IN 4 HOURS (mg/kg body weight)
-2	- urine POTASSIUM EXCRETED IN 4 HOURS (mg/kg body weight)
-8	- urine MAGNESIUM EXCRETED IN 4 HOURS (mg/kg body weight)
0	- urine SODIUM EXCRETED IN 4 HOURS (mg/kg body weight)
-38*	- urine CREATININE EXCRETED IN 4 HOURS (mg/kg body weight)
-24	- urine VOLUME OF URINE EXCRETED IN 4 HOURS (ml)
-16	- urine NITRATE EXCRETED IN 4 HOURS (mg/kg body weight)
13	- urine ANTI-AFLATOXIN REACTIVE METABOLITES EXCRETED IN 4 HOURS (ng) (AFB, AFM and other reactive metabolites)
8	- urine IODINE EXCRETED IN 4 HOURS (ug/kg body weight)
7	- food composite PLANT PROTEIN INTAKE (g/day)
-3	- food composite TOTAL FIBRE INTAKE (g/day)
3	- food composite TOTAL NEUTRAL DETERGENT FIBRE INTAKE (g/day)
8	- food composite HEMI-CELLULOSE FIBRE INTAKE (g/day)
-3	- food composite CELLULOSE FIBRE INTAKE (g/day)
11	- food composite INTAKE OF LIGNINS REMAINING AFTER CUTIN REMOVED (g/day)
-26	- food composite CUTIN INTAKE (g/day)
12	- food composite STARCH INTAKE (g/day)
-2	- food composite PECTIN INTAKE (g/day)
16	- food composite RHAMNOSE INTAKE (g/day)
9	- food composite FUCOSE INTAKE (g/day)
14	- food composite ARABINOSE INTAKE (g/day)
5	- food composite XYLOSE INTAKE (g/day)
25	- food composite MANNOSE INTAKE (g/day)
7	- food composite GALACTOSE INTAKE (g/day)
4	- food composite BORON INTAKE (mg/day)
27	- food composite CALCIUM INTAKE (g/day)
-9	- food composite COPPER INTAKE (mg/day)
27	- food composite IRON INTAKE (mg/day)
3	- food composite POTASSIUM INTAKE (g/day)
2	- food composite MAGNESIUM INTAKE (g/day)
28	- food composite SODIUM INTAKE (mg/day)
2	- food composite PHOSPHORUS INTAKE (g/day)
0	- food composite SELENIUM INTAKE (ug/day)
4	- food composite ZINC INTAKE (mg/day)
5	- food composite ARSENIC INTAKE (ug/day) (measured with a lower limit of detectability of 0.08 ug/g)

-24	- food composite CADMIUM INTAKE (ug/day) (measured with a lower limit of detectability of 0.03 ug/g)
4	- food composite MERCURY INTAKE (ug/day) (measured with a lower limit of detectability of 0.01 ug/g)
9	- food composite LEAD INTAKE (ug/day) (measured with a lower limit of detectability of 0.04 ug/g)
0	- food composite HEXACHLOROCYCLOHEXANE (ppm) (alpha, beta, gamma (lindane) and delta isomers of BHC)
-21	- food composite 1,1,1-TRICHLORO-2,2-BIS(4-CHLOROPHENYL)ETHANE (ppm) (DDE,p,p'; DDT,o,p'; DDT,p,p'; TDE ,p,p' metabolites of DDT)
-15	- food composite 0,0-DIETHYL-0-(2-ISOPROPYL-6-METHYLPYRIMIDIN-4-YL)PHOSPHOROTHIOATE (ppm) (common name: diazinon)
-25	- food composite ISOMER OF POLYCHLORINATED BIPHENYL (ppm)
3	- diet survey TOTAL PROTEIN INTAKE (g/day) (measured as Kjeldahl N multiplied by 6.25)
2	- diet survey NON-FISH ANIMAL PROTEIN INTAKE (g/day)
2	- diet survey FISH PROTEIN INTAKE (g/day)
1	- diet survey PLANT PROTEIN INTAKE (g/day)
-11	- diet survey TOTAL LIPID INTAKE (g/day)
8	- diet survey 'SOLUBLE' CARBOHYDRATE (nitrogen free extract) INTAKE (g/day)
-2	- diet survey CALORIC INTAKE (kcal/day)
-16	- diet survey PERCENTAGE OF CALORIC INTAKE FROM LIPIDS
-4	- diet survey CRUDE FIBRE INTAKE (g/day)
9	- diet survey CALCIUM INTAKE (mg/day)
4	- diet survey PHOSPHORUS INTAKE (mg/day)
6	- diet survey IRON INTAKE (mg/day)
2	- diet survey RETINOL (VITAMIN A) INTAKE (retinol equivalents/day; animal sources only)
8	- diet survey TOTAL CAROTENOID INTAKE (retinol equivalents/day)
0	- diet survey THIAMINE (VITAMIN B1) INTAKE (mg/day)
4	- diet survey RIBOFLAVIN (VITAMIN B2) INTAKE (mg/day)
-4	- diet survey NIACIN INTAKE (mg/day)
9	- diet survey VITAMIN C (ASCORBIC ACID) INTAKE (mg/day)
7	- diet survey RICE INTAKE (g/day air-dry basis)
1	- diet survey WHEAT FLOUR INTAKE (g/day air-dry basis)
-7	- diet survey OTHER CEREAL INTAKE (g/day air-dry basis)
-4	- diet survey STARCHY TUBER INTAKE (g/day fresh weight)
0	- diet survey LEGUME AND LEGUME PRODUCT INTAKE (g/day fresh weight)
4	- diet survey LIGHT COLOURED VEGETABLE INTAKE (g/day fresh weight)
28	- diet survey GREEN VEGETABLE INTAKE (g/day fresh weight)
-17	- diet survey SEA VEGETABLE INTAKE (g/day fresh weight)
-15	- diet survey SALT PRESERVED VEGETABLE AND DRIED VEGETABLE INTAKE (g/day as-consumed basis)
7	- diet survey FRUIT INTAKE (g/day fresh weight)
-19	- diet survey PROCESSED STARCH AND SUGAR INTAKE (g/day as-consumed basis)
-17	- diet survey NUT INTAKE (g/day as-consumed basis)
4	- diet survey MILK AND DAIRY PRODUCTS INTAKE (g/day as-consumed basis)
-32*	- diet survey EGG INTAKE (g/day as-consumed basis)
-10	- diet survey MEAT INTAKE (g/day as-consumed basis)

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 -5     - diet survey FISH INTAKE (g/day as-consumed basis)
 15     - diet survey RAPESEED OIL INTAKE (g/day)
-32*    - diet survey OIL INTAKE OTHER THAN RAPESEED (g/day) (includes peanut, sesame, cotton seed and soybean oil)
-24     - diet survey SOY SAUCE INTAKE (g/day)
 38**   - diet survey SALT INTAKE (g/day)
-11     - diet survey OTHER FOODS INTAKE (g/day as-consumed basis)
                    (other than specified in 5019-5038)
  2     - diet survey ASCORBATE + plasma ASCORBATE + urinary ASCORBATE = ASCORBATE INDEX
                    (Average of rank for each: 1=lowest, 65=highest)
 37*    - diet survey SALT INTAKE (gm/day: mean=15,sd=5) + urinary SODIUM (where available) (mg/kg/4 hours: mean=15,sd=4)
                    AVERAGE=SALT INDEX
 13     - questionnaire AGE IN 1983 (years)
  0     - questionnaire PERCENTAGE NEVER MARRIED
-33*    - questionnaire PERCENTAGE BORN OUTSIDE COUNTY
  0     - questionnaire HEIGHT (cm.)
  5     - questionnaire WEIGHT (kg.)
-54***  - questionnaire PERCENTAGE WHO HAVE EVER SMOKED ANY FORM OF TOBACCO DAILY FOR MORE THAN 6 MONTHS
-49***  - questionnaire PERCENTAGE WHO CURRENTLY SMOKE (and have done so daily for more than 6 months)
 -9     - questionnaire PERCENTAGE WHO HAVE EVER SMOKED MANUFACTURED CIGARETTES DAILY FOR MORE THAN 6 MONTHS
-31*    - questionnaire PERCENTAGE WHO HAVE EVER SMOKED HOMEMADE CIGARETTES DAILY FOR MORE THAN 6 MONTHS
 20     - questionnaire PERCENTAGE WHO HAVE EVER SMOKED A PIPE DAILY FOR MORE THAN 6 MONTHS
 -5     - questionnaire PERCENTAGE WHO HAVE EVER SMOKED A WATER PIPE DAILY FOR MORE THAN 6 MONTHS
 -6     - questionnaire PERCENTAGE WHO HAVE EVER SMOKED CIGARS DAILY FOR MORE THAN 6 MONTHS
  5     - questionnaire PERCENTAGE WHO HAVE EVER USED SNUFF DAILY FOR MORE THAN 6 MONTHS
-15     - questionnaire CURRENT DAILY CONSUMPTION OF MANUFACTURED CIGARETTES (no. per person)
-25     - questionnaire CURRENT DAILY CONSUMPTION OF "OTHER" TOBACCO (EXCLUDING MANUFACTURED CIGARETTES)
                    (g per person)
-47**   - questionnaire TOTAL CURRENT DAILY CONSUMPTION OF TOBACCO (g per person)
 31     - questionnaire AGE STARTED SMOKING (years)
-20     - questionnaire PERCENTAGE WHO HAVE EVER DRUNK BEER MORE THAN ONCE A WEEK FOR 6 MONTHS
-20     - questionnaire CURRENT DAILY CONSUMPTION OF BEER (g per person)
 25     - questionnaire PERCENTAGE WHO HAVE EVER DRUNK WINE MORE THAN ONCE A WEEK FOR 6 MONTHS
 15     - questionnaire CURRENT DAILY CONSUMPTION OF WINE (g per person)
-21     - questionnaire PERCENTAGE WHO HAVE EVER DRUNK LIQUOR MORE THAN ONCE A WEEK FOR 6 MONTHS
-23     - questionnaire CURRENT DAILY CONSUMPTION OF LIQUOR (g per person)
 -7     - questionnaire TOTAL CURRENT DAILY CONSUMPTION OF ALCOHOL (g per person)
-14     - questionnaire PERCENTAGE WHO MAKE STEAMED BREAD OR PANCAKES DAILY
  8     - questionnaire PERCENTAGE WHO HAVE EVER EATEN STICKY OR MOULDY STEAMED BREAD OR PANCAKES
 -2     - questionnaire TIMES PER YEAR EAT STICKY OR MOULDY STEAMED BREAD OR PANCAKES
-26     - questionnaire TIMES PER YEAR EAT MOULDY SALT PRESERVED VEGETABLES
 13     - questionnaire TIMES PER YEAR EAT MOULDY CORN
-23     - questionnaire PERCENTAGE WHO HAVE EVER EATEN MOULDY PEANUTS
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-11	- questionnaire TIMES PER YEAR EAT MOULDY SWEET POTATOES
9	- questionnaire TIMES PER YEAR EAT SALT PRESERVED VEGETABLES
8	- questionnaire TIMES PER YEAR EAT GREEN VEGETABLES
-23	- questionnaire TIMES PER YEAR EAT CARROTS
-8	- questionnaire TIMES PER YEAR EAT POTATOES (excluding sweet potatoes)
9	- questionnaire TIMES PER YEAR EAT SWEET POTATOES
-16	- questionnaire TIMES PER YEAR EAT FRUIT
-8	- questionnaire TIMES PER YEAR EAT FISH
-26	- questionnaire TIMES PER YEAR EAT MEAT
-13	- questionnaire TIMES PER YEAR EAT EGGS
3	- questionnaire TIMES PER YEAR DRINK MILK
3	- questionnaire 1982 RATION OF RICE (g/day air-dry basis)
2	- questionnaire 1982 RATION OF WHEAT (g/day air-dry basis)
-11	- questionnaire 1982 RATION OF CORN (g/day air-dry basis)
-28	- questionnaire 1982 RATION OF SORGHUM (g/day air-dry basis)
-1	- questionnaire 1982 RATION OF MILLET (g/day air-dry basis)
11	- questionnaire 1982 RATION OF LEGUMES (mostly soya beans) (g/day air-dry basis)
8	- questionnaire 1982 RATION OF FRESH AND DRIED SWEET POTATOES (g/day fresh weight)
3	- questionnaire 1982 RATION OF OIL AND FAT (g/day)
10	- questionnaire AGE STARTED MENSTRUATION (years)
-14	- questionnaire AGE AT FIRST PREGNANCY (years)
14	- questionnaire NUMBER OF PREGNANCIES
7	- questionnaire NUMBER OF LIVEBIRTHS
14	- questionnaire NUMBER OF FAILED PREGNANCIES
-7	- questionnaire ESTIMATED MEDIAN AGE AT MENOPAUSE (years)
-20	- questionnaire PERCENTAGE WHOSE DRINKING WATER SUPPLY IS SHALLOW WELL WATER
5	- questionnaire PERCENTAGE WHOSE DRINKING WATER SUPPLY IS DEEP WELL WATER
17	- questionnaire PERCENTAGE WHOSE DRINKING WATER SUPPLY IS SURFACE WATER (pond, lake, river or rain)
14	- questionnaire PERCENTAGE WHO HAVE CANCER PATIENTS IN FAMILY
14	- nitrosamine study URINE VOLUME (ml excreted in 12 hours after ingesting 500mg L-proline)
19	- nitrosamine study URINE VOLUME (ml excreted in 12 hours after ingesting 500mg L-proline and 200mg ascorbic acid)
-62*	- nitrosamine study N-NITROSOSARCOSINE (ug excreted in 12 hours after ingesting 500mg L-proline)
-19	- nitrosamine study N-NITROSOSARCOSINE (ug excreted in 12 hours after ingesting 500mg L-proline and 200mg ascorbic acid)
-21	- nitrosamine study N-NITROSOPROLINE (ug excreted in 12 hours after ingesting 500mg L-proline)
-28	- nitrosamine study N-NITROSOPROLINE (ug excreted in 12 hours after ingesting 500mg L-proline and 200mg ascorbic acid)
-35	- nitrosamine study N-NITROSO-2-METHYL-THIAZOLIDINE-4-CARBOXYLIC ACID (ug excreted in 12 hours after ingesting 500mg L-proline)
-26	- nitrosamine study N-NITROSO-2-METHYL-THIAZOLIDINE-4-CARBOXYLIC ACID (ug excreted in 12 hours after ingesting 500mg L-proline and 200mg ascorbic acid)

-14 - nitrosamine study N-NITROSO-THIAZOLIDINE-4-CARBOXYLIC ACID
(ug excreted in 12 hours after ingesting 500mg L-proline)
-16 - nitrosamine study N-NITROSO-THIAZOLIDINE-4-CARBOXYLIC ACID
(ug excreted in 12 hours after ingesting 500mg L-proline and 200mg ascorbic acid)
36 - nitrosamine study NITRITE (mg excreted in 12 hours)
(Mean of amounts excreted after ingesting 500mg L-proline with and without 200mg ascorbic acid)
-17 - nitrosamine study NITRATE (g excreted in 12 hours)
(Mean of amounts excreted after ingesting 500mg L-proline with and without 200mg ascorbic acid)
-48 - nitrosamine study THIOETHERS (ug excreted in 12 hours)
(Mean of amounts excreted after ingesting 500mg L-proline with and without 200mg ascorbic acid)
2 - nitrosamine study ASCORBATE-INHIBITABLE PROLINE NITROSATION (ug/12 hours)
(Calculated from nNPRO-nNPROa)
12 - general features ARIDITY
(1=Humid, 2=Subhumid, 3=Subarid, 4=Arid)
-7 - general features HEAT ZONE (1=North Temperate,2=Middle Temperate,3=South Temperate,4=North Subtropical,
(5=Middle Subtropical,6=South Subtropical,7=North Tropical,8=Middle Tropical,9=South Tropical)
7 - general features ELEVATION (meters)
-1 - general features LATITUDE (degrees N)
-9 - general features LONGITUDE (degrees E)
-3 - general features 1973-5 AVERAGE SEX-SPECIFIC POPULATION AGED 0-14 YEARS (in 1000s)
(Note: value to be used for MF is AVERAGE of M and F values)
-3 - general features 1973-5 AVERAGE SEX-SPECIFIC POPULATION AGED 0-64 YEARS (in 1000s)
(Note: value to be used for MF is AVERAGE of M and F values)
-7 - general features 1982 AVERAGE OF SEX-SPECIFIC POPULATIONS (in 1000s)
(Note: sex-specific population = half total population)
-27 - general features GROSS VALUE OF INDUSTRIAL AND AGRICULTURAL OUTPUT (yuan per person, 1982)
-4 - general features POPULATION DENSITY (persons/sq km, 1982)
-7 - general features 1981 BIRTH RATE (per 1000 total population)
22 - general features INFANT MORTALITY RATE (per 1000 livebirths in 1981)
(cf. 1973-5 death rates for age <1)
-6 - general features PERCENTAGE WHO ARE UNIVERSITY GRADUATES (1982)
-5 - general features PERCENTAGE WITH JUNIOR/MIDDLE SCHOOL EDUCATION (1982)
-12 - general features PERCENTAGE OF POPULATION AGED 12 YEARS OR MORE WHO ARE LITERATE (1982)
4 - general features PERCENTAGE OF EMPLOYED POPULATION WHO ARE IN AGRICULTURE (1982)
0 - general features PERCENTAGE OF EMPLOYED POPULATION WHO ARE IN INDUSTRY (1982)

Table 2

H.pylori IgG antibody prevalence* by age in rural China

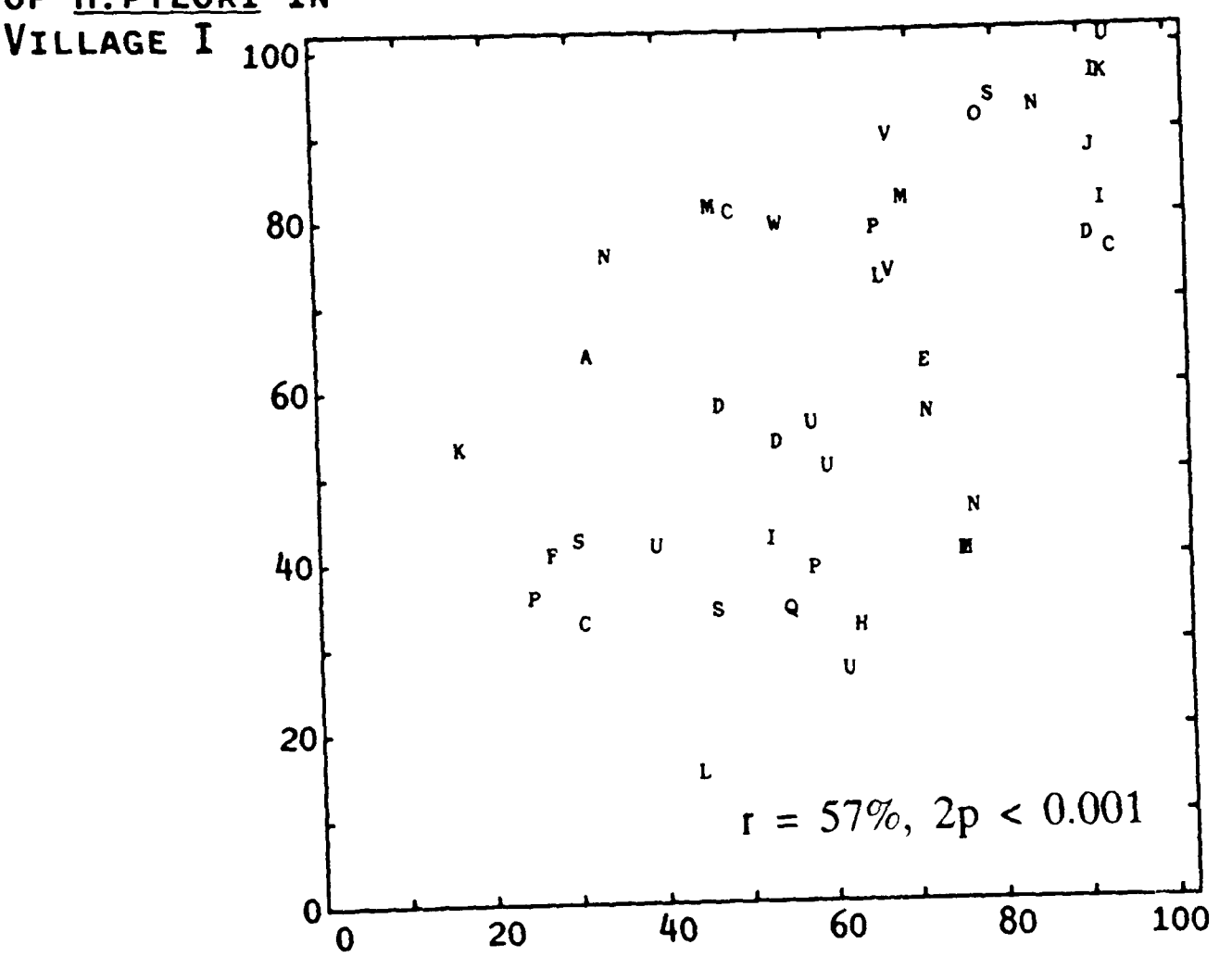
Age-range (yrs)	Total	No. positive	%
35-39	382	213	55.8
40-44	271	150	55.4
45-49	312	180	57.7
50-54	319	214	67.1
55-59	318	205	64.5
60-64	280	175	62.5
35-64	1882	1137	60.4

X^2_{Trend} = 9.67; 1 df; p = 0.002

*Positive antibody response defined by ELISA as more than 10 μg/ml of specific IgG antibody.

FIGURE 2

PREVALENCE OF H.PYLORI VILLAGE I VS VILLAGE II IN 46 COUNTIES

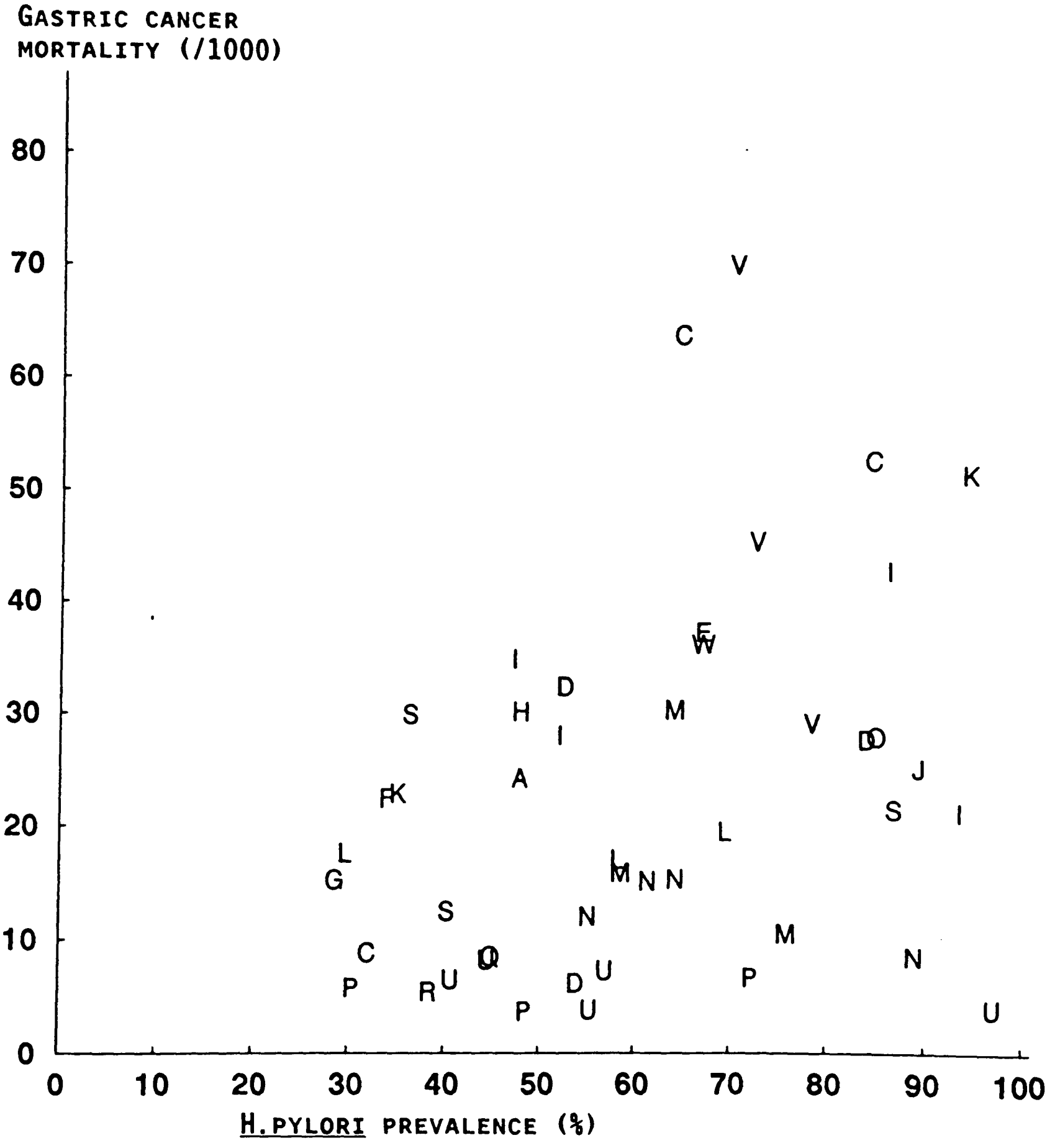


PREVALENCE (%) OF H.PYLORI IN VILLAGE II

LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY

FIGURE 3

CORRELATION OF GASTRIC CANCER MORTALITY# AND
H.PYLORI ANTIBODY PREVALENCE IN 46 COUNTIES



CUMULATIVE MORTALITY UP TO 64 YEARS OF AGE
LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY
R = 0.34; 2P = 0.02

Table 3

H-pylori IgG antibody prevalence and selected cancer mortality rates+ in 46 rural Chinese counties

Type of Cancer	Correlation
Nasopharyngeal	-0.22
Oesophageal	0.08
Gastric	0.34*
Colorectal	-0.16
Liver	0.16
Lung (male)	-0.20
Breast	-0.07
Cervical	0.03
Penis	-0.23
Bladder	0.19
Brain	0.04
Lymphoma	0.32
Leukaemia	0.04
All cancers	0.17
All other causes	0.12
All causes	0.20

+ Cumulative rates, 0-64 years, 1973-75, males and females combined (except for sex-specific cancers and for male lung cancer).

* 2p=0.02

3.4 Correlation of H.pylori and gastric cancer

The analyses presented here have generally used combined male and female mortality rates. Figure 3 shows a scatter plot for the relationship between H.pylori prevalence rates and cumulative gastric cancer mortality rates in those aged 0-64 years.

Table 3 shows the correlation coefficients between the estimated county H.pylori prevalence rates and the (0-64 years) cumulative mortality rates for 13 specific types of cancer, together with those for all cancers, all non-neoplastic causes, and all causes of death. Only the correlation with gastric cancer was statistically significant ($r = 0.34$, $2p = 0.02$). Use of male specific mortality rates did not appreciably alter the correlations, except the correlation with male lymphoma became statistically significant. In particular, the correlation between H.pylori and gastric cancer remained exactly unaltered ($r = 0.34$, $2p = 0.02$).

The correlation between H.pylori infection at different age groups and gastric cancer remained approximately the same (35-44 years $r = 0.29$, $2p > 0.05$; 45-54 years $r = 0.31$ $2p < 0.05$; 55-64 years $r = 0.29$ $2p > 0.05$).

3.5 Correlation with other known associations

In order to put this correlation between H.pylori and gastric cancer into perspective, Table 4 lists a range of

Table 4

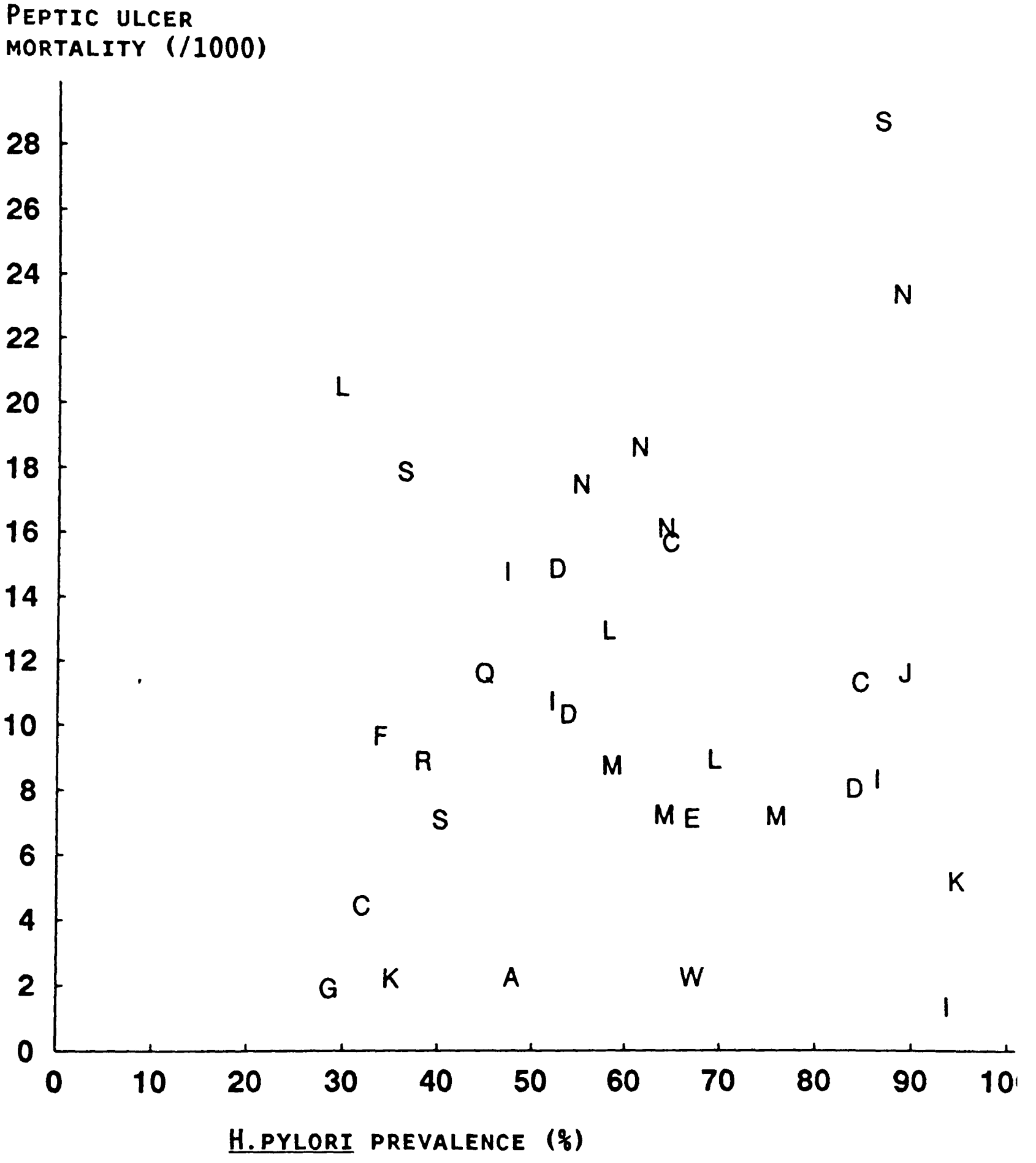
Other important associations

Schistosomiasis and colon cancer	0.72**
Fish consumption and nasopharyngeal cancer	0.56***
Hepatitis B virus antigen and liver cancer	0.46**
Green vegetable consumption and stomach cancer	-0.35*
Cigarette smoking and lung cancer	0.31*

* $2p < 0.05$
** $2p < 0.01$
*** $2p < 0.001$

FIGURE 4

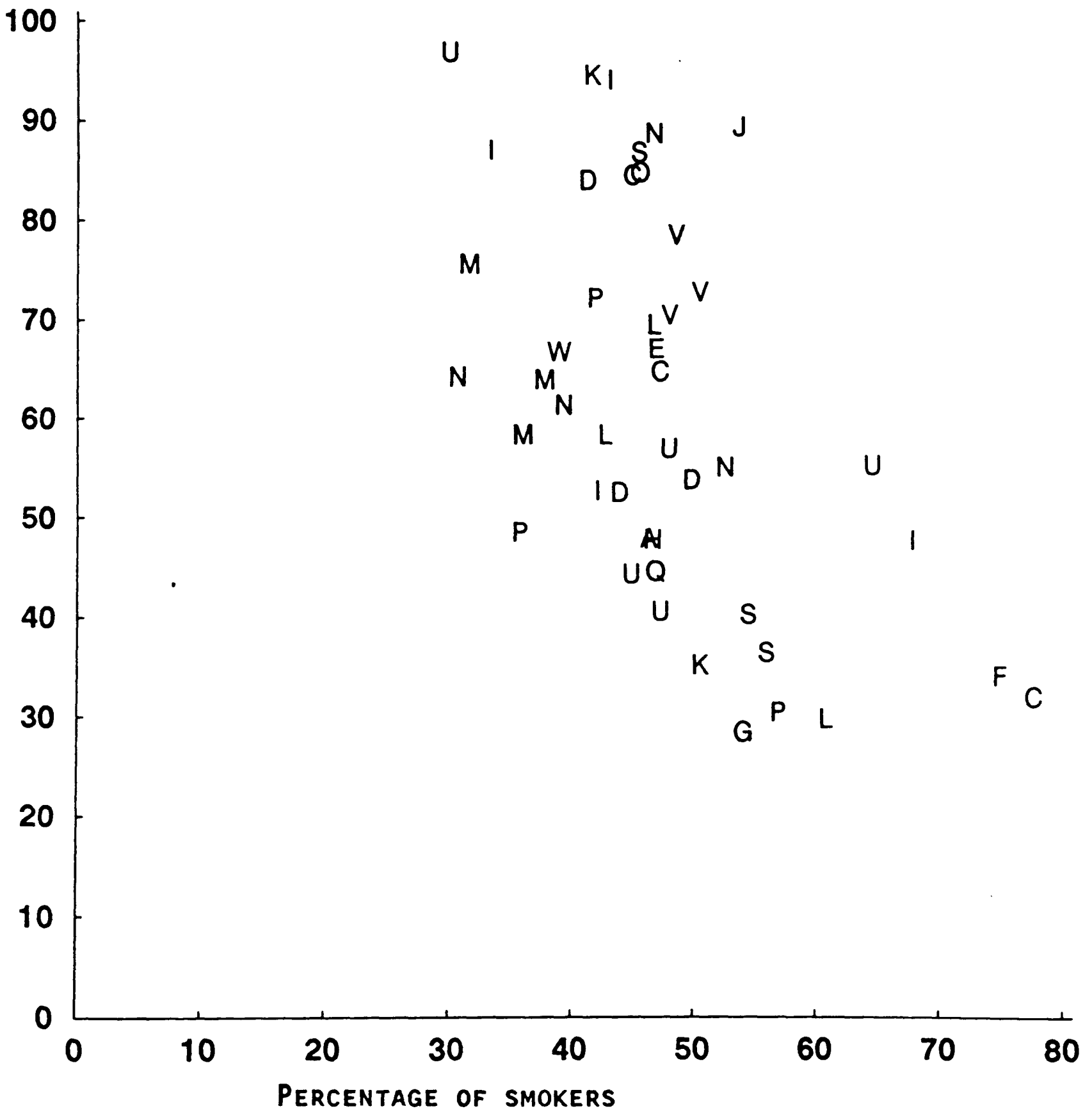
CORRELATION OF PEPTIC ULCER MORTALITY# AND H.PYLORI ANTIBODY PREVALENCE IN 46 COUNTIES



CUMULATIVE MORTALITY UP TO 64 YEARS OF AGE
LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY
$r = 0.11$; $2p = 0.55$

FIGURE 5

CORRELATION OF *H.PYLORI* ANTIBODY PREVALENCE AND PERCENTAGE WHO HAVE EVER SMOKED ANY FORM OF TOBACCO FOR MORE THAN 6 MONTHS IN 46 COUNTIES



LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY

other statistically significant correlations for other better known associations. The correlation between Hepatitis B virus antigen and liver cancer had a correlation coefficient of similar magnitude, $r = 0.46$, $2p < 0.001$. Smoking and lung cancer had a correlation of 0.31 ($2p < 0.05$) . Other strong correlations included green vegetable consumption and gastric cancer (-0.35, $2p < 0.01$).

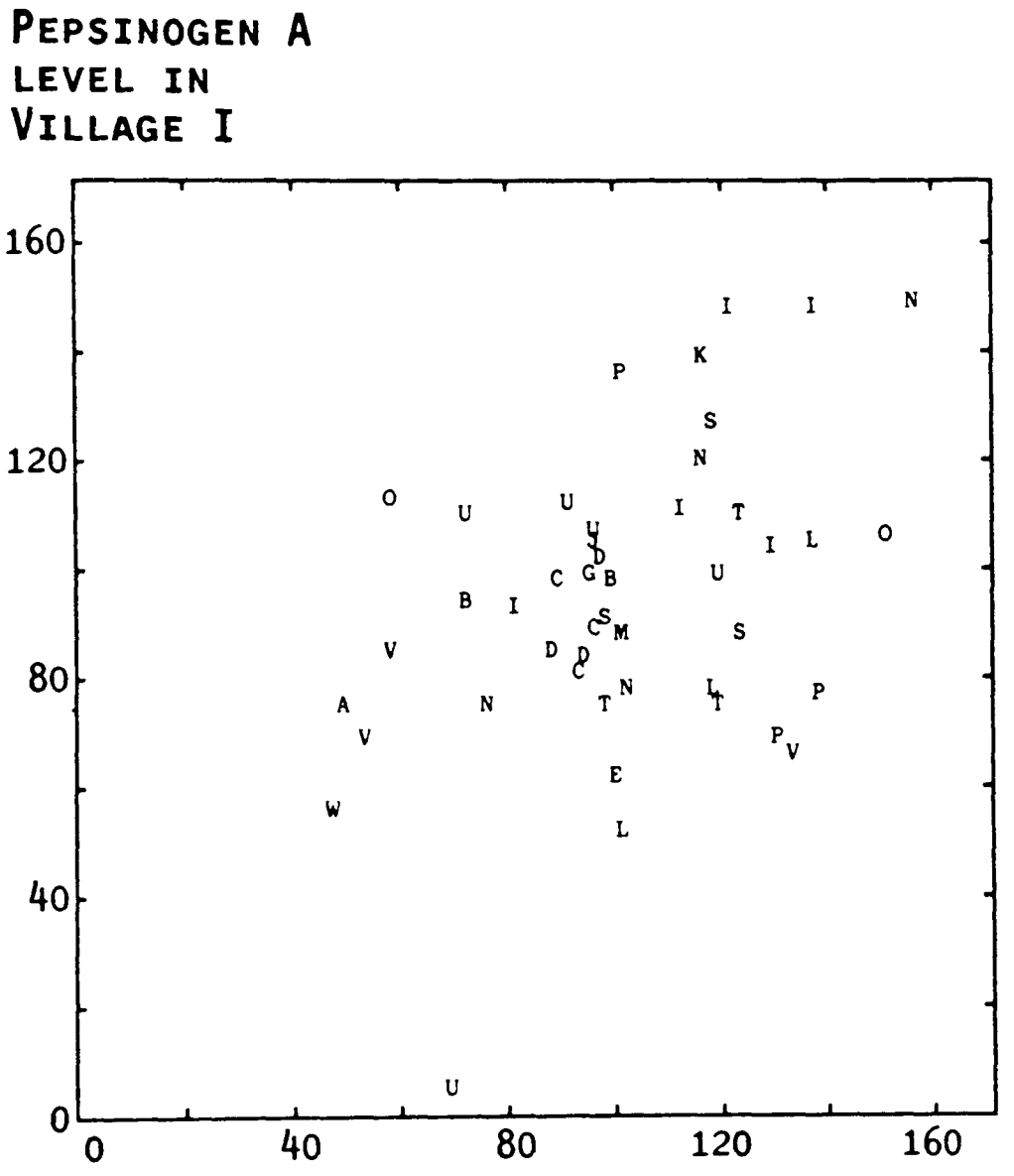
3.6 Correlation of H.pylori and other causes of death

Correlation coefficients were also calculated for 37 non-neoplastic causes of death or groupings of causes of death (Table 1). Of these the only cause for which there was prior expectation of an association with H.pylori infection was peptic ulcer. The correlation coefficient in this case was 0.11, which was not significant ($2p = 0.11$, Figure 4).

By chance alone at least two or three of the remaining correlations would be expected to be conventionally ($2p < 0.05$) significant; in fact, three were. All involved perinatal causes of death ($r = 0.36$ for congenital defects, excluding heart diseases; $r = 0.43$ for prematurity; and $r = 0.36$ for other perinatal conditions, excluding tetanus). None of the other causes or groups of causes had a conventionally significant association with H.pylori antibody prevalence.

FIGURE 6

POOLED PEPSINOGEN A LEVELS IN MEN 55-64 YEARS VILLAGE I VS VILLAGE II IN 63 COUNTIES



$\overline{R = 0.41,\ 2P < 0.01}$

LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY

3.7 Other correlations

H.pylori prevalence was also correlated with 110 blood components, 26 urinary components, 41 dietary nutrient levels, 33 dietary components analysed from food samples, and 59 lifestyle questions, a total of 286 factors. The results of this correlation are shown in Table 1. Again, by chance alone about 14 correlations would be expected to be significant ($p < 0.05$), and in fact, 19 were. These included Herpes simplex virus antibody prevalence ($r = 0.33$), egg consumption ($r = -0.32$), salt consumption ($r = 0.38$) and notably, several indices of current smoking as shown in Figure 5 (plasma cotinine $r= -0.40$, $2p <0.001$, prevalence of those ever smoked $r = -0.55$; $2p < 0.001$, prevalence of current smokers $r = -0.49$; $2p < 0.001$, total tobacco consumption $r = -0.47$; $2p < 0.01$).

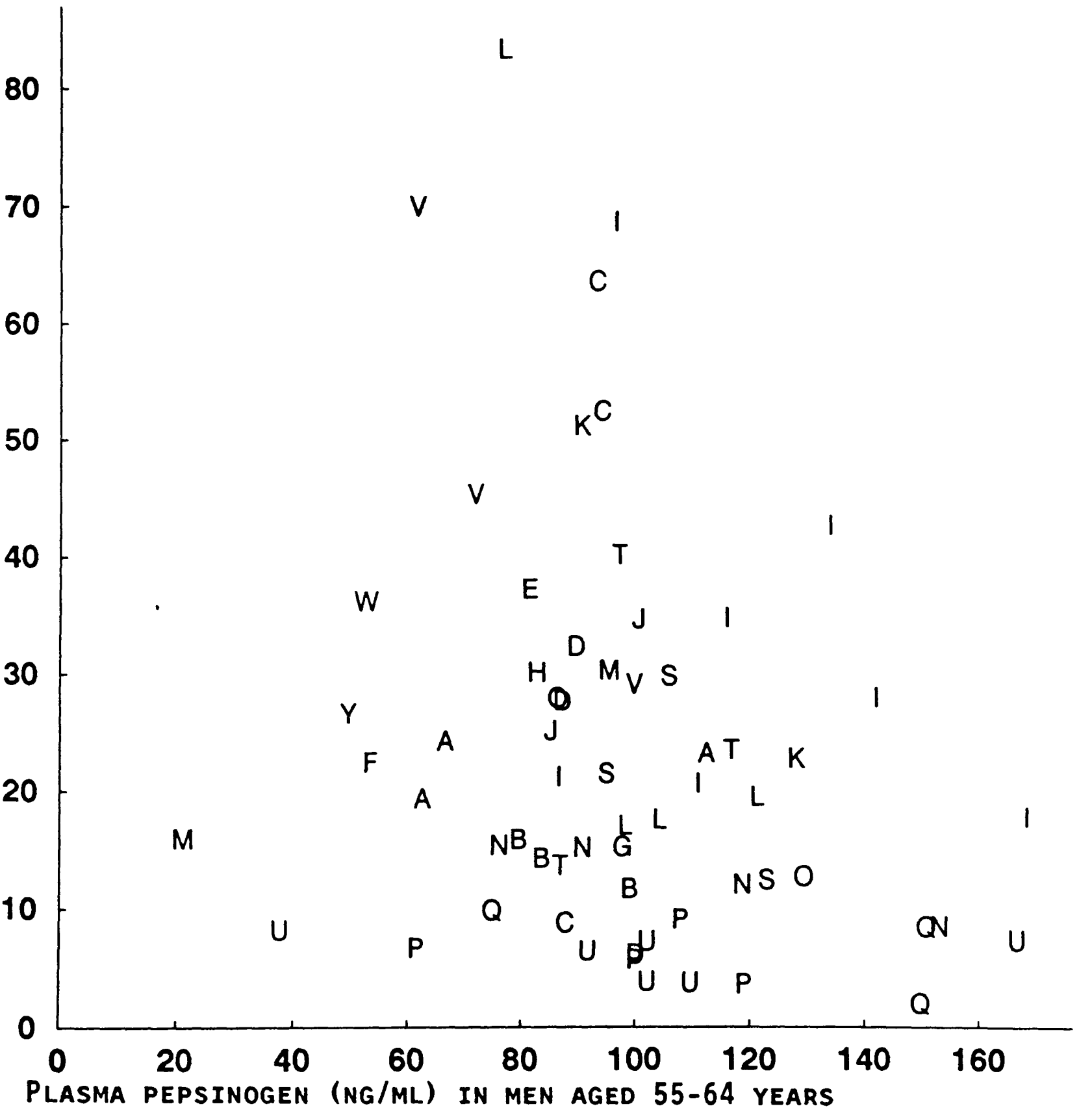
3.8 Pepsinogen A and gastric cancer

Mean pooled pepsinogen levels in the 46 counties ranged from 46.0 ng/ml to 155.0 ng/ml (mean 100.4 ng/ml, sd 26.4), a 3.8-fold variability.

In the 46 counties for which there was pooled plasma available from both villages, the correlation coefficient for the comparison of the mean pooled levels of pepsinogen between the two villages was 0.41 ($p < 0.01$, Figure 6). As for the data on H.pylori, this demonstrates that the variation in pepsinogen levels between these counties represents real geographic variation.

FIGURE 7

CORRELATION OF GASTRIC CANCER MORTALITY # AND PEPSINOGEN A LEVELS IN POOLED SAMPLES MEN AGED 55-64 IN 63 COUNTIES



CUMULATIVE MORTALITY UP TO 64 YEARS OF AGE
LETTERS IN SCATTERPLOT ARE THE INITIALS OF EACH COUNTY
R = -0.22; 2P = 0.09

There was a negative correlation between pepsinogen (PG) A levels and gastric cancer ($r = -0.22$, Figure 7) but this was statistically of borderline significance ($2p = 0.09$, Figure 7). Other disease associations with PGA included mortality due to digestive diseases other than peptic ulcer in those aged 0-14, and in those aged 0-64 (0.29, 0.32 respectively, $2p < 0.01$). The correlation between PGA levels and mortality due to peptic ulcer was not significant ($r = 0.22$, $2p > 0.5$). Plasma PGA levels were significantly correlated with dietary vitamin C intake ($r = 0.4$ $2p < 0.01$), but not with plasma or urinary vitamin C levels, nor with dietary carotene ($r = 0.32$ $2p < 0.05$).

4 DISCUSSION

4.1 Validity of study

This study had some features that are missing from many other ecological comparisons. Chinese counties offer considerable variability in both the exposure and in the disease of interest. There are few other areas of the world where one could identify populations displaying a twenty five-fold range in gastric cancer mortality and about a three-fold range in H.pylori prevalence.

Another advantage of studying rural Chinese populations was that these populations are relatively stable, unlikely to be affected by factors like migration.

Although with larger numbers of blood samples per county the scatter of H.pylori prevalence rates might have been somewhat reduced, it would still have been substantial. Having a reasonably large number of data points (46 counties for H.pylori determination and 63 for PGA determination) was an obvious advantage, as was the ability to examine the extent to which there was within-county heterogeneity for the variables concerned. If the correlation between paired communes had been non-significant this would have indicated that the calculated county mean was not a reliable estimate of the true county mean, but since it was highly significant, the H.pylori antibody levels (or pooled PGA levels), and hence their relationship with disease, are reasonably reliable.

4.2 H.pylori and gastric cancer

This study provided the first systematic epidemiological evidence of an association between H.pylori infection and the aetiology of gastric cancer. The correlation was moderately significant ($2p = 0.02$) and may therefore have been inflated (or, equally, reduced) by the play of chance in a study of only 46 counties. Gastric cancer was, however, specified as being of interest prior to the study, and it was the only neoplastic cause of death that was significantly associated with H.pylori.

The observed correlation between H.pylori prevalence and gastric cancer mortality of 0.34 was not a particularly strong association and showed considerable scatter (Figure 3). Part of this scatter must have been due to purely statistical fluctuations between one village and another. The correlation between Hepatitis B antigen virus (for which there is a large international effort to promote vaccination) and liver cancer was of the same magnitude (0.46, $2p < 0.001$), and the increased significance was probably due to 65 county rates being available instead of the 46 available from this study.

One would expect only a limited degree of association if, as seems plausible, H.pylori infection acted synergistically with other factors that are generally required for the development of gastric cancer. Also, because the onset of CAG occurs several decades before cancer development,

H.pylori antibody prevalence should ideally have been correlated with cancer rates 20-30 years later, not 10 years earlier.

Another feature of the correlation between *H.pylori* and gastric cancer was that there are no counties with high gastric cancer rates and low *H.pylori* prevalence rates. In contrast, there were several counties where *H.pylori* prevalence rates were high but gastric cancer rates were low. This could imply that *H.pylori* infection was a necessary but not a sufficient step in gastric carcinogenesis.

4.3 *H.pylori* and peptic ulcer mortality

Peptic ulcer mortality might also have been expected to show a significant association with antibody prevalence as there is now considerable evidence to suggest that it is associated with *H.pylori* infection (Graham 1989, Wyatt 1989). Peptic ulcer is, however, rarely fatal and the patterns of mortality from this cause in rural China may have been distorted by variations in the availability of appropriate medical services, as well as by variations in any cofactors for the production or progression of peptic ulcer.

4.4 Other disease associations

The significant associations between *H.pylori* antibody prevalence and the three categories of perinatal mortality

may well have been chance findings. There is no obvious pathological mechanism whereby H.pylori could directly influence foetal or perinatal development, and two or three conventionally significant correlations between disease and H.pylori were expected just by chance. It might be speculated that H.pylori infection in adults may act as some kind of general marker of poor social conditions that have an adverse effect on birth outcome, but other more direct markers of this kind (such as the infant mortality rate, the literacy rate, or the proportion with junior school education) were not significantly related to H.pylori antibody prevalence.

4.5 Other associations

Although the correlations between H.pylori and smoking appeared striking, some of the smoking variables were strongly correlated with each other. For example, the correlation between the prevalence of current smoking and and total tobacco used was 0.61 ($2p < 0.01$). If all the smoking categories are combined into one then the number of significant associations ($2p < 0.05$) are reduced to 12, as opposed to 14 expected to occur purely by chance and therefore the relationship between H.pylori and smoking may also have been a chance finding. Smoking of bought cigarettes is a recent development in China, and it could be hypothesised that those who smoke have higher levels of disposable income. However there was no obvious correlation between H.pylori and more direct indices of socioeconomic

status such as literacy rates, infant mortality or gross value of agricultural and industrial output (Table 1). In an unrelated survey of smoking habits in China, Tomson and Coulter (1987) found that 80% of peasant farmers smoked, but so did 50-60% of factory workers and 30% of doctors. Also, many peasant farmers smoked home grown tobacco.

4.6 Plasma pepsinogen A levels

Although only of borderline significance (2p = 0.09), the negative correlation (-0.22) between pepsinogen A levels and gastric cancer was in the expected direction. Although of poor sensitivity in detecting those with CAG (see Chapters 1-3), low PGA levels have been associated with increased gastric cancer incidence in other studies (Burr et al 1987, Stemmermann et al 1978, 1987). Low PGA levels have also been associated with lower levels of plasma ascorbate, suggesting an association between vitamin C intake and CAG (Burr et al 1987). The data in this study was probably too sparse to reach any definite conclusions without PGA measurements for in a larger number of people in the older age groups. If pepsinogen is to be used as a marker of CAG, further studies of its usefulness are indicated, especially in China.

In conclusion, a moderately significant geographical association between gastric cancer mortality and the prevalence of H.pylori infection was demonstrated in rural China. Although such a relationship is biologically

plausible given the known role of H.pylori in the causation of gastritis, more data will be needed before it can be considered to be reliably established. This would include not only geographic correlation studies but also prospective studies of individuals and perhaps even randomised intervention trials of the effects of the elimination of H.pylori from the stomach (by a short course of antibacterial treatment) either on cancer or, more practically, on the development, regression or progression of "precancerous" gastric lesions.

There is no doubt that several other agents, especially dietary components, are involved in the aetiology of gastric cancer (Howson et al 1986) but, if substantiated, the involvement of H.pylori may help to explain certain epidemiological features of the disease, notably the strong inverse association with socio-economic status (Howson et al 1986). If H.pylori is transmitted from person to person (Mitchell et al 1989), then one might expect its prevalence to be high in populations with poorer living conditions. It will be necessary, however, to test the association between H.pylori and gastric cancer in other epidemiological studies before deciding whether there is a real aetiological relationship.

5 SUMMARY

To examine the geographic association between *Helicobacter pylori* infection and gastric cancer, the prevalence of IgG antibodies to *H.pylori* was assessed in plasma samples taken in 1983 from 1,882 men, aged 35-64 years, in 46 rural counties of the People's Republic of China. The gastric cancer mortality rates in these counties in 1973-75 varied from 3 per 1,000 (cumulative rate, 0-64 years) to 69 per 1,000 while the proportions of the population positive for *H.pylori* antibodies (based on an average of about 41 men per county) varied from 28% to 96%.

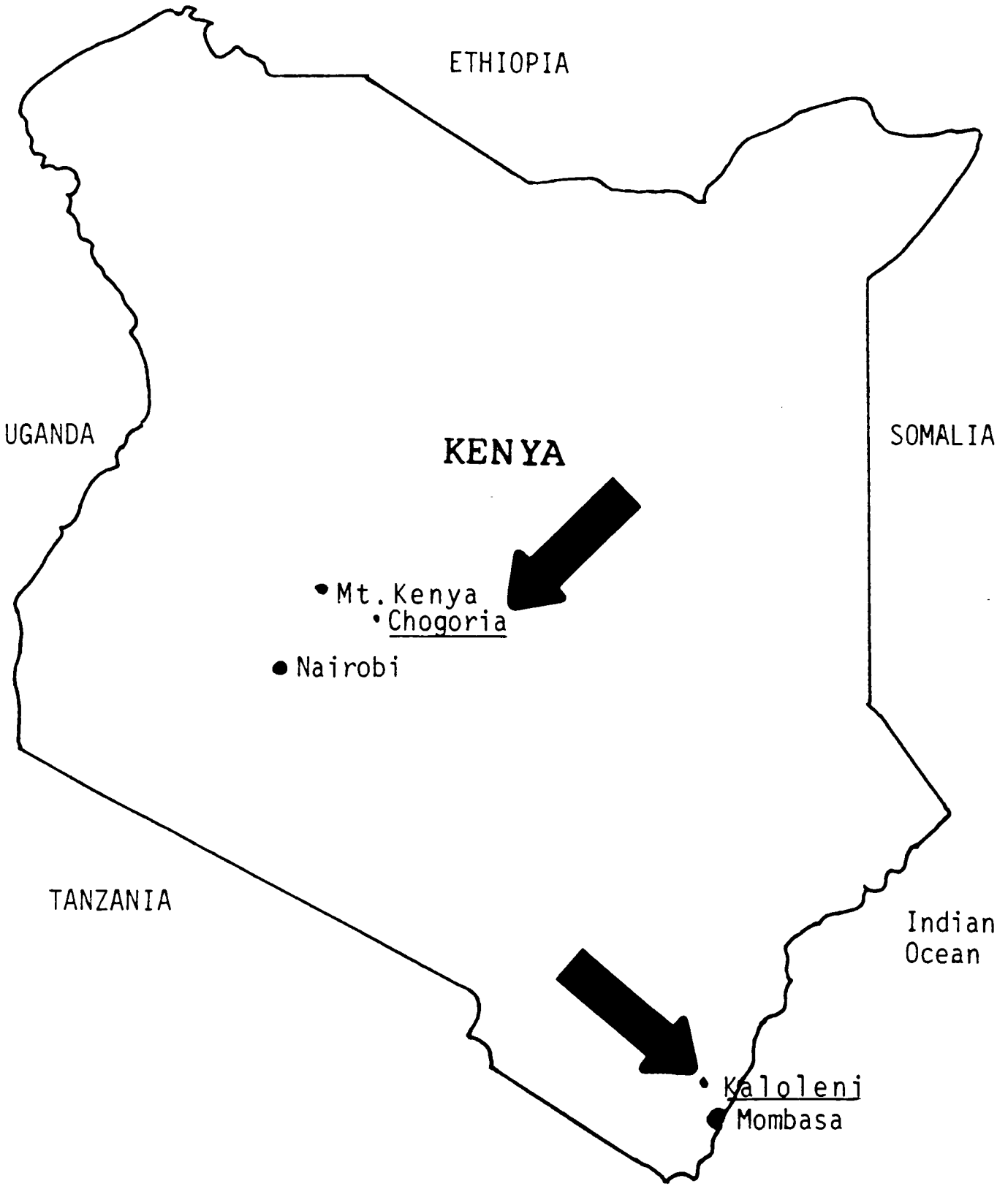
The estimated correlation between *H.pylori* antibody prevalence and gastric cancer mortality was 34% (p=0.02). No other type of cancer showed a significant association with *H.pylori*.

CHAPTER 6

EPIGASTRIC PAIN, HELICOBACTER PYLORI AND MYCOTOXINS IN KENYA

Figure 1.

Map of Kenya showing study areas



1 INTRODUCTION

Acute epigastric pain associated with dyspepsia, nausea and vomiting, affecting mainly young people, accounts for up to 20% of total outpatient attendances in the Meru district of Kenya. It is also the fourth most common reason for admission into the medical ward at the Presbyterian Church of England Hospital (PCEA) in Chogoria, a Meru village (Fig 1). Duodenal ulcer has been reported as uncommon in this region (Tovey and Turnstall 1975) but a high prevalence of *Helicobacter pylori*-associated gastritis has been reported in this hospital and it was thought that this organism was playing a role in the aetiology of this acute form of dyspepsia (Lachlan *et al* 1988).

Recent changes in maize storage from traditional stores to plastic sacks have been observed in this region for some time. It was thought that these changes in storage methods may have favoured the growth of mycotoxin-producing fungi such as *Fusarium spp.* and *Aspergillus spp.*. Aflatoxins (AF), produced by *Aspergillus spp.* have been associated with epigastric pain in other regions in Kenya (Ngindu *et al* 1982). T-2-Trichothecenes (T-2-T), produced by *Fusarium spp.* on contaminated flour have been associated with epigastric pain and vomiting in India (Bhat *et al* 1989). It was therefore thought possible that ingestion of these mycotoxins may have also played a role in the development of gastric symptoms in this area of Kenya.

An additional anecdotal observation at the PCEA hospital

showed an apparent increase in the proportion of gastric cancer cases amongst young people, with over 40% of cases diagnosed at laparoscopy being younger than 45 years (G.W. Lachlan, pers. comm.). However, an endoscopy unit was established at this hospital in 1986, which led to the suspicion that this observed increase in gastric cancer may have caused by improved diagnostic facilities and recent specialist interest in this condition.

This chapter describes a small pilot study designed to test the association between H.pylori, chronic atrophic gastritis (CAG) and gastric symptoms; and to test the significance of aflatoxins and T-2-Trichothecenes in the development of gastric symptoms. In addition, an attempt was made to estimate the incidence of gastric cancer in this region by searching hospital records for diagnoses of gastric cancer from 1965 to 1988.

2 SUBJECTS AND METHODS

2.1 Subjects (Table 1)

A consecutive group of 25 patients aged 19-30 years, complaining of upper abdominal pain and dyspepsia attending the endoscopy clinic of the PCEA hospital (the dyspepsia group), was compared with a group of 20 symptom free healthy volunteers aged 19 to 30 years, recruited from the hospital staff of the PCEA Hospital (the Chogoria volunteers).

Little was known about the geographical distribution of H.pylori, mycotoxins and epigastric pain in Kenya and for this reason another group of healthy, symptom-free volunteers was recruited from the hospital staff of St Luke's Hospital in Kaloleni, (Figure 1) on the east coast near Mombasa (the Kaloleni volunteers) for comparison with the Chogoria volunteers.

A fourth and final group comprised 96 inpatients at the PCEA hospital. These were children and young adults aged 3-19 years who were hospitalised for conditions other than gastric symptoms. All subjects in the dyspepsia and the two volunteer groups completed a brief questionnaire regarding their symptoms, occupation and conditions in which maize was stored at home.

2.2 Serum and urine tests

Five ml of blood was taken from each person in the dyspepsia group, the two volunteer groups and from the children. Serum was separated, on site, for the measurement

of H.pylori IgG antibody levels and for the determination of serum aflatoxin-albumin adduct (AF) levels. AF levels were measured using an ELISA technique described by Wild et al 1990 and in Appendix 1. A cut-off of 5 picogrammes (pg) aflatoxin albumin adduct/ml serum or greater was considered as a positive exposure to AF (Wild et al 1990).

H. pylori IgG antibody levels were measured using the same standardised enzyme linked immunosorbent assay described by Newell et al 1987 and in Appendix 1. An IgG antibody concentration of 10 microgrammes μg/ml or greater was regarded as a positive antibody response to H.pylori infection (Chapter 2).

Subjects in the dyspepsia and volunteer groups provided a spot urine sample for the determination of urinary aflatoxin levels and (T-2-T) adduct levels. T-2-T levels were measured using an ELISA technique described elsewhere (Lee et al 1989, Appendix 1). A cut-off of 0.5 ng/ml or greater was considered as a positive indicator of T-2-T exposure.

2.3 Endoscopy and histology

Subjects in the dyspepsia and the two volunteer groups, but not the children, underwent an endoscopy. Five mucosal biopsies were collected from each subject: two from the antrum, one from the incisura and two from the fundus. Biopsies were taken from the same sites as in the Oxford endoscopy study described in Chapter 2. Biopsies were fixed in formalin, embedded and sectioned using standard

histological procedures. A Giemsa stain (Bancroft and Stevens 1982) was used to identify Helicobacter-like organisms on the gastric mucosa. Presence of Helicobacter-like organisms in at least one out of the five biopsy sites was considered as a positive indicator of infection.

Other sections were stained using Haematoxylin and Eosin (Bancroft and Stevens 1982) for classification of the gastric mucosa. Biopsies were classified into normal (N), chronic superficial gastritis (CSG) and CAG using Whitehead's (1973) classification. The presence of Helicobacter-like organisms (HLO) and the grading of gastritis was assessed by one histopathologist.

2.4 Estimation of gastric cancer incidence rates

A search for gastric cancer cases was undertaken by examining the ward books, surgery and medical records, death certificates, and gastroscopy records for the period 1965-1988. For the period 1965-1974, data on gastric and other cancers was already collected from this hospital as part of another survey (P.Cook-Mozzafari and D. Burkitt unpublished data) were also made available. Information gathered comprised name, inpatient number, age, sex and year of first diagnosis. Duplicate entries (same name or same inpatient number) were eliminated.

The 1969 and 1979 Kenyan National Census data (Kenya: Ministry of Finance 1970, 1980) for the Meru district were used to estimate the age specific population size for this

region from 1965-1988, using linear intrapolation and extrapolation techniques (Smith and Zoph 1970).

Sub-Saharan African age specific gastric (and other) cancer incidence rates have been calculated by P. Cook-Mozzafari and D. Burkitt (unpublished data) using what were considered to be the most reliable registries at the time. These were registries were Johannesburg and Natal, South Africa; Ibadan, Nigeria; and Kaydondo, Uganda (Doll et al 1966, Doll et al 1972). These rates were used to derive the expected number of gastric cancer cases for the Meru population. The ratio of observed:expected gastric cancer cases was multiplied by the average crude incidence rate for gastric cancer of 7.09 per 100,000 (of the Johannesburg, Natal, Ibadan and Kaydondo registries (P. Cook-Mozzafari and D. Burkitt, unpublished data) to yield an indirectly adjusted incidence rate for each year for gastric cancer in this region. Incidence rates for 1988 were directly standardised using the world standard population (Muir et al 1987) for comparison with rates from other regions.

2.5 Statistical methods

Chi-squared tests (Armitage and Berry 1987) were used to test for the association between the presence or absence of H.pylori, CAG and mycotoxins in the dyspepsia group and the Chogoria volunteers. As recommended by Armitage and Berry (1987), the Fisher's exact test was chosen as an alternative to the Chi-squared to test for associations when the expected cell frequency in any cell was less than five. For all the

statistical analyses regarding mucosal histopathology, those with CAG were grouped into one category, irrespective of severity. A Chi-squared test for linear trend (Armitage and Berry 1987) was used to analyse trends in the prevalence of H.pylori infection in relation to mucosal histopathology and in relation to age in the children. Pearson's (r) correlation coefficient, and a 2-sided p-value was used to test the significance of the correlation between urinary and serum levels of aflatoxin (Armitage and Berry 1987). All Chi-squared , Fisher's exact and correlation statistical tests were done using the SAS-PC (1985) statistical package.

A poisson regression model was used to test for significant trends in the observed against expected number of gastric cancers over the 23 year period, using the Generalised Linear Interactive Modelling statistical package, GLIM (Healy, 1987).

3 RESULTS

3.1 Description of population groups

Mean ages of the dyspepsia, Chogoria volunteers and Kaloleni volunteers were 23.8 (s.d. = 4.0), 23.0 (s.d. = 2.5) and 21.6 (s.d. = 1.5) years, respectively. The dyspepsia group comprised students (48%), farmers (24%), salaried workers (16%), and 'housewives' (12%) as shown in Table 2. The combined volunteer groups comprised student nurses (67.5%), trained nurses (20%) and salaried hospital staff (12.5%).

A total of 65 persons were endoscoped, 20 from each of the volunteer groups and 25 from the dyspepsia group. Serum was taken for the determination of H.pylori IgG antibodies in all 65 subjects, but AF levels were measured in only 60 subjects. Urine samples were taken from 65 subjects for the determination of AF levels. Fifty-six subjects had urine tested for the determination of T-2-T levels.

3.2 Mucosal histopathology

Gastric histological findings in the dyspepsia and the two volunteer groups are shown in Table 3. In the three groups combined, 14% had a normal gastric mucosa, 17% had CSG, and 70% had CAG. The prevalence of CAG was statistically marginally higher in the dyspepsia group when compared to the Chogoria volunteers, 88% versus 60% respectively (X^2 = 3.3; 1df; p = 0.07). The difference in CAG between the dyspepsia and the Kaloleni volunteers was statistically significant (88% versus 55%, X^2 = 6.2; 1df; p

Table 1

Description of Kenyan study populations

	Population	Description	N	Age range	Gastric biopsies*	Blood sample+	Urine sample#	Brief Quest$
1.	Dyspepsia group	Endoscopy patient series	25	19-30	Yes	Yes	Yes	Yes
2.	Chogoria comparison group	Symptom free hospital staff	20	19-30	Yes	Yes	Yes	Yes
3.	Kaloleni comparison group	Symptom free hospital staff	20	18-30	Yes	Yes	Yes	Yes
4.	Children	Inpatient wards	96	3-19	No	Yes	No	No

* Biopsies for the histological assessment of gastritis and Helicobacter-like organisms.
\+ Serum for the measurement of H.pylori IgG antibodies and aflatoxin-albumin bound adducts.
\# Urine for the measurement of urinary aflatoxin levels.
$ Questions on dyspeptic symptoms, storage conditions.

Table 2

Occupational distribution of dyspepsia group and volunteers

Occupation	Volunteers		Dyspepsia group	
	N	(% of total)	N	(% of total)
Student	27	(67.5)	12	(48.0)
Farmer	0	(0.0)	6	(24.0)
Salaried	5	(12.5)	4	(16.0)
Nurse	8	(20.0)	0	(0.0)
Housewife	0	(0.0)	3	(12.0)
Total	40	(100.0)	25	(100.0)

Table 3

H.pylori and mucosal histopathology in dyspepsia group and volunteers

Mucosal histology	Kaloleni volunteers			Chogoria volunteers			Dyspepsia group			Total		
		% with			% with			% with			% with	
	N	HLO*	HP+	N	HLO	HP	N	HLO	HP	N	HLO	HP
N	4	0.0	100.0	3	0.0	66.7	2	0.0	50.0	9	0.0	77.7
CSG	5	80.0	80.0	5	100.0	100.0	1	100.0	100.0	11	90.9	90.9
CAG mild	10	90.0	100.0	9	88.9	100.0	17	82.4	100.0	36	86.1	100.0
CAG moderate	1	100.0	100.0	3	33.3	100.0	4	75.0	100.0	8	62.5	100.0
CAG severe	0	0.0	0.0	0	0.0	0.0	1	100.0	100.0	1	100.0	100.0
CAG overall	11	90.9	100.0	12	75.0	100.0	22	81.8	100.0	45	82.3	100.0
Total	20	70.0	95.0	20	70.0	95.0	25	70.0	96.0	65	72.3	95.4
% with CAG		55.0				60.0			88.0			
		#				$						

N = Normal mucosa, CSG = Chronic superficial gastritis, CAG = Chronic atrophic gastritis

* *Helicobacter*-like organisms

\+ *Helicobacter pylori* IgG antibody positive

~ X^2_{Trend} (Hp IgG) = 7.1; 1df; p = 0.008

X^2_{Trend} (HLO) = 10.6; 1df; p = 0.001

$ X^2 = 3.3; 1 df; p = 0.07

\# X^2 = 6.2; 1 df; p = 0.001

Table 4

Seroprevalence of H.pylori IgG antibodies in Kenyan populations

Population	Age	N	% Positive	
Children	<5	21	4.8	*
	5-9	25	16.0	
	10-14	25	40.0	
	15-19	25	48.0	
Volunteers:				
Kaloleni	19-25	20	95.0	+
Chogoria	19-30	20	95.0	
Dyspepsia group	18-30	25	96.0	

* X^2_{Trend} = 13.0; 1df; p = 0.0003

+ N/S

= 0.001).

3.3 Mucosal histopathology and H.pylori

Table 3 also shows the relationship between HLO, H.pylori and mucosal histopathology. HLOs were found in 72.3% (47) of the total 65 subjects endoscoped. An extra 15 subjects were HLO negative on histological assessment but showed a positive serological H.pylori response.

HLOs were observed in greater frequency in the dyspepsia group than the Chogoria volunteers (76% versus 70%, respectively), but the difference was not significant (X^2 = 0.7; 1df; p = 0.4). Looking at the combined data for all the dyspepsia and volunteer groups combined, none of those with a normal mucosa had HLO, but 77.7% of those with a normal mucosa showed elevated H.pylori antibody levels. 90.9% of those with CSG and 82.3% of those with CAG also had HLO (X^2_{Trend} N, CSG, CAG = 10.6; 1df; p = 0.001). Similarly, H.pylori infection rates (IgG antibody levels > 10 μg/ml) in relation to CSG and CAG were 90.9% and 100% respectively (X^2_{Trend} = 7.1; 1df; p = 0.008).

3.4 Seroprevalence of H.pylori

Table 4 shows the seroprevalence of H.pylori in relation to age in the group of 96 children and young adults, and the seroprevalence of H.pylori in the dyspepsia group and in the two volunteer populations. Among the children, seroprevalence of H.pylori infection increased with age from 4.8% in those aged less than five years, increasing to 48%

Figure 2

Albumin–bound serum aflatoxin levels (pg/ml) in Kenyan children

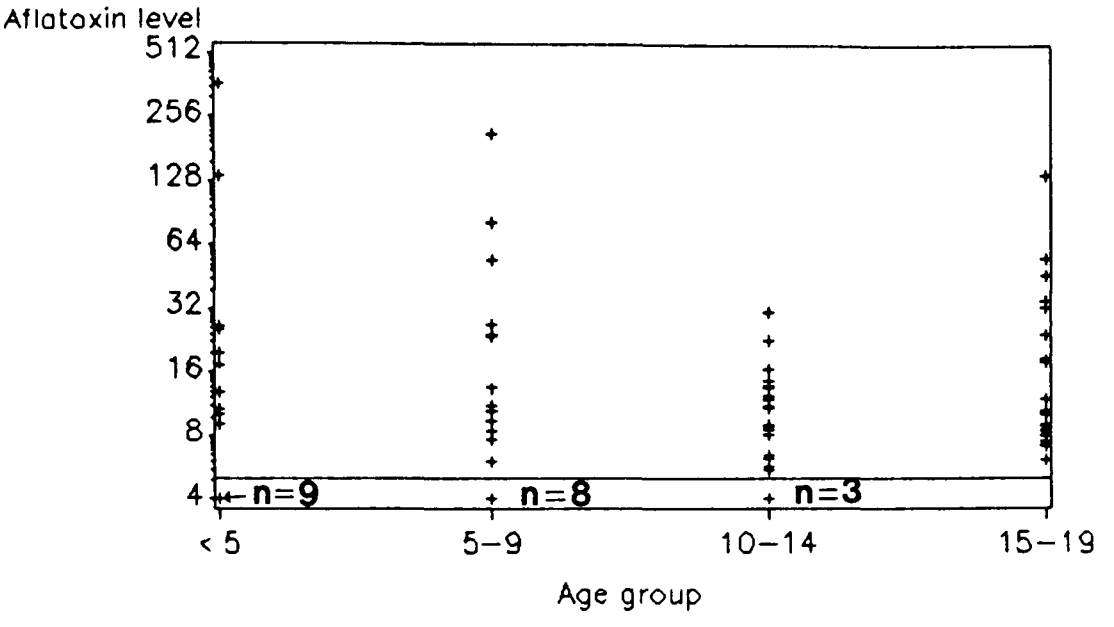


Figure 3

Albumin–bound serum aflatoxin levels (pg/ml) in Kenyan populations

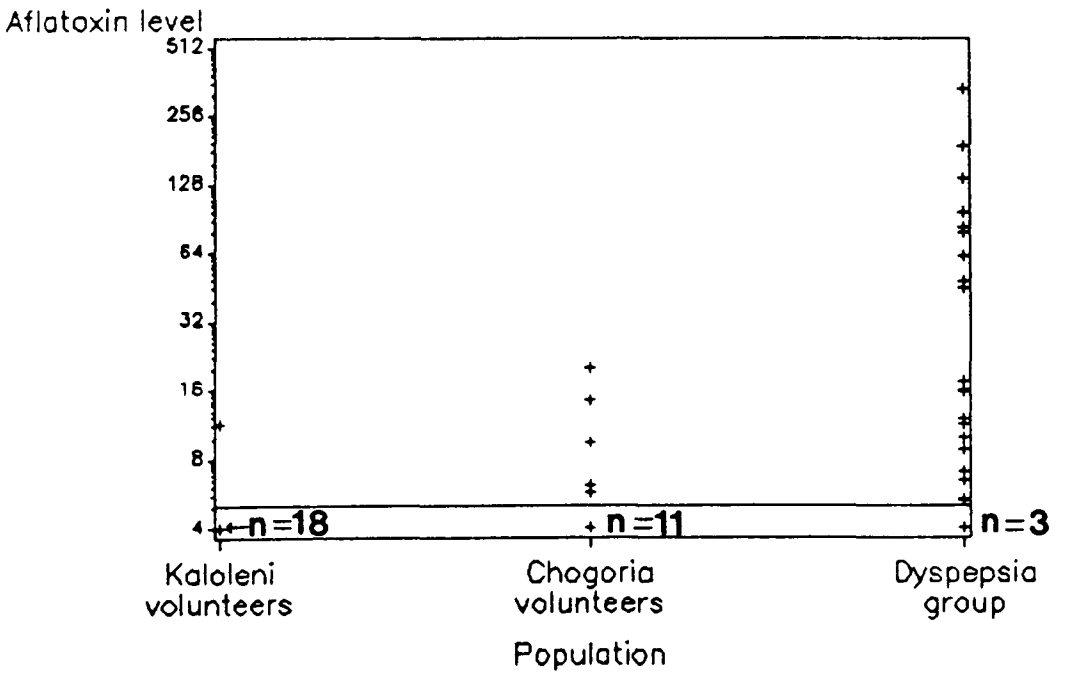


Table 5

Presence of serum albumin-bound aflatoxin adducts, urinary T2-trichothecences in Kenyan populations

Area	Mucosal histology	N	% AF +ve	N	% T-2-T +ve
Kaloleni controls	N/CSG	9	11.1	8	37.5
	CAG	11	9.1	11	36.4
	Total	20	10.0	19	36.8
Chogoria controls	N/CSG	6	33.3	7	0.0
	CAG	10	40.0	9	0.0
	Total	16	37.5*	16	**0.0
Dyspepsia group	N/CSG	3	100.0	3	66.7
	CAG	21	85.7	18	27.8
	Total	24	87.5$	21	33.3+
All populations	N/CSG	18	27.7	18	27.7
	CAG	42	54.8#	38	23.7~

* Fisher's 2p = 0.06 (Kaloleni vs Chogoria volunteers)
**Fisher's 2p = 0.007 (Kaloleni vs Chogoria volunteers)

$\$X^2 = 8.8$; 1df; p = 0.003

$+X^2 = 4.5$; 1df; p = 0.01

$\#X^2 = 0.0$; 1df; p = 0.8

$\sim X^2 = 0.0$; 1df; p = 1

in those aged 15-19 years (X^2_{Trend} = 13.0; 1df; p = 0.0003). Prevalence rates in the dyspepsia group and the two volunteer groups were all very similar (95-96%).

3.5 Exposure to mycotoxins

3.5.1 Aflatoxin levels in the study population

Figures 2 and 3 show the distribution of serum aflatoxin albumin adduct levels (AF) in the study populations. In the children, the prevalence of exposure to AF (5 pg/ml and greater) was 52.6% in those aged less than five years, increasing to 100% in those aged 15-19 years.

Evidence of AF exposure was found in 10.5% of the Kaloleni volunteers, in 37.5% of the Chogoria volunteers and in 87.5% of the dyspepsia patients (Table 5). The difference in AF exposure between the dyspepsia group and the Chogoria volunteers was statistically significant (X^2 = 8.8; 1 df; p < 0.003). The difference in AF exposure between the Chogoria and the Kaloleni volunteers was statistically marginally significant (Fisher's 2p = 0.06). Aflatoxin albumin adduct levels exceeding 20 pg/ml were found in 41.2% of those in the dyspepsia group in contrast to 0% in the Kaloleni volunteers (Fisher's 2p = 0.0009), 6.3% in the Chogoria volunteers (Fisher's 2p = 0.03) and an average of 20.5% in the children and young adult population (X^2 = 4.5; 1 df; p = 0.03).

Table 6
Relationship between detectable aflatoxin albumin adduct level and type of maize storage container

Storage container	N	% AF positive
Plastic bags	8	50.0*
Other	52	46.2*

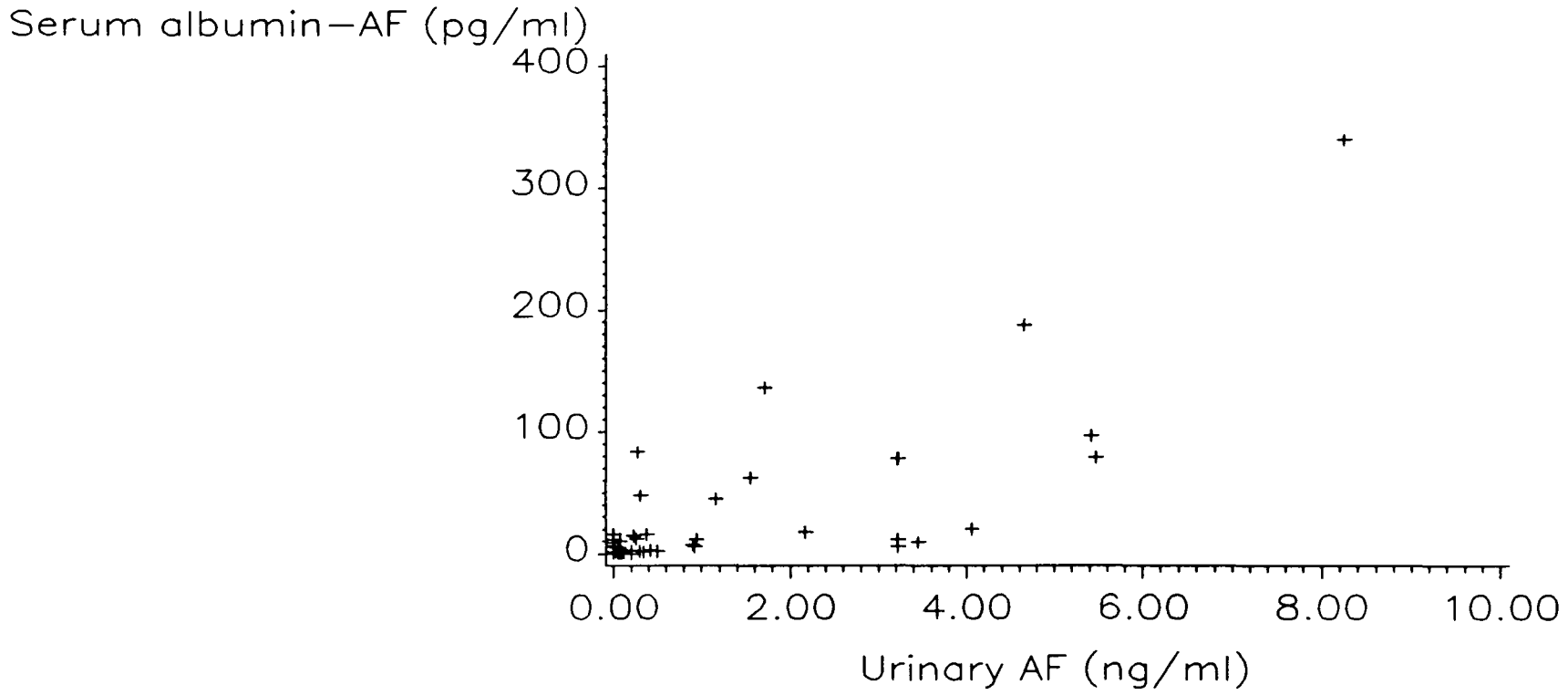
* X^2 = 0.04; 1df; p = 0.8

Table 7
Age specific incidence rates (per 100,000) of gastric cancer in Chogoria

	Year							
	1965-1970		1971-1976		1977-1982		1983-1988	
Age group	N	I.R.*	N	I.R.	N	I.R.	N	I.R.
<45	3	0.10	6	0.16	5	0.11	50	0.69
45-64	13	3.64	17	4.31	15	3.46	74	11.68
65+	1	0.75	22	14.77	12	7.29	53	23.83

* I.R. Incidence rate per 100,000

Figure 4
Relationship between serum albumin and urinary AF adduct levels in Kenyan populations



Pearson's r = 0.77, 2p < 0.0001, N = 59

3.5.1.1 Relationship between aflatoxin levels and mucosal histopathology

Looking at the dyspepsia and volunteer groups combined, detectable AF levels were found in a marginally higher proportion of those with CAG (54.8%) when compared with those with N/CSG (27.7%), as shown in Table 5 (X^2 = 3.6; 1df; p = 0.055).

3.5.1.2 Relationship between serum and urinary AF levels

There was a strong correlation between serum albumin bound and urinary AF (r = 0.77, 2p = 0.0001), as shown in Figure 4. Because of the close correlation between serum-albumin bound and urinary AF levels and because serum albumin bound AF are a measure of more long-term exposures than urinary AF (about 20 days versus 24-48h Wild et al 1989) only the serum aflatoxin results are discussed.

3.5.1.3 Aflatoxins in relation to storage methods

As shown in Table 6 there was no significant difference in the prevalence of exposure to AF between those who used plastic bags to store maize and those who did not (50% versus 46.2%, respectively (X^2 = 0.04; 1df; p = 0.8).

3.5.2 T-2-Trichothecene exposure in the study population

Table 5 shows evidence of detectable exposure to T-2-T in 33.3% of the dyspepsia group, in none of the Chogoria volunteers but in 38.0% of the Kaloleni volunteers. There

was a significant difference in the proportions exposed to T-2-T between the dyspepsia group and the Chogoria volunteers (X^2 = 4.6; 1df; p = 0.03), and between the Chogoria and Kaloleni volunteers (Fisher's 2p = 0.007). T-2-T exposure in the dyspepsia group was not significantly different when compared to the volunteers from Kaloleni (X^2 = 0.1; 1df; p = 0.8).

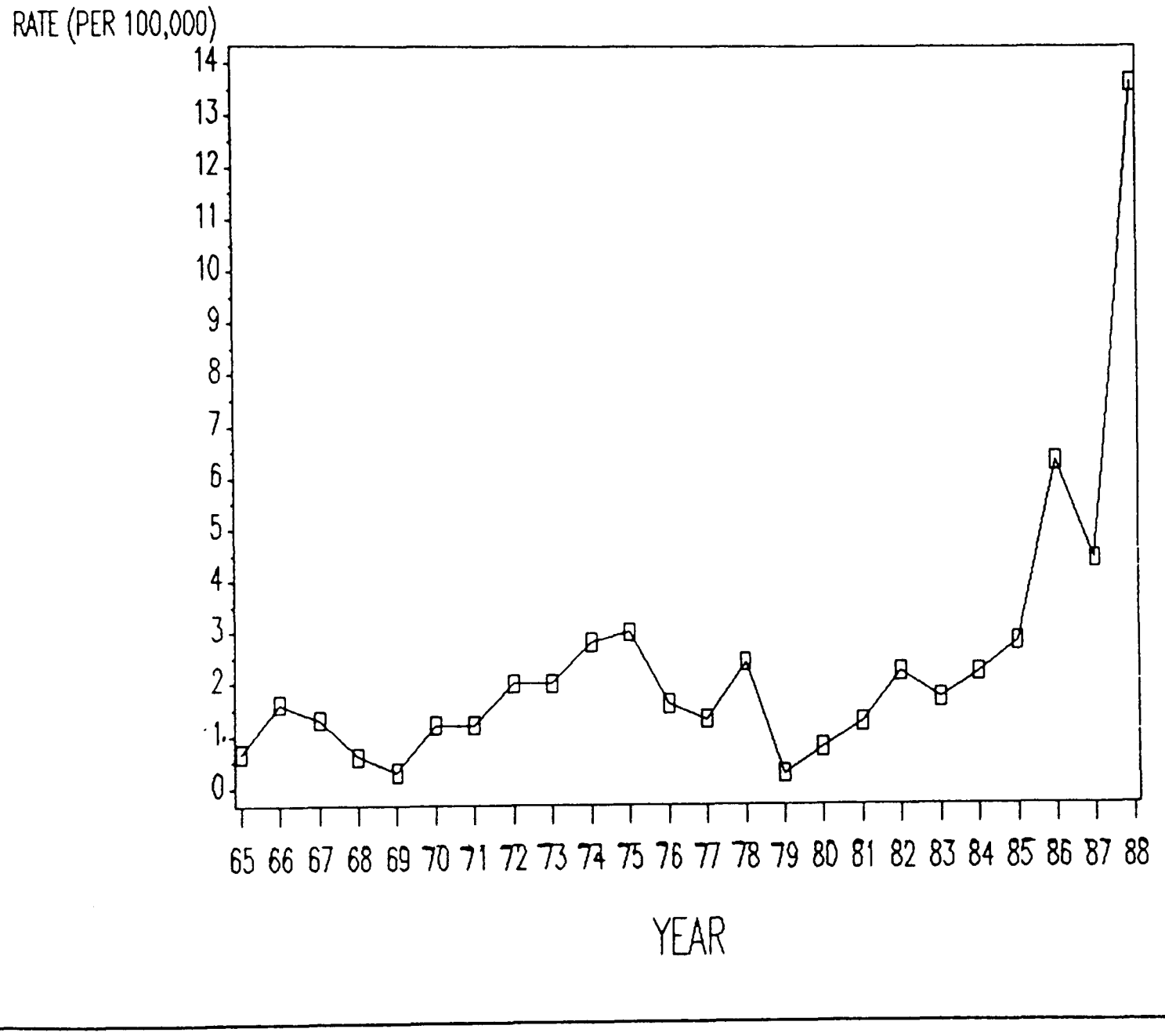
Looking at the dyspepsia and the volunteer populations combined, T-2-T were present in 45% of those with N/CSG, and in 50% of those with CAG; the difference between these two groups was not significant (X^2 = 0.0; 1df; p = 1).

3.6 Gastric cancer rates

Over the time period 1965-1988, a search of hospital records yielded a total of 228 gastric cancer cases diagnosed at the hospital, five of which had no information on age. Of the 228 cases, 36 were originally recorded by P. Cook-Mozzafari and D. Burkitt for 1965-1975 (excluding 1971, unpublished data).

Figure 5 shows the age standardised incidence rates (ASIR) for gastric cancer for each year for the period 1965-1988. ASIR for gastric cancer appeared to be below about 3 per 100,000 for the period 1965-1985, but increased steeply in the years 1986-1988 to a peak of 13.7 per 100,000 in 1988, corresponding to a World Standardised Rate of 10.4 per 100,000. An endoscope was acquired at the hospital in 1986, but excluding those diagnosed endoscopically reduced the

FIGURE 5
TRENDS IN AGE ADJUSTED INCIDENCE RATES OF GASTRIC CANCER
CHOGORIA HOSPITAL, MERU DISTRICT, KENYA
MALES AND FEMALES 1965-1988



ASIR only by a small amount, to 12.5 per 100,000.

Table 7 shows the number of gastric cancers and the estimated age specific rates for gastric cancer per 100,000 for each age group (<45, 45-64, 65+ years) for 1965-70, 1971-6, 1977-82 and 1983-8. For those aged under 45 years, rates were approximately 0.1 to 0.2 per 100,000 for the period 1965-1982, but increased to 0.7 per 100,000 in the period 1983-1988. Age specific rates for those aged 45-64 years increased from about four per 100,000 in the period 1965-1982 to 12 per 100,000 in the period 1983-88. Rates for those over 65 years were low (0.7 per 100,000) in 1965-1970, intermediate in the periods 1971-76 and 1977-82 (15 and 7 per 100,000 respectively), and high (24 per 100,000) in the period 1983-88.

4 DISCUSSION

4.1 Comparability of study groups

In this study, the difficulty in sampling randomly selected community based controls was partly overcome by choosing healthy volunteers as a comparison group. However, salaried hospital or nursing staff were most likely of a higher social class than an outpatient group drawn from the general population. These occupational differences may have been important confounders may in part have been responsible for differences between the dyspepsia and the volunteer populations. In an exploratory study of this small size, the confounding effect of occupation could be properly estimated but it ought to be considered when planning other larger studies.

4.2 Seroprevalence of H.pylori

The steep rise in seroprevalence rates, especially at a young age, was consistent with the infection pattern seen in other studies of young adults in other African countries (Chapter 1). In the Ivory coast and in Algeria, the seroprevalence rate in those aged 0-9 years was about 50% and over 70% in those aged 20-29 years (Megraud et al 1989). Infection of H.pylori at a young age in Western or wealthier countries is rare amongst children. For example in a UK study, seroprevalence of H.pylori amongst children inpatients aged 6-20 years was about 4% (Jones et al 1986).

Because of the ubiquitous distribution of H.pylori infection (over 70%) in the dyspepsia and volunteer

populations, other factors, in addition to an initial infection, may have been responsible for epigastric pain in this area.

4.3 Relationship between H.pylori and mucosal histopathology

Seven out of the nine subjects identified as positive for H.pylori on serology but H.pylori negative on histology had a normal mucosa. It is possible that this group were only recently infected, with too low a number of microorganisms to be detected histologically and too recent an infection to result in CSG or CAG. Another reason why HLO was not detected on a normal mucosa could have been due to the lack of independence in the assessment of gastritis and HLO status. If this bias was operating, one would expect HLO to be missed on normal biopsies, and this could explain the discrepancy found between histology and serology.

Almost all (80-90%) of those with CSG had either histological or serological evidence of H.pylori infection. This pattern was different from that presented in Chapter 2 on an endoscopy series from Oxford, where, using the same methodology, only 20-30% of those with CSG had evidence of H.pylori infection. CSG in the UK may have been associated with factors other than H.pylori, such as consumption of spirits, aspirin and other gastric irritants that were absent in Kenya. However, spirits, aspirin and other gastric irritants such as emetics and purgatives are freely available in Kenya, but their relationship to gastritis in

this district has not been investigated. Although gastric irritants may be consumed in Kenya, H.pylori may have been be the more important factor associated with CSG at a young age. But, given that the majority of the study population was infected with H.pylori, there was insufficient heterogeneity to test the association between H.pylori and gastritis, and other factors in Kenya. An analogy to this situation was made by Rose (1985) who suggested that if everyone was exposed to a necessary agent the distribution of a disease would be determined by its genetic susceptibility.

4.4 Prevalence of CAG

The prevalence of CAG was elevated in the dyspepsia group when compared to the Chogoria volunteer group, suggesting that this epidemic of epigastric pain may have been associated with CAG. However, CAG has not been shown to be associated with any specific clinical symptoms (Taylor 1986, Villako et al 1984).

The prevalence of CAG in the dyspepsia and volunteer populations was high (over 50%), but this is consistent with recent studies from other countries in Africa, reporting prevalence rates of 'gastritis' in adult populations of over 90% (Wyatt et al 1987, Rouvroy et al 1988, Holcombe et al 1989, Glupczynski et al 1989). The high prevalence of CAG is uncommon in populations of such a young age outside Africa (Chapter 1). High prevalence rates of CAG, especially at a young age, have usually been associated with

a higher gastric cancer risk (Chapter 1, Correa et al 1976).

4.5 Exposure to T-2-Trichothecenes and upper epigastric pain

T-2-T have been implicated in an epidemic of epigastric pain and vomiting in India following consumption of infected wheat flour (Bhat et al 1989). T-2-T were present in similar proportions in the Kaloleni volunteers when compared with the dyspepsia group but were absent in the Chogoria volunteers. Epigastric pain amongst young people has also been reported in Kaloleni, albeit anecdotally. If T-2-T were associated with epigastric pain one would expect at least a small proportion of the Chogoria volunteers to have been exposed to this mycotoxin. Because of the absence of T-2-T in the Chogoria volunteers it is unlikely that T-2-T were responsible for epigastric pain in the dyspepsia group.

4.6 Sources of aflatoxins

The half-life of albumin in humans is about 20 days, (Wild et al 1989) thus serum albumin-bound aflatoxin levels are a measure of longer term exposure than urinary aflatoxin levels. The strong correlation ($r = 0.77$; $2p < 0.0001$) between urinary and serum albumin-bound aflatoxin levels found in this study implies that exposure to aflatoxin occurred on a frequent basis, implicating consumption of contaminated staple foods. This study found no relationship between AF and maize storage conditions, but this does not exclude maize or other staples from being vehicles of AF exposure. Further studies, designed to trace the source of

AF in this population are indicated.

4.7 Prevalence of aflatoxin exposure and epigastric pain

The symptoms of this acute form of epigastric pain seen in this study were different from acute hepatitis reported from other parts of Kenya (Ngindu et al 1982). There was neither tenderness in the right upper quadrant nor jaundice. Whereas exposure to AF in these populations appeared to be ubiquitous, with all of the children aged 15-19 having evidence of AF exposure, absolute levels of AF exposure were highest in the dyspepsia group, with 48% showing levels exceeding 20 pg/ml.

H.pylori has been shown to lower the viscosity and increase the permeability of the gastric mucus layer (Sarosiek et al 1988) and it could be possible that this damage facilitates the diffusion and binding of AF on the gastric epithelium. It could be speculated that in conjunction with H.pylori infection, high AF levels were responsible for epigastric pain in the dyspepsia group.

Chronic active gastritis resulting from H.pylori infection may eventually progress to CAG. Albeit of borderline significance, AF was also associated with CAG. It might be possible that AF in the presence of CAG was responsible for the symptoms of epigastric pain seen in Chogoria. The association between AF and CAG could also explain why the children, (who probably have lower prevalence rates of CAG than the older groups) were not

affected with epigastric pain. Indirect evidence of a relationship between AF and CAG was found in China, where fungal strains of Aspergillus sp. producing sterigmatomycin (a chemical relative of AF) were more common in gastric samples from those with CAG when compared to those with a normal mucosa or CSG (Zhang et al 1984).

Differences in occupations and/or lifestyle characteristics between the dyspepsia and the volunteer groups may have been important confounders of AF exposure. Hospital staff may have been protected from AF by eating a more varied diet, low on staple foods such as maize. Those in the dyspepsia group may have used emetics more frequently than hospital staff. If this was the case, the epigastric symptoms seen in Chogoria which appeared to be due to AF may have been due to some other unidentified factor(s).

This is the first time an association between AF and epigastric pain has been reported and for this reason, the relevance of AF, H.pylori and CAG in the aetiology of epigastric pain needs to be confirmed in other studies using comparable groups as controls.

4.8 Is there a relationship between AF, CAG and gastric cancer?

AF has been associated with an increased risk of hepatocellular carcinoma, especially against a background of chronic hepatitis due to Hepatitis B virus infection (IARC 1976, Yeh et al 1989). Although not directly comparable

with other studies, the prevalence of CAG in this study seems higher than the prevalence of CAG in populations with traditionally high gastric cancer rates (See table 10 in Chapter 1). AF has been implicated in gastric cancer in the rainbow trout (Bogovskii et al 1987) and in laboratory rats (Newberne and Connor 1980). Aspergillus sp. has been found in greater frequency in gastric samples of people living in high gastric cancer risk areas in China (Zhang et al 1984). A carcinogen such as AF in the presence of chronic inflammation of the gastric mucosa (CAG) may have also played a role in gastric carcinogenesis.

Cancer incidence has only been sporadically reported from other African countries. From the information available, (world standardised) gastric cancer rates (per 100,000) were 1.8(M) 0.2(F) in Swaziland 1979-1983 (Peers and Keen 1986), 5.3(M) 1.9(F) in Gambia 1986-1988 (Gambia Hepatitis Intervention Study 1988) and 20.4(M) 10.5(F) in Bamako, Mali 1987-1988 (Bayo et al 1990). Reported rates from Kenya in 1976 ranged from about 2 to 14 per 100,000, and the highest gastric cancer rates recorded in East Africa in 1976 were 23 per 100,000 in Rwanda-Burundi (P.Cook-Mozzafari and D. Burkitt unpublished data).

The (highest) world standardised rate for gastric cancer in Chogoria (1988) was 10.4 per 100,000, moderately high for African rates but still considerably lower than those reported from high gastric cancer risk areas of Colombia (190 per 100,000), Europe (about 50 per 100,000), or

Nagasaki in Japan (82 per 100,000) (Muñoz et al 1988).

The increase in gastric cancer rates over the period 1983-1988 is noteworthy, and affected all age groups. However, it ought to be noted that this is the best estimate on the data that was made available. There are a number of reasons for suspecting the accuracy of this estimate. Firstly, the Meru population may not have grown in a linear fashion as assumed by the extrapolation of the census data since 1979. Using a logarithmic extrapolation however reduced the 1988 peak incidence rate by less than one percentage point.

Secondly, this increase may have been due to specialist interest in gastric cancer by a surgeon who arrived at the hospital in 1984 and who introduced fibre-optic endoscopy in 1986. Specialist interest in oesophageal cancer in another hospital in Kenya led to an artificial 6-fold increase in oesophageal cancer rates (Ahmed and Cook 1969), and for this reason the recent increase in gastric cancer rates in this hospital must be interpreted with caution. However, excluding nine cases diagnosed after 1986 by gastroscopy alone had little effect on the 1988 ASIR, reducing it from 13.6 to 12.5 per 100,000.

The third reason for the increase in gastric cancer incidence in this hospital could have been a change in patients' choice of hospital over time. There are 5 other hospitals in the Meru district and another two in the

adjoining Embu district. If for any reason there was a preference in recent years by the population in the district for the hospital in Chogoria, then this may have led to an artificial increase in gastric cancer seen at this hospital. One way of looking at changes in referral patterns would be to compare the spatial distribution of the gastric cancer cases over time. The addresses of those diagnosed with gastric cancer over the study period are currently being collected, so this question can only be addressed at a later date.

For the reasons above, it is not possible to say with any certainty whether there was a real increase in gastric cancer in this region. However, recent anecdotal reports of increases in gastric cancer amongst young adults in Zimbabwe (Dent et al 1977, Nkanza 1988), and Nigeria, (Atoba et al 1989) indicate the need to maintain surveillance of gastric cancer incidence trends in this area.

5 SUMMARY

This study attempted to investigate the causes of epigastric pain in young adults by assessing H.pylori status, AF and T-2-T exposure levels and CAG in a group of young symptomatic patients and in two groups of healthy volunteers. AF and H.pylori antibody levels were also measured in a group of young inpatients for unrelated conditions.

Whilst the prevalence of H.pylori and the prevalence of CAG was high in both the volunteer and dyspepsia populations, AF levels were found to be highest in the dyspepsia group. A combination of H.pylori infection, CAG and AF may have been responsible for epigastric pain in the dyspepsia group. This is the first time that such an association has been documented and for this reason it needs to be reconfirmed in other studies.

CAG is an important pathological precursor of gastric cancer. Because of the unusually high prevalence of CAG and AF exposure in this population, it may be possible that this combination was involved in the recent increase in gastric cancer incidence observed in this region. Diagnostic bias and changes in referral patterns over time may also have accounted for the recent increase in gastric cancer rates and further studies including measuring the changes in referral patterns in this district over time are indicated.

CHAPTER 7

CONCLUSION

1 Introductory remarks

The reason for focussing on the relationship between H.pylori and CAG in this thesis is because CAG is a major risk factor for gastric cancer. Despite declining incidence, gastric cancer is the second most common cancer worldwide, only recently superseded by lung cancer (Muñoz, 1988). High social class and dietary factors such as the consumption of vitamin C, fresh fruit and vegetables, and allium vegetables have been shown to be protective factors; while consumption of salt and smoked foods were shown to be risk factors in retrospective case-control studies of gastric cancer (Howson et al, 1986, Muñoz, 1988).

However, a persistent problem in case-control studies of gastric cancer (and other chronic diseases) has been the long time period, of 2-3 decades, thought to occur between exposure to relevant aetiological factors and the presentation of cases with the disease.

By focussing on the epidemiology of H.pylori and its relationship to CAG it may be possible to gain a better understanding of some of the early processes involved in the transition of a normal gastric mucosa to gastric cancer, before the appearance of symptoms.

2 Relationship between H.pylori and mucosal histopathology

H.pylori has been found to be associated with chronic active gastritis in studies of deliberate or accidental infection, in the endoscopy-based studies and in treatment studies reviewed in Chapter 1. In the Oxford study (Chapter 2), H.pylori was found to be associated with both chronic active and chronic atrophic gastritis.

The precise mechanism by which chronic active gastritis becomes atrophic is yet to be established but it is possible that H.pylori and other factors are responsible. H.pylori produces a luminal immunological response, thought to be in part immunopathogenic (Newell and Stacey 1989). H.pylori is a producer of mucinase which degrades the gastric mucus layer (Sarosiek et al 1988) and urease, which causes the accumulation of excess ammonia in the gastric lumen, leading to possible damage of the gastric epithelium (Megraud 1989). Chronic inflammation has also been associated with increased production of acids and proteolytic enzymes such as pepsins (Samloff 1989), which might have a deleterious effect on the gastric mucosa. Dietary irritants such as alcohol, salt, aspirin and coarse grains described in Chapter 1 were shown to be risk factors for CAG. Vitamin C, and fruit and vegetable consumption were inversely associated with CAG.

Data from the endoscopy study conducted in Oxford (Chapter 2) showed that an enzyme-linked immunosorbent assay (ELISA) to determine serum levels of H.pylori antibodies was

a sensitive (84.8%) and specific (92.7%) marker of H.pylori infection. Using this assay for detecting H.pylori antibodies, it was therefore possible to conduct several seroprevalence studies of H.pylori infection in a number of non-clinical populations.

H.pylori antibodies were less sensitive and less specific as markers of CAG (sensitivity = 71.4%, specificity = 90.9%) and the addition of serum pepsinogen to H.pylori as a combined marker did not lead to any marked improvements in detecting those with and without CAG except for an increased specificity at the expense of a reduced sensitivity. One of the limitations of the Oxford study was that there was only one case with severe CAG, probably because the incidence for gastric cancer in Oxford is low. This study ought to be repeated in a population with a higher prevalence of severe CAG, most likely to be found in a region of high gastric cancer incidence. Besides measuring the sensitivity and specificity of pepsinogen and H.pylori in detecting those with severe CAG, other markers such as serum gastrin could also be assessed.

3 Relationship between H.pylori and sociodemographic variables

A common feature of the studies from Caerphilly, (Chapter 3), Italy (Chapter 4) and China (Chapter 5) was the pronounced association of H.pylori prevalence with age. Prevalence of H.pylori infection by age rose steeply up to about 45 years and tended to level off in those older than 45 years. In contrast, almost all of the Kenyan health worker volunteers and patients aged 18-30 years were infected with H.pylori (Chapter 6).

The increasing prevalence of infection rates by age observed in cross-sectional studies of H.pylori has led Blaser (1989), Parsonnett (1989) and Graham (1988) to comment that H.pylori is gradually acquired as one gets older, at about 1% per year. If a) the hypothesis of gradual acquisition is true, and b) H.pylori is transmitted from person to person, then one might expect household density to be strongly associated with H.pylori prevalence rates.

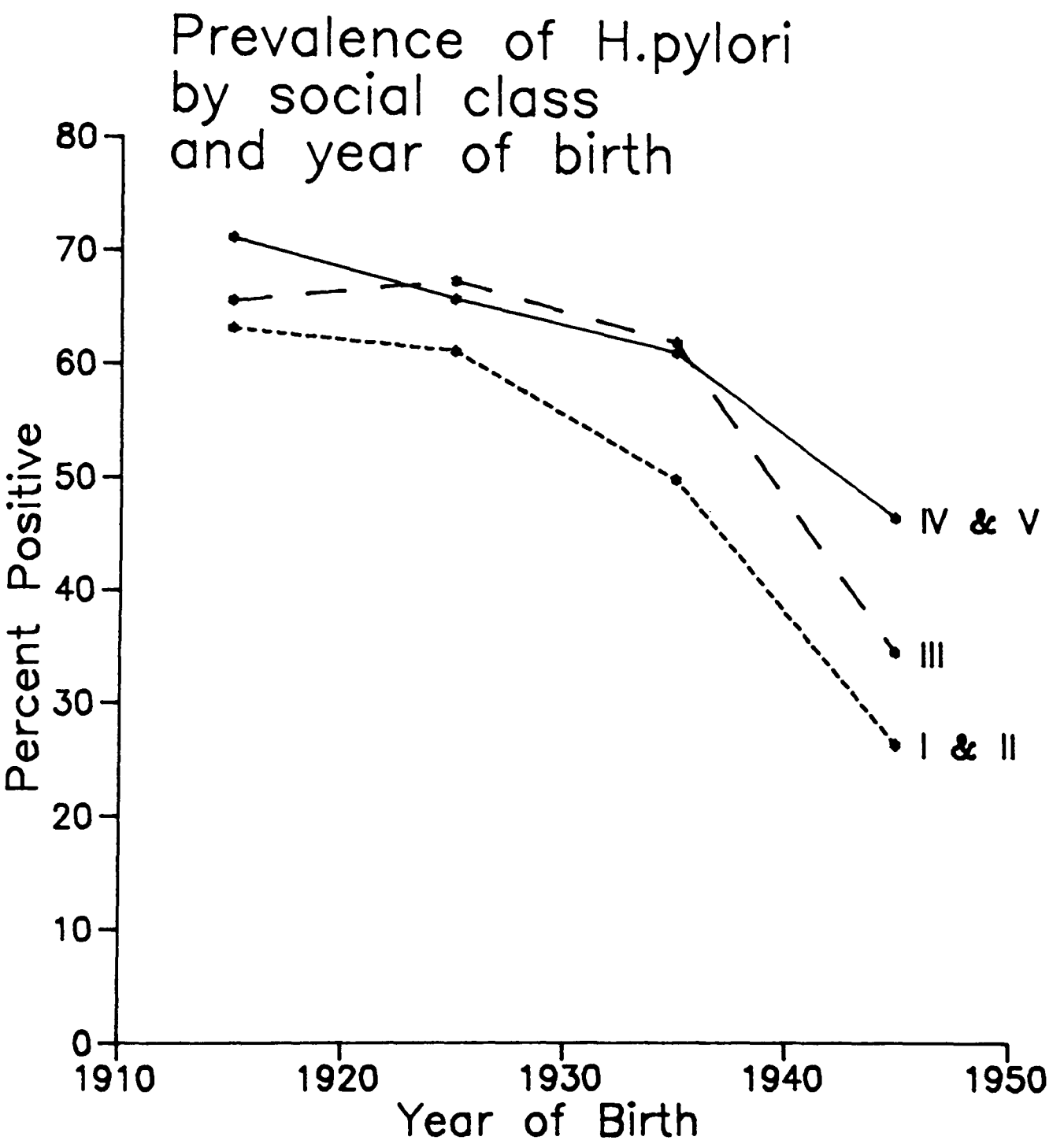
The data from Caerphilly shows that H.pylori infection is not related to number of adults, nor with the number of children in the subject's household. In the UK, those in the lowest social classes have children at a younger age than those in the higher social classes (Werner 1985). A relationship with current household density (number of children or adults) would have provided an explanation for

the different prevalence rates, by social class, in those in the youngest age group, and would have supported the hypothesis that H.pylori is gradually acquired as one gets older.

Instead, a strong association was found with the number of the subject's siblings suggesting that environmental influences early in life may be of significance in relation to H.pylori infection. If it is confirmed that infection occurs at a young age, this would imply that a cohort effect may explain observed relationship between prevalence and age (Marshall, 1990). The prevalence of infection would be highest in those born at the beginning of the century, decreasing during successive birth cohorts in keeping with improvements in housing and environmental conditions (by social class) in the UK. This is illustrated in the data from Caerphilly (Figure 1), which simply replots the data from Chapter 3 Figure 1, and which shows the distribution of H.pylori by decade of birth and social class. A cohort effect that differs by social class offers a more plausible explanation of the social class trends than attempting to postulate a differential age acquisition curve in the different social class groups.

In the Caerphilly study the trend with social class was predominated by those in the youngest age group, aged 30-34 years (that is, those born between 1944 and 1948). There was a trend by social class in the Italian study (Chapter

FIGURE 1



DATA FROM CAERPHILLY MEN (CHAPTER 3) AGED 30-74

4), but this was not statistically significant. There were only seven subjects under the age of 35 in the Italian study compared with 106 in Caerphilly. If the differences in H.pylori infection in relation to social class were primarily due to differences in the youngest age groups, it is possible that the data in Italy were numerically too sparse to show a relationship with social class, or with any other variable.

In order to unravel the secular trends (cohort and age effects) of H.pylori infection, serum or plasma taken from individuals approximately every 10 (or so) year intervals needs to be analysed for the presence of H.pylori antibodies. This study could utilise serum or plasma collected from a cohort which has been followed up in the past.

4 Relationship of H.pylori to diet and lifestyle characteristics

Another aim of this thesis was to measure the association of H.pylori in relation to dietary and lifestyle characteristics. Those with H.pylori infection in the Caerphilly study (Chapter 3) were shown to consume less vitamin C than those without, but in the Italian study (Chapter 4) vitamin C was positively associated with the presence of H.pylori.

Alcohol consumption was found to be positively associated

with H.pylori infection in Caerphilly (Chapter 3) but not in Italy (Chapter 4).

Smoking was found to be inversely associated with H.pylori in Italy and China (Chapters 4 and 5, respectively) but not in Caerphilly (Chapter 3).

In Italy, vitamin C consumption was higher in the poorer southern regions than in the affluent north (Buiatti et al 1985); by contrast, in the Caerphilly study and in the UK in general, vitamin C consumption was lowest in the lowest social classes (Fehilly et al 1984). Given these discrepancies it seems more likely that the dietary and other lifestyle associations found in relation to H.pylori infection were due to socioeconomic differences in the different countries, rather than being of aetiological importance.

Dietary and lifestyle factors were implicated as being important in the aetiology of CAG and gastric cancer in several studies (Chapter 1, Howson et al 1986) and it would be important to elucidate the relationship between H.pylori, diet, lifestyle and CAG in other studies.

Clearly, more studies of risk factors to H.pylori infection are indicated. By establishing more sensitive and specific markers of CAG it may be possible to conduct large-scale aetiological studies of 'serological markers' of CAG

in non-clinical populations, circumventing the need for invasive endoscopic procedures.

5 Relationship between H.pylori and gastric cancer

Infection by H.pylori can cause chronic antral gastritis, which may eventually progress to CAG. Because CAG is a precursor of gastric cancer, another aim of this thesis was to test the association between H.pylori prevalence and gastric cancer.

Whilst individual studies of people attending endoscopy clinics have shown a weak association between H.pylori and gastric cancer, the pooled odds ratio of the endoscopic studies (calculated in Chapter 1) showed a significant positive association of 3.2. The data presented from the Chinese study (Chapter 5) provided the first systematic evidence in a non-clinical population of a geographic correlation of 0.34 between H.pylori prevalence and gastric cancer mortality.

At first glance, a correlation of 0.34 on the basis of 45 correlation points (counties) may not appear impressive. However, the correlation between other previously established risk factors and cancer, for example, Hepatitis B antibodies and liver cancer was 0.46, (based on 65 correlation points), a difference of only 8%. Also, no other cancer in the Chinese study (Chapter 5) showed a significant correlation with the prevalence of H.pylori.

There were several counties in the Chinese study (Chapter 5) which showed high prevalence rates of H.pylori, exceeding 80% but with very low gastric cancer mortality rates. By contrast, there were no counties with high mortality rates of gastric cancer and low rates of H.pylori infection. If reconfirmed in other studies, a lack of counties with low H.pylori prevalence but high gastric cancer mortality rates implies that H.pylori may be a necessary first step in a multistage model of gastric carcinogenesis. After an initial infection, dietary, lifestyle environmental and genetic factors could influence the rate of transition from chronic active gastritis to CAG, and later to gastric cancer.

In the Caerphilly study (Chapter 3), the number of siblings, an indirect measure of overcrowding at birth, showed a strong association with H.pylori infection. Overcrowding at birth was also found to be an important risk factor for gastric cancer in the UK by Barker et al, (1990) who suggested that part of the epidemiology of gastric cancer might have an infectious aetiology. The Caerphilly study and the study by Barker et al (1990) provided indirect evidence that H.pylori infection in early life is a risk factor for gastric cancer in later life. In addition, the parallel decrease in H.pylori infection in each social class category and decade of birth in the Caerphilly study is consistent with the decrease in mortality in gastric cancer by social class observed in the UK this century (Logan

1982).

Data from the Italian study (Chapter 4) showed that those living in high gastric cancer risk areas had slightly (but not significantly) higher H.pylori prevalence rates. Similarly, those born in Caerphilly (which has a higher SMR when compared to UK rates) had higher (but not significant) prevalence rates of H.pylori infection than those born elsewhere (Chapter 3). One of the limitations of studies in Europe (Chapters 3 and 4) could be that the variation of gastric cancer was too low to show any geographical differences in H.pylori infection. By contrast, the Chinese study had a 25-fold variation in gastric cancer mortality.

A striking feature of African populations was the extremely high prevalence rates of H.pylori infection and CAG (Chapters 1 and 6), but low gastric cancer incidence rates. The highest gastric cancer rates recorded in Africa were 20/100,000 in Mali in 1988-1989 (Bayo et al 1990) and 23/100,000 in Rwanda Burundi in 1976 (P. Cook Mozzafari and Burkitt, unpublished data). These African rates are low when compared to other regions such as Japan (Nagasaki = 82/100,000, Muñoz 1989). A reason for these low African rates might be underreporting due to poor diagnostic facilities.

Whilst still requiring confirmation, the Kenyan study presented in Chapter 6 was the first to document a recent

rapid increase in gastric cancer in a rural population. The increase in gastric cancer rates could be due to improvements in diagnostic practice at the hospital in Chogoria, or to changes in referral patterns over time.

If the African gastric cancer rates are genuinely low, this contrast of high H.pylori and low gastric cancer rates could be similar to that found in some of the counties in the Chinese study, and would be in keeping with H.pylori being the first of a series of events leading to gastric cancer. Dietary or other environmental factors offering protection against gastric cancer (or the lack of promoting factors) might explain the relatively low gastric cancer rates found in other African countries. However, cancer incidence has only been sporadically recorded in the African continent. With the absence of information on gastric cancer incidence rates in Sub-Saharan African countries it is difficult to gauge the relevance of these high levels of H.pylori and associated CAG reported from these countries.

The Kenyan study (Chapter 6) was also the first to show an association between aflatoxins and epigastric pain, with aflatoxin levels being highest in the dyspepsia group. Aflatoxins are important co-factors, against a background of chronic hepatitis (inflammation), in the aetiology of liver cancer (Wild et al 1989). It was therefore speculated that, in the Kenyan study, aflatoxins might also be implicated in the aetiology of gastric cancer, in the presence of CAG.

6 H.pylori and other gastroduodenal diseases

Although not the main focus of this thesis, duodenal and gastric ulcers have been reported as important sequelae of H.pylori infection (Blaser 1989, Graham 1988). Positive but statistically non-significant associations were found between H.pylori and with those with a history of gastric, duodenal and unspecified ulcers in the Italian study (Chapter 4). In the Caerphilly study (Chapter 3) information on gastric and duodenal ulcers was not collected. However, the prevalence of H.pylori infection by year of birth, and the prevalence and mortality due to gastric and duodenal ulcer in the UK showed a similar secular pattern in relation to social class (Susser 1982).

In the Chinese study there was a statistically weak but positive correlation between H.pylori prevalence and peptic ulcer mortality. In both the Chinese and Italian studies, information on the frequency of ulcers was limited for several reasons: firstly, medical histories sometimes did not distinguish between gastric and duodenal ulcers; secondly, gastric and duodenal ulcers are rarely fatal (Langman 1979) and thirdly, many ulcers might remain undiagnosed (Jones and Lydeard 1989).

7 Public health implications

H.pylori infection of the gastric mucosa seems to be responsible for initiating events that may in the long term lead to gastric cancer and other gastroduodenal disease in a

subsection of the population. Therefore, in theory, eradication of H.pylori may lead to a delay in the onset of CAG or if already infected, lead to a normalisation of the gastric mucosa and a lowering of the long-term risk of developing gastric cancer. A short course of safe antibacterial treatment or a vaccine might therefore be developed to prevent or delay the onset of H.pylori infection, and presumably CAG.

An inherent difficulty in eradicating H.pylori is the unique niche in which this organism is found. H.pylori resides in the buffered environment of the gastric mucus gel and does not invade gastric tissue. H.pylori is sensitive to many common antibiotics in vitro (McNulty 1989). However most luminal and systemic antibacterial agents do not appear to penetrate the gel in sufficient concentration to eradicate all the organisms in vivo, and the activity of some antibiotics, for example Cephalexin, is affected by the low pH of the stomach lumen (McNulty 1989).

The use of single antibacterial agents such as penicillins has been ineffective against H.pylori (Glupczynski et al 1988, McNulty et al 1988, McNulty 1989) and in some cases has resulted in the emergence of antibiotic resistant H.pylori strains (Marshall 1989, Glupczynski et al 1988, McNulty et al 1988).

Short-term elimination has been achieved by a combination

of antibiotics and bismuth salts (Marshall et al 1990, Tytgat and Rauws 1990). Recurrence has been common however, either due to reinfection (Oderda et al 1989, Drumm et al 1990) or to inadequate eradication of the organism (Blaser 1990). For this reason there has been concern whether long-term elimination of H.pylori can be achieved with current treatment regimes (Glupczynski 1989, Blaser 1990).

Due to the aggressiveness of current therapies (usually requiring a combination of bismuth and an antibiotic such as metronidazole or amoxycillin) H.pylori treatment has been restricted only to those with recurrent duodenal and gastric ulcers. There is currently considerable debate concerning the efficacy of these current treatment regimes in treating ulcers (Tytgat et al 1989) and several controlled clinical trials are in progress. The treatment of H.pylori-associated gastritis has also been debated (Rauws and Tytgat 1989) but, given current uncertainties about the effectiveness of the therapy, has not been seriously advocated.

If a safe and effective treatment against H.pylori becomes available then, at least in principle, it could be used to treat asymptomatic populations, especially those living in areas of high gastric cancer mortality. However, in practice, the large-scale treatment of asymptomatic non-clinical populations is unlikely to be feasible due to costs, possible long term toxicity and build-up of

antibiotic resistance (Blaser 1990),

There may be, in the future, sufficient grounds to justify randomised intervention trials in areas of high gastric cancer mortality. For example, the protective efficacy of a course of treatment could be measured in relation to the subsequent incidence of gastric cancer, or more practically on the regression or progression of precancerous lesions such as CAG.

In addition, a vaccine against H.pylori could be developed. The precedent for a vaccine against an infectious agent preventing cancer already exists. A hepatitis B vaccine is already undergoing field trials in the Gambia and in China (Gambia Hepatitis Study Group 1987, Yeh et al 1989) in an attempt to reduce mortality due to liver cancer. In the Chinese study, the correlation between hepatitis B and liver cancer was of a similar magnitude as the correlation between H.pylori and gastric cancer. In theory therefore an H.pylori vaccine could be developed to reduce mortality due to gastric cancer, gastroduodenal morbidity or the prevalence of gastric cancer precursors such as chronic atrophic gastritis. However it is worth noting that H.pylori appears to be unaffected by host luminal immunological responses (Newell and Stacey 1989) and therefore the development of an effective vaccine may not necessarily be straightforward.

An additional approach might be to screen asymptomatic populations for the presence of markers of CAG by using, for example, a combination of H.pylori antibodies and serum pepsinogen levels. It might then be possible to treat and follow-up endoscopically those with a 'positive' serology. In Japan, pepsinogen A and C are being used to screen asymptomatic populations for the detection of those with CAG for follow-up by endoscopy (Miki et al 1989).

Even if a vaccine or a safe antibacterial treatment regime were developed, nothing is known about the long term risks and benefits of H.pylori treatment, the target groups to be treated or screened; or about the minimum period of bacterial suppression required to achieve significant reductions in gastric cancer mortality.

The data in the Caerphilly study suggest that H.pylori is transmitted from person-to-person, and that new generations in industrialised countries are being less exposed to the organism. The most likely explanation for a decrease in the prevalence of H.pylori by successive generations is because of improvements in sanitary and living standards seen in Western populations this century.

In order to isolate specific risk factors for public health interventions, it would also be important to study the relevance of various routes of transmission of H.pylori such as salivary versus faecal-oral. Isolating specific risk

factors of infection/reinfection would also be of importance in treatment strategies. For example, recurrence rates could be compared in subjects whose close family contacts were treated, with recurrence in subjects whose close contacts were not treated.

From almost total obscurity, H.pylori has been shown to be an important factor in the aetiology of chronic gastritis, and gastroduodenal ulcers. Chronic inflammation and cellular proliferation are important risk factors in the aetiology of epithelial cancers, including gastric cancer (Wright and Alison 1984, Lipman et al 1986, Correa 1988, Chapter 1). The series of studies presented has shown a relationship with CAG and indirectly, gastric cancer. The importance of H.pylori in relation to other factors implicated in the multistage model of gastric carcinogenesis is yet to be established. Focussing on events in the stomach that predispose to gastric cancer, well before the appearance of symptoms, however, does offer some hope for preventing a disease which usually has a poor prognosis.

APPENDIX 1

LABORATORY METHODS

1 INTRODUCTION.

This chapter describes the laboratory methods that were used to analyse the serum and plasma samples obtained from subjects in the studies described in Chapters 2 to 6. All the laboratory methods utilised enzyme linked immunosorbent assay (ELISA) techniques. H.pylori antibody concentrations were measured in all the studies, pepsinogens in the Oxford and Chinese study (Chapters 3 and 5) and serum albumin bound and urinary aflatoxins and urinary T-2 trichothecene concentrations were measured in the Kenyan study (Chapter 7).

2 ENZYME LINKED IMMUNOSORBENT ASSAY FOR ANTI-H.PYLORI ANTIBODIES

The method used for the determination of H.pylori antibodies has been described elsewhere (Steer et al 1987, Newell et al 1988,1989). It involves the absorption of the antigen onto the microELISA plates. Excess antigen is washed off and serum / plasma is then incubated with the bound antigen. After a wash cycle, antigen bound-human IgG is incubated with anti-human IgG couples to an enzyme which is visualised using a coupled chromogenic enzyme substrate. The optical density reading is proportional to the amount of antibody present.

The H.pylori assays were carried out by Dr D. Newell and Ms A. Stacey, Public Health Laboratory Services, Porton Down, (Chapters 2, 5 and 6) and by Mr S. Pedley and Ms. K. Marks of University Diagnostics, University College, London (Chapters 3 and 4).

Antigen

Acid extract antigen from H.pylori (NCTC 11638) solubilised in 50 µl of 0.2 M glycine/HCl buffer was diluted to give 5 µg protein/ml in 0.1 M carbonate buffer (pH 9.6). Antigen was coated to Dynatech microELISA plates (100 µg/well) by leaving overnight at room temperature.

ELISA

MicroELISA plates were washed 3 times with 150 µl of ELISA wash (8.5 g/l NaCl, 0.5 ml/l Tween 20). Each wash was

allowed to stand for 10 min, and wells were patted dry on absorbent paper after each wash.

Human sera were stored in 50% glycerol at -20°C. In the wells, duplicate samples of 100 μl of human serum or plasma samples were diluted in 1:100 or 1:200 0.05 M TRIS-Acetate buffer (pH 7.6) with 1% globulin-free bovine serum albumin. In each microELISA plate, control positive and negative antibody sera were also added.

Plates were incubated in humidified boxes for 2 hours at 37°C and washed 3 times, allowing to stand for 10 minutes between washes. 100 μl of 1:2000 dilution of sheep anti human IgG $(Fab)_2$ Peroxidase conjugate (Amersham International), diluted 1:100 was added per well. and plates were incubated at 37°C for 30 min, and washed 3 times as before.

100 μl of substrate (58 mg tetra methyl benzidine substrate dissolved in 10 ml DMSO-stock solution: 100 μl stock diluted to 10 ml 0.1 M sodium Acetate buffer, pH 6.0, with 1 μl hydrogen peroxide) was added to each well, for 10 min at 37°C. Reaction was stopped with 50 μl 2 M sulhphuric acid and the optical density was measured at 450 nm.

A cut off of 10 μg H.pylori IgG / ml gave the best discrimination between culture, urea test and histology H.pylori positive and negative tests (Newell et al 1990). At a 1:200 sample dilution, an optical density of 0.6 or

more corresponded to 10 μg H.pylori IgG /ml serum. At a 1:100 sample dilution 10 μg/ml corresponded to an optical density of 0.7 or more.

3 ELISA METHOD FOR THE DETERMINATION OF PEPSINOGEN A AND PEPSINOGEN C

These standardised ELISA assays for PGA and PGC were carried out in the laboratories where they were developed. Dr A. Zwiers and Professor S. Meuwissen, Department of Gastroenterology, Free University of Amsterdam, performed the ELISA tests for pepsinogen A and C in serum from subjects in the Oxford study (Chapter 2) and pepsinogen A from samples in the Chinese study (Chapter 5).

Antigen

PGA and PGC were purified from surgical specimens of normal gastric mucosa. The mucosa was homogenised in 0.1 M sodium phosphate buffer solution (pH 8.0) and the first purification step was done using a sephadex column (G75 100X5 cm, 0.025 M BISTRIS/Hcl, pH 6.0). A second purification of PGA and PGC was done using fast performance liquid chromatography on an ion exchange column using an 0-0.5 M NaCl gradient, followed by a Superose 12 gel filtration column. Antibodies to PGA and PGC were raised in goats as described elsewhere (Pals and Westerveld 1986).

For PGA, standard series and samples were diluted 1:20 in PBS (0.1% Tween) and non-immune goat serum. Non-immune goat serum was added to avoid crossreaction with goat IgG. For PGC, standards and samples were diluted 1:2 in PBS (0.1% Tween) and 3% BSA.

ELISA method

MicroELISA plates were coated overnight at 4°C with 5 µg/ml (in 0.1 M sodium carbonate buffer pH 9.6) purified goat anti-human PGA, (or anti-PGC) conjugated with horse radish peroxidase type IV (RZ = 3.0, Sigma USA). Plates were then incubated with 0.1% BSA in PBS for 30 min at 20°C and washed 3 times (with 5 min incubations) in 0.1% tween 20 in PBS.

After washing, plates for the PCA assay were incubated with the goat anti-human PGA for 1 h at 37°C. The PGC assay used rabbit anti PGC for 1 h at 37°C and then by incubation with horse radish peroxidase and pig anti-rabbit IgG. Plates were washed 3 times as above and incubated at 20°C for 30 min in substrate (2.2 mM o-phenylene diamine and 3.5 mM hydrogen peroxide in 0.1 M citric acid / phosphate buffer pH 5.5). The reaction was stopped by adding 4M HCl and optical density was read at 492 nm.

The ELISA technique appeared to be reliable and sensitive for the determination of both PGA and PGC, with inter- and intra-assay variation similar to radioimmunoassay. A correlation coefficient between the two methods was 0.95 (Westerveld et al 1987). Addition of weighed standards to samples resulted in 96.5% being recovered (Pals et al 1987).

Cut off levels

Receiver operating characteristic curves of PGA and

PGA/PGC levels diagnosing chronic atrophic gastritis were constructed by Pals et al (1987), and showed that 25 μg PGA/ml had a sensitivity and specificity of 50.4% and 92.7% in detecting those with CAG. A PGA/PGC ratio of 1.5 had a sensitivity and specificity of 66.4 and 83.0 in detecting those with CAG (Westerveld et al 1987).

4 ELISA METHOD FOR AFLATOXIN-ALBUMIN BOUND ADDUCTS

Introduction

In contrast to previous methods of determining biological AF exposure, attempts were made to quantify AF in human urine, milk, and serum, but in all these samples, excretion was rapid and AF levels would reflect only 2-3 day's exposure (Wild et al 1990). Recently, Wild et al (1990) demonstrated that AF binds quantitatively to albumin and upon repeated exposure, albumin binding parallels the binding to liver DNA (Wild et al 1987, Wild et al 1990). The half life of albumin in humans is about 20 days and therefore albumin bound AF appeared to be a useful marker of longer term (weeks/months) biological AF exposure than was previously available.

Both albumin-bound serum and urinary aflatoxin levels from the Kenyan study were measured by Dr. C. Wild, International Agency for Research on Cancer, Lyon, France.

Antigen

Polyvinyl Micro-ELISA plates (Dynatech, Plochingen FRG, 100 µg/well) were coated with 2.5 ng purified aflatoxin B1 (Sigma, St Louis), diluted in 50 µl of phosphate buffer solution.

Albumin isolation

0.5 ml of plasma or serum were heat treated to inactivate HIV at 55°C for 45 min and cooled on ice for 10 min. 750 µl of saturated ammonium sulphate was added and the mixture was

vortexed. In order to precipitate immunoglobulins, the mixture was centrifuged at 9,000g for 15 min at 0°C, and the supernatant removed. 100 µl of 1 M acetic acid was added to the supernatant, adjusted to pH 5 and the precipitated albumin was collected after centrifugation at 9,000 g for 10 min at 0°C. The albumin pellet was dissolved in 0.5 ml phosphate buffer solution (pH 7.4) and a 20 µl sample was diluted 1:50 double distilled water.

ELISA

Standards

Albumin, isolated from male BDIV rats treated with 3 mg/kg [^{14}C]-aflatoxin B1 (0.75 µCi) sacrificed after 24 h, was used for the standard inhibitors for aflatoxin-albumin ELISA. Albumin was isolated by precipitation (as above) and the modification level was calculated from the specific activity of [^{14}C]-aflatoxin B1 as 0.78 ng aflatoxin/mg albumin. Rat albumin was diluted in human serum albumin solution of 4 mg/ml in phosphate buffer solution (pH 7.4) to yield aflatoxin concentrations of 3.3 to 330 pg/ml albumin.

In order to prevent the aflatoxin binding onto the plastic microELISA wells, 100 µl of 0.1% ovalbumin, incubated at room temperature for 60 min was first added to each Micro-ELISA well, followed by serum samples or standards, and finally purified aflatoxin B1 antibody. Samples were assayed in quadruplicate, on at least 2 separate days and a mean inhibition value of <20% was regarded as negative.

For urinary aflatoxin analysis, 10-25 ml urine samples were loaded onto activated Sep-pak C18 cartridges (Waters, Millipore Corp., UK) and stored at 4°C. Cartridges were washed with 5 ml distilled water and the aflatoxins were eluted with 5 ml 80% methanol and dried overnight. The eluate was reconstituted in distilled water (1 ml per 5 ml of the original urine volume) for affinity column purification. Aflatoxin antibody affinity columns (Easi-extract, Oxoid, Basingstoke, UK) were first washed with 10 ml PBS (pH 5.4) and subsequently 1 ml of sample was diluted to 50 ml in PBS and loaded onto the column at a rate of about 25 ml/min. Columns were washed in double distilled water and bound aflatoxins were then collected by elution with 5 ml 80% ethanol. Eluates were dried and reconstituted in 500μl PBS before diluting 1:25 in PBS for ELISA quantification against an aflatoxin B1 standard curve.

Cut-off levels

Levels of 5 pg AF-albumin adduct /ml serum, or greater were considered as a positive exposure to AF (Wild et al 1990).

5 ENZYME LINKED IMMUNOSORBENT ASSAY FOR T-2 TOXIN METABOLITES IN URINE

T-2 Toxin is rapidly metabolised into H-T-2 toxin, T-2 tetraol, and into several hydroxylated derivatives. Antibodies produced against T-2 toxin have been found to be most specific to T-2-tetraol-tetracetate (T-2-4ol-4Ac) (Lee et al 1989). In order to measure the concentration of T-2 toxin and its metabolites in urine, all the T-2 metabolites were converted into T-2-4ol-4Ac.

A direct competitive ELISA was developed by coating anti-3-Ac-neosolaniol-hemisuccinate-bovine serum albumin conjugate (Anti-3Ac-NEOS-HS-BSA) to the ELISA plates and using 3-Ac-NEOS-HS-peroxidase as the enzyme marker.

Measurements for T-2-Toxin and metabolites on the urine samples from Kenya (Chapter 6) were done by Dr. C. Wild, International Agency for Research on Cancer.

Sample preparation

To recover T-2 toxin and its metabolites from a urine sample, first a 10 ml distilled water was passed through an XAD-2 column (Sigma A-7643) was passed. This was followed by 1 ml of sample at a flow rate of 1.5 ml/min and a wash with 20 ml water. Toxin and metabolites were eluted twice with 5 ml acetone and air dried. Dried sample extract was then hydrolysed using 0.5 ml of 5% 0.01 KOH in methanol. Sample was air dried to remove ethanol and water. Sample was then acetylated by adding 0.2 ml acetic anhydride (5% solution in

acetonitrile) and 0.2 ml 4-dimethylaminopyridine (DMP) solution and incubated for 1 h. Sample was again air dried, and dissolved in 0.1 ml ethanol and 0.01 M PBS. Hydrolysation and acetylation converts T-2 toxin and metabolites into T-2-4ol-4Ac.

NUNC microELISA wells were coated with 100 µl (1:2000) 3-Ac-NEOS-HS-BSA in antibody coating buffer (0.05 M/l of bicarbonate-carbonate buffer pH 9.5). Plates were incubated overnight at 4°C and washed 4 times in 350 µl Tween-PBS. 350 µl BSA-PBS (0.1 g bovine serum albumin in 100 ml PBS) was then added and plates were incubated at 37°C for 30 min. Plates were washed 4 times as before. 50 µl T-2-4ol-4Ac standards and samples, and 50 µl 3-Ac-NEOS-HS-HRP conjugate were added and incubated at 37°C for 30 min. Plates were washed as before and 1 N HCl was added to stop the reaction, and the optical density was read at 490 nm.

The correlation between RIA and ELISA technique for recovering T-2 toxins and metabolites was 87%. The average analytical recovery of 50 ng/ml samples of T-2 Toxin and metabolites was identical to a radioimmunoassay RIA For 200 ng/ml samples of T-2 toxin and metabolites, the average recovery was 94% using ELISA and 87% using an RIA (Lee et al 1989). This high level of recovery makes the ELISA method a useful analytical tool for measuring exposure to T-2 toxins in individuals.

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