THE INTER-RELATIONSHIP BETWEEN ALCOHOL
CONSUMPTION AND LIVER DISEASE ON THE PREVALENCE
AND DISEASE MANIFESTATIONS OF *HELICOBACTER*
*PYLORI*: A CLINICAL, HISTOLOGICAL AND *IN VITRO* STUDY.

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Peptic ulceration of the upper gastrointestinal tract, typically secondary to infection with *H.pylori*, is a significant cause of morbidity and mortality in the general population. Patients with liver disease have a higher prevalence of peptic ulceration than that observed in the normal population, yet the association between it, and *H.pylori* infection, is much less clearly defined.

In chapter 1, following a discussion about the clinical significance of peptic ulcer disease, the metabolism of alcohol and its effects on the upper gastrointestinal tract are reviewed. Then the discovery, epidemiology, diagnostic techniques and effects of *H.pylori* on the upper gastrointestinal tract are outlined. The role of *H.pylori* in gastric alcohol metabolism introduces a review of how alcohol and *H.pylori* might interact to cause upper gastrointestinal pathology in man.

The current literature in relation to *H.pylori* prevalence in liver disease in general, and alcohol related liver disease in particular, is conflicting. Studies to date generally fail because of small numbers, inadequate demographic data, inadequate or inappropriate control comparisons and failure to accurately characterize the level of liver injury or drinking history. A detailed review of the current state of knowledge in this area is presented which attempts to analyze the prevalence of *H.pylori* after controlling for the important variables of degree of liver injury, alcohol consumption and demography, where possible. Studies suggesting that, in the otherwise normal population, the prevalence of *H.pylori* declines with increasing alcohol consumption and that this effect might be partially beverage dependent are discussed. In patients with liver disease in general, and cirrhosis in particular, no firm conclusion about the prevalence of *H.pylori* in these patient populations can be drawn and the reasons for this are discussed in detail.

The presented literature review highlights areas for further work concerning *H.pylori* prevalence in patients misusing alcohol both with and without significant liver disease. The need to carefully control for extent of liver injury, demography and alcohol consumption is emphasized.

Chapter 2 examines the seroprevalence of *H.pylori* in four carefully defined populations of healthy volunteers (n=142), hospital inpatients (n=55) and patients with non-alcoholic (n=151) and alcoholic liver disease (n=176). The overall *H.pylori* seroprevalence in the four groups was healthy volunteers (18.3%), hospital patients (47.3%), non-alcoholic liver disease (40.4%) and alcohol misusers (35.2%). The lower overall seroprevalence observed in healthy volunteers was due to this group being significantly younger than the three patient groups and this apparent effect was lost following correction for age. No significant differences existed in overall *H.pylori* seroprevalence between the three patient groups.

In healthy volunteers, the seroprevalence of *H.pylori* increased by 7%/year of increasing age and in hospital patients by 11%/year of increasing age. This age-related increase in *H.pylori* seroprevalence is in broad agreement with other published work. A unique finding was however a 5%/year of increasing age decline in *H.pylori* seroprevalence in both liver injury groups. In minimal injury, non-alcoholic liver disease, the *H.pylori* seroprevalence increased by 3%/year of increasing age. But, in cirrhotic non-alcoholic liver disease, the seroprevalence declined by approximately 20%/year of increasing age. The net effect being the 5%/year of increasing age decline in *H.pylori* seroprevalence observed overall for non-alcoholic liver disease.

In alcohol related minimal liver injury, *H.pylori* seroprevalence declined by 10%/year of increasing age. But, in significant alcohol related liver injury, the seroprevalence remained
constant for all decades. Again, combining these two seroprevalence rates for H.pylori gave the overall 5%/year of increasing age decline in seroprevalence in the alcohol related injury group.

Putative explanations for the observed differences in age related seroprevalence between alcoholic and non-alcoholic liver injury are discussed including differences in mechanism of liver injury and alcohol consumption.

Chapter 3 prospectively assesses the prevalence of H.pylori in 100 consecutively recruited alcoholic misusers: These individuals were carefully assessed in terms of extent of liver injury (92 had diagnostic liver biopsy), drinking history, recent drug ingestion and socioeconomic circumstances. Daily median (range) ethanol consumption was 196 (48-800) g ethanol/day, with a lifetime dose of 1138 (40-7884) Kg ethanol. Men consumed significantly higher daily ethanol than women (224 vs 120 g ethanol/day: p<0.001). But no significant difference between men and women was observed for age of first drink, onset of alcohol misuse or years of misuse. 80 patients had minimal injury (normal or fatty change, 72 based on biopsy) and 20 significant liver injury (alcoholic hepatitis and/or cirrhosis-all on biopsy).

The overall H.pylori prevalence (sensitivity; specificity) by diagnostic modality was serology 43.6% (64.4%; 75.5%), UBT 66.7% (92.9%; 90.0%), urease 25.5% (52.2%; 98.2%) and histology 33.0% (71.7%; 100.0%). The 'gold-standard' used for determining sensitivity and specificity was either histology positive or histology negative but at least two non-histological tests positive. Using this gold-standard, the overall H.pylori prevalence was 46.0%.

No significant association between H.pylori status and smoking, caffeine consumption, recent drug ingestion in the previous three months or level of alcohol misuse could be determined. Similarly, no significant difference in H.pylori prevalence could be detected using any diagnostic modality between significant and minimal liver injury groups (50.0% vs 45.0% respectively, with gold-standard)

The H.pylori prevalence by age, using the gold-standard was 56.3% (<40yr), 40.5% (41-50yr), 52.2% (51-60yr) and 66.6% (>61yr). These differences between decades were not significant. It is the lack of an apparent age related increase in H.pylori prevalence that emphasizes the unique features of this patient population. Broadly similar results were obtained with other diagnostic modalities except urease testing. Here, H.pylori prevalence was significantly lower at the extremes of age when compared with the gold-standard (21.0% vs 56.3% [<40yr: p=0.03]; 31.3% vs 66.6% [>61yr: p=0.04]). It is hypothesized that the apparently lower H.pylori prevalence obtained with urease testing might be accounted for by a direct alcohol toxicity effect on H.pylori itself. This would be analogous to the false negative urease tests produced following recent PPI or antibiotic ingestion.

Chapter 4 examines the relationship between symptoms, endoscopic findings, H.pylori status, alcohol consumption, and gastroduodenal pathology in the 100 prospectively assessed alcoholic misusers: 36% had no dyspeptic symptoms but 24% had symptoms scored as ‘severe.’ No significant relationship existed between dyspepsia scores and daily alcohol consumption, period of abstinence, any drug usage (PPI, H2 blockers, antibiotics or other), current smoking or H.pylori status. Similarly, following initial classification of the 100 alcohol misusers by H.pylori status (33 H.pylori +) no relationship between either presence or severity of symptoms and any other risk factors for dyspepsia could be determined.

Of the 100 alcohol misusers, 60% had ‘any abnormality’ at endoscopy. Within the sub-set of patients with dyspepsia, 59.4% had an abnormality at endoscopy and 61.1% of the asymptomatic patients also had an endoscopic abnormality. 58.8% of the H.pylori positive alcohol misusers had dyspepsia (66.7% H.pylori negative). Exactly half had an abnormality at endoscopy (63.6% H.pylori negative) and in the 41.2% of the H.pylori positive patients who
were asymptomatic (33.3% \textit{H.pylori} negative), 46.1% had an endoscopic abnormality (68.2% \textit{H.pylori} negative). None of these differences either within, or between groups was significant.

Histological changes in the gastric mucosa of the 100 alcohol misusers were classified according to the Sydney system as either antral (A) or body (B): normal (13%A; 22%B), \textit{H.pylori} associated gastritis (31%A; 29%B), acute gastritis (3%A; 3%B), reactive gastritis (7%; 3%B), intestinal metaplasia/atrophy (4%A; 7%B). The commonest histological abnormality was chronic gastritis (41%A; 35%B). None of these differences of histological abnormalities between sites was significant.

The mean gastric inflammatory scores for the 100 alcohol misusers was 1.07 and significantly higher scores were observed in \textit{H.pylori} positive alcohol misusers when compared with negative misusers (Hp+; 2.37, Hp-; 0.411: p<0.0001).

Comparison between 50 age and sex matched pairs of alcohol misusers and controls were undertaken: A non-significantly lower prevalence of \textit{H.pylori} was found in the antrum and body of the alcohol misusers in comparison with matched controls. A significantly lower prevalence of chemical gastritis and antral intestinal metaplasia was observed in the alcohol misusers (6% vs 22%; p=0.021). Explanations for this finding, apparently in conflict with the Sydney system predictions, are discussed. A speculative explanation involving direct alcohol toxicity against \textit{H.pylori} in turn allowing temporary healing of \textit{H.pylori} associated gastritis is discussed.

Chapter 5 examines the effects of different alcoholic beverages on the motility and survival of \textit{H.pylori in vitro}. All alcoholic beverages were diluted to 0.05g ethanol/ml as this is approximately equal to the ethanol content of table wine. A profound effect on \textit{in vitro} motility and survival of \textit{H.pylori} was observed following incubation with both red and white wines. No other beverage, at this ethanol concentration, produced a similar effect. Evidence is presented to support a ‘congeners hypothesis’ for the mode of action of alcoholic beverages in affecting \textit{in vitro} \textit{H.pylori} motility and survival. The nearer the beverage is to wine in the production process the more profound the effects on \textit{H.pylori}. Thus, non-alcoholic grape juice has an effect that is approximately intermediate between wine and laboratory ethanol. Conversely, sake, which is rice based, has much less effect on both the \textit{in vitro} motility and survival of \textit{H.pylori} at this ethanol concentration.

Chapter 6 discusses the congener hypothesis in more detail. It speculates that whereas liver injury is dependent on lifetime ethanol dose, \textit{H.pylori} related gastric injury may depend not only on ethanol but also other beverage congeners. How a complex cycle in the alcohol misuser of infection with \textit{H.pylori}, gastric irritation, partial clearing of the organism due to beverage congener consumed only to be followed at a later date by resurgence of infection is presented.

In conclusion, the relationship between \textit{H.pylori}, liver injury, alcohol consumption and peptic ulceration is complex. The ‘congeners-hypothesis’, developed during this work, is discussed and the importance of considering not just the ethanol content of consumed alcoholic beverages in future studies but also the non-ethanol congeners is emphasized.
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STATEMENT OF ORIGINALITY

The projects described in this thesis were designed and performed by myself except as stated below:

In Chapter 2, the ELISA assay for *H. pylori* was performed by Mrs Louise Newport in the Department of Microbiology, Royal Free Hospital London. The multi-variate statistical analysis for this chapter was performed with help from Dr Fiona Lample in The Department of Primary Care and Population Sciences, Royal Free and University College Hospital Medical School, London.

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# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NSAIDs</td>
<td>Non steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>PPI</td>
<td>Proton pump inhibitor drugs</td>
</tr>
<tr>
<td>H2 blocker</td>
<td>Histamine receptor antagonist drugs</td>
</tr>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>ALDH</td>
<td>Acetaldehyde dehydrogenase</td>
</tr>
<tr>
<td>ABV</td>
<td>Alcohol by volume</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunoabsorbent assay</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>UBT</td>
<td>Urea Breath test</td>
</tr>
<tr>
<td>CLO*</td>
<td>‘Campylobacter (H. pylori) Like Organisms’ test (for urease).</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
</tr>
<tr>
<td>PUD</td>
<td>Peptic Ulcer Disease</td>
</tr>
<tr>
<td>DU</td>
<td>Duodenal Ulcer</td>
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<tr>
<td>GU</td>
<td>Gastric Ulcer</td>
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INTRODUCTION: THE EFFECTS OF ALCOHOL AND HELICOBACTER PYLORI ON THE UPPER GASTROINTESTINAL TRACT.

1.1 Introduction

Both excessive use of alcohol and infection with *H. pylori* impact significantly on the upper gastrointestinal (GI) tract in man. It is unclear, however, whether these two factors work independently, synergistically, or even contrary to one another as pathogenic agents.

In this chapter, the metabolism of alcohol and definitions of drinking levels will be described. The effects of alcohol *per se* on the upper GI tract will then be delineated. Then, details of the discovery, epidemiology and risk factors for infection with *H. pylori* will be detailed and its role in the pathogenesis of upper GI tract disease discussed. Finally, the literature on the interrelationships between alcohol use/misuse, *H. pylori* infection and upper GI disease will be surveyed taking into account the confounding effects of any associated alcohol-related liver injury. Finally, the aims of the thesis will be detailed based on the gaps in our knowledge of the interplay of these two major pathogenic factors in the development of upper GI tract disease in man.

1.2 The Clinical Significance of Peptic Ulcer in Gastroenterology

Peptic ulceration is the term used to describe the clinical and pathological changes associated with ulceration either in the stomach or duodenum of man (Fig 1.1 A and B). The clinical features vary considerably; patients might be asymptomatic or else suffer indigestion and epigastric pain, or else life threatening haematemesis, perforation or rarely, intestinal obstruction.

It is difficult to assess the exact prevalence of the condition as many individuals are asymptomatic and diagnosis can only reliably be made if the patient undergoes endoscopy, surgery or at necropsy. In the UK, the overall prevalence of peptic ulcers is of the order of 6-13% in men and 2-5% for women (Shearman 1989, cited at www.ebando.co.uk/). This is in broad agreement with the 8.5% prevalence in Spain (Vaira et al. 1994), but is ten times lower than the 82% prevalence in a Greek series of patients with dyspepsia (Archimandritis et al...
These differences may be methodological but also reflect geography and socio-economic conditions. Improving socio-economic conditions are cited as a major reason to account for a declining peptic ulcer prevalence in developed countries (Tovey et al. 1975).

The annual adult incidence of peptic ulcers overall in the UK is 1.8/1000/year. For gastric ulceration the incidence is 0.5 for men and 0.3 for women/1000 same sex individuals/year. This compares with an incidence of duodenal ulceration of 2.2 for men and 0.6 for women/1000 same sex individuals/year (Shearman 1989).

The commonest life-threatening complication of peptic ulcer disease is bleeding. This may occur in up to 20% of ulcer patients and accounts for half the cases of upper gastrointestinal bleeding in some studies (Laine et al. 1994). Despite developments in endoscopy over the last 20 years, the overall mortality from upper gastrointestinal bleeding has remained constant at approximately 1 per 100,000 since the 1980s (Bloom 1991). This probably reflects an increasing burden of ‘complex’ ulcer disease in an elderly population with multi-organ disease (Hasselgren et al. 1998).
Figure 1.1A: Gastric Ulceration

A gastric ulcer (black arrow), approximately 4mm in diameter with surrounding blood (thin white arrow). There is a surrounding gastritis (thick white arrow). This patient was *H. pylori* negative and had been consuming 1 bottle of spirits (240g ethanol)/day.

Figure 1.1B: Duodenal Ulceration

A duodenal ulcer (black arrow), approximately 6mm in diameter, in the first part of the duodenum. There is a surrounding duodenitis (thick white arrow). This patient was *H. pylori* positive and consumed less than 20g ethanol/week.
1.3 The Metabolism of Alcohol

1.3.1 Absorption and Metabolism of Alcohol in the Mouth and Oesophagus
Alcohol can be absorbed from the whole gastrointestinal tract by passive diffusion, there being no active transport of ethanol across mucosal membrane. Consequently, absorption from the buccal mucosa and oesophagus, under normal circumstances, is minimal due to rapid transit and minimal 'contact-time' with the mucosa (Bode 1980; Kalant et al 1971).

1.3.2 Absorption and Metabolism of Alcohol in the Stomach
The absorption of alcohol from the stomach is variable and depends on several factors. It is most rapid when taken on an empty stomach and when the alcohol concentration is between 20-30%. Thus, sherry with an alcohol concentration by volume (ABV) of 20% produces a more rapid rise in blood alcohol concentration than either beer with an ABV of 8% or spirits with an ABV of 40%. The conventional explanation for the slow absorption of spirits is that they reduce gastric emptying (Paton 1998).

In the post-prandial state, alcohol absorption characteristics are altered with up to 39.4+/-.1% being absorbed through the stomach in the first hour and only 24.0+/-3.0% through the duodenum for the full time needed for complete oral absorption (Corto et al. 1986). The remaining absorption takes place through the stomach after the first post-prandial hour. Other factors known to affect ethanol absorption include drugs probably by interfering with gastric emptying (Paton 1998).

The gastric mucosa makes a contribution to final blood ethanol concentration following oral ingestion by metabolism of ethanol using a mucosal associated alcohol dehydrogenase (ADH). The significance of this and its contribution to final blood ethanol concentration is however contentious. Caballeria et al (1987) found negligible gastric ADH activity in rat stomach when compared with hepatic ADH under 'physiological alcohol conditions.' But, gastric ethanol concentrations are typically higher than hepatic ethanol concentrations. When ethanol concentrations were increased to be 'physiologically appropriate' for the stomach, as opposed to the liver, gastric ADH activity increased. At the same time, hepatic ADH activity declined due to substrate inhibition, so at approximately 700 mM alcohol concentration gastric ADH activity was approximately equal to hepatic ADH activity. Even this concentration of alcohol is relatively low as it is roughly equal to the concentration of ethanol found in '50:50'diluted Table wine [Table wine at 8% alcohol by volume (ABV) is 1.35mM].
Introduction: Alcohol, *H. pylori* and the Upper GI Tract

Studies using comparisons between oral and intravenous ‘area under the curve’ plots for alcohol metabolism suggest gastric ADH may contribute between 20-30% of ‘first-pass’ alcohol metabolism (Julkunen *et al.* 1985). Interpretation is however difficult because alcohol metabolism does not follow ‘first-order’ kinetics (Morgan *et al.* 1998). As a consequence, it is uncertain if these differences ‘truly represent first pass metabolism or simply reflect slower absorption’. In addition, ‘is it mainly gastric or hepatic in origin?’ (Lieber 1998).

Gastric ADH is present in parietal cells, which in turn are found predominately in the gastric antrum (Julkunen *et al.* 1985). It is therefore the loss of gastric ADH that is probably a more significant factor in producing higher peak ethanol concentrations following oral alcohol consumption than a loss of surface area for passive absorption in partial gastrectomy patients (Frezza *et al.* 1997). Similarly, gastric ADH activity in non-alcoholic women being 59% of men caused a first-pass metabolism of alcohol of only 23% of the equivalent male value (Frezza *et al.* 1990). While this has been cited as a potential explanation for higher rates of alcohol related injury in females (Saunders *et al.* 1981), body composition, and in particular differences in fat-water ratios are likely to be much more significant at determining peak alcohol levels and hence toxicity (Morgan *et al.* 1998).

The importance of gastric ADH in the first-pass metabolism of alcohol is not universally accepted. For example, Brown *et al.* (1994) while demonstrating significant variation in gastric ADH between individuals, were unable to correlate this in any way to pharmacokinetic profiles of alcohol metabolism. A possible explanation for the lack of correlation between alcohol and gastric ADH may lie with the biopsy protocol used; only antral biopsies were taken to assess ADH and these might not accurately reflect total gastric ADH. In addition, it is difficult to fully control for weight of biopsy specimens and as a consequence total enzymatic activity—a factor acknowledged by the authors. In addition, four of the 33 subjects studied had ‘peptic ulcer disease.’ The implication of this finding is that they were also *H. pylori* positive although this data was not available. Failure to assess *H. pylori* status must be considered of critical importance when assessing ethanol first-pass metabolism for reasons to be discussed later (see section 1.8).

The animal model data to support the importance of gastric metabolism in the first-pass metabolism of alcohol has been succinctly reviewed by Lieber (1998). In rats, infusion of ethanol directly into the duodenum or portal circulation to effectively by-pass the stomach,
produces a blood alcohol curve almost identical to that seen after peripheral intravenous injection. Similarly, the alcohol pharmacokinetic curves are equivalent between sham operated and portal vein ligated rats, reinforcing the point that the predominant metabolic first-pass metabolism step is gastric and not hepatic.

Probably the best study on gastric ADH and demography was by Seitz et al (1993). Until the 4th-5th decade, gastric ADH activity is significantly higher in men than women. In the 5th-6th decades however male gastric ADH declines to similar values as that seen in females. There is then a ‘recovery’ in subsequent decades, with no significant difference between the two sexes (Figure 1.2). This distribution of gastric ADH may be a factor in explaining higher peak alcohol concentrations observed in women although, as previously discussed, differences in body composition are likely to prove more significant. The decline in gastric ADH in the 5th decade may represent an ageing phenomenon with the development of atrophic gastritis. Certainly atrophic gastritis is to be expected in the 5th decade in individuals who are infected with H.pylori. But, the subjects in this study were all said to be H.pylori negative.

For gastric ADH to be effective, alcohol must remain in the stomach for as long as possible. As expected, metaclopramide that speeds gastric emptying reduces the first-pass metabolism of alcohol and buscopan, which delays gastric emptying, has the opposite effect (Oneta et al. 1998). This is analogous to the earlier observations on delayed gastric emptying with spirits and post-prandially. This process is interrupted further if drugs that suppress gastric ADH, such as H2 antagonists, are given (Bode et al. 1999).

From the above discussion it can be seen that the metabolism of alcohol is critically dependent on the upper gastrointestinal tract before any alcohol even reaches the liver for further metabolism. Key to this process is gastric emptying which is typically slowed by drugs such as buscopan and increased with metaclopramide The level of gastric ADH to metabolise alcohol is in turn critically dependent on age, sex, previous alcohol use or misuse and recent drug ingestion.
1.3.3 Absorption and Metabolism of Alcohol in the Duodenum

Absorption of ethanol from the duodenum is mainly by passive diffusion and is critically dependent on the prandial state as discussed above. The duodenum has an alkaline pH due to pancreatic secretions. It has been suggested that these produce the optimum pH for \textit{H. pylori} ADH to metabolise ethanol to acetaldehyde. This is however, probably irrelevant, since the bacterium has an exclusive gastric habitat except when gastric metaplasia has developed in the duodenum (Wyatt \textit{et al.} 1990; see section 4.3.1)

Changes in small intestinal motility have been observed following both oral and intravenous alcohol administration to healthy volunteers and alcohol misusers. In the jejunum, there is a decrease in type 1 (impending) wave activity but no effect on type 3 (propulsive) wave activity. In the ileum, the opposite is observed with no effect on type 1 waves but an increase in type 3 activity (Bode 1980). While these changes may account for the diarrhoea commonly suffered by alcohol misusers, the absence of ADH in the small intestine, means that it is unlikely to influence alcohol metabolism in a way analogous to that seen with changes in gastric emptying.
1.3.4 Metabolism of Alcohol in the Liver

The metabolism of alcohol in the liver has been reviewed by Morgan and Ritson (1998). The following is a summary of this work. The three hepatic enzymes involved in alcohol metabolism are ADH, located in the cytosol, CYP2E1, an isoform of cytochrome P450, located in the smooth endoplasmic reticulum and catalase located in peroxisomes. All three enzymes catalyse the conversion of ethanol to acetaldehyde. ADH is quantitatively the most important enzyme for alcohol metabolism with CYP2E1 only being induced in habitual drinkers at increasing alcohol concentrations. The role of catalase in ethanol metabolism appears to be minor.

Ethanol is metabolised by all three enzymes to acetaldehyde, which in turn, is metabolised by acetaldehyde dehydrogenase (ALDH) to the less toxic acetate. Two isoforms of ALDH have been described and the second mitochondrial isoform (ALDH2) is the more important. Inherited abnormalities of this enzyme account for the marked facial flushing, seen after alcohol ingestion, in some Orientals. The acetate produced as a result of ALDH metabolism is rapidly metabolised to carbon dioxide and water.

1.4 Alcohol Use and Misuse

1.4.1 Levels of Use and Misuse

It is not possible to define truly ‘safe’ and ‘non-safe’ levels of alcohol consumption as these depend on time and situation. For example, the risk of alcohol-related injuries begin to increase with blood ethanol concentrations as low as 20mg/100ml (4.3mmol/L) (Morgan and Ritson 1998). For alcohol related physical harm, excluding injuries, a consensus view from the Royal Colleges evolved throughout the 1980s and this has again been summarised by Morgan and Ritson (1998). In men, intakes of alcohol <21 units a week and, in women, of <14 units a week are associated with ‘low’ risk. Intakes of between 22-50 units/week in men and between 15-35 units/week in women are described as ‘hazardous’ and carry an ‘intermediate’ risk. ‘Harmful drinking’ or ‘alcohol misuse’ are defined as >50 units/week in men and >35 units/week in women.

1.4.2 The Alcohol Dependence Syndrome

Before assessing any putative role of alcohol as a risk factor for peptic ulcer disease it is first necessary to distinguish between ‘use’ and ‘misuse’ of alcohol. These two groups are not defined in terms of a specific daily alcohol consumption. Instead, the ‘alcohol misuser’ is
identified as someone who has a drinking pattern that meets certain diagnostic criteria, whereas alcohol users do not have features of the alcohol misuse syndrome.

The 'alcohol dependence syndrome' has 7 specific features which include:
1) The subjective awareness of compulsion to drink alcohol. Typically described as 'craving' or 'necessity to drink.'
2) Narrowing of drinking repertoire. Not just in terms of 'preferred beverage,' but also in terms of more stereotyped and predictable drinking behaviour.
3) Primacy of drinking over other behaviour. At its extreme, drinking takes over the misusers life which is devoted to obtaining and consuming alcohol.
4) Altered tolerance to alcohol. Boasts of 'never being drunk' reflect induction of liver enzymes and increased clearance of ethanol. A more 'lay-definition' is 'it costs an alcohol misuser twice as much money to get drunk as a non drinker.'
5) Repeated withdrawal. Classical delerium tremens is the most outward sign of alcohol withdrawal. Often described by the misuser as 'bad-nerves'.
6) Relief and/or avoidance of withdrawal by further drinking. The classical morning 'DTs' are 'cured' by an 'eye-opener.'
7) Reinstatement of drinking behaviour after abstinence. The alcohol misuser may be able to be abstinent for a few weeks 'without problem' but then, by re-experimenting with alcohol rapidly escalates 'back to square one' in terms of consumption.

1.5 The Effect of Alcohol on The Upper Gastrointestinal Tract.

1.5.1 Alcohol and the Mouth

Salivary flow is increased when alcohol is applied to the buccal mucosa or tongue but not stomach and this effect is observed with both laboratory alcohol and beverage alcohol (Bode 1980; Martin et al 1971). Patients with alcoholic liver disease, may have different salivary characteristics than normal individuals with both an increased salivary flow after stimulation with citric acid and possibly an increased protein content (Durr et al. 1982). The exact relation between alcohol misuse, salivary flow and, an increased prevalence of parotitis, glossitis and paradontitis in alcohol misusers is not clear and may relate more significantly to secondary malnutrition (Larato 1973; Bode 1980).

The effects of alcohol on the oral mucosa have recently been reviewed by Jennings and Howdle (2000): Alcohol causes mucosal trauma predisposing to Candida spp infection and
promotes pre-malignant change such as leukoplakia. Frank malignant change may develop years after the cessation of active misuse and may occur at multiple oral sites. This suggests that alcohol causes a dysplastic ‘field-change’ in the oral mucosa before frank malignancy develops. A significant correlation between mean daily alcohol consumption and cancers of the tongue and oral region is well recognised and does appear to be independent of the increased risk observed in smokers (Schwartz et al. 1962). But, since most alcohol misusers are often heavy smokers, it is difficult to quantify the relative effects of these two agents.

1.5.2 Alcohol and The Oesophagus
Following intoxication in normal individuals, a decrease in lower oesophageal sphincter pressure is observed (Mayer et al. 1978; Weinbeck et al. 1981) and this appears, at least in part, to depend on blood ethanol concentration (Mayer et al. 1978). This in turn promotes gastro-oesophageal reflux by up to five-fold in healthy volunteers (Kaufman et al. 1978) and is likely to be higher in alcohol misusers due to a secondary autonomic neuropathy (Villako et al. 1995). These changes in turn, have been proposed as a mechanism to account for a higher prevalence of oesophagitis and Barretts oesophagus often found in alcohol misusers (Messian et al. 1978; Weinbeck et al. 1981).

1.5.3 Alcohol and the Stomach
Interest in the upper gastrointestinal tract, dyspepsia and alcohol is not new. In 1661 Robert Lovell wrote about the ‘imbecility of the stomach’ in relation to dyspepsia or ‘dyspepsy’ as it was then called (Gibbs 1997). Much was written subsequently and distilled into an aetiological definition by 1811 in John Quincy’s Lexicon Physico-Medicum; dyspepsia was defined as giving ‘great grief and uneasiness of mind, intense study, profuse evacuations and excess in venery’. An association with the ‘drinking of spiritous liquors’ as an aetiological factor was noted, but typical of the day ‘tea, tobacco, opium and other narcotics’ were also included in the list of aetiologies. Much emphasis was also placed on the ‘nervousness’ element of dyspepsia aetiology.

A more scientific approach to dyspepsia in general, and specifically the role of alcohol on gastric mucosa, was taken by William Beaumont in his study of the fistulous stomach of Alexis Martin. Direct observation of gastric mucosa was possible in this individual and the erythema associated with alcohol ingestion described. The intra-mucosal capillary dilation causing the erythema has been subsequently characterised and may be associated with protein
Introduction: Alcohol, *H. pylori* and the Upper GI Tract

Deposition (Dinoso *et al*. 1976; Silen *et al*. 1974). This in turn is associated with inter-cellular tight junction disruption (Dinoso *et al*. 1976).

A ‘haemorrhagic gastritis’ in alcohol misuse has been described (Dagradi *et al*. 1973; Gottfried *et al*. 1976; Pötzi *et al*. 1982). The level of alcohol misuse needed to produce this change is high as it is not seen in otherwise healthy individuals consuming low or moderate alcohol doses (Bode 1980; Dinoso *et al*. 1972).

The exact role of alcohol on gastric emptying is not clear with studies supporting both a delay and an acceleration of gastric emptying depending on exact experimental circumstance (Bode 1980; Sanders *et al*. 1982). These differences may not be due to experimental technique and may represent opposite ends of a physiological spectrum. A wide variation in gastric emptying would be one explanation for the wide inter-individual variation in blood-ethanol concentration-time curves following standard doses of alcohol (Stefenelli *et al*. 1977). *H. pylori* does not appear to influence gastric emptying based on radio-nuclide studies, but advanced cirrhosis of non-alcoholic aetiology does appear to delay gastric emptying using the same technique (Kao *et al*. 1994; Kao *et al*. 1996).

The role of alcohol in the aetiology of gastric carcinoma is controversial. Alcohol is known to produce gastritis and gastritis is in turn, believed to be the precursor of intestinal metaplasia and later dysplasia. A plausible biological mechanism for a role of alcohol in the development of gastric cancer therefore exists (Correa *et al*. 1983). An association also exists between gastric carcinoma and cigarette consumption as well as low vegetable consumption (Hirayama 1981). But, since alcohol misusers are also likely to fall into these particular categories, interpretation becomes difficult.

### 1.5.4 Alcohol and the Duodenum

The effects of alcohol on duodenal mucosa appear to be less marked than those observed in the stomach. On a microscopic level, however, interference with the absorption of monosaccharides (Dinda *et al*. 1981), glucose (Ghirardi *et al*. 1971), amino acids (Bode 1980; Kuo *et al*. 1974) and vitamins (Bode 1980; Hermos *et al*. 1972) have all been recognised.
1.6 The Discovery of *Helicobacter pylori*

*Helicobacter pylori* is a spiral or curved, flagellated, microaerophilic, Gram-negative rod, which has a unique ability to colonise and survive in the acid environment of the stomach (Goodwin *et al* 1989) (Figure 1.3). The first sighting of spiral organisms in the stomachs of mammals was made by Bottcher in 1874. Further reports confirmed these findings (Kreinitz 1906), but in 1954 Palmer found no evidence of this bacteria in over a thousand human stomach biopsies examined histologically. Progress was inevitably slowed after this and as late as 1975 culture of gastric aspirates only yielded Pseudomonas aeruginosa (Steer 1975).

Introduction: Alcohol, *H. pylori* and the Upper GI Tract

Figure 1.3: *Helicobacter pylori*

False colour electron micrograph showing flagellae (large white arrow), filamentous nucleoid (black arrow), plasma membrane surrounding the outer cell wall (curved white arrow) and cytoplasm containing ribosomes (small white arrow).

X 36,000.

1.7 The Epidemiology of *H. pylori*

1.7.1 Risk Factors for Acquiring Infection.

*H. pylori* is largely acquired in childhood, ostensibly by person-to-person spread and demonstrates a cohort effect. It is associated with poor socio-economic circumstances such as poor education, poverty, crowded living conditions and lack of hot water (Fiedorek et al. 1991; Mendall et al. 1992; Webb et al. 1994). A history of ‘gastric disease’ in either parent also increases infectivity risk (Brenner et al. 1998). As living conditions have improved in the population overall the number of children infected has declined; this helps explain the falling prevalence rates in Western populations. Positive serology is present in approximately 50% of those over 60 years of age but only 20% of those under 40 (Anon. 1993). These figures are likely to reflect changes in childhood acquired infection rates as the incidence of newly
acquired or re-acquired infection in adults may be as low as 0.5% (Parsonnet et al 1995). In developing countries, the prevalence of \textit{H.pylori} is higher with poor living conditions being the major factor to explain adult prevalence rates of up to 70%, although the incidence of adult acquired re-infection is also higher than in the West at 3-10% (Pounder et al 1995; Parsonnet et al 1995).

1.7.2 Geographical Variations in Prevalence of \textit{H.pylori}

In general, less developed countries have higher prevalence rates of \textit{H.pylori} as a consequence of poorer socio-economic conditions promoting infection as already discussed (section 1.7.1). Thus, the overall \textit{H.pylori} prevalence in England is no more than 34% (Megraud et al. 1989), but overall prevalence rates of 60-80% are found in Vietnam and Algeria (Megraud et al 1989).

Figure 1.4 shows \textit{H.pylori} seroprevalence for several different countries by decade. The increasing seroprevalence up to approximately the 5\textsuperscript{th} or 6\textsuperscript{th} decade in most countries is well illustrated: This is explained by the cohort effect with currently older individuals not having enjoyed such good socio-economic conditions in childhood as later generations. The decline in seropositivity with increasing age after approximately the 6\textsuperscript{th} decade is an ageing phenomenon. A combination of generalised ‘anergy’ as well as progressive loss of \textit{H.pylori} due to atrophic gastritis after many years of colonisation is the most likely explanation for this phenomenon.

Differences in socio-economic conditions and ethnicity influence \textit{H.pylori} prevalence not just between different regions of the World, but also within those individual countries as well. For example, while the overall \textit{H.pylori} prevalence may be approximately 34% in England, in a Southern Asian community the rate is higher at 47% (Stone et al. 1998). While acknowledging that a complex interplay of socio-economic and ethnic differences probably account for this disparity it serves to illustrate the importance of accurately characterising study populations.
1.8 *H. pylori* and Alcohol Metabolism

In addition to gastric parietal ADH, *H. pylori* also contains active alcohol dehydrogenase. Active cytosolic ADH activity has been demonstrated in 32 strains of *H. pylori* tested in an ex-vivo assay by Salmela et al (1994). The ADH activity increased approximately threefold with increasing ethanol concentration from 50mM to 1.5M. Also of interest is the corresponding increase in acetaldehyde—the end product of ethanol metabolism and known to be highly toxic to biological systems. Assuming that *H. pylori* cytosolic ADH is exposed to ethanol at the same concentration as that found in the stomach, then the ethanol concentrations used are of physiological relevance with 1.5M ethanol being approximately equal to the ethanol concentration found in wine at 8% ABV [Wine at 8% ABV is 1.35M].

It is unclear why *H. pylori* evolved the ability to produce ADH and consequently metabolise alcohol. Presumably, ADH provides a survival advantage for the bacterium by metabolising toxic acetaldehyde to less toxic ethanol using the reverse of the reaction outlined for the metabolism of alcohol in section 1.3.4. This is however a teleological argument.
With the discovery that *H. pylori* contains significant ADH activity the obvious, but important, question about its role in the first-pass metabolism of ethanol, was also addressed by Salmela *et al.* (1994). These authors again assayed ADH in an *ex-vivo* system using gastric biopsies obtained at endoscopy. There was no significant difference in gastric *body* biopsy ADH activity between patients who were *H. pylori* positive versus those that were negative, at any concentration of ethanol assayed. However, when *antral* biopsies were assayed, ADH activity was significantly lower in patients who were *H. pylori* positive. This effect was observed at the three concentrations of ethanol used in the assay.

The apparent contradiction of lower antral ADH activity and presence of *H. pylori*, is explained by the presence in these patients of antral gastritis: While *H. pylori* may be contributing some ADH activity, it is small when compared with the normal ADH activity of the gastric mucosa. Gastritis-a common consequence of *H. pylori* infection-leads to loss of gastric mucosal ADH and in turn leads to reduced assayable ADH activity. The authors were able to show a trend towards significance with lower ADH activity observed in *antral* biopsies with severe gastritis when compared with moderate gastritis. No difference in ADH activity was observed however between *body* biopsies in the presence of moderate and severe gastritis (Salmela *et al.* 1994).

Other authors have also confirmed the association between *H. pylori* infection and reduced gastric ADH. In addition, a return towards normal ADH values after *H. pylori* eradication has also been demonstrated (Kechagias *et al.* 1999; Simanowski *et al.* 1998). Similarly, an inverse relationship between ADH activity and increasing alcohol consumption up to 0.8g ethanol/Kg body weight/day and also in alcohol misusers has also been demonstrated (Bode *et al.* 1999; Seitz *et al.* 1993). The loss of gastric ADH in alcohol misusers, causes the expected reduction in first pass metabolism of alcohol which returns towards control values after 6 weeks abstinence (DiPadova *et al.* 1987). This in turn, may help to explain increasingly the damaging effects of ethanol in recidivist misusers. To compound this effect, alcohol misusers are more likely to be heavy smokers—a further factor associated with reduced gastric ADH activity (Bode *et al.* 1999).

It is therefore possible to conclude that *H. pylori* probably plays only a small part in the *overall* metabolism of ethanol through its ADH, although local acetaldehyde toxicity may play a part
in disease pathogenesis. With the development of gastritis due to H. pylori infection, there is loss of gastric ADH and a consequent reduction in the first-pass metabolism of ethanol.

1.9 The Diagnosis of H. pylori

1.9.1 H. pylori Diagnosis in the General Population

The diagnostic tests for H. pylori are summarised in Table 1.1. There is no definitive 'gold standard' diagnostic test for H. pylori. Culture, the standard test for most infectious diseases, is highly specific but historically has lacked sensitivity (Anon. 1994). Experienced centres now achieve sensitivities of more than 95% with the polymerase chain reaction (PCR) performed on gastric biopsy homogenate. In centres experienced at performing both tests, culture and PCR are equally sensitive and specific (Van Zwet et al. 1993; Bickley et al. 1998). Serological tests are less specific than other tests for diagnosing current infection as they are usually directed against IgG antibody which is known to persist after clearance of H. pylori (Veenendaal et al. 1991; Prasad et al. 1995). Also, the sensitivity and specificity of commercially available ELISA kits may be affected by geographical antigenic variation between H. pylori strains, subject age, ethnicity and the use of NSAIDs (Bodhiatta et al. 1993; Glupczynski et al. 1993; Taha et al. 1993). Endoscopic biopsy methods include histology and the rapid urease tests (commercially as a CLO\textsuperscript{R} test, Ballard Medical Products, Utah, USA). The latter is dependent on H. pylori urease to produce a colour change in a test well into which a biopsy specimen is placed. Both methods detect current infection but may be subject to sampling error and variations in bacterial density, due to the presence of gastric atrophy or intestinal metaplasia (Louw et al. 1993), or else the use of antibiotics, bismuth or proton pump inhibitors (Logan et al. 1995; Atherton 1995). Interobserver variation, even when special H. pylori stains are used may affect the sensitivity and specificity of histological examination (Christensen et al. 1992). But, samples analysed by an 'expert histologist' have a sensitivity and specificity of 99% and 100% respectively (Maconi et al. 1999).

The urea breath test (UBT) is non-invasive, reproducible (Steen et al. 1995) and detects current infection. It is a test of whole stomach and therefore not subject to sampling error. Commercial breath tests such as Pylobactell\textsuperscript{R} (BSIA ltd, Brentford, UK) rely on the presence of H. pylori urease to metabolise $^{13}$Carbon-urea into breath $^{13}$CO\textsubscript{2}: After a baseline breath $^{13}$CO\textsubscript{2}, the subject is asked to drink a $^{13}$Carbon-urea test meal. In the presence of H. pylori urease, $^{13}$CO\textsubscript{2} is produced, and the increase, in breath $^{13}$CO\textsubscript{2} detected by mass spectrometer, indicates active H. pylori infection. Sensitivity of $^{13}$C-breath tests is affected by the recent use
of antibiotics, bismuth and proton pump inhibitors, because of an associated reduction in bacterial load and/or inhibition of \textit{H. pylori} urease (Perri \textit{et al.} 1995; Chey \textit{et al.} 1996).

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Author</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Bickley (1998)</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR</td>
<td>Bickley (1998)</td>
<td>95%</td>
<td>100%</td>
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<tr>
<td>Serology</td>
<td>Laheij (1998)</td>
<td>92%</td>
<td>93%</td>
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<tr>
<td>CLO\textsuperscript{R}</td>
<td>Dye (1988)</td>
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<td>Histology</td>
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<tr>
<td>UBT</td>
<td>Menegatti (1997)</td>
<td>98%</td>
<td>100%</td>
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Abbreviations: PCR: polymerase chain reaction; CLO\textsuperscript{R}: urease testing; UBT: urea breath testing

\textbf{Table 1.1: Sensitivity and Specificity of Different Test Methods for \textit{H. pylori}.}
1.9.2 H. pylori Diagnosis in Alcohol Misusers and Patients with Liver Disease

The factors that affect diagnostic sensitivity and specificity for \textit{H. pylori} in the context of alcohol misuse and/or liver disease are less clearly defined. Again no ‘gold-standard’ exists, but histological examination of biopsy specimens remains the best method for diagnosing presence or absence of \textit{H. pylori}. It can not however determine bacterial viability. This is clearly an important consideration in examining the hypothesis that alcohol and/or liver disease may influence the natural history of infection and is of critical importance in interpreting the results of a study by Weston \textit{et al} (1997). These authors compared the sensitivity and specificity of CLO\textsuperscript{R} testing for \textit{H. pylori} against results from histological biopsises. A single antral CLO\textsuperscript{R} test had a sensitivity for \textit{H. pylori} detection of 72.7%. This increased to 82% if an additional CLO\textsuperscript{R} test was taken from the body of the stomach. The specificity for either of these procedures for diagnosing \textit{H. pylori} was in excess of 98% when compared with gastric biopsies. Using a univariate analysis, a significant association between overall CLO\textsuperscript{R} test concordance and histological sampling was the absence of alcohol consumption, absence of previous \textit{H. pylori} eradication attempts and ethnicity. The possibility that alcohol consumption might reduce \textit{H. pylori} urease activity and that this in turn reflects a relative decline in bacterial viability is interesting. This would be analogous to the reduction in sensitivity of urease based tests seen after the administration of antibiotics and/or PPIs. It can at best be considered a ‘working-hypothesis’ because of the multiplicity of other factors that need consideration, as this study emphasises.

In a study comparing serology with CLO\textsuperscript{R} testing, histology and culture combined, Luthra \textit{et al} (1998), found a 63.3% seroprevalence of \textit{H. pylori} but only a 44.9% prevalence using all three combined techniques. Although a number of demographic characteristics including age, sex, smoking, drugs and alcohol were examined, only recent use of antibiotics was associated with the non-concordance between serology and combined diagnostic techniques. These authors raise the possibility of an \textit{H. pylori} ‘suppressive-factor’ to account for the discrepancy between serological testing and combined tests, even if their results were only able to demonstrate antibiotic usage as a possible factor.

High sensitivity for the detection of \textit{H. pylori} in patients with cirrhosis using serological testing has also been demonstrated by Nardone \textit{et al} (1996). Using an IgG assay, a sensitivity of 85% for detection of \textit{H. pylori} was obtained in 48 non-alcoholic cirrhotic patients. The specificity was however low at 38% when compared with urease testing and histology.
combined. Again there is a problem of interpretation with urease testing being dependent on the presence of viable bacteria and histology being operator dependent. It may, therefore, be of significance that the specificity of the assay was also low at 56% in patients with non-cirrhotic liver disease. What this work did clearly show, however, was the very low sensitivity of IgA based serological assays, with IgA testing for \textit{H. pylori} sensitivity for all liver disease groups no better than 25%.

The influence of alcohol drinking and the $^{13}$C urea breath test has been investigated by Bielanski \textit{et al} (1998). Under control conditions, peak $^{13}$C values occurred between 10 to 20 minutes in healthy volunteers or recovering alcoholics who were \textit{H. pylori} positive. But, after consuming 58g of alcohol, only 27% of subjects, from either group, had a $^{13}$C peak at 10-20 minutes. Instead, the $^{13}$C peak occurred at 35-45 minutes and was reduced in magnitude by 30-55%. If, however, the breath test was performed 1 hour after the alcohol dose, the $^{13}$C curve was identical to control values. The authors therefore concluded that \textit{acute} alcohol consumption is associated with a reduction in \textit{H. pylori} urease activity as assessed by the urea breath test and recommend at least a one-hour delay between acute consumption and breath testing. This work raises the possibility that alcohol may be an ‘inhibitory’ factor of \textit{H. pylori} \textit{in vivo} although a methodological problem is equally plausible.

1.10 \textit{H. pylori} and Peptic Ulcer Disease

1.10.1 Peptic Ulcer disease after the discovery of \textit{H. pylori}

The isolation of \textit{H. pylori} produced a ‘paradigm shift’ in concepts about the causation and treatment of peptic ulcer disease (Marshall \textit{et al} 1984; Marshall 1986). No longer was mere acid suppression a central intellectual and therapeutic goal but antibiotics to treat the underlying \textit{H. pylori} infection and subsequently heal peptic ulcer rapidly became the accepted norm.

The critical importance of \textit{H. pylori} in the pathogenesis of peptic ulcer disease in the majority of patients is reviewed by Kuipers \textit{et al} (1995): The cumulative prevalence of \textit{H. pylori} in 1695 patients with duodenal ulcer was 94.5%. While the prevalence in 1395 patients with gastric ulcer was 83.9%. The majority of \textit{H. pylori} negative peptic ulceration, both in the duodenum and stomach, appears to be related to direct irritants, the commonest of which are NSAIDs (Borody \textit{et al}. 1991, 1992).
1.10.2 The Role of \textit{H.pylori} in Complex Ulcer Disease

The strong association between \textit{H.pylori} and peptic ulcer disease in the otherwise normal population, has already been discussed (section 1.10.1). A minority of patients with peptic ulceration do not have \textit{H.pylori} and in nearly all of these cases, a direct irritant effect, typically due to NSAID usage is implicated in pathogenesis. In complex ulcer disease however, \textit{H.pylori} may play a more limited role in pathogenesis. For example, the prevalence of \textit{H.pylori} in patients with bleeding ulcers may be as low as 70\% (Laine \textit{et al} 1996) and this raises the possibility that, at least in complex clinical situations, other factors take-on an ever increasing significance in ulcer pathogenesis.

1.11 \textit{H.pylori}, Alcohol and Malignancy of the Upper Gastrointestinal Tract

1.11.1 Alcohol, \textit{H.pylori} and Oesophageal Malignancy

The relationship between Barretts oesophagus, alcohol and \textit{H.pylori} has been examined in a study by Weston \textit{et al} (2000): These authors found an approximate 15.0\% prevalence of \textit{H.pylori} infection in patients with Barretts oesophagus and high grade dysplasia or cancer. This was lower than the \textit{H.pylori} prevalence in low-grade dysplasia (36.2\%) or in patients with simple reflux symptoms (44.2\%). These differences in dysplasia only correlated with \textit{H.pylori} status and were independent of gender, smoking, alcohol or drug usage. The authors concluded that \textit{H.pylori} had a protective effect against the development of high grade dysplasia/carcinoma in patients with Barretts oesophagus. The presumptive mechanism is that \textit{H.pylori} causes atrophic gastritis (see below), which in turn, reduces gastric acid and damage secondary to gastro-oesophageal reflux.

Other changes recognised in the oesophagus that may relate to alcohol and \textit{H.pylori} are changes of intestinal metaplasia at the gastro-oesophageal junction. Hiroto \textit{et al} (1999) demonstrated an increased dysplasia risk in long segment Barretts when compared with shorter segments. Of perhaps more interest, however, was the demonstration that gastro-oesophageal intestinal metaplasia was significantly associated with less alcohol and a higher prevalence of \textit{H.pylori} infection.

It can, therefore, be seen that while the precise mechanism and sequence of events is not entirely clear, \textit{H.pylori}, alcohol consumption and smoking are factors determining the severity and outcome of acid reflux disease on the oesophageal mucosa.
1.11.2 Alcohol, H. pylori and Gastric Malignancy

The most clearly established aetiological factor for gastric carcinoma is chronic H. pylori infection (Blok et al. 1997). Indeed, the epidemiological evidence for the association is so strong that the WHO classified H. pylori as a group 1 carcinogen in 1994 (Broutet et al. 1998). Therefore, if alcohol or smoking are risk factors for gastric cancer, then a distinction needs to be made between them being co-factors with H. pylori in carcinogenesis or alternatively factors that facilitate H. pylori infection, which in turn leads to gastric cancer. For example, a study from Moscow demonstrated a positive correlation between cancer of the cardia in men and other gastric sites in women with increasing vodka consumption (Zaridze et al 2000). But, an interaction was also found between alcohol consumption and smoking as well as gastric cancer and H. pylori infection. Therefore, at least three interacting factors were responsible for the increased prevalence of gastric carcinoma in this patient population. It is not possible from these data to determine if smoking and alcohol were co-factors for carcinogenesis with H. pylori or simply facilitated H. pylori infection.

Broutet et al (1999) demonstrated differences in H. pylori prevalence in seven administrative regions of France: In the South-West region the standardised prevalence was 15.2% and in the North-West region it was 24.8%. The respective gastric cancer mortality rates were 37 and 51.8/100,000 inhabitants. The correlation between H. pylori prevalence and gastric cancer could not clearly be shown for all regions in France although the authors discuss the probable methodological flaws for this apparent lack of correlation. Why the prevalence of H. pylori is lowest in the South-West of France is not clear, but it is interesting to note that this is the Bordeaux region where wine consumption is higher than in any other part of France (Megraud F personal communication).

1.12 Inter-Relationships between H. pylori, Alcohol, Liver Injury and Peptic Ulcer

To this point the influence of H. pylori and alcohol on the upper gastrointestinal tract have been considered largely in isolation. This separation, however, is artificial because frequently both risk factors for upper gastrointestinal pathology coexist. In addition, increasing alcohol misuse is associated with worsening liver injury. Thus, complex interactions between H. pylori, alcohol and liver injury might be anticipated to further influence upper gastrointestinal pathology.
As discussed previously (section 1.10.2), *H. pylori* may play a less significant role in complex ulcer disease especially if irritant agents such as NSAIDs are also present. Since bleeding and NSAID usage make the association between ulcer and *H. pylori* less clear it could be hypothesised that other irritants to gastric mucosa either alone, or in combination with *H. pylori* and drugs might further influence the natural history and prevalence of peptic ulceration. Clearly alcohol is a potential ‘gastric irritant’ especially if taken in excess.

A further level of complexity in studying a putative role for alcohol as a risk factor for peptic ulcer disease is the question of any underlying liver disease itself. In advanced liver disease, not necessarily due to alcohol, changes in immunoreactivity are recognised (Runyon *et al* 1993). This might be expected to alter the ability to clear *H. pylori*-a phenomenon that has already been discussed in terms of prevalence decline in the ageing population (section 1.7.2). Similarly, the development of ‘non-*H. pylori* gastritis', be this due to direct toxicity of alcohol or secondary to portal hypertension, could reasonably be expected to influence *H. pylori* clearance and/or increase the likelihood of peptic ulceration in its own right.

Finally, the influence of alcohol on *H. pylori* itself needs to be considered; if alcohol in some way altered the natural history of the infection then an influence on ulcer prevalence as a consequence might be anticipated. The loss of diagnostic accuracy of several tests for *H. pylori* in the context of alcohol use and misuse discussed in section 1.9.2, may simply be methodological or due to a real effect of alcoholic beverages inhibiting the organism.

From the above discussion it can be seen that any study looking at peptic ulceration in patients with liver disease must consider the following factors:

- The background prevalence of peptic ulceration and *H. pylori* in an appropriate age and sex matched control population.
- The accuracy of any diagnostic method for *H. pylori*.
- The influence of confounding factors for both *H. pylori* and peptic ulceration including alcohol consumption, recent drug usage, smoking and associated medical conditions.
- The aetiology and severity of any liver injury.
- Factors unique to gastric pathophysiology in liver disease including presence or absence of varices and/or portal hypertensive gastropathy.
Introduction: Alcohol, \textit{H.pylori} and the Upper GI Tract

To this end, the prevalence of \textit{H.pylori} will first be examined in relation to drinking behaviour and the presence or absence of liver disease. Then, the interaction between alcohol, liver disease, \textit{H.pylori} and alcohol will be examined in relation to peptic ulcer prevalence and pathogenesis.

1.13 Prevalence of \textit{H.pylori} in Non-habitual Drinkers with Minimal or No Liver Injury

The best study to date examining the relationship between non-habitual alcohol consumption, \textit{H.pylori} and minimal or no liver injury is by Brenner \textit{et al} (1997): The study population comprised 447 patients, carefully controlled for age and sex, attending a single German general practice. Patients were excluded if they presented with a gastrointestinal complaint, a history of peptic ulcer disease or previous treatment for \textit{H.pylori}. The urea breath test was used to diagnose \textit{H.pylori} and the relationship between prevalence and several putative risk factors for \textit{H.pylori} infection assessed. Alcohol consumption showed a negative dose response relation with odds ratios, when compared with non-drinkers, of 0.9 and 0.33 for the consumption of <75g and >75g of alcohol/week respectively (p=0.005). The authors commented that the majority of patients consumed the local beer (German lager) or wine but no specific sub-set analysis by ‘preferred beverage’ was attempted as these data were not collected (Rothenbacher D, personal communication).

Sub-group analysis, by preferred beverage, was however performed by the same authors in a subsequent and similar study involving 425 insurance workers (Brenner \textit{et al.} 1999). Using similar methods, the inverse relationship between \textit{H.pylori} and alcohol consumption was again demonstrated. The population was classified as either ‘non-drinker’, ‘ever-drinker’ of <75g/week or ‘ever-drinker’ of >75g/week of either beer or wine. The odds ratio (95% confidence limit) of being \textit{H.pylori} positive in the ‘ever-drinkers’ when compared with non-drinkers was 0.47 (0.26-0.88) for <75g/week ethanol as wine and 0.27 (0.05-1.44) for >75g/week ethanol as wine. (p=0.010) When a similar comparison was performed for beer drinkers, lower odds ratios of \textit{H.pylori} infection in ‘ever-drinkers’ was observed with a trend to significance for consumption >75g ethanol/week as beer (p=0.259).

Several criticisms of the studies described above can be made: These include the assumption that those individuals consuming >75g alcohol/day did not have significant liver injury. Also, information on the full drinking history was not obtained so it is not possible to determine what percentage of individuals were consuming significantly more than 75g alcohol weekly. The use of the urea breath test to diagnose \textit{H.pylori} does, however, have a high level of
sensitivity and specificity for detecting infection and most other confounding factors were avoided. Similarly, although not explicitly stated, it is unlikely that the breath test was performed within an hour of the last alcoholic drink-a factor known to affect sensitivity and specificity as discussed in section 1.9.2. Overall, therefore, evidence is provided for a reduced prevalence of *H. pylori* in a population consuming ‘more than desirable’ levels of alcohol without symptoms or apparent significant liver injury. There is also a suggestion that some beverages may have a more significant effect on *H. pylori* viability and/or survival than others.

The finding of reduced *H. pylori* prevalence with increasing alcohol consumption has also been demonstrated in a Danish seroprevalence study (Rosenstock *et al.* 2000). A total of 2,913 individuals were studied, and an inverse relationship observed between *H. pylori* seroprevalence and consumption of more than 13 drinks/week (OR 0.8). The implicit assumption is that this approximates to 13 ‘standard measures’ or 130g ethanol/week although this data is not provided. When analysis by ‘preferred beverage’ was performed, the inverse relationship between *H. pylori* seroprevalence and consumption was again demonstrated; this effect was most marked with drinkers of more than 4 glasses of wine/week (OR 0.5). This work again supports the concept that alcoholic beverage consumption might be associated with a lower prevalence of *H. pylori* and that the beverage consumed may also play a significant role.

Ogihara *et al.* (2000) also found a negative dose response curve between *H. pylori* seroprevalence and increasing alcohol consumption in 8837 individuals in Japan (OR 0.88: 0.79-0.98). A similar negative dose response was also seen in relation to smoking and the authors speculate that increased gastric acidity secondary to smoking helps eliminate *H. pylori*. An alternative speculation is that smokers may also consume more alcohol and that this may act as an additional ‘clearing’ agent.

A criticism that can be made of the later two studies described is that they depended on serological diagnosis for *H. pylori*. Serology does however have widespread acceptance in epidemiological work especially if ingestion of drugs known to affect *H. pylori* are excluded (Cutler *et al.* 1995). In addition, the broad agreement between serological studies and the studies by Brenner *et al.* (Brenner *et al.* 1997, 1999) using a different diagnostic technique for the diagnosis of *H. pylori*, help to support each other in terms of conclusion and validity of method.
The apparent negative association between \textit{H.pylori} prevalence and alcohol consumption in the otherwise normal population is not supported by all work. For example, in France, Pateron \textit{et al} (1990) showed a 45\% prevalence of \textit{H.pylori} in individuals with no clinical liver disease consuming \textless 80g alcohol/day and a 65\% prevalence in individuals consuming \textgreater 80g/day also with no apparent liver injury. The diagnosis of \textit{H.pylori} was made by histological examination of gastric biopsies which should confer a high level of diagnostic accuracy. But, it is not possible to clearly draw conclusions from this work because the two populations studied were not comparable. The alcohol misusers were selected on consumption criteria while the ‘control’ patients comprised individuals with dyspepsia undergoing upper endoscopy. The latter can not be considered an appropriate control group in view of the associations between \textit{H.pylori} and dyspepsia. It is also quite possible that the ‘control’ individuals were limiting their alcohol consumption prior to endoscopy to relieve dyspeptic symptoms. In addition, ‘control’ alcohol consumption was approximately 560g alcohol/week or approximately three times recommended UK alcohol limits for men (see section 1.4.1).

Battaglia \textit{et al} (1993) also studied \textit{H.pylori} prevalence in a large prospective series of 286 non-habitual drinkers. \textit{H.pylori} infection was diagnosed by examination of at least four gastric biopsies, and the overall prevalence was 59.8\%. Significant associations were identified between the prevalence of \textit{H.pylori} infection, the symptom score, presence of gastritis and of active duodenal ulceration. However, no association was observed between \textit{H.pylori} prevalence and either smoking or alcohol consumption at either less than, or more than, 40g ethanol/day. This study is potentially significant because of the large number of subjects studied, consideration of recent drug usage and good diagnostic accuracy for \textit{H.pylori}. But, just as for the study by Pateron \textit{et al} (1990), discussed above, it is weakened by its reliance on an exclusively dyspeptic population.

Considering all of the above studies together, a pattern of results appears to emerge. In studies performed on largely \textit{asymptomatic} individuals, \textit{H.pylori} prevalence apparently declines with increasing alcohol consumption and this appears to be independent of diagnostic technique used and might also be partially beverage dependent (Brenner \textit{et al} 1997, 1999; Rosenstock \textit{et al} 2000; Ogihara \textit{et al} 2000). If however, the individuals have \textit{symptoms} the relationship between alcohol consumption and \textit{H.pylori} prevalence becomes more complex. In the \textit{dyspeptic} patients described by Battaglia \textit{et al} (1993), no association between alcohol consumption and \textit{H.pylori} prevalence was observed. But, this population was, to an extent,
'self-selected' because all the patients studied had dyspepsia. It is quite possible, therefore, that symptoms caused a change in alcohol consumption behaviour. Or, alternatively, the H. pylori infection was sufficiently 'virulent' not to be affected by any putative 'anti-H. pylori' effect of the consumed alcohol. This may also help reconcile the higher H. pylori prevalence observed with increasing consumption observed by Pateron et al (1990). Again, control populations were in fact patients with dyspepsia and these individuals were also consuming quite high levels of alcohol. Similarly, the influence of any underlying liver injury in affecting H. pylori prevalence is also not clear. On clinical grounds the patients had no apparent liver injury, but this was not formally assessed and so it is not possible to exclude this as a potential confounding factor.

1.14 Prevalence of H. pylori in Habitual Drinkers with Minimal or No Liver Injury

Virtually no published studies exist on the prevalence of H. pylori in habitual drinkers with minimal liver injury. This is because most studies either concentrate on H. pylori prevalence in patients with alcoholic cirrhosis (section 1.15) or are epidemiological studies as already discussed in section 1.7 where alcohol history data is typically rudimentary or difficult to fully interpret. As already discussed in relation to the work by Brenner et al (Brenner et al. 1997, 1999), in the group of volunteers consuming >75g alcohol/week, it is not clear how many subjects exceeded this amount and could be more appropriately classified as 'habitual drinkers' or 'misusers.' Similarly, the problems of control, as discussed in relation to the study by Pateron et al (1990), apply equally to the alcohol misusers as well as to 'controls.'

1.15 Prevalence of H. pylori in Patients with Alcoholic Cirrhosis

Table 1.2 summarises the studies undertaken to date, that look at the prevalence of H. pylori in patients with both alcoholic and non-alcoholic cirrhosis. Many difficulties arise in interpretation of these data and in drawing appropriate conclusions. First, purity of the population under study; typically, populations of patients with both alcoholic and non-alcoholic cirrhotic patients are grouped together as a single, mixed, 'cirrhotic-population'. Consequently, trying to determine any potential influence on H. pylori prevalence due to the aetiology of the liver disease is impossible. Second, even in studies that specifically include patients with alcoholic cirrhosis, there is a paucity of drinking data. Of the 20 studies that include patients with alcoholic cirrhosis, summarised in Table 1.2, only five give any alcohol consumption data (Pateron et al. 1990; Prigent-Delecourt et al 1994; Kirchner et al 1996; Calvet et al. 1997; Marshall et al 1998). Of these, only two compare a true abstinent and
actively misusing group of patients with alcoholic cirrhosis (Pateron et al. 1990; Marshall et al 1998). No studies provide alcohol data on the patients with non-alcoholic cirrhosis or for other controls, if included.

The third problem of interpretation relates to the control population. In all 22 studies examining *H. pylori* prevalence in patients with cirrhosis, of any aetiology and summarised in Table 1.2, only 11 include any form of control population. This typically comprises healthy volunteers (Imoto et al. 1989; Pateron et al. 1990; McCormick et al. 1991; Guslandi et al 1993; Parikh et al. 1994; Wu et al 1995; Wang et al 1996; Cakaloglu et al 1997; Siringo et al. 1997; Marshall et al 1998) although one study used hospital inpatients (Kirchner et al 1996) and another patients with dyspepsia (Misra et al. 1990). Of the studies that include alcoholic cirrhotic patients, 11 have a control population.

The fourth difficulty in the interpretation of *H. pylori* prevalence figures in cirrhosis is the lack of age stratification. This is of critical importance in view of the relationship between increasing age and *H. pylori* prevalence discussed in section 1.7.1. Only three of the studies in Table 1.2 stratify subjects by age (Chen et al 1994; Calvet et al. 1997; Siringo et al. 1997).

The fifth problem arises due to lack of precise diagnosis of the underlying liver disease. For example, of the 22 studies in Table 1.2, only 7 use the ‘gold-standard’ of liver biopsy to confirm the diagnosis of cirrhosis (McCormick et al 1991; Gubbins et al 1993; Parikh et al 1994; Prigent-Delecourt et al 1994; Balan et al 1996; Schmulson et al 1997; Marshall et al 1998) and two use mixed clinical and biopsy criteria (Chen et al 1994; Siringo et al 1997). In the remaining 13, only clinical assessment is relied upon for assessing cirrhosis (see Table 1.2).

A lack of precise diagnostic tests for *H. pylori* also leads to difficulties in interpretation. For example, multiple diagnostic tests are not often used with seven studies relying only on a single serological diagnosis for *H. pylori*. (Gubbins et al. 1993; Chen et al 1994; Wu et al 1995; Cakaloglu et al 1997; Calvet et al. 1997; Siringo et al. 1997; Marshall et al 1998). As previously discussed (section 1.9.1) single diagnostic test reliance introduces increased error, especially if less precise serological techniques are used.
With all the assumptions and caveats outlined above, four studies compare \textit{H.pylori} prevalence in cirrhosis with a non-cirrhotic control population. In two of these studies show a higher \textit{H.pylori} prevalence in alcoholic cirrhosis when compared with control (Kirchner \textit{et al.} 1996; Siringo \textit{et al.} 1997), and one a lower prevalence (Marshall \textit{et al.} 1998). The final study shows a higher prevalence in alcoholic cirrhosis when compared with control in those patients consuming $> 80$g alcohol daily, but the opposite in those individuals consuming $< 80$g daily (Pateron \textit{et al.} 1990).

In the studies that compare \textit{H.pylori} prevalence between a mixed population of alcoholic and non-alcoholic patients with control, five show a higher prevalence in the cirrhotic group (Imoto \textit{et al.} 1989; McCormick \textit{et al.} 1991; Wu \textit{et al.} 1995; Kirchner \textit{et al.} 1996; Siringo \textit{et al.} 1997) and four a lower prevalence (Misra \textit{et al.} 1990; Guslandi \textit{et al.} 1993; Wang \textit{et al.} 1996; Cakaloglu \textit{et al.} 1997). Similarly, if the overall mixed cirrhotic group \textit{H.pylori} prevalence is compared against the sub-set of patients with alcoholic cirrhosis, then one study (Schmulson \textit{et al.} 1997) shows a higher prevalence of \textit{H.pylori} in alcoholic cirrhosis and three a lower prevalence (Chen \textit{et al.} 1994; Mion \textit{et al.} 1994; Prigent-Delecourt \textit{et al.} 1994). One study demonstrates a higher \textit{H.pylori} prevalence in drinking alcoholic cirrhotic patients when compared to the mixed group, but a lower prevalence in the abstinent group (Marshall \textit{et al.} 1998).

Thus, no clear conclusion about the prevalence of \textit{H.pylori} in patients with alcoholic cirrhosis can be made. The interesting observation that active alcohol consumption \textit{might} influence \textit{H.pylori} prevalence in cirrhosis will be examined in the next section.
<table>
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<th>First author (year)</th>
<th>Region</th>
<th>Aetiology</th>
<th>(n)</th>
<th>Diagnosis</th>
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<th>Age</th>
<th>H. pylori</th>
<th>Summary</th>
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<td>no</td>
<td>0</td>
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<td>no</td>
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<td>no</td>
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<td>no</td>
<td>49</td>
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<td>no</td>
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<td>no</td>
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<td>no</td>
<td>45.5</td>
<td>cirrhotics&lt;control</td>
</tr>
<tr>
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<td>153</td>
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<td>no</td>
<td>41.8</td>
<td>cirrhotics&lt;control</td>
</tr>
<tr>
<td>Marshall (1998)</td>
<td>Britain</td>
<td>alcohol</td>
<td>81</td>
<td>healthy</td>
<td>yes</td>
<td>no</td>
<td>16</td>
<td>cirrhotics&lt;control</td>
</tr>
</tbody>
</table>

Table 1.2: *H. pylori* Prevalence in Cirrhosis

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1.16 Alcohol Consumption and *H. pylori* Prevalence in Cirrhotic Patients

A study undertaken by Prigent-Delecourt *et al* (1996) specifically addressed the role of active alcohol consumption and its relation to *H. pylori* prevalence in alcoholic cirrhosis: These authors showed a 62.5% *H. pylori* prevalence in the drinking group and only a 27.5% prevalence in the abstinent group. This impressive difference between the two groups is, however, difficult to interpret because of the absence of a control group. A similar conclusion was reached by Paterson *et al* (1990), where the *H. pylori* prevalence was 48% in cirrhotic patients consuming >80g alcohol/day, but only 26% in those cirrhotic patients consuming <80g/day. The control prevalence was 45%. But the 'control' population were undergoing upper endoscopy for dyspepsia. It is therefore quite possible that the lower *H. pylori* prevalence observed in 'modestly drinking' cirrhotic patients was simply equivalent to the background control prevalence of *H. pylori* in 'non-dyspeptic' and *H. pylori* negative normal individuals.

In contrast, Marshall *et al* (1998) demonstrated a 29% *H. pylori* seroprevalence rate in abstinent alcoholic cirrhotic patients and a significantly lower seroprevalence of 7% in actively drinking alcoholic cirrhotic patients consuming a median (range) of 188 (10-640) g alcohol/day. The seroprevalence rate in the control population of healthy volunteers was 16%.

These conclusions are supported by a study performed by Calvet *et al* (1997): In all, 220 patients with alcoholic cirrhosis were studied. Multivariate analysis demonstrated a lower *H. pylori* seroprevalence in alcoholic cirrhotic patients consuming increasing quantities of alcohol, which, in this area of Catalonia, is the local red wine as 'preferred-beverage'. While this effect could be a manifestation of liver cirrhosis, it is difficult to resist making comparisons between the lower *H. pylori* prevalence observed in this predominantly wine drinking population with cirrhosis and the lower *H. pylori* prevalence described in healthy volunteer wine drinkers described by Brenner *et al* (Brenner *et al*. 1997, 1999; section 1.13).

While it is not possible to draw firm conclusions based on these studies, there is a 'recurring-theme' of reduced *H. pylori* prevalence with increasing alcohol consumption in patients with alcoholic cirrhosis. Whether this is in turn 'liver injury dependent' and/or 'beverage-dependent' is not clear.
1.17 *H. pylori* Prevalence in Patients With Non-Cirrhotic, Non-Alcoholic Liver Disease.

No studies have been performed, to date, that explore the prevalence of *H. pylori* in patients with non-cirrhotic, non-alcoholic liver disease.

1.18 The Prevalence of *H. pylori* in Patients With Non-Alcoholic Cirrhosis

A number of studies on the prevalence of *H. pylori* in patients with non-alcoholic cirrhosis have been undertaken to date (Table 1.2).

Similar problems of interpretation exist with the studies involving patients with non-alcoholic cirrhosis as encountered with the studies involving patients with alcoholic cirrhosis which are discussed in section 1.15. In the majority of studies, a mixed cirrhotic population of alcoholic and non-alcoholic cirrhotic patients is used. Only two studies look at an exclusively non-alcoholic population but rely only on clinical methods for assessment of liver injury and serology for diagnosis of *H. pylori* (Wu et al. 1995; Cakaloglu et al. 1997). The prevalence of *H. pylori* in the non-alcoholic cirrhotic patients was higher than in healthy control subjects in one study (Wu et al. 1995), but lower than healthy controls in the other (Cakaloglu et al. 1997).

Thus, no clear conclusion can be made about the prevalence of *H. pylori* in patients with non-alcoholic cirrhosis.

1.19 The Prevalence of Peptic Ulcer in Alcohol Users and Misusers

1.19.1 Peptic Ulcer in Non-habitual Drinkers with Minimal or No Liver Injury

The relationship between non-habitual alcohol consumption and the outcome once *H. pylori* infection has become established is far from clear. There is evidence that alcohol may either facilitate the development of significant upper gastrointestinal pathology or help protect against it. For example, Archimandaitis *et al.* (1995) endoscopically examined 295 patients who presented with dyspepsia. A total of 241 individuals (82%) showed evidence of gastric or duodenal ulceration and of these 197 (82%) were infected with *H. pylori* diagnosed by gastric biopsy. A significant relationship was found between the presence of ulceration and smoking as well as alcohol consumption. The authors suggested that once *H. pylori* infection is acquired the irritant effects of smoking and alcohol might contribute to ulcer development. While it is probably reasonable to assume that this patient group did not have significant liver disease, even though this was not explored, the alcohol data is rudimentary. While an association between alcohol and peptic ulceration was seen, the authors simply classified patients as either
drinkers or non-drinkers. It is not therefore possible to correlate levels of consumption with pathology or \textit{H. pylori} prevalence. Another problem of interpretation, relates to the population under study itself. Patients who drink, smoke and have dyspepsia, severe enough to warrant endoscopy, are likely to represent a more extreme end of the spectrum of peptic ulcer disease and conclusions based on studying this group may not be readily applicable to the population as a whole.

In contrast, Schubert \textit{et al} (1993) showed that alcohol consumption was associated with significantly lower rates of duodenal ulceration in patients who were infected with \textit{H. pylori}, again diagnosed by gastric biopsy, who were undergoing endoscopy for dyspepsia. In all, 1086 patients were examined and risk factors significantly associated with duodenal ulceration included \textit{H. pylori} infection, previous ulcer history, male sex, bleeding and pain. Similarly, gastric ulceration was associated with \textit{H. pylori} infection, aspirin ingestion and bleeding. Alcohol consumption was associated with a decreased risk (p=0.026) of duodenal ulceration but was not a significant risk factor for gastric ulceration. Again the assumptions about liver injury and alcohol history apply equally to this study as to the one by Archimandaitis \textit{et al} (1995) discussed above.

The relationship between alcohol consumption and peptic ulcer disease is, therefore, far from clear. It is tempting to speculate that alcohol consumption may reduce the prevalence of \textit{H. pylori} infection and thus reduce one risk factor for peptic ulceration in its own right, but at the same time serve as a mucosal 'irritant' accelerating ulcer development in those individuals where \textit{H. pylori} infection persists. This might explain the observed discrepancies between the different studies discussed above but cannot in itself be considered as a 'unifying hypothesis'.

\subsection*{1.19.2 Peptic Ulcer Prevalence in Habitual Drinkers with Minimal or No Liver Injury}

There are no studies that specifically look at this group of individuals.

\subsection*{1.19.3 Peptic Ulcer Prevalence in Alcoholic Cirrhotic Patients}

Table 1.3 summarises studies to date on the prevalence of peptic ulceration in cirrhotic patients. Similar problems of interpretation are encountered as those found in studies examining the relationship between \textit{H. pylori} prevalence and cirrhosis discussed in sections 1.15-1.18. Typically either the aetiology of the cirrhosis is not stated (Imoto \textit{et al.} 1989; Calvet \textit{et al.} 1998) or else a population of mixed aetiology is evaluated (Rabinovitz \textit{et al} 1990;
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Chen *et al* 1994, 1995; Wu *et al* 1995; Prigent-Delecourt *et al* 1996; Wang *et al*. 1997). Only one study attempts to compare duodenal ulcer prevalence between alcoholic and non-alcoholic cirrhotic male patients (Rabinovitz *et al* 1990). The prevalence of duodenal ulcer was 12.2% in alcoholic cirrhotic patients and ranged from 6.6% to 9.5% in the patients with the non-alcoholic cirrhosis. The authors conclude that there is a significantly higher prevalence of duodenal ulceration in patients with cirrhosis of viral or alcoholic aetiology when compared with cirrhotic patients of autoimmune or cryptogenic origin and also a healthy population taken from other published work. But, specific comparison of duodenal ulcer prevalence between drinking and abstinent alcoholic cirrhotics was not made. Therefore, any potential role of alcohol in the aetiology of duodenal ulcer in patients with cirrhosis may have been missed. Similarly, no assessment of gastric ulceration was made and no attempt was made to assess *H. pylori* status in any of the patients. Thus, it is unclear whether the higher prevalence of duodenal ulceration observed in cirrhotic patients was due to cirrhosis *per se*, current alcohol misuse or *H. pylori* infection.

As the only study that specifically looks at ulcer prevalence in alcoholic cirrhosis is flawed, it is not possible to specifically discuss peptic ulcer prevalence in alcoholic cirrhotic patients in isolation. A general review of peptic ulceration in cirrhosis with emphasis on the role of alcohol in its aetiology can however be attempted.

The prevalence of peptic ulceration in cirrhotic patients ranges from 4.3-16.7% (Table 1.3). The difficulties of accurately assessing prevalence in the general population have already been discussed (section 1.2). But, with an estimate of 6% overall peptic ulceration in the UK population as an ‘upper limit estimate’, the prevalence in patients with cirrhosis is either equal to this upper limit or approximately three times higher.

In only one study was a positive correlation observed between *H. pylori* infection and peptic ulceration in this patient group (Calvet *et al*. 1998). There was also an association with male sex and the presence of ulcers. The study, however, had both a relatively low rate of peptic ulceration (GU:4.3%; DU:5.8%) and of *H. pylori* infection (10.5%). In contrast, in four studies no correlation was observed between peptic ulceration in patients with cirrhosis and *H. pylori* infection (Chen *et al*. 1995; Wu *et al*. 1995; Prigent-Delecourt *et al*. 1996; Wang *et al*. 1997).
The prevalence of gastric ulceration in patients with cirrhosis ranges from 4.3-14.8% and of duodenal ulceration from 5.8-16.7% (Table 1.3). In those studies where prevalence data for duodenal and gastric ulceration is available the rates are approximately equal (Table 1.3). Therefore, although absolute prevalence rates for peptic ulceration vary widely between studies, in nearly all cases there is an equal prevalence of duodenal and gastric ulceration. Since the incidence of duodenal ulceration in the normal population is between 2-4 times higher than the incidence of gastric ulceration (section 1.2), it raises the possibility that the mechanisms responsible for the development of peptic ulceration in patients with cirrhosis differ from those found in the normal population. This in turn is supported by the apparent lack of correlation between the presence of peptic ulceration and \textit{H.pylori} infection as already discussed.
<table>
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<tr>
<th>Aetiology</th>
<th>Cirrhosis</th>
<th>H. pylori</th>
<th>GU</th>
<th>DU</th>
<th>H. pylori</th>
<th>Comment</th>
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<tr>
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<td>n/a</td>
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<td>n/a</td>
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<tr>
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<td>Bx,culture</td>
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</table>

Abbreviations: HVWP: hepatic vein wedge pressure; DU: duodenal ulcer; GU: gastric ulcer; Bx: biopsy; UBT: urea breath test
1.20 Other Risk Factors for Peptic Ulceration in Patients with Cirrhosis

With no clear correlation between the presence of *H. pylori* infection and peptic ulcer disease in patients with cirrhosis (section 1.19.3), alternative potential risk factors for ulcer disease need to be considered.

Only three studies have looked at putative risk factors, other than *H. pylori*, for peptic ulceration in cirrhotic patients and these are summarised in Table 1.4 (Prigent-Delecourt et al 1994; Calvet et al. 1998; Rabinovitz et al 1990). From this summary it can be seen that presence of varices, age, current smoking and Childs grade do not correlate with an increased prevalence of peptic ulceration. The presence of portal hypertensive gastropathy has been associated with both an increased (Rabinovitz et al 1990) and no-increased risk of peptic ulceration (Prigent-Delecourt et al 1994). One study supported male sex as being a risk factor for peptic ulceration in cirrhosis (Calvet et al. 1998), another did not (Prigent-Delecourt et al 1994) while the remaining study only included men (Rabinovitz et al 1990). As previously discussed (section 1.19.3), Rabinovitz et al, (1990) did show a significantly lower prevalence of duodenal ulceration in the range 6.6-9.5% in patients with non-alcoholic cirrhosis of autoimmune origin and a 12.2% prevalence in patients with cirrhosis of alcoholic or viral origin. But, no assessment of *H. pylori* status was made.

One study showed that recent alcohol consumption was a risk factor for the development of peptic ulceration in cirrhosis, but no quantification of consumption was provided (Prigent-Delecourt et al 1994). In contrast, Calvet et al (1998) did not show any similar association. (Table 1.4)
### Introduction: Alcohol, *H. pylori* and the Upper GI Tract

<table>
<thead>
<tr>
<th>Author (year)</th>
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<th>Sex</th>
<th>Varices</th>
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<th>Aetiology cirrhosis</th>
<th>Childs grade</th>
<th>Alcohol</th>
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<td>no</td>
<td>no</td>
<td>no</td>
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<td>n/a</td>
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<td>no</td>
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<td>no</td>
<td>n/a</td>
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</tr>
</tbody>
</table>

no = no association found; yes = association found; n/a = data not available.

*association of PUD with male sex; **only men studied

+ association of PUD with recent alcohol consumption

++significantly higher prevalence of PUD in alcoholic and viral cirrhosis than cirrhosis of autoimmune or cryptogenic origin

Abbreviations: PHG: portal hypertensive gastropathy; PUD: peptic ulcer disease

**Table 1.4: Risk Factors for Peptic Ulceration in Patients with Cirrhosis.**

**1.21 Summary**

Peptic ulceration is a common condition with an associated high mortality and morbidity. In the otherwise normal population it is invariably associated with *H. pylori* infection. In patients with cirrhosis, the prevalence of peptic ulceration may be at least three times that observed in the normal population and the associated morbidity and mortality even higher. But, the
increased prevalence of peptic ulceration observed in cirrhotic patients cannot be correlated with an increased prevalence of *H. pylori*. This can be interpreted as either a methodological failure in assessment of *H. pylori* status and/or another mechanism contributing to the high prevalence of peptic ulceration in this patient population.

Studies that have tried to establish the prevalence of *H. pylori* in patients with liver disease are largely inadequate. This is due to a number of factors including, failure to use accurate diagnostic techniques for *H. pylori*, inadequate, if any, control groups with appropriate age stratification and failure to accurately characterise the extent of liver injury. The other factor, usually only given cursory notice, is current or past drinking behaviour. This must be of critical importance in view of the increasing evidence that alcohol consumption influences *H. pylori* prevalence and that *H. pylori* infection, in turn, affects alcohol metabolism by interfering with gastric ADH. It is therefore quite possible that a complex interaction exists between *H. pylori*, alcohol and the progression of alcoholic liver disease.

To support an interaction between alcohol consumption and *H. pylori*, at least in the otherwise normal population, are the negative dose response curves observed by several studies between *H. pylori* and increasing alcohol consumption. There is also a suggestion that some beverages may have a more profound effect at inhibiting *H. pylori* than others. This raises the further interesting possibility that it is not necessarily the ethanol component of alcoholic beverages *per se* that might affect *H. pylori* but some other component or congener in the beverage that may play an equal or more important role.

1.22 Aims

- To determine prevalence of *H. pylori* in a population of carefully characterised alcohol misusers.
- To determine the sensitivity and specificity of diagnostic tests for *H. pylori* in the context of alcohol misuse.
- To examine the relationship between alcohol misuse, liver disease and changes in the upper gastrointestinal mucosa.
- To determine the major risk factors for peptic ulceration in patients with liver disease.
- To examine *H. pylori* motility and survival *in vitro* following incubation with alcoholic beverages.
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- To examine the 'congener-hypothesis' *in vitro*: This hypothesis is that alcoholic beverages do affect *H. pylori* both *in vivo* and *in vitro* and this effect, is at least in part, due to the non-ethanol congeners.
CHAPTER 2: SEROPREVALENCE OF Helicobacter pylori IN ALCOHOL MISUSERS IN RELATION TO DRINKING BEHAVIOUR AND LIVER INJURY.

2.1 Introduction

The role of alcohol in the natural history of H. pylori infection needs to be further elucidated. To date, a number of studies have shown both an increased and decreased prevalence of H. pylori in patients consuming varying quantities of alcohol and with different levels of liver injury (Paton et al. 1990; Gubbins et al. 1993; Chen et al. 1994; Mion et al. 1994; Kirchner et al. 1996; Brenner et al. 1997; Calvet et al. 1997; Schmulson et al. 1997; Marshall et al. 1998).

Several possible explanations exist to account for the discrepancy in these findings: Firstly, few studies have been adequately controlled, with this data either missing or inappropriate; in some studies the control population comprises healthy volunteers, blood donors or hospital patients (Kirchner et al. 1996; Cakaloglu et al. 1997; Siringo et al. 1997; Manes et al. 1998; Marshall et al. 1998). But in other studies, indeed the majority, the control population comprises individuals presenting with dyspepsia (Misra et al. 1990; Paton et al. 1990; McCormick et al. 1991; Guslandi et al. 1993; Parikh et al. 1994; Wang et al. 1996; Bahnacy et al. 1997). In many studies no control population is included or the ‘control’ is obtained from other published work (Imoto et al. 1989; D'amico 1990; Misra et al. 1990; Balzano et al. 1991; McCormick et al. 1991).

Secondly, the study population and control populations, if available, are often not matched for important characteristics such as age, gender or background history. Only a handful of studies attempt to age match study populations despite the critical importance of age in relation to H. pylori prevalence (Paunio et al. 1994; Calvet et al. 1997; Siringo et al. 1997).

Thirdly, populations are generally poorly characterised in relation to their drinking behaviour and the degree and severity of any accompanying liver disease with few giving any information at all for either control or patient groups. (Paton et al. 1990; Gubbins et al. 1993; Parikh et al. 1994; Prigent-Delecourt et al. 1994; Schmulson et al. 1997; Marshall et al. 1998)
Fourthly, the number of patients studied is typically small at 60 or less allowing for the possibility of type II error when interpreting results (Balzano et al. 1991; Mosca et al. 1991; Mion et al. 1994; Cakaloglu et al. 1997; Schmulson et al. 1997). This error is likely to be further compounded when only one diagnostic method for *H. pylori* is used. This particular problem is common in many published studies to date (Misra et al. 1990; Gubbins et al. 1993; Guslandi et al. 1993; Parikh et al. 1994; Cakaloglu et al. 1997; Schmulson et al. 1997; Siringo et al. 1997).

### 2.2 Aims

To assess the seroprevalence of *H. pylori* in a large representative population of alcohol misusers controlling for potential confounding variables such as age, gender, recent and lifetime drinking behaviour and the degree and severity of any associated liver injury.

### 2.3 Patients and Methods

The study population comprised a total of 524 individuals (259 men: 265 women) who were assigned to one of four distinct population subgroups (Table 2.1).

#### 2.3.1 Group 1: Healthy volunteers

Healthy volunteers (n=142: 60 men; 82 women), recruited from among laboratory personnel and relatives of patients. None had a history or clinical evidence of alcohol misuse or of chronic liver disease and none currently consumed alcohol in excess of 20g/day. All had normal liver function tests except two individuals with Gilbert’s syndrome in whom serum bilirubin concentrations were elevated but to less than twice the upper laboratory reference range. None was on regular medication or had received antibiotics or proton pump inhibitors in the preceding 3 months.

#### 2.3.2 Group 2: Hospital inpatients

Hospital inpatients (n=55: 23 men; 32 women), recruited from the general medical wards, following admission with a variety of medical disorders, for example, myocardial infarction, pyelonephritis or chronic obstructive pulmonary disease. None had a history or clinical evidence of alcohol misuse or of chronic liver disease and none currently consumed alcohol in excess of 20g/day. All patients had normal liver function tests except four patients with an elevated aspartate transaminase (AST) of cardiac origin. Five patients (9.1%), all of whom had a non-gastroenterological primary diagnosis, had findings such as a history of dyspepsia or anemia suggestive of an additional gastrointestinal disorder. Most patients were on long-term medication
for a variety of underlying conditions, or else prescribed for their current complaint. None, however, had received antibiotics or proton pump inhibitors in the previous 3 months.

2.3.3 Group 3: Non-alcoholic liver disease

Individuals with non-alcoholic liver disease (n=151: 64men; 87 women), recruited from both the inpatients and outpatients service of the University Department of Medicine at the Royal Free Hospital. The diagnosis of non-alcoholic liver disease was based on historical, clinical, laboratory, radiological and histological findings. Patients were further categorised as ‘non-cirrhotic’ (n=60: 26men; 34 women) or ‘cirrhotic’ (n=91: 38men; 53women) None gave a history of alcohol misuse or consumed alcohol in excess of 20g/day. The majority were receiving medication, mainly vitamin supplements and diuretics but none had received antibiotics or proton pump inhibitors in the preceding 3 months.

2.3.4 Group 4: Alcohol Misusers

Alcohol misusers (n=176: 112men; 64women) recruited from both the inpatient and outpatient service of the University Department of Medicine at The Royal Free Hospital. The diagnosis of alcohol misuse was based on a history of alcohol consumption in excess of 60g/day for men and 40g/day for women for at least one year. The median (range) length of the drinking history for this group was 30 (1-60) yr with median daily intake of 100 (40-500) g alcohol and a median lifetime dose of 483 (54-5606) Kg. Overall, 57.4% of patients had been abstinent from alcohol for more than 1 month prior to the study and 53.4% for 3 months. (Table 2.3)

The degree of liver injury was categorised following histological examination of needle biopsy material, as minimal to include minor non-specific changes and steatosis (n=44: 34men; 10 women), alcoholic hepatitis (n=11: 4men; 7 women) and cirrhosis (n=121: 74men; 47 women)

Sera were collected and stored at -20°C. Analysis for \textit{H.pylori} IgG antibody was performed in batches using a microwell-based ELISA (Premier \textit{H.pylori}, Launch Diagnostics, Kent, UK). Sonicated cell lysate was used as the capture antigen. The method was automated using an automatic immunodiagnostic analyser (Biomaster, Launch Diagnostics, Kent, UK.) The results were interpreted spectrophotometrically at 450nm and classified as positive, negative or equivocal.
2.4 Statistical analysis

For the purpose of analysis, the alcohol misusers were classified as either 'minimal injury'-patients with minimal or fatty change on liver biopsy and 'significant injury'-patients with alcoholic hepatitis and/or cirrhosis on biopsy. Total lifetime dose of alcohol was classified as 'low', 'medium' and 'high' based upon tertiles of distribution.

Population data were tested for normality and analysed using Wilcoxon, Mann-Whitney and Chi squared tests. For analysis of *H. pylori* seroprevalence, subjects were classified as either *H. pylori* positive or *H. pylori* negative. Subjects with an equivocal result were excluded from the final analysis.

A multivariate logistic regression analysis with interaction term was used to determine the independent effects of sex, age, lifetime alcohol dose and diagnosis in relation to *H. pylori* prevalence.
### Table 2.1: Demographic Details of the Study Populations.

<table>
<thead>
<tr>
<th>Population subgroup</th>
<th>Number</th>
<th>Gender (M:F)</th>
<th>Median(range)age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>142</td>
<td>60:82</td>
<td>40.5(18-82)^^^</td>
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<tr>
<td>Hospital inpatients</td>
<td>55</td>
<td>23:32</td>
<td>67.5(26-90)**</td>
</tr>
<tr>
<td>Non-alcoholic liver disease</td>
<td>151</td>
<td>64:87</td>
<td>53.0(21-83)*^</td>
</tr>
<tr>
<td>non-cirrhotic</td>
<td>60</td>
<td>26:34</td>
<td>51.0(22-83)</td>
</tr>
<tr>
<td>cirrhotic</td>
<td>91</td>
<td>38:53</td>
<td>56.0(21-82)</td>
</tr>
<tr>
<td>Alcohol misusers</td>
<td>176</td>
<td>112:64</td>
<td>51.0(21-77)*^</td>
</tr>
<tr>
<td>minimal change</td>
<td>44</td>
<td>34:10</td>
<td>47.0(21-71)</td>
</tr>
<tr>
<td>alcoholic hepatitis</td>
<td>11</td>
<td>4:7</td>
<td>50.0(28-71)</td>
</tr>
<tr>
<td>cirrhosis</td>
<td>121</td>
<td>74:47</td>
<td>52.0(31-77)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>524</td>
<td>259:265</td>
<td>51.0(18-90)</td>
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</table>

Significance of difference in age between healthy volunteers and other groups: * p<0.05, ** p<0.01.
Significance of difference in age between hospital inpatients and other groups: ^ p<0.05, ^^ p<0.01.
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<table>
<thead>
<tr>
<th>Population subgroup</th>
<th>(n)</th>
<th>Drinking history (yr)</th>
<th>Alcohol intake (g/day)</th>
<th>Lifetime alcohol dose (Kg)</th>
<th>Abstinent &gt;1month n(%)</th>
<th>Abstinent &gt;3months n(%)</th>
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<td>Minimal change</td>
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<td>100</td>
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<td>(1.5-4.9)</td>
<td>(51-280)</td>
<td>(109-2847)</td>
<td>(38.6)</td>
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<td>Alcoholic Hepatitis</td>
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<td>613</td>
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<td>(1-40)</td>
<td>(64-500)</td>
<td>(117-5606)</td>
<td>(45.5)</td>
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<td>Cirrhosis</td>
<td>121</td>
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<td>98*</td>
<td>350*</td>
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<td>(54-2803)</td>
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<td>483</td>
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<tr>
<td></td>
<td></td>
<td>(1-60)</td>
<td>(40-500)</td>
<td>(54-5606)</td>
<td>(57.4)</td>
<td>(53.4)</td>
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</table>

Data presented as median (range) *n=78

Table 2.2: Drinking Behaviour in Alcohol Misusers, by Degree of Liver Injury.

2.5 Results

2.5.1 Patient Demography

Age distributions for all sample populations were found to be non-Gaussian. The healthy volunteers were significantly younger than the patients in the other three groups whilst the hospital inpatients were significantly older; there was no significant difference in the ages of the alcohol misusers and the patients with non-alcoholic liver disease (Table 2.1)

There were significantly more men than women in the alcohol misuse group when compared to healthy volunteers and hospital patients (p<0.01). The apparent excess of women in the non-alcohol liver disease group when compared with healthy volunteers and hospital patients was not significant (Table 2.1)
2.5.2 Overall *H. pylori* Seroprevalence
The overall *H. pylori* seroprevalence rate in the healthy volunteers was 18.3%. This was significantly lower than in hospital in-patients (47.3%; p<0.0001), the patients with non-alcoholic liver disease (40.4%; p<0.0001) and the alcohol misusers (35.2%; p<0.002). The prevalence rates in the three patient groups were comparable (Figure 2.1).

2.5.3 Effect of Gender on *H. pylori* Seroprevalence
The overall *H. pylori* seroprevalence rate in males was 17.7% and in females 15.7%. This gender difference was not significant (p=0.11). Similarly, no significant gender effect, on *H. pylori* seroprevalence, was observed within the individual subpopulations. Thus the male to female imbalance between groups had no effect on overall *H. pylori* seroprevalence rates.

2.5.4 Effect of Age on *H. pylori* Seroprevalence
A significant effect of age on *H. pylori* seroprevalence was observed in all four populations studied (p<0.001): The average increase in the odds of *H. pylori* seroprevalence was 7%/year of increasing age when age was fitted as a continuous variable in a logistic regression analysis. The corresponding figure in hospital patients was 11%/year of increasing age. Comparing seroprevalence below and above the 5th decade, for both populations, showed no significant difference between healthy volunteers and hospital patients (p=0.328).

In contrast to healthy volunteers and hospital patients, an opposite effect of age on *H. pylori* seroprevalence was observed in patients with liver disease: In both non-alcoholic and alcoholic liver disease, *H. pylori* seroprevalence rates decreased, with increasing age, by 5%/year of increasing age (Figure 2.2). Comparison of *H. pylori* seroprevalence below and above the 5th decade again showed no significant difference between the two liver disease groups (p=0.07). However, comparing *H. pylori* seroprevalence rates below and above the 5th decade for either liver disease group with either hospital patients or healthy volunteers showed a significant difference (Hospital vs Alcohol p<0.0001; Hospital vs Non-Alcohol p<0.0001: Healthy vs Alcohol p<0.01; Healthy vs Non-Alcohol p<0.01).
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**Figure 2.1: Overall *H. pylori* Seroprevalence in the Four Study Subgroups**

![Graph showing seroprevalence in different population subgroups.]

**Figure 2.2: *H. pylori* Seroprevalence in the Four Population Sub-groups by Age**

![Graph showing seroprevalence by age and population subgroup.]

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2.5.5 Effect of the Degree of Liver Injury and Age on *H. pylori* Seroprevalence.

Overall there was no significant difference in *H. pylori* seroprevalence rates between the patients with non-alcohol related liver disease and the alcohol misusers (40.4 vs 35.2%). Both within and between groups there was no significant difference in *H. pylori* seroprevalence rates in relation to the degree of liver injury (non-alcoholic liver disease: non-cirrhotic 41.7%, cirrhotic 39.6%; alcohol misusers: minimal injury 36.4%; significant injury 34.8%).

In the patients with non-alcoholic liver disease, *H. pylori* seroprevalence showed an overall inverse relationship to increasing age. The average decrease in the odds of *H. pylori* seroprevalence was 5%/year of increasing age (Figure 2.2). This relationship was also observed within the subgroup of patients with cirrhosis in whom an average decrease in the odds of *H. pylori* seroprevalence was observed, but of greater magnitude, at 20%/year of increasing age. In contrast, in the patients with non-cirrhotic injury, the seroprevalence rate increased with age; the average increase in the odds of *H. pylori* seroprevalence being 3%/year of increasing age (Figure 2.3). This difference for interaction between age and disease sub-group for *H. pylori* seroprevalence in non-alcoholic liver disease was highly significant (p<0.001).

In the alcohol misusers, there was also an overall inverse relationship between *H. pylori* seroprevalence and increasing age; the average decrease in the odds of *H. pylori* seroprevalence was 5%/year of increasing age (Figure 2.1). In the patients with minimal alcohol related liver injury, a decline in odds of *H. pylori* seroprevalence rate of 10%/year of increasing age was observed. This contrasted with the fixed prevalence rate, in relation to age, of approximately 30% (but check figure) observed in the patients with significant alcohol-related liver injury (Figure 2.4). Again, these difference for the interaction between age and disease sub-group for *H. pylori* seroprevalence were highly significant (p<0.001).
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Figure 2.3: *H. pylori* Seroprevalence in Patients with Non-Alcoholic Liver Disease

![Graph showing seroprevalence of *H. pylori* in NALD patients by age group.]

Figure 2.4: *H. pylori* Seroprevalence in Patients with Alcoholic Liver Disease

![Graph showing seroprevalence of *H. pylori* in ALD patients by age group.]

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2.5.6 Effect of Lifetime Alcohol Dose and Liver Injury on *H. pylori* Seroprevalence

In the alcohol misusers overall, the median (range) of drinking history was 30(1-60) years, with a median daily alcohol consumption of 100 (40-500) g/day. Total median lifetime dose was 483(54-5606) Kg (Table 2.2). No significant differences were observed in any of these variables in relation to the degree of liver injury (Table 2.2).

Overall, the seroprevalence of *H. pylori* tended to be lower with increasing lifetime alcohol dose (OR for increasing dose tertiles 1, 1.7, 0.5 p=0.06: Fig 2.5). In patients with significant liver injury, this trend was again observed (OR for tertiles 1, 0.97, 0.3 p=0.08), with the difference between low and high tertile lifetime dose being significant (p=0.03). No relationship was observed however between *H. pylori* seroprevalence rates and lifetime alcohol dose in the patients with minimal liver injury.

No significant difference in age existed between patients in the different tertiles of lifetime dose for either minimal (mean age low tertile lifetime dose 48.5yr vs 44.3yr high tertile dose) or significant liver injury (mean age low tertile 51.5yr vs 56.5yr high tertile). As lifetime alcohol dose is a derived variable, patients who are older at the time of study might be expected to have a higher lifetime dose due to potentially more years of misuse. The lack of age differences between high and low tertile groups is however evidence against lifetime dose being a ‘surrogate’ marker for increased patient age.
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2.5.7 Effect of Abstinence and Liver Injury on *H. pylori* Seroprevalence

In alcohol misusers who were drinking up to one month before the study the overall *H. pylori* seroprevalence rate was 33.3% independent of the degree of liver injury (Table 2.3). In the individuals who had been abstinent from alcohol for more than one month the overall seroprevalence rate was 36.6%, again independently of the degree of liver injury. No significant change in seroprevalence rate overall or in relation to the degree of liver injury was observed after periods of abstinence in excess of 3 months (Table 2.3).
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<table>
<thead>
<tr>
<th></th>
<th>overall</th>
<th>&lt;1mo. abstinence</th>
<th>&gt;1mo. abstinence</th>
<th>&gt;3mo. abstinence</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Minimal injury</td>
<td>44</td>
<td>36.4</td>
<td>9</td>
<td>33.3</td>
</tr>
<tr>
<td>Significant injury</td>
<td>132</td>
<td>34.8</td>
<td>16</td>
<td>33.3</td>
</tr>
<tr>
<td>All misusers</td>
<td>176</td>
<td>35.2</td>
<td>25</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Table 2.3: \textit{H.pylori} Seroprevalence in Relation to Abstinence and Liver Injury

2.6 Discussion

The overall \textit{H.pylori} seroprevalence rates were 18.3\% in healthy volunteers, 47.3\% in hospital inpatients, 40.4\% in non-alcoholic liver disease patients and 35.2\% in alcohol misusers. The lower overall seroprevalence rate in the healthy volunteers was due to this population being significantly younger than the other three groups. Similarly, overall \textit{H.pylori} seroprevalence in the three patient groups were comparable despite the hospital patients being significantly older.

The increase in \textit{H.pylori} seroprevalence of 7\%/year of increasing age in healthy volunteers and of 11\%/year in hospital inpatients, is in general agreement with other published figures. The steady increase in \textit{H.pylori} prevalence up to the 5th decade was first reported by Marshall \textit{et al} in 1984b. Subsequent studies support the generally accepted view, that \textit{H.pylori} prevalence increases by approximately 1\%/year (Graham \textit{et al} 1989; Parsonnet \textit{et al}. 1992; Kuipers \textit{et al}. 1993). While this studies' results are higher than this, they are between the 4.2\%/year quoted in a Spanish study (Gisbert \textit{et al}. 1998) and 13\%/year from Korea (Kim \textit{et al}. 1998). The increasing prevalence with age is likely to be a cohort effect (Marshall \textit{et al} 1984b), and this has recently been elegantly demonstrated by Gause-Nilsson \textit{et al} (1998).
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An unusual feature observed both in the alcohol misuse group and non-alcohol liver disease group is the very high H. pylori seroprevalence observed in the younger age decades. This is the opposite of that observed in hospital inpatients, healthy volunteers and other published work-consistently showing a lower H. pylori prevalence in the earlier decades (Marshall et al 1984b; Graham et al 1989; Parsonnet et al. 1992; Kuipers et al. 1993). These findings are difficult to explain: While it is possible to speculate that alcohol misusers might live a more deprived existence—an association with increased H. pylori prevalence, the same can not be applied to patients with non-alcoholic liver disease. Similarly, H. pylori infection is acquired in childhood presumably before the onset of alcohol misuse or the development of any liver injury.

The average 5%/year of increasing age decline in H. pylori seroprevalence observed in both liver disease groups is also a novel finding. While several studies have shown a reduced overall prevalence of H. pylori in patients with alcohol related liver injury, none have convincingly controlled for population age (Chen et al 1994; Mion et al 1994; Brenner et al. 1997; Calvet et al. 1997; Schmulson et al 1997; Marshall et al 1998).

The average 5%/year decline in H. pylori seroprevalence in patients with non-alcohol related liver disease has two components. These two components, in turn, reflect the degree of liver injury and are very different both in magnitude and direction: The first component is the average 3%/year increase in H. pylori seroprevalence observed in non-cirrhotic patients. This, in turn, is offset by the average 20%/year decrease seen in the cirrhotic group. This latter figure, however, must be interpreted with caution because it is an average of a very 'left-skewed' distribution. Up to, and including the 5th decade, H. pylori seroprevalence in non-alcoholic cirrhotic patients is 92.9% or higher. But, in the 6th decade, seroprevalence falls to 5.5% and declines even further in subsequent decades (Figure 2.3). Thus, it can be seen that the overall decline in H. pylori seroprevalence of 5%/year in the non-alcohol related liver disease patient group is an average of two distinct sub-population seroprevalence rates defined by liver injury.

The increase in H. pylori seroprevalence with age observed in the non-alcoholic, non-cirrhotic patients could be explained by this group only having mild liver injury and therefore could be expected to behave like healthy volunteers or 'ordinary' hospital inpatients. The decline in H. pylori seroprevalence observed in non-alcoholic cirrhotic patients, and in particular the dramatic fall in seroprevalence seen at the 5th decade, is harder to explain. A possible explanation would include the development of atrophic gastritis after the 5th decade. As discussed in section
1.3.2, a decline in *H. pylori* prevalence after the 5-6\textsuperscript{th} decade, in the healthy population is thought to be due to the development of atrophic gastritis after years of infection. The *H. pylori* is then no longer able to survive in this environment and is 'cleared.' In favour of this hypothesis is the observation that the majority of non-alcoholic cirrhotic patients in this study were not of viral aetiology but due to conditions generally regarded as either 'autoimmune' or having an autoimmune component such as primary biliary cirrhosis. This population could therefore be expected to have a higher rate of other 'autoimmune' conditions such as pernicious anemia that might accelerate and/or potentiate the atrophic change due to *H. pylori*. Indirect evidence to support the importance of immune status in influencing *H. pylori* disease progression was recently reviewed by Nedrud et al (1999). These authors emphasised the importance of both host immunity and *H. pylori* CagA status at triggering an immune response and influencing disease progression. CagA status was not however available in this study.

The average overall decline in *H. pylori* seroprevalence of 5%/year observed in alcohol misusers is similar in magnitude and direction to that observed in non-alcohol related liver injury patients. Again, this overall figure has two distinct components, in turn, reflecting the degree of liver injury: In significant alcoholic liver injury, the *H. pylori* seroprevalence remains constant through the decades, at approximately 35%. But, in minimal injury, the *H. pylori* seroprevalence declines by 10%/year of increasing age. It can therefore be seen that the minimal injury group is not only the major determinant of overall *H. pylori* seroprevalence in the alcohol misusers, but also that the seroprevalence distribution is opposite in direction to that observed with non-alcohol related minimal liver injury.

It is again difficult to explain the 'fixed' *H. pylori* seroprevalence observed in alcoholic cirrhotic patients. An explanation based on the possibility of atrophic gastritis developing in the 5\textsuperscript{th}-6\textsuperscript{th} decades as discussed for non-alcoholic cirrhosis does not fit with the data. Conceivably, any decline in *H. pylori* seroprevalence that might occur due to the development of atrophic gastritis in the later decades might be 'offset' by re-acquisition of infection. As already discussed, alcohol misusers do frequently live a deprived existence and this is often cited as an explanation for acquiring infections in general. Against this hypothesis, however, is the known low re-acquisition rate of *H. pylori* infection of typically <1%/year observed in the otherwise normal population (Parsonnet et al. 1992; Kuipers et al. 1993).
A putative role of portal hypertensive gastropathy in influencing the natural history of *H. pylori* infection in patients with cirrhosis has been discussed previously (Section 1.20). It is difficult, however, to see how this could explain the differing patterns of *H. pylori* seroprevalence observed in alcoholic and non-alcoholic cirrhosis observed in this study because portal hypertensive gastropathy reflects portal hypertension and not the aetiology of the liver disease *per se*. Data on portal hypertensive gastropathy was not however available for this study and it can only be assumed that it occurred equally in both the alcoholic and non-alcoholic cirrhotic patient groups.

An alternative explanation for the differences observed in *H. pylori* seroprevalence observed between alcoholic and non-alcoholic cirrhotic patients might relate to drug usage: For this reason, use of antibiotics in the previous three months was an exclusion criteria for study entry. It is possible, however, that once a diagnosis of ‘cirrhosis’ is made clinicians are more likely to prescribe antibiotics for incidental infections and so secondarily treat *H. pylori*. This might conceivably explain the fall in *H. pylori* seroprevalence in the 5th decade seen in the non-alcoholic cirrhotic patients, but not the static seroprevalence observed in the alcoholic cirrhotics. To suggest that alcoholic cirrhotic patients have poor compliance to treatment and therefore do not complete antibiotic courses and achieve secondary *H. pylori* eradication appears tenuous.

A more attractive hypothesis to explain differences in *H. pylori* seroprevalence between non-alcoholic and alcoholic cirrhotic patients focuses on alcohol consumption—the main difference between the two groups. ‘Alcoholic gastritis’ is a recognised feature of alcohol misuse and this, in turn, might influence survival of *H. pylori in vivo*. If alcoholic gastritis was also found in association with portal hypertensive gastropathy then the local environment for the bacteria would be significantly different from that observed in the otherwise normal population. If it is also hypothesised that alcohol itself may be directly influencing *H. pylori in vivo*, then a further level of complexity is introduced. Evidence to support an effect of alcohol consumption on the natural history of *H. pylori* seroprevalence comes from the significant difference observed between low and high tertiles of lifetime dose observed in the alcoholic cirrhotic patients (figure 2.5). Whether this is due to direct toxicity against *H. pylori*, or through the development of alcoholic gastritis or some other mechanism is unclear. Support of a ‘direct-toxicity’ effect of alcohol on *H. pylori*, albeit indirect, comes from in vitro studies, that have demonstrated a general anti-bacterial effect of alcoholic beverages, although not all have specifically studied *H. pylori* (Weisse *et al* 1995; Marshall *et al* 1999).
Indirect evidence for a ‘direct-toxicity’ hypothesis of alcohol consumption on \textit{H. pylori}, albeit in the normal population rather than alcohol misusers \textit{per se}, comes from the studies discussed in the introduction (section 1.13). Rosenstock \textit{et al} (2000) demonstrated a negative \textit{H. pylori} seroprevalence dose response curve with wine consumption and Ogihara \textit{et al} (2000) demonstrated a similar phenomenon in Japan. While these studies in no way provide direct evidence for direct toxicity of alcoholic beverage against \textit{H. pylori}, they do help to support a ‘working hypothesis’ that alcohol might influence the natural history of \textit{H. pylori} infection.

The patients with minimal change provide further evidence, albeit indirect, that alcohol misuse might influence the natural history of \textit{H. pylori} infection. The fall in \textit{H. pylori} seroprevalence from 100\% in the 4\textsuperscript{th} decade to 20\% in the 5\textsuperscript{th}, is 10 years earlier than that generally expected in the otherwise normal population or that observed in the non-alcoholic cirrhotic group (Marshall \textit{et al} 1984b; Graham \textit{et al} 1989; Parsonnet \textit{et al.} 1992). This ‘left-shift’ of the \textit{H. pylori} seroprevalence distribution might reflect the earlier onset of atrophic gastritis and \textit{H. pylori} clearance perhaps accelerated by a chronic alcoholic gastritis. Alternatively, alcohol may be playing a more direct role in \textit{H. pylori} clearance the natural history of which is in turn influenced by the absence of cirrhosis-related portal hypertensive gastropathy. While theoretically attractive, a hypothesis of increased \textit{H. pylori} clearance due to alcohol is not directly supported by the observation that lifetime dose did not influence \textit{H. pylori} seroprevalence in this patient group.

2.7 Summary and Conclusions

The \textit{overall} \textit{H. pylori} seroprevalence rates were 18.3\% in healthy volunteers, 47.3\% in hospital inpatients, 40.4\% in non-alcoholic liver disease patients and 35.2\% in alcohol misusers. The \textit{H. pylori} seroprevalence increased with age in both healthy volunteers and hospital inpatients-a pattern similar to that observed in other published studies. Patients with liver disease however had much higher \textit{H. pylori} seroprevalence rates in the early decades than either healthy volunteers or hospital patients. In addition, seroprevalence decreased with increasing age-the opposite to that observed in the non-liver disease groups.

The \textit{average 5\%/year decrease} in \textit{H. pylori} seroprevalence observed in both liver injury groups had two distinct components that in turn, reflected the degree of liver injury. In non-alcoholic liver disease, an \textit{increase} in \textit{H. pylori} seroprevalence in the minimal injury group was offset by a \textit{decline} in seroprevalence observed in the cirrhotic group. Possible explanations for this include
the minimal injury group behaving like 'normal' patients and the cirrhotic patients clearing \textit{H.pylori} in the 5\textsuperscript{th} decade due to the development of atrophic gastritis.

The same average overall 5%/year decrease in \textit{H.pylori} seroprevalence was also observed in alcohol misuse patients. This average figure was again comprised of two distinct \textit{H.pylori} seroprevalence distributions. These, again reflected the extent of liver injury. The alcoholic cirrhotic patients had a constant \textit{H.pylori} seroprevalence of 35\%, whereas the seroprevalence in minimal injury patients declined on average by 10%/year. Speculation about the potential roles of atrophic and alcoholic gastritis, portal hypertensive gastropathy and direct alcohol toxicity is presented. The importance of alcohol consumption in influencing \textit{H.pylori} serorevalence, even if the mechanism awaits elucidation, is emphasised by the significant association between high tertile of lifetime consumption and lower seroprevalence observed in alcoholic cirrhotic patients.

\textbf{2.8 Future Directions}

This study has demonstrated the importance of accurately characterising study populations in terms of age, drinking history and liver injury when trying to determine the seroprevalence of \textit{H.pylori}. One weakness of the study is the reliance on a single diagnostic method for \textit{H.pylori} and this needs to be addressed in future work. Similarly, it is unclear what effect, if any, liver disease or alcohol misuse might have on the overall accuracy of diagnostic method chosen. It is known that recent drug usage interferes with diagnostic methods for \textit{H.pylori} and this study has presented some evidence to support the concept that alcohol might also be important, but other factors including socio-economic status and presence or absence of portal hypertensive gastropathy also needs to be considered.

In view of these factors, future work needs to concentrate on all known risks for \textit{H.pylori} infection and prospectively collect this data. In addition, several diagnostic methods need to be used to determine the relative sensitivity and specificity in the context of alcohol misuse and liver disease. Since we have also speculated that alcoholic gastritis may be a factor influencing \textit{H.pylori} disease progression, this also needs to be simultaneously assessed to determine its relative role in the natural history of infection.

Finally, consideration to the alcoholic beverages consumed needs to be considered. It has been assumed in this study, like in other published work, that any putative role of alcoholic beverage
on the natural history of *H. pylori* is due to its ethanol content. This draws on the analogy with liver disease, where, other things being equal, liver damage is proportional to total lifetime ethanol dose. While it appears logical to extend this concept to any putative role of alcoholic beverages on *H. pylori*, it is quite possible that a non-ethanol component(s), or congener, may have a more significant influence on the natural history of *H. pylori* infection. The relative influence of ethanol and congener on *H. pylori* will also be addressed in future work.
Chapter 3: Diagnosis and Risk Factors for H. pylori in Alcohol Misusers

CHAPTER 3: PROSPECTIVE ASSESSMENT OF DIAGNOSTIC METHODS AND RISK FACTORS FOR HELICOBACTER PYLORI INFECTION IN ALCOHOL MISUSERS.

3.1 Introduction

The data presented in chapter 2 provided evidence for a complex interaction between alcohol misuse and the severity of the associated liver injury in influencing the seroprevalence of H. pylori. The relative importance of each factor in determination of overall H. pylori seroprevalence was, however, only partially addressed. It is, for example, unclear whether the effects of alcohol consumption and the extent of liver injury affect H. pylori seroprevalence in an independent, dependent or even more complex fashion. Similarly, the role of other potential risk factors for H. pylori infection in addition, or in combination with, alcohol and liver disease were not fully investigated.

The interpretation of the seroprevalence data presented in chapter 2 was hampered because little or no information is currently available on the relative accuracy of the various tests for determining H. pylori status in the context of alcohol misuse and liver disease. While quoted values for the sensitivity and specificity of serological testing for H. pylori often exceed 90% in the otherwise normal population (section 1.9), no studies have specifically assessed this in the context of active alcohol misuse. Clearly, performance of assay method is of critical importance in the evaluation of these data. To assess the validity, or otherwise, of serology as a diagnostic method for H. pylori in alcohol misusers requires a prospective assessment in this patient population of the diagnostic methods for H. pylori.

3.2 Aims

- To prospectively determine the prevalence of H. pylori in a population of alcohol misusers carefully controlled for alcohol consumption, degree of liver injury and other risk factors for H. pylori infection including social and demographic background.
- To assess sensitivity and specificity of individual tests for the diagnosis of H. pylori in this clinical context.
3.3 Overall Study Design

One hundred alcohol misusers attending as inpatients or outpatients were recruited sequentially for the study. A detailed drinking and demographic profile was collected for each patient and the extent of liver injury determined by percutaneous liver biopsy. Several non-invasive and invasive diagnostic tests for *H. pylori* were performed to allow comparison of different test methods in the context of alcohol misuse and liver injury. All patients underwent diagnostic upper endoscopy during which systematic biopsies were obtained according to the modified Sydney system to allow histological diagnosis of *H. pylori* (Section 4.2.3). Patients received active medical and psychological support both during and after the study to attain and maintain abstinence from alcohol.

3.4 Ethics

The study received ethical approval from the institutional ethics board of University College London Medical School (Royal Free Campus) and The Royal Free Hospital NHS Trust. All patients provided written informed consent.

3.5 Recruitment

3.5.1 Patient Recruitment and Eligibility

Patients were recruited sequentially from the outpatient and inpatient services of the Centre for Hepatology at The Royal Free Hospital, London. After full assessment, all patients were offered either in-patient or out-patient detoxification dependent on clinical need.

3.5.2 Inclusion Criteria

Any patient actively misusing alcohol was potentially eligible for the study. For the purposes of recruitment, ‘alcohol misuse’ was defined as any patient whom asked for help with their current drinking and had associated alcohol-related physical or psychological problems. This broad definition was chosen to help make the study population as representative of the general population of alcohol misusers as possible.

3.5.2 Exclusion Criteria

Exclusion criteria were again limited to make the population as representative of day-to-day clinical practice as possible and included:

- Refusal or inability to give informed consent.
3.6 Clinical Assessment and Data Collection

3.6.1 Patient Demography

Standard demographic data were collected on all patients. In addition, current and previous employment histories were obtained and classified according to major groups as defined by the OECD Standard Occupational Classification (1991). Previous employment histories were considered particularly relevant to provide an indirect marker of childhood socio-economic status in view of the increased risk of *H. pylori* infection in the young and socially disadvantaged (section 1.7). In addition, alcohol misuse is typically associated with a decline in ‘social-status’ and recording current socio-economic circumstances might not fairly reflect those circumstances previously enjoyed.

3.6.2 History, Examination and Clinical Management

All patients underwent a standard history and physical examination in addition to specialist assessment as part of the study. Particular emphasis was placed on elucidating signs of chronic liver disease, alcohol misuse and hepatic decompensation. All information was recorded on a standard pro-forma for future analysis.

All patients were offered active medical and psychological support to help attain and then maintain abstinence from alcohol. This took the form of regular clinic review, active participation in alcohol support groups, contact with appropriate agencies and pharmacological treatments including acamprosate to help manage alcohol 'cravings'.

- No contraindication to upper gastrointestinal endoscopy or liver biopsy as defined by local guidelines. Typical contraindications included severe cardio-respiratory compromise or uncontrolled bleeding diathesis.
- Requirement, because of their clinical condition, for any emergency procedure, but most typically endoscopy for upper gastrointestinal haemorrhage. These patients were not initially eligible for inclusion but were later recruited if their clinical condition permitted.
- History of non-compliance with investigational or therapeutic regimes.
- Homeless or of no fixed abode.
3.6.3 Alcohol History
The following were obtained by direct questioning and, where possible, confirmed by collateral history from family members:

- Age of first alcoholic drink
- Dates of regular drinking, daily drinking, binge episodes, misuse and abstinent periods.
- Preferred beverage(s)
- Time of last drink
- Periods of abstinence prior to endoscopy
- Previous detoxification attempts
- Physical dependence including withdrawal fits and delerium tremens
- Administration of the 'CAGE' questionnaire, with a final score out of four for each positive response: ‘Have you ever been told to Cut down your drinking?’, ‘Has anyone Annoyed you telling you about your drinking?’, ‘Have you felt Guilty about your drinking?’ ‘Have you ever had an Eye-opener- a drink first thing in the morning?’(Paton 1994).
- Family history of alcohol misuse.
- Lifetime alcohol dose: This is a derived variable from daily alcohol consumption in g/day multiplied by number of years drinking at that consumption level. The final result is a lifetime alcohol dose in Kg.

3.6.4 Drug History
A complete current drug history was obtained from all patients. In addition, specific information about use of drugs known to influence *H pylori* such as proton pump inhibitors (PPi), H2 receptor antagonists, antibiotics and bismuth compounds taken in the last three months was sought.

3.6.5 Liver Disease Assessment
In addition to clinical evaluation, patients also underwent full haematological, biochemical, virological and immunological evaluation to exclude any other factor for liver injury other than alcohol. All patients had basic ‘liver function tests’ including aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), albumin and prothrombin time.

In addition, a diagnostic liver biopsy was performed to assess the extent of liver injury. These biopsies were processed as part of routine care. Patients were classified for study purposes as either having ‘minimal’ or ‘significant’ alcohol-related liver injury. ‘Minimal injury’ included
either a normal biopsy, minimal changes or steatosis. Whereas 'significant liver injury' included those individuals with alcoholic hepatitis and/or cirrhosis.

3.7 Non-Invasive Tests for H. pylori

3.7.1 Serology
All patients had a serological test for H. pylori using the assay system described in section 2.3.4. These tests were assessed as positive, negative or equivocal. Patients testing equivocal were considered negative for the purposes of this study.

3.7.2 Urea Breath Tests
Standard $^{13}$C-urea breath tests (Pylobactell™, BSIA Ltd, Brentford, Middlesex, UK) were performed on all compliant patients who met local criteria for use; these included absence of antibiotic usage in the previous three months or use of proton pump inhibitors in the previous two weeks. These tests were assessed as positive, negative or equivocal. Patients testing equivocal were again considered negative for the purposes of this study.

3.8 Endoscopy

3.8.1 Endoscopic Technique
All patients underwent a standard upper endoscopy as soon as possible after admission to hospital. The procedure was deferred in those patients who were experiencing alcohol withdrawal until established on an adequate drug regimen. Olympus™ endoscopic equipment was used for all procedures (XQ10, XQ20 and IT10). At endoscopy, biopsies were obtained according to the modified Sydney System (Section 4.2.3-4.2.4) using standard biopsy forceps (Olympus™ XTF230X). Biopsies were placed on strips of cellulose-acetate paper (Whatmann, UK) and then placed in standard formalin preservative. All endoscopies were performed by a single operator (JCM) to avoid inter-observer variation. Reports were issued using a standard format (Endoscribe™, UK).

3.9 Invasive Endoscopic Tests for H. pylori

3.9.1 Histological Analysis for H. pylori
All samples were processed routinely using standard histological techniques. Sections were stained using both Haemotoxilin and Eosin staining and Giemsa stain to aid identification of H. pylori. Assessment of sections was undertaken by a histologist with a special interest in
Chapter 3: Diagnosis and Risk Factors for *H. pylori* in Alcohol Misusers

*H. pylori* and gastro-duodenal histology. The pathologist had no knowledge of any clinical details of the patients except that they were being assessed as part of a study on gastro-duodenal pathology in alcohol misuse.

### 3.9.2 Urease testing

At endoscopy, in addition to systematic biopsies, a single antral biopsy was taken to test for *H. pylori* urease (CLO test, Ballard Medical Products, Utah, USA) as described in section 1.9. The slide was ‘read’ at 1 hour by endoscopy staff trained to interpret CLO tests, but not directly involved in the care of the patient at the time of the procedure. Tests were classified as either positive (any pink discolouration) or negative (yellow colour persisting). Tests were re-checked at 24 hours before final classification as either positive or negative. In equivocal cases, an independent opinion from a gastroenterologist, not involved with the study, was sought.

### 3.10 Statistics

Patient demographic data was analysed using simple descriptive statistics and found to be non-normally distributed. Comparison between different discontinuous variables was by Chi squared analysis. All statistical calculations were performed using Astute (University of Leeds 1993).

The sensitivity and specificity of individual diagnostic tests was calculated using the formulae:

\[
\text{Sensitivity} = \frac{TP}{TP+FN}; \quad \text{Specificity} = \frac{TN}{TN+FP}
\]

Where TP = ‘True Positive’ rate, TN = ‘True negative’ rate, FP the ‘False positive’ rate and FN the false negative rate.

For this analysis, any patient in whom *H. pylori* was identified by histology at any gastric site biopsy was classified ‘*H. pylori* positive’. Patients in whom *H. pylori* was not identified after histological analysis of all gastric biopsy sites but in whom two or more other tests for *H. pylori* were positive (serology, urease or UBT) were also classified as ‘*H. pylori* positive’. Patients who were histology negative and in whom only one other test was positive were classed ‘*H. pylori* negative’, as were all patients with all negative tests.
3.11 Results

3.11.1 Completeness of Data Collection

Full demographic, medical and alcohol data were obtained on all 100 patients. Serological testing for *H. pylori* was available in 94 patients and 21 patients had a urea breath test. Urease testing (CLO^6^) testing was performed in 98 patients. In one patient no antral biopsy was available and in another patient, no posterior body biopsy was available. 98 patients had anterior gastric body biopsies for *H. pylori*. For the assessment of liver injury, 92 patients had successful liver biopsy (Table 3.1).

<table>
<thead>
<tr>
<th>Data Parameter</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics:</td>
<td></td>
</tr>
<tr>
<td>Medical and surgical history</td>
<td>100</td>
</tr>
<tr>
<td>Alcohol history</td>
<td>100</td>
</tr>
<tr>
<td><em>H. pylori</em> Diagnosis:</td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td>94</td>
</tr>
<tr>
<td>C^{13} Urea breath test (UBT)</td>
<td>21</td>
</tr>
<tr>
<td>Urease Testing (CLO^{6^})</td>
<td>98</td>
</tr>
<tr>
<td>Antral biopsy</td>
<td>99</td>
</tr>
<tr>
<td>Posterior gastric biopsy</td>
<td>99</td>
</tr>
<tr>
<td>Anterior gastric biopsy</td>
<td>98</td>
</tr>
<tr>
<td>Liver Diagnosis:</td>
<td></td>
</tr>
<tr>
<td>Complete database*</td>
<td>100</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>92</td>
</tr>
</tbody>
</table>

*screening for virological, immunological and serological markers of non-alcohol related liver injury

Table 3.1: Completeness of Data Collection for Major Study Parameters

3.11.2 Patient Demographics

The 100 alcohol misusers [71 male and 29 female] had a median (range) age of 48 (28-77) yr. There was no significant difference between the median ages of the men [median 50 (34-66) yr] and women [median 50 (28-77) yr].
The frequency distribution of socio-economic class for the alcohol misusers based upon the OECD classification of ‘best-ever’ employment is shown in Figure 3.1: 37% of the patients were unemployed (OECD 10) and 28% were employed/engaged in unskilled manual work (OECD 9). A similar distribution of OECD classes was observed for ‘current employment’ and no significant difference between ‘best-ever’ and ‘current’ OECD classification was observed.

Figure 3.1: Socio-Economic Patient Profile Based on ‘Best-Ever’ Employment

At the time of study, 83 patients were current smokers with a median (range) of 20 (4-80) pack-years. 15 patients consumed no caffeinated beverages (regular tea or coffee) at the time of study. The remaining 85 patients consumed a median (range) of 3 (1-35) cups of regular caffeinated tea or coffee/day.

In the three months prior to study, 10 patients had taken proton pump inhibitors either alone or in combination with other drugs known to influence *H. pylori* (Figure 3.2). Similarly 8 patients had taken H2 receptor antagonists either singularly or in combination and 12 patients had received antibiotic therapy in the previous three months. But only one patient had received antibiotic therapy in combination with other agents conventionally used for *H. pylori* eradication. No patient had received formal *H. pylori* eradication therapy at any time prior to study.
3.11.3 Alcohol Consumption

The drinking behaviour of the alcohol misusing patients is summarised in Table 3.2. No significant differences between men and women in terms of age of first drink, age of onset of alcohol misuse or total years of misuse was observed. Men, however, consumed significantly more alcohol/day than women [M:224 (48-800); F:120 (48-400) g ethanol/day: p<0.001]. Similarly, the lifetime ethanol dose was significantly higher in men [1497 (40-7884) Kg] than in women [832 (42-3547) Kg: p<0.001].
Chapter 3: Diagnosis and Risk Factors for *H. pylori* in Alcohol Misusers

### Table 3.2: Alcohol Misuse Data for 100 Alcohol Misusers [median (range)]

<table>
<thead>
<tr>
<th></th>
<th>Age 1st drink (yr)</th>
<th>Age Misuse (yr)</th>
<th>Years of Misuse</th>
<th>Daily Consumption (g ethanol/d)</th>
<th>Lifetime ethanol dose (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=100)</td>
<td>16 (6-30)</td>
<td>20.5 (9-59)</td>
<td>22.5 (0-50)</td>
<td>196 (48-800)</td>
<td>1138 (40-7884)</td>
</tr>
<tr>
<td>Male (n=71)</td>
<td>16 (6-30)</td>
<td>20.0 (9-50)</td>
<td>24.0 (0-50)</td>
<td>224 (48-800)*</td>
<td>1497 (40-7884)*</td>
</tr>
<tr>
<td>Female (n=29)</td>
<td>18 (10-30)</td>
<td>22.0 (12-59)</td>
<td>19.0 (2-45)</td>
<td>120 (48-400)*</td>
<td>832 (42-3547)*</td>
</tr>
</tbody>
</table>

* difference men vs women for daily consumption and lifetime ethanol dose p<0.001

### 3.11.4 Liver Disease

Of the 100 alcohol misusing patients, 92 had a diagnostic liver biopsy. The eight patients who did not have a diagnostic liver biopsy were classified as having 'minimal' liver injury based on clinical, laboratory and radiological findings. The basis for this assessment included:

- Absence of cutaneous and other superficial features of alcohol misuse and of clinical signs of chronic liver disease.
- Normal liver ‘synthetic-function’ (albumin and prothrombin time).
- Absence of oesophageal varices and/or portal hypertensive gastropathy at endoscopy.
- A smooth, homogenous liver edge on ultrasound and/or CT scanning.

Overall, 80 patients had minimal injury (72 on biopsy, 8 on clinical criteria) and 20 patients significant liver injury (all on biopsy).

No significant differences existed between the ages of patients with significant liver injury when compared with minimal injury. Although men consumed significantly higher amounts of alcohol than women, there was no significant difference in consumption either in g ethanol/day consumed...
or lifetime dose when comparing minimal with significant injury either by sex or overall (Table 3.3).

<table>
<thead>
<tr>
<th>Liver Injury</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number Patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>20</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Minimal</td>
<td>80</td>
<td>58</td>
<td>22</td>
</tr>
<tr>
<td><strong>Age (yr) [median (range)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>49</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>(40-66)</td>
<td>(40-59)</td>
<td>(42-66)</td>
</tr>
<tr>
<td>Minimal</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>(32-71)</td>
<td>(32-71)</td>
<td>(35-66)</td>
</tr>
<tr>
<td><strong>Daily consumption (g/ [median (range)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>176</td>
<td>200*</td>
<td>120*</td>
</tr>
<tr>
<td></td>
<td>(48-480)</td>
<td>(48-480)</td>
<td>(96-240)</td>
</tr>
<tr>
<td>Minimal</td>
<td>200</td>
<td>240*</td>
<td>124*</td>
</tr>
<tr>
<td></td>
<td>(48-800)</td>
<td>(48-800)</td>
<td>(64-400)</td>
</tr>
<tr>
<td><strong>Lifetime Dose (Kg) [median (range)]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant</td>
<td>1270</td>
<td>1350*</td>
<td>1086*</td>
</tr>
<tr>
<td></td>
<td>(491-3548)</td>
<td>(491-2275)</td>
<td>(526-3548)</td>
</tr>
<tr>
<td>Minimal</td>
<td>1314</td>
<td>1497*</td>
<td>788*</td>
</tr>
<tr>
<td></td>
<td>(1-7884)</td>
<td>(1-7884)</td>
<td>(234-2190)</td>
</tr>
</tbody>
</table>

* difference men vs women daily and lifetime alcohol consumption: p<0.001.

Table 3.3: Demographic Data and Alcohol Consumption by Liver Injury

3.11.5 Overall \textit{H.pylori} Prevalence

The overall \textit{H.pylori} prevalence in this patient population by diagnostic method, is shown in Table 3.4. The overall \textit{H.pylori} prevalence using the gold-standard, as previously defined (section 3.10), was 46% and with histology alone 33%. This difference was not significant. The overall \textit{H.pylori} seroprevalence was 43.6%. This was non-significantly different from that obtained either with the gold-standard or with histology alone.
The 66.7% overall \textit{H. pylori} prevalence obtained using $^{13}$C-urea breath testing was significantly higher than the prevalence determined by histology alone (p=0.03), but not significantly higher than the prevalence using the gold-standard.

Overall \textit{H. pylori} prevalence using urease testing was 25.5%. This was non-significantly lower than the prevalence with histology, but significantly lower than \textit{H. pylori} prevalence using the gold-standard (p=0.04).

<table>
<thead>
<tr>
<th>Diagnostic Technique</th>
<th>Serology</th>
<th>$^{13}$UBT</th>
<th>Urease (CLO$^R$)</th>
<th>Histology</th>
<th>Gold-Standard$^\star\star$</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{H. pylori} prevalence</td>
<td>43.6%</td>
<td>66.7%</td>
<td>25.5%</td>
<td>33.0%</td>
<td>46.0%</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>94</td>
<td>21</td>
<td>98</td>
<td>100*</td>
<td>100</td>
</tr>
</tbody>
</table>

*2 patients had only 2 of 3 possible sites biopsied for \textit{H. pylori} (antrum, anterior body or posterior body).

$^\star\star$Gold-standard = either histology positive or histology negative and 2 or more non-histological tests positive

Table 3.4: \textit{H. pylori} Prevalence in Alcohol Misusers by Diagnostic Technique

3.11.6 Overall Sensitivity and Specificity of Diagnostic Tests for \textit{H. pylori}

The sensitivity and specificity of both individual and combined diagnostic tests for \textit{H. pylori} were obtained by comparing test results with those obtained from the gold-standard. The sensitivity and specificity for \textit{H. pylori} diagnosis by technique are summarised in Table 3.5.
Table 3.5: Sensitivity and Specificity of Different Diagnostic Techniques for *H. pylori*.

For *H. pylori* diagnostic tests used singularly, histology had a sensitivity of 71.7%. This was higher than the sensitivity achieved with either CLO⁸ testing (52.2%) or serology (64.4%) but lower than the 92.9% sensitivity achieved with ¹³C urea breath testing.

In contrast to the lowest sensitivity achieved for *H. pylori* diagnosis of any test using CLO⁸ testing, the specificity of this test was high at 98.2%. ¹³C-urea breath testing achieved a specificity of 90.0% and serology 75.5%. The specificity of histological diagnosis alone was 100%, but, this
in turn reflects the definition of gold-standard for *H. pylori* diagnosis which defines any patient as ‘*H. pylori* positive’ if histology is positive. Using a comparison between histology alone with the gold-standard, false positive results can not exist by definition.

Using paired tests for *H. pylori* diagnosis, serology and $^{13}$C-urea breath testing combined provided maximum sensitivity for *H. pylori* diagnosis at 85.7%. A combination of serology and CLO$^R$ testing had low sensitivity for diagnosis at 45.7% and a combination of histology and one other diagnostic test reduced sensitivity for *H. pylori* diagnosis to between 30-34.8% (see Table 3.5). All paired tests were however able to provide specificity of 100% for the diagnosis of *H. pylori*.

Using non-histological ‘triple-testing’, for *H. pylori*, a sensitivity of diagnosis of 57.1% could be achieved using all three tests. This increased to 69.6% if just one test positive was accepted as a positive result but at the expense of specificity falling from 100% to 74.1%.

Using all diagnostic tests for *H. pylori* (serology, $^{13}$C-UBT, CLO$^R$ and histology), maximum sensitivity of diagnosis was achieved using one of four tests positive at 100%. This is however a reflection of the gold-standard which defines any patient as *H. pylori* positive if histology is also positive as previously discussed. Specificity using one test positive was 74.1% and for other test combinations 100% specificity was achieved. Two of four tests positive provided the next best sensitivity of *H. pylori* diagnosis at 68.9% (Table 3.5).

### 3.11.7 Influence of Demographic Factors on *H. pylori* Prevalence

Significantly more men were included in the study than women (M=71; F=29; p<0.05), but, as previously discussed (section 3.11.2), no significant differences existed in the ages between men and women. Similarly, after first classifying patients by *H. pylori* status, there was no significant difference either in the proportion of men to women or in their age distribution. [*H. pylori* positive: 33M 71.7% age 47 (32-77); *H. pylori* negative: 38M 66.7% age 47 (28-74)].

There was no significant relationship between current smoking and *H. pylori* status with 76.1% of *H. pylori* positive patients smoking and 84.8% of *H. pylori* negative patients being current smokers. Similarly, no significant relationship existed between *H. pylori* status and caffeine consumption with median (range) for tea and coffee consumption combined being 2 (0-11) cups/day in *H. pylori* positive patients and 2 (0-22) in *H. pylori* negative patients.
In the three months prior to study, 4.3% of the *H. pylori* positive patients had received antibiotics and 8.5% of the *H. pylori* negative patients had received antibiotics. This difference showed a trend to significance (p=0.09). 4.3% of the *H. pylori* positive patients had received proton pump inhibitors in the previous three months and 13% of the *H. pylori* negative patients had taken these medicines. This difference was not however significant. Similarly, no significant difference existed between *H. pylori* positive and negative patients in regards consumption of either H2 receptor antagonists or NSAIDs.

3.11.8 Influence of Liver Injury to Overall *H. pylori* Prevalence

The overall *H. pylori* prevalence by diagnostic technique and degree of liver injury is presented in Table 3.6. Using the gold-standard for diagnosis, the overall *H. pylori* prevalence was 50.0% in the significant liver injury group and 45.0% in the minimal liver injury group. The equivalent figures using histology were 40.0% (significant) vs 31.3% (minimal); urease testing, 20.0% (significant) vs 26.3% (minimal); serology, 40.0% (significant) vs 44.6% (minimal) and for urea breath testing 57.1% (significant) vs 58.8% (minimal). For all of the diagnostic methods, no significant difference existed between the overall prevalence of *H. pylori* in the significant liver injury group when compared with the minimal injury group.

<table>
<thead>
<tr>
<th>Diagnostic Technique</th>
<th>Gold-Standard</th>
<th>Histology</th>
<th>Urease (CLO\textsuperscript{H})</th>
<th>Serology</th>
<th>\textsuperscript{13}C-UBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant Injury</td>
<td>50.0%</td>
<td>40.0%</td>
<td>20.0%</td>
<td>40.0%</td>
<td>57.1%</td>
</tr>
<tr>
<td>Minimal Injury</td>
<td>45.0%</td>
<td>31.3%</td>
<td>26.3%</td>
<td>44.6%</td>
<td>58.8%</td>
</tr>
<tr>
<td>Sample Size (n)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>94</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 3.6: *H. pylori* Prevalence by Diagnostic Technique and Extent of Liver Injury
3.11.9 Alcohol Consumption and Overall H. pylori Prevalence

After first classifying patients by $H. pylori$ status, no significant difference in alcohol consumption between $H. pylori$ positive and negative individuals existed for any parameter of alcohol consumption including daily and total lifetime dose (Table 3.7).

<table>
<thead>
<tr>
<th>$H. pylori$ Status</th>
<th>Age 1st Drink</th>
<th>Age Misuse</th>
<th>Years of Misuse</th>
<th>g ethanol/d</th>
<th>Lifetime dose (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive (n=46)</td>
<td>16 (8-30)</td>
<td>22.0 (8-30)</td>
<td>20.0 (2-43)</td>
<td>200 (48-640)</td>
<td>1402 (175-7008)</td>
</tr>
<tr>
<td>negative (n=54)</td>
<td>16 (8-29)</td>
<td>20.0 (17-55)</td>
<td>14.5 (0-50)</td>
<td>172 (48-800)</td>
<td>1095 (40-7884)</td>
</tr>
</tbody>
</table>

Table 3.7: Alcohol Consumption and $H. pylori$ Status

The $H. pylori$ prevalence by ‘favourite beverage’ consumed at the time of study is summarised in Figure 3.3. No significant difference in $H. pylori$ prevalence by beverage consumed could be determined.
Chapter 3: Diagnosis and Risk Factors for *H. pylori* in Alcohol Misusers

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**Figure 3.3: Preferred Beverage(s) and *H. pylori* Status**

![Graph showing preferred beverages and *H. pylori* status](image)

*2 beverages* = 2 of spirits, beer or wine regularly consumed at time of study.

**3 beverages** = spirits, beer and wine regularly consumed at time of study.

---

### 3.11.10 Age and *H. pylori* Prevalence

The prevalence of *H. pylori* by age and diagnostic technique is presented in Figure 3.4. The *H. pylori* prevalence using the gold standard was 56.3% in those aged 40 years or less. In subsequent decades *H. pylori* prevalence was 40.5% (41-50yr), 52.2% (51-60yr) and 66.6% (61+yr). No significant difference existed either overall, or after comparison between any two decades for *H. pylori* prevalence. When similar comparisons were made using the other diagnostic tests for *H. pylori*, again no significant differences in *H. pylori* prevalence could be detected between decades except for a trend towards significance in comparing *H. pylori* seroprevalence below the age of 40 years (42.0%) with prevalence at ages older than 61 years (62.5%: p=0.075).
The prevalence of *H. pylori*, diagnosed by breath testing, was 60% in each of the three decades for which data were available (4th, 5th and 6th). This had a tendency to significance when compared with the 40.5% *H. pylori* prevalence obtained by gold-standard in the 4th decade (p=0.08), but was not significantly different from the gold-standard prevalence in either the 5th or 6th decades.

In patients aged 40 years or below, the *H. pylori* prevalence obtained with serology and histology was identical at 42.0%. This was not significantly lower than the 56.3% gold-standard *H. pylori* prevalence for the same decade. The 21.0% *H. pylori* prevalence obtained in this decade using urease testing was however significantly lower than that obtained using the gold-standard (p=0.03).

At the opposite extreme of age in patients aged 61 years or above, no significant difference existed in *H. pylori* prevalence between the gold standard (66.6%) and either serology (62.5%) or histology (43.8%). However, *H. pylori* prevalence using urease testing was significantly lower than that obtained with the gold-standard at 31.3% (p=0.04).
Again, using comparisons confined to the extremes of age (<40 and >61yr), \textit{H.pylori} prevalence using urease testing was also significantly lower than that obtained with serology (p=0.05) but not histology.

In the 4\textsuperscript{th} and 5\textsuperscript{th} decades, no significant differences existed between the gold-standard and either serology or histological diagnosis of \textit{H.pylori}. In the 4\textsuperscript{th} decade however, the difference between the \textit{H.pylori} prevalence using the gold-standard (40.5\%) had a trend to be significantly higher than the prevalence using urease testing (17.5\%; p=0.07).

### 3.11.11 Sensitivity and Specificity of Different Diagnostic Tests by Age

The sensitivity and specificity of the different diagnostic tests for \textit{H.pylori} by age is presented in figure 3.5A and B. The sensitivity of \textit{H.pylori} diagnosis using histology ranged from 70.0-88.9\% and showed no significant variation with age. The sensitivity of \textit{H.pylori} diagnosis using serology ranged from 33.3-70.0\%. No significant difference existed in the sensitivity of seroprevalence when comparing any of the decades from the 4\textsuperscript{th} onwards. However, the lower sensitivity of diagnosis using serology of 33.3\% observed in ages below the 4\textsuperscript{th} decade was significant when compared with the sensitivity of diagnosis in all other decades (p<0.05). A similar pattern of sensitivity of diagnosis was seen with urease testing with no significant difference observed in the 4\textsuperscript{th}-6\textsuperscript{th} decades but a significantly lower diagnostic sensitivity seen up to the 4\textsuperscript{th} decade (33.3\%; p<0.05). No significant variation in diagnostic sensitivity for \textit{H.pylori} with age was seen for breath testing for the decades that data were available (4\textsuperscript{th}-6\textsuperscript{th}; Figure 3.5A).

The specificity of histological diagnosis of \textit{H.pylori} was 100\% for all decades. As previously discussed, this reflects the gold-standard definition whereby any patient who is histology positive is classed as \textit{H.pylori} positive regardless of any other test result (Figure 3.5A).

For the three earlier decades studied (<4\textsuperscript{th}, 4\textsuperscript{th} and 5\textsuperscript{th}), no significant difference existed in the specificity of diagnosis of \textit{H.pylori} using serology. In the 6\textsuperscript{th} decade or older however, specificity of serological diagnosis was reduced to 40\%. This was significantly lower than the specificity of diagnosis in the two preceeding decades (p<0.05) and tended to significance for ages younger than the 4\textsuperscript{th} decade (p=0.07) (Figure 3.5B).

No significant variation with age was observed in the specificity of diagnosis of \textit{H.pylori} using urease testing with 100\% specificity of diagnosis achieved at all ages above 41 years. Similarly,
no significant variation with age for the specificity of *H.pylori* diagnosis with age was observed for breath testing within the confines of limited available data (Figure 3.5B).
Figure 3.5(A): Sensitivity of Diagnostic Techniques for *H. pylori* by Age

Figure 3.5(B): Specificity of Diagnostic Techniques for *H. pylori* by Age
3.11.12 Influence of Age and Endoscopy Timing on Diagnostic Tests for *H.pylori*

The prevalence of *H.pylori* as assessed by urease testing is significantly lower than the gold-standard at the extremes of age (<40 and >61 year Section 3.11.10). It is therefore possible to formulate the hypothesis that alcohol consumption was influencing these test results by inhibiting *H.pylori* since the urease test is dependent on both presence and viability of *H.pylori*. While age differences might influence serological test methods due to putative variation in antibody response, the urease test would not be similarly affected due to its reliance on bacterial urease.

To test this hypothesis, the time interval from the patient stopping drinking and having endoscopy was examined: If alcohol was causing inhibition of *H.pylori* in vivo then the earlier the endoscopy and urease test was performed, the more likely that the urease test would produce a false negative result. Patients were first classified into three age groups of ‘young patients’ (<40 years), ‘middle age patients’ (41-60 years) and ‘older patients’ (>61 years) to correspond with the ages in which variations in *H.pylori* prevalence by urease testing were observed. Then comparisons were made between these three groups in terms of endoscopy timing (Table 3.8). Older patients tended to have endoscopy later than either middle aged or younger patients but this difference was not significant.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>All</th>
<th>&lt;40</th>
<th>41-60</th>
<th>61+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>100</td>
<td>25</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Endoscopy Interval median (range) days</td>
<td>6(1-490*)</td>
<td>5.5(1-90)</td>
<td>6(1-490*)</td>
<td>7(1-150*)</td>
</tr>
</tbody>
</table>

*2 patients were entered into the study on the basis of a history and clinical picture of recent alcohol misuse, but later stated that they had not drank alcohol for 150 and 490 days respectively. Median time to endoscopy with these patients excluded 6 (1-90).*

**Table 3.8: Endoscopy Timing and Age**
3.12 Discussion

The population of 100 alcohol misusers in this study were chosen to be as representative of the wider alcohol misusing population as possible. As in general alcohol misusing populations, and the population described in chapter 2 (section 2.3.4), there was an excess of males to females. But, no significant differences existed between the ages of the male and female patients. Similarly, there was no significant difference between men and women in terms of age of onset of drinking, age of misuse or length of overall drinking history. Men did however consume significantly more alcohol/day than women. As a consequence, the derived variable of total lifetime alcohol dose was also significantly higher in men than women. This did however reflect the increased daily consumption in men as opposed to the number of years of misuse as this was non-significantly different between the two sexes.

Following classification by *H. pylori* status, there was no significant difference between *H. pylori* positive and negative patient groups either in terms of age, sex distribution, alcohol history or overall consumption.

The recruitment area for patients around The Royal Free Hospital covered several socially disadvantaged areas as well as Hampstead itself. This area is one of the most affluent and economically privileged in the United Kingdom. But, 37% of the study population were unemployed and 28% performed only manual labour as ‘best-ever’ employment. While these figures are not surprising using anecdotal comparison with the general alcohol misusing population, they are important as they demonstrate that the study was unlikely to have any ‘Hampstead-effect’ bias.

Of the 100 alcohol misusers studied, 83% had minimal liver injury and 17% ‘significant-injury’. No significant difference existed between the two liver disease groups in terms of sex distribution, alcohol history or *H. pylori* prevalence. These findings are not surprising because inevitably some selection bias in patient recruitment had to take place to ensure safety during gastric biopsy. Therefore, patients with good synthetic function were studied by ‘default’ even if it later transpired that their liver biopsy showed cirrhosis.

The overall *H. pylori* prevalence using the ‘gold-standard’ as defined by positive histology or two other positive diagnostic tests was 46.0%. Overall *H. pylori* prevalence using histology alone was
Chapter 3: Diagnosis and Risk Factors for *H. pylori* in Alcohol Misusers

33.0% but this was not significantly different from the gold standard. Similarly, the overall *H. pylori* seroprevalence of 43.6% was not significantly different from that obtained with either the gold-standard or histology alone. It was also similar to the overall *H. pylori* seroprevalence of 35.2% described in chapter 2 for an equivalently aged population of alcohol misusers.

Using urease testing, the overall *H. pylori* prevalence was 25.5%. This lower prevalence was not significantly different from the prevalence using histology alone but was significantly lower than the prevalence using the gold-standard.

In contrast, the 66.7% overall *H. pylori* prevalence using breath testing was significantly higher than the prevalence obtained with histology alone but not significantly higher than the gold-standard *H. pylori* prevalence.

Using the 'gold-standard' for *H. pylori* diagnosis, no significant differences in prevalence either between individual decades or with increasing age could be detected. Of particular note was the *H. pylori* prevalence in ages below 40 years (56.3%) being virtually identical to the prevalence observed in patients over 61 years of age (66.6%). The striking feature of these results is the constant prevalence of *H. pylori* with increasing age. It is the lack of an increase in *H. pylori* prevalence with increasing age that singles out the uniqueness of this patient population of alcohol misusers when compared with the otherwise normal population. Clearly, the most obvious mechanism to account for this difference is the level of alcohol misuse and/or liver injury.

The *H. pylori* prevalence of 60% at all decades for which data were available using breath testing appears to be very high. Indeed, when comparison was made between breath testing and the gold-standard by individual decades, a tendency to significantly higher *H. pylori* prevalence in the 4th decade was observed although this effect was not seen in the older age groups. This result must however be interpreted with extreme caution because breath testing was only performed on 24 patients and selection bias was also highly likely. This is because breath testing had to be performed on an out-patient basis and attendance for testing was generally poor. Inevitably patients who were compliant with detoxification and/or were told that other diagnostic modalities had suggested infection with *H. pylori* were more likely to attend for further testing. Thus, the higher prevalence of *H. pylori* both overall and by individual decade had a very high 'positive selection bias' which helps to explain these results. It is however interesting to note that again the expected increase in *H. pylori* prevalence with increasing age did not occur. So while the actual
prevalence assessed with this method is likely an over-estimate due to selection bias, the lack of an age related increase in \textit{H.pylori} prevalence again supports the observation that the alcohol misusing population is behaving in a unique manner.

Serological diagnosis for \textit{H.pylori} tended to non-significantly underestimate \textit{H.pylori} prevalence when compared with the gold-standard and non-significantly overestimate the true prevalence when compared with histology for all ages. Serological assessment of \textit{H.pylori} prevalence was also the only diagnostic test modality that had a tendency to significance for higher \textit{H.pylori} seroprevalence at older ages when compared with younger ages-a phenomenon not seen with any other test modality. The increasing seroprevalence with age is however not surprising in view of the fact that antibody titres persist after successful eradication of \textit{H.pylori}. Therefore the relative increase in \textit{H.pylori} seroprevalence with age may reflect persistence of antibody titres rather than presence of the bacterium itself.

Histological analysis for \textit{H.pylori} is considered a 'near-gold-standard' for diagnosis because of its non-reliance on bacterial viability or host 'immunological responsiveness'. It is therefore interesting that \textit{H.pylori} prevalence using urease testing was lower than that observed with histology in three out of four decades even if the result did not reach statistical significance. Similarly, \textit{H.pylori} prevalence as assessed by urease testing, was significantly lower at the extremes of age than that obtained with the gold-standard-a combined test with a high bias to histological diagnosis. If alcohol was able to inhibit \textit{H.pylori}, then urease testing might be most affected as an assay method due to its requirement for \textit{H.pylori} to be present \textit{and} viable. It might also be expected to be the assay most affected by alcohol misuse since the number of \textit{H.pylori} tested is relatively small when compared with the 'whole stomach' assay of \textit{H.pylori} used during a breath test.

A significant difference was detected between seroprevalence rates for \textit{H.pylori} and prevalence rates using urease testing. This reflects the tendency of serology to over-estimate prevalence due to persistence of an antibody response after eradication of infection and the tendency for urease testing to under-estimate prevalence due to a putative direct effect of alcohol. This might, in turn, be seen as being analogous to the loss of urease testing sensitivity and specificity seen after recent use of antibiotics or proton pump inhibitors. The high prevalence of \textit{H.pylori} observed with breath testing has already been discussed and is, to some extent, a selection bias phenomenon.
To examine the hypothesis that alcohol might be causing inhibition of \textit{H.pylori} urease and false negative urease tests, the timing of endoscopy in relation to age was examined. No significant relationship could be determined however between the interval before endoscopy and age of the patient. The lower \textit{H.pylori} prevalence as determined by urease testing at the extremes of age can not therefore explained by the recency of drinking in relation to endoscopy and urease testing.

As already discussed the \textit{overall} prevalence of \textit{H.pylori} using histology alone (33.0\%) and serology (43.6\%) was similar to the overall seroprevalence determined for alcohol misusers in chapter 2 (35.2\%). The \textit{constant} prevalence of \textit{H.pylori} using all diagnostic methods with age in this study was similar to the pattern of \textit{seroprevalence} observed in \textit{significant} liver injury described in chapter 2. But, in this study 79\% of the patients had \textit{minimal} liver injury using the same criteria used for classification in chapter 2. Thus, it might have been expected for \textit{H.pylori} prevalence to \textit{decline} with increasing age in the same way that seroprevalence declined in the minimal injury group previously described.

These differences are unlikely to be due to the serological assay itself as the same assay was used in both studies. Similarly, blood samples were processed in a similar manner although samples used for the study in chapter 2 had generally been frozen for longer periods than the samples collected for this study. In addition, this study has shown a close correlation between \textit{H.pylori} seroprevalence and prevalence using other diagnostic methods at least in pattern of distribution even if not in identical magnitude. It is therefore unlikely that the seroprevalence distributions noted in this study and the study in chapter 2 are due to some unidentified failure of \textit{H.pylori} serological assay.

There are, however, a number of differences between the two populations of alcohol misusers in the two studies. In the current study, the median (range) lifetime ethanol dose was 1138 (40-7884) Kg. In the seroprevalence study presented in Chapter 2 however, lifetime alcohol dose was between approximately one third and half this level at 483 (54-5606) Kg for all patients and 525 (109-2847) Kg for patients with minimal injury (See Tables 2.2 and 3.1). Similarly, median daily alcohol consumption in the previous seroprevalence study was approximately 100 (40-500) g/day regardless of liver disease diagnosis. But in the current study, men, who comprised 71\% of the population, consumed approximately double the amount of daily alcohol at 224 (48-800) g ethanol/day (Tables 2.2 and 3.1).
If it is accepted that any methodological differences between the assessment of seroprevalence and extent of liver injury when comparing the current study with the minimal injury group in the previous seroprevalence study are small, then alcohol consumption is the only major difference between the two. It therefore appears that minimal liver injury and more 'modest' alcohol misuse is associated with a declining *H. pylori* prevalence with increasing age as discussed in chapter 2. But, when minimal liver injury is associated with even higher levels of misuse, as in the current study, a change in the prevalence distribution of *H. pylori* is seen with a constant prevalence with increasing age being observed. The increasing level of alcohol misuse appears to cause a change in the minimal liver disease population *H. pylori* prevalence characteristics so that it more closely resembles the 'significant' injury group with a constant *H. pylori* prevalence as discussed in chapter 2. It is interesting to note that in the seroprevalence study described in chapter 2 there was no significant difference in level of alcohol misuse regardless of extent of liver injury with patients having advanced liver disease consuming similar quantities of alcohol as patients with minimal injury. It is therefore possible to hypothesise that a combination of significant liver injury and more 'modest' levels of alcohol misuse accounted for the constant *H. pylori* prevalence described in chapter 2. But, if the liver injury is minimal and the level of misuse more 'modest' *H. pylori* prevalence declines with age again as previously described. But, if as in the current study, minimal liver injury is associated with very high levels of misuse, the *H. pylori* prevalence behaves as if the liver disease is significant with a constant prevalence observed with increasing age.

Indirect evidence for this hypothesis comes from the observation that the minimal liver disease population described in chapter 2 had a 100% *H. pylori* seroprevalence at ages below 40 yr, but the equivalent figure for the same age in this study was only 43.6% (see figure 2.4 and 3.4). If one assumes that the populations are directly comparable, then it could be hypothesised that the lower seroprevalence observed in younger patients in this study is due to their extreme levels of alcohol misuse causing a 'clearing' of the bacterium. If this was the case, then it would follow that the 'constant' *H. pylori* prevalence observed in the current study, at least in the early decades, was due to the prevalence in the early years being reduced due to even more extreme levels of alcohol misuse.

It can therefore be speculated that in younger patients daily alcohol consumption is often very high which in turn tends to suppress *H. pylori*. Evidence for this comes from the low apparent prevalence of *H pylori* in the early decades as detected by urease testing as well as low apparent
sensitivity of this assay in this population. In addition the extreme levels of alcohol misuse seen in this study produced an \textit{H.pylori} seroprevalence, confirmed with the gold-standard, of less than half the seroprevalence of the previous study looking at patients with similar liver injury but more 'modest' levels of misuse. With increasing age, liver disease advances and the patient is unable to drink to the same level that they used to 'enjoy'. The suppressant effect of the daily very high levels of alcohol on the \textit{H.pylori} is less marked. But no increase in prevalence is observed perhaps because of the long-term suppressant effect of lifelong alcohol misuse. Or because the immunological response to the bacterium is 'blunted' due to the development of advancing liver disease and/or as a consequence of the development of atrophic gastritis. The relationship between alcohol misuse and gastric pathology as a potential factor influencing the natural history of \textit{H.pylori} infection will be examined in the next chapter.

\subsection*{3.13 Summary and Conclusions}

This chapter has presented data to support the hypothesis that alcohol misuse changes the natural history of \textit{H.pylori} infection. Instead of the expected increasing prevalence with age, \textit{H.pylori} prevalence remains constant throughout the decades at approximately 46.0\%. This phenomenon is independent of \textit{H.pylori} assay method used with the exception of urease testing. With this assay, especially at the extremes of age, significant under-estimation of \textit{H.pylori} prevalence is observed. A hypothesis is developed suggesting that alcohol misuse may cause direct inhibition of \textit{H.pylori} urease to account for these experimental results. Alternatively, the effect may be mediated by an effect on the gastric mucosa-an effect that will be examined in the next chapter.
4.1 Introduction

The previous chapters have shown that the prevalence of H. pylori in alcohol misusers is unique with an apparently constant prevalence at all ages in contrast to the increasing prevalence with age observed in other populations. This phenomenon is likely to be due to a complex interaction between age, extent of liver injury and alcohol consumption. Evidence was presented, albeit indirect, to suggest that alcohol misuse might be associated with inhibition of H. pylori urease and by implication, bacterial inhibition and/or death. On an intuitive basis, inhibition of H. pylori due to the ethanol contained in alcoholic beverages is attractive in view of the widespread use of ethanol as an antiseptic. But an alternative hypothesis could equally relate to changes in the gastric mucosal environment caused by alcohol damage in turn leading to a secondary effect on H. pylori.

In support of a hypothesis relating to mucosal change due to alcohol in turn causing an effect on H. pylori is the high prevalence of dyspeptic symptoms in alcohol misusers. This is in turn attributed by anecdote to the quantity and quality of beverage consumed. However if alcohol misuse has a suppressant effect on H. pylori, as indirectly suggested in the previous two chapters, then it is possible to envisage that there may be symptom-led changes in alcohol consumption. It could be speculated that the H. pylori infected alcohol misuser may moderate consumption due to unpleasant dyspeptic symptoms only for resurgence of suppressed infection to give back the original or new symptoms. Changes in H. pylori prevalence may therefore reflect changes in symptom-led alcohol consumption throughout life. Central to this concept would be that there are modifications in the gastric and duodenal mucosa as a consequence of alcohol misuse and/or H. pylori infection. Until now, the implicit assumption has been that any putative effect of alcohol misuse on H. pylori is due to a directly toxic effect on the bacterium. However it is equally possible that alcohol related mucosal changes also have an effect on the bacterium and so any putative effect of alcohol on H. pylori may be secondary to mucosal pathology. Similarly, these two mechanisms of direct toxicity of alcohol on H. pylori and alcohol mucosal change are not mutually exclusive and may combine to a greater or lesser extent depending on individual circumstances.
To take this hypothesis further it is clearly essential to examine the changes that occur in the upper gastrointestinal mucosa in relation to *H. pylori*, liver injury and alcohol misuse.

4.2 Gastric Pathology

4.2.1 Gastritis - Historical Overview

Inflammation in the stomach – gastritis - in response to noxious stimuli has been recognised for centuries. Towards the end of the nineteenth century extensive changes in the gastric mucosa following various traumas were described. Magnus reviewed this pathology in the 1930s and dismissed these findings as post-mortem autolysis (Avery-Jones 1952). Although it is ironic that in the same paragraph that Magnus dismissed earlier work as artefact, gastritis is defined as ‘all non-neoplastic lesions of the gastric mucosa in which direct invasion by pathogenic organisms can be eliminated.’ Another dogma similar to ‘no acid no ulcer’ was therefore enshrined until the discovery of *H. pylori* (section 1.6). This too demanded a paradigm-shift in thought regarding concepts of aetiology of gastritis similar to that of peptic ulceration.

In the 1950s, prior to the development of endoscopic biopsy, specimens were obtained either at gastrectomy or post-mortem. Gastritis was classified as either ‘inflammatory’ or ‘non-inflammatory’ (Avery-Jones 1952). The latter was recognised as being associated with pernicious anaemia and it would now be described as autoimmune type gastritis. The inflammatory forms of gastritis were classified as either acute or chronic and described by location as either pyloric region or generalised. Acute gastritis was associated with a neutrophil infiltrate and chronic gastritis with mononuclear cells—a distinction that we recognise today. Similarly, inflammation either in the pylorus, body or both is a feature recognised in modern classifications of gastritis (see below-section 4.2.4).

Gastroenterologists showed little interest in the histology of gastritis prior to the discovery of *H. pylori*. This was due to both a poor understanding of the aetiology and pathogenesis and to poor correlation of the histopathology with symptoms. Moreover treatment was not available (Tytgat 1994). Even with the development of endoscopy, it was apparent that there was little concordance between endoscopic mucosal change and histological abnormality (Cronstedt & Simpson 1973; Myern & Serck-Hanssen 1974)

4.2.2 Gastritis in the *H. pylori* Era

With the discovery of *H. pylori* by Warren and Marshall in 1983 and the demonstration that it was the leading cause of non-autoimmune type gastritis in man, interest in this area was reawakened (Marshall & Warren 1984; Morris *et al.* 1988). Advances and widespread use of endoscopic biopsy techniques allowed further elucidation of the fundamental disease processes
that occur with *H. pylori* associated gastritis. In addition it was becoming increasingly apparent that a uniform terminology and diagnostic technique was needed to ensure maximum consistency and diagnostic accuracy.

### 4.2.3 The Sydney System for the Classification of Gastritis

The Sydney System for the classification of gastritis was developed by an international Working Party at the eponymous World Congress of Gastroenterology in 1990 (Ogihara *et al.* 2000). The two basic divisions are endoscopic and histological. As previously discussed (section 4.2.1) there is little correlation between the endoscopic and histological appearance of gastritis. However it was deemed useful to have systematic classifications of both if ‘prospective correlation between endoscopy and histopathology are ever to become meaningful’ (Misiewicz *et al.* 1994). The basic outline of the Sydney Classification is summarised in Figure 4.1. In 1994 the Modified Sydney System was produced following a further meeting of the international group and reported by Dixon *et al.* (1996). The following is a summary of this work.

#### 4.2.4 Topography and Grading of The Sydney System

Topography, or site of gastritis, is classified as either antral, body or pangastritis. This is an important feature of the Sydney System. Therefore a biopsy from both antrum and body is essential and two from both sites preferred. The additional biopsies have been shown to increase the diagnostic yield for *H. pylori* especially in patients who have been taking proton pump inhibitor and treatments known to suppress *H. pylori* (Hunt 1993; Solcia *et al.* 1989). A fifth gastric biopsy from the incisura angularis is also considered useful, as it is in this area that maximum degrees of atrophy and intestinal metaplasia are most consistently found (Stemmermann 1994). The five biopsy sites for the modified Sydney System are shown in Figure 4.2.

The updated Sydney recommendations state the best sites to assess *H. pylori* associated gastritis. These advocate five biopsy sites: two from the middle antrum (within 2-3 cm from the pylorus on the lesser and greater curves), two from the body (from the lesser curve of the body about 4 cm proximal to the angulus and from the mid portion of greater curve approximately 8 cm from the cardia) and one from the incisura. The rationale of multiple sites is that topography determines outcome in *H. pylori* infection, antral gastritis and high acid output indicative of duodenal ulcer disease, pangastritis with low acid output and *H. pylori* infection, gastric ulcer/carcinoma risk.

Histologically, the modified Sydney System grades gastritis by activity (neutrophils), chronic inflammation (lymphocyte infiltration), *H. pylori* load, presence of atrophy and intestinal
metaplasia by use of a visual analogue scale. The system used is semi-quantitative and the
density of *H. pylori* or severity of inflammation graded as either ‘mild, moderate’ or ‘marked’
(formally ‘severe’). For research purposes, the graded variables can be conveniently assigned a
numerical score as 0 (not present) through to 3 (marked change).
Figure 4.1: The Sydney System

From Tytgat (1994) based on Misiewicz (1991)

Figure 4.2: Biopsy Sites for the Sydney System

One specimen should be obtained from the lesser (A1) and greater curvature (A2) of the antrum, both within 2-3cm from the pylorus; from the lesser curve of the corpus about 4cm proximal to the angulus (B1); from the middle portion of the greater curve of the body, approximately 8cm from the cardia (B2); and one from the incisura angularis (IA).

Based on Dixon et al (1996)
4.2.5 The Sydney System: Histology.

The term *chronic active gastritis* refers to the presence of neutrophil polymorphs on a background of chronic inflammation (Figure 4.3). *Active* refers to the presence of neutrophils, as opposed to other inflammatory cells and it is a graded variable (section 4.2.4). The presence of neutrophils is important, as it is a universal phenomenon in *H. pylori* gastritis. Indeed, the density of the intraepithelial neutrophils has been correlated with the degree of mucosal damage and with the intensity of *H. pylori* infection (Fiocca et al. 1992; Stolte & Eidt 1992). Typical sites for neutrophils in *H. pylori* gastritis include lamina propria, epithelium and within the foveolar lumen. Neutrophil infiltrates are characteristic in *H. pylori* infection and persistence for more than a few days after eradication treatment is nearly always associated with treatment failure (Graham & Genta 1994). In contrast, successful eradication of *H. pylori* is associated with an increase in eosinophils the significance of which is unclear (McGovern et al. 1991). It is interesting to note that other *Helicobacter* species such as *H. heilmannii* are not associated with neutrophil infiltration and is generally associated with chronic gastritis (see below and Figure 4.4).

*Chronic gastritis* is characterised by an increase in mononuclear cells, typically lymphocytes, usually in the lamina propria (Figure 4.5). These changes are a particularly sensitive marker for chronic inflammation because normal individuals have less than five mononuclear cells per high power (X40) field. The changes in chronic inflammation are less specific than those encountered for chronic active gastritis and tend to reflect any cause of chronic gastric inflammation. In the specific case of *H. pylori*, a chronic mononuclear inflammatory infiltrate may persist for up to a year following eradication in contrast to the associated neutrophil infiltrate, which rapidly recedes following effective therapy (Genta et al. 1993; Solcia et al. 1994).

*Glandular atrophy*, loss of specialised glandular cells, reflects severe long lasting chronic inflammation from any cause. If the inflammatory process is autoimmune in nature, then glandular atrophy is typically seen throughout the body and antrum and without any associated intestinal metaplasia. In contrast, chronic inflammation due to *H. pylori* infection is often associated with both glandular atrophy and intestinal metaplasia (Figure 4.6).

*Intestinal metaplasia* can be recognised morphologically by the presence of mucin containing goblet cells (Figure 4.6). Intestinal metaplasia is recognised as a pre-malignant risk factor, some authors would comment that complete type of intestinal metaplasia types carries a higher risk than incomplete types (Rokkas et al. 1991; Tosi et al. 1993). Thus, at least on a simplistic level, it can be seen that chronic inflammation due to any insult results in glandular atrophy and
associated metaplastic change to protect against continuing acid and inflammatory attack. The presence of metaplasia in turn predisposes to the dysplasia-neoplasia sequence resulting in gastric carcinoma.

*Lymphoid follicles* are a characteristic feature of *H.pylori* gastritis and consist of aggregates of B-lymphocytes with germinal centres. Very dense infiltrates are associated, albeit rarely, with the development of mucosa-associated T-cell lymphoma (MALT).

*Chemical or reactive gastritis* is a response to a chemical irritant. It is characterised by *foveolar hyperplasia* or corkscrew glandular epithelial hyperplasia. Other features include oedema, smooth muscle proliferation in the lamina propria and only a minor, if any, increase in inflammatory infiltrate (Figure 4.7). This latter feature is especially useful in distinguishing a chemical irritant damage from that due to *H.pylori*. Chemical gastritis has been associated with bile reflux following gastric surgery, NSAID use and alcohol consumption. But in the majority of cases no specific association can be made (Sobala *et al.* 1990, 1993).

*Acute gastritis* is not routinely seen on biopsy specimens because, as the name implies, it is the acute response to an irritant such as NSAIDs, alcohol or acid in stress-ulceration. Endoscopically erosions throughout the stomach are typical, and histologically, oedema and submucosal congestion predominate. Inflammation, as such, is typically sparse or absent which has led to the preferred term of *acute gastropathy*.

### 4.3 Duodenal Pathology

#### 4.3.1 Duodenal Biopsy Topography and Histology

In proven endoscopic duodenitis it is arguable that biopsy of the duodenum is not necessary as there is both good correlation between endoscopic appearances of moderately severe duodenitis and the histological changes seen (Joffe *et al.* 1978; Elta *et al.* 1987).

Peptic duodenitis and gastric metaplasia (GM) occur in the bulb and are rare in the second part (Leonard *et al.* 1997; Walker & Dixon 1996). However if a biopsy is considered necessary then it should be remembered that duodenitis is a stage of duodenal ulcer disease which may be focal and can be missed on one biopsy only. Two biopsies are therefore the minimal requirement, and if taken from the anterior wall and roof and more than 10mm distally from the pylorus to avoid sampling errors, will also detect gastric metaplasia in 95% of cases (Walker & Dixon 1996).
Histological assessment of duodenitis should include a systematic examination of the biopsy for surface erosions, regeneration, intraepithelial lymphocytes (IELs), neutrophils (IENs) and pathogens. Gastric metaplasia is restricted to the surface epithelium, and is best seen on a periodic acid schiff (PAS) stain. In the lamina propria chronic inflammation is seen as a definitive increase in lymphocytes and any neutrophils are abnormal (Wyatt et al. 1990).

Duodenitis is characterised by acute inflammation with a neutrophil infiltrate in the lamina propria and epithelium (Figure 4.8). With more chronic inflammation a shift towards lymphocytic infiltrates and gastric metaplasia are observed in a way analogous to the changes observed in the gastric mucosa. Similarly, active and chronic inflammation is assessed and can be graded semi-quantitatively as in the stomach. It is, however, more difficult to classify milder forms of duodenitis than gastritis because unlike gastric mucosa, lymphoid tissue is the norm in the duodenum and a mild increase in lymphocytes can be difficult to determine. The histological description of duodenitis also includes surface erosion, regeneration and appraisal of gastric metaplasia, including extent which is assessed semi-quantitatively using a <5%, 5-20% or >20% schema which shows good inter-observer agreement. The presence of gastric metaplasia appears to be essential for the colonisation of the duodenum by *H. pylori*, as without this change the bacteria cannot exist (Wyatt et al. 1990).
Figure 4.3: Chronic Active Gastritis.

\( H. pylori \) seen within glandular mucosa (straight black arrow) with neutrophils forming part of the neutrophil infiltrate of chronic active gastritis (white arrow) X120

Figure 4.4: Helicobacter heilmannii

\( H. heilmannii \) (straight arrow) showing characteristic straighter morphology than \( H. pylori \) and lack of neutrophil inflammatory response.
Figure 4.5: Chronic Gastritis

Note the intense mononuclear cell infiltrate (black arrow), absence of *H. pylori* and intra-mucosal haemorrhage (white arrow) X 120

Figure 4.6: Glandular Atrophy and Intestinal Metaplasia

Note the increased goblet cell mucin (curved arrow) and intestinal type epithelium (straight arrow)
Figure 4.7: Reactive (Chemical) Gastroitis

Note the foveolar hyperplasia (corkscrew-shape) glands (curved arrow), inflammatory response (fat arrow) and smooth muscle hyperplasia (long thin arrow)

Figure 4.8: Duodenitis

Note the neutrophil infiltrate (black arrow) similar to that seen in the gastric mucosa in chronic active gastritis (figure 4.5)
4.4 The Influence of Alcohol on Gastric and Duodenal Pathology

4.4.1 Alcohol and Gastritis

In the updated Sydney System reactive or chemical gastritis is associated with irritants including NSAIDs and alcohol consumption. The histological changes of chemical gastritis are not specific to any particular agent and in many cases no definite risk factor can be identified (section 4.2.5). The problem of assessing any putative role of alcohol consumption in the genesis of gastritis is well illustrated in a study, albeit in the pre H. pylori era, performed by Roberts et al (1972): These workers showed a significant correlation between the severity of gastritis and the degree and persistence of alcohol consumption. Alcohol misusers who were hospitalised had the highest inflammatory scores, then heavy drinkers and finally ordinary dyspeptic patients. However, there was no correlation between symptoms of dyspepsia and severity of observed histological change. The study can be criticised from two viewpoints—only a single site gastric biopsy obtained by Crosby capsule was assessed and the subjects studied were unusual in that the alcohol misusers hospitalised for detoxification apparently had no dyspeptic symptoms and of the control group, 21% consumed alcohol to misuse levels. Nevertheless, it does support an intuitively attractive argument, recognised by the Sydney classification, that alcohol is associated with gastritis in its broadest sense.

Other studies have looked for a definitive association between reactive gastritis and putative irritant factors (Sobala et al. 1990, 1993): A strong association between reactive gastritis and bile reflux in either the normal or post-surgical stomach has been demonstrated. Similarly, an association with NSAID use has also been shown. However, there was only a tendency to significance for an association between reactive gastritis and alcohol use. (Sobala et al. 1990). There are several interpretations of these findings which can be made; one possibility is that the study had inadequate power and the data subjected to a type two error. Another is that alcohol is not as clearly associated with reactive gastritis as was first thought despite the intuitively attractive argument that alcohol is an irritant. A final problem of interpretation is that the alcohol misusers in the study by Sobala et al (1990) consumed 30 units (240g) alcohol per week. While this definition is in excess of UK government guidelines for drinking (<21 units/week for men and <14 units/week women) and so is strictly correct, it is a relatively conservative definition of misuse. For example, it is approximately a third of the weekly median consumption (700g) of the alcohol misusers presented in chapter 2 and a sixth of the weekly median of the alcohol misusers in chapter 3 (1372g). No other data on alcohol consumption or beverage type were provided.
Therefore it is only possible to conclude that there is a tendency to significance between heavier alcohol consumption and reactive gastritis based on these data.

4.4.2 Alcohol and Duodenal Pathology

Very few studies have been undertaken on the relationship between alcohol consumption and duodenal pathology, the focus typically being on the relationship between duodenal ulceration and alcohol consumption (Sections 1.5.4 and 1.10).

Wyatt et al (1990) examined the relationship between gastric metaplasia in the first part of the duodenum (D1) and several putative risk factors in a large series of 471 adults and 47 children: Overall, the prevalence of gastric metaplasia in D1 was 31%. It was commoner in adults than children, in men more than women and in those individuals with lower gastric pH. No association could be determined between D1 gastric metaplasia and cigarette consumption, NSAID use or alcohol consumption. Using logistic regression analysis, D1 gastric metaplasia and gastric \textit{H. pylori} were independent risk factors for the development of active duodenitis. The authors hypothesised that once D1 gastric metaplasia is established, \textit{H. pylori} can colonise this epithelium. This may lead to duodenal inflammation and ultimately to ulcer formation. It is interesting to note that no clear support for an association between alcohol and inflammation, in this case duodenitis, could be established despite the intuitive attraction of this argument.

4.5 Aims

To establish the prevalence of upper gastrointestinal symptomatology, endoscopic appearances and histology in a group of alcohol misusers and to ascertain the relationship between these variables, taking into account the confounding factors of \textit{H. pylori} status and the presence of liver disease.

4.6 Methods

4.6.1 Patient and Control Populations

Patient recruitment and population characteristics of the 100 alcohol misusers used for this study have already been described in sections 3.3 to 3.11. Control subjects consisted of otherwise healthy individuals undergoing upper endoscopy for a variety of reasons but typically for the investigation of dyspepsia (Table 4.1). None had a past or present history of alcohol misuse and were excluded if they regularly consumed more than 30g ethanol/day for men or 20g/day for women. None had taken antibiotics, proton pump inhibitors or H2 antagonists in the previous 4 weeks prior to study.
Chapter 4: *H. pylori* and Upper GI pathology

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<table>
<thead>
<tr>
<th>Sex (M:F)</th>
<th>Age median (range) yr</th>
<th>Current Smoker (n)</th>
<th>Tea-Total* (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31:19</td>
<td>53 (28-77)</td>
<td>26</td>
<td>12</td>
</tr>
</tbody>
</table>

* 12 patients consumed no alcohol. 38 patients consumed no more than 32-160g/week

Table 4.1: Demographic Characteristics of 50 Age Matched Control Subjects.

4.6.2 Endoscopic Biopsy and Assessment

The endoscopic techniques used to obtain upper-gastrointestinal biopsies have been described in section 3.8.1. At endoscopy, paired biopsies from the antrum (A) and body (B) were taken according to the modified Sydney system (Section 4.2.4 and Figure 4.2). In addition, paired biopsies were also taken from the anterior and posterior walls of the first (D1) and second (D2) parts of the duodenum. As previously discussed (section 3.9.1), all samples were assessed by a single histopathologist who had no prior knowledge of any clinical details other than participation in the study.

Gastric biopsies were assessed according to the modified Sydney system (section 4.2.5) and duodenal biopsies as described in section 4.3.1. Following this analysis, each patient was assigned a histological diagnosis according to the Sydney system and an inflammatory score. The scores for both chronic and active inflammation (mild =1, moderate =2, severe = 3) were combined to give an overall inflammatory score for the antrum and body. Congestion of small vessels and oedema in the stomach was also noted if present. The activity score was also calculated separately for antrum and body (mild =1, moderate =2, severe = 3) in a subgroup of 50 patients to be compared with the control group of patients. Similarly, the scores from the first and second parts of the duodenum were also combined to give an overall duodenal inflammatory score.

Patients were classified as either *H.pylori* positive or negative dependent on histological analysis of biopsy samples. The presence of *H.pylori* in any biopsy specimen from any site classed a subject as *H.pylori* positive. Histology was used in preference to the previous gold-standard used in chapter 3 (section 3.10) for several reasons including:
This chapter intended to study primarily histological changes in alcohol misusers in relation to presence or absence of \textit{H. pylori} bacteria.

Chapter 3 did not demonstrate any significant differences in \textit{H. pylori} prevalence when assessed by the gold-standard and compared with histology alone.

Tests for \textit{H. pylori} other than histology were not available for the control patient group so an \textit{H. pylori} prevalence based on the gold-standard could not be determined in this group.

\subsection*{4.6.3 Dyspepsia Assessment}

A standard dyspepsia questionnaire was administered to all patients. The symptoms chosen for assessment have previously been validated by Svedlund \textit{et al} (1988) and include:

- Epigastric pain
- Heartburn
- Acid reflux
- Sucking sensation
- Nausea and vomiting
- Eructation
- Odynophagia
- Dysphagia
- Weight loss

Symptoms were scored as never present (0), occasionally present (1) and frequently present (2). A total numerical value for dyspepsia could therefore be derived for each patient ranging from 0 (asymptomatic) through to 18 (all symptoms at maximum severity). For analysis, a final derived score was obtained with 0 being equivalent to a symptom score total of zero, 1 for a total symptom score of 1-6, 2 for a total score of 6-12 and 3 for a score of 13 or more.

\subsection*{4.6.4 Previous and Current Gastrointestinal Disease}

Information about previous gastrointestinal disease was obtained by patient questioning and notes review where available. Particular emphasis was placed upon obtaining information about previous endoscopy, treatment for \textit{H. pylori} or significant upper gastrointestinal events such as episodes of haematemesis and/or cardiovascular collapse as indicators of potential peptic ulcer disease.

Specific data were collected on risk factors for gastrointestinal disease including current or previous smoking, family history of peptic ulcer disease or caffeine consumption as described in sections 3.6.2-3.11.2.
4.6.5. Presentation of Results and Statistical Analysis

Data from the 100 alcohol misusers were analysed using descriptive statistics to allow comparison between histological abnormalities and symptoms in addition to endoscopic changes and putative risk factors for mucosal change including \textit{H.pylori} status.

Comparisons were then made between a sub-set of 50 of the alcohol-misusing patients with 50 age and sex matched control individuals. Comparisons could then be made between two distinct populations differing only in amounts of alcohol consumed.

4.7 Results: 100 Alcohol Misusers:

4.7.1 Alcohol misuse, Dyspepsia and \textit{H.pylori} Status

In the 100 alcohol-misusing patients, 36\% were asymptomatic (score 0) and 64\% had any (score 1 or above) dyspeptic symptoms. 24\% of all alcohol misusers had severe dyspeptic symptoms (score 3): see Table 4.2.

<table>
<thead>
<tr>
<th>Dyspepsia Score</th>
<th>% Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36 (36)</td>
</tr>
<tr>
<td>1</td>
<td>18 (18)</td>
</tr>
<tr>
<td>2</td>
<td>22 (22)</td>
</tr>
<tr>
<td>3</td>
<td>24 (24)</td>
</tr>
</tbody>
</table>

Table 4.2: Dyspepsia Score in the 100 Alcohol Misusers

Thirty-three alcohol misusers were \textit{H.pylori} positive, median (range) age of 47 (32-77) years. The prevalence of any dyspeptic symptoms was 58.6\% and the mean dyspepsia score was 1.1.

Sixty-seven alcohol misusers were \textit{H.pylori} negative, median (range) age 48 (28-74) years. Within this group, the prevalence of dyspepsia was 66.7\%, with a mean dyspepsia score of 1.5.

None of these differences were significant.

No significant relationship existed between dyspepsia scores and daily alcohol intake, period of abstinence from alcohol any drug usage or individual drug usage (proton pump inhibitor, H2 antagonist, NSAID or antibiotics) or current cigarette smoking either in the group of alcohol misusers as a whole or in the sub-groups defined by \textit{H.pylori} status.
4.7.2 Overall Endoscopic Appearances, Alcohol Misuse, Dyspepsia and *H. pylori* Status

Of the 100 alcohol misusers, 60% had an abnormality at endoscopy in the oesophagus, stomach or duodenum. The remaining 40% had an entirely normal endoscopy. Within the sub-group of 64 alcohol-misusing patients with dyspeptic symptoms, 59.4% (n=38) had an abnormality at endoscopy. Similarly, 61.1% (n=22) of the patients who were asymptomatic also had an abnormality at endoscopy. These differences were not significant.

58.8% (n=20) of the *H. pylori* positive alcohol misusers had dyspepsia symptoms. Of these, 50% (n=10) had an abnormality at endoscopy and 50% had a normal endoscopy. Similarly, in the 41.2% (n=13) *H. pylori* positive patients who were asymptomatic, 53.9% (n=7) had a normal endoscopy and 46.1% (n=6) an abnormality.

In the *H. pylori* negative alcohol misusers, 66.7% (n=44) had dyspepsia. Of these, 63.6% (n=28) had an abnormality at endoscopy. In the 33.3% (n=22) asymptomatic *H. pylori* negative patients, 68.2% (n=15) had an abnormality at endoscopy. These differences were again not significant.

Similarly, no significant difference could be detected between normal and an abnormality at endoscopy when *H. pylori* positive patients were compared with *H. pylori* negative patients after initial classification as dyspepsia or asymptomatic.

4.7.3 Oesophageal Endoscopic Appearances, Alcohol Misuse and *H. pylori* Status

Analysis of endoscopic findings was also performed by region in the upper gastrointestinal tract: 16% of all patients had oesophagitis at endoscopy. No other endoscopic abnormalities were detected including the presence of oesophageal varices. After classification by *H. pylori* status, 17.6% of *H. pylori* positive patients and 15.1% of *H. pylori* negative patients had endoscopic oesophagitis.

4.7.4 Gastric Endoscopic Appearances, Alcohol Misuse and *H. pylori* Status

In the stomach, 45% of all alcohol misusers had a gastric endoscopic abnormality (gastritis at any location, erosions or other pathology). After classification by *H. pylori* status the prevalence of any gastric endoscopic abnormality was 38.2% in the *H. pylori* positive alcohol misusers and 48.5% in *H. pylori* negative misusers. These differences were not significant.

The individual endoscopic gastric diagnoses were also examined in relation to *H. pylori* status in view of the classical association between *H. pylori* colonisation and antral gastritis. Antral gastritis was present in 19% of all alcohol misusers (*H. pylori* positive 17.6%; *H. pylori* negative 19.7%). Body gastritis was present in 1.5% (*H. pylori* positive 0%; *H. pylori* negative 1.5%),
generalised gastritis in 17% (\emph{H. pylori} positive 14.7%; \emph{H. pylori} negative 18.2%) and gastric erosions in 8% (\emph{H. pylori} positive 5.9%; \emph{H. pylori} negative 9.1%). None of these differences were significant.

4.7.5 Duodenal Endoscopic Appearances, Alcohol Misuse and \emph{H. pylori} Status

In the first part of the duodenum, 19% of all alcohol misusers had a duodenal endoscopic abnormality (duodenitis, erosions, ulceration or other). These abnormalities were present in 8.8% of the \emph{H. pylori} positive patients and 24.3% of the \emph{H. pylori} negative alcohol misusers. In the second part of the duodenum, the only abnormality encountered was duodenitis. The overall prevalence of duodenitis was 3%, and interestingly was present in 4.4% of the \emph{H. pylori} negative patients only and in none of the \emph{H. pylori} positive alcohol misusers. None of these endoscopic differences between \emph{H. pylori} positive and negative alcohol misusers was significant.

In the 36% of all alcohol misusers who were asymptomatic, 75% had a normal first part of duodenum (D1) at endoscopy, 22.2% had duodenitis and 2.8% duodenal ulceration. In the second part of the duodenum (D2), 94.4% were normal endoscopically and 5.6% had duodenitis.

In the 64% of all alcohol misusers who had any symptoms, 84.4% had a normal D1 (98.4% normal D2), 14.1% duodenitis (1.6% in D2) and 1.6% duodenal ulceration (none in D2).

No significant difference between endoscopic findings when comparing asymptomatic with any symptoms in alcohol misusers was observed. Similarly, no difference was observed in endoscopic findings after first classifying patients as either \emph{H. pylori} positive or negative. In the whole series of 100 patients, two had duodenal ulceration and these patients were both \emph{H. pylori} positive, but this was not a statistically significant excess over the \emph{H. pylori} negative patients with no ulceration.

4.7.6 Gastric Histology by Site in Alcohol Misusers

Figure 4.9 A and B shows the histological findings in the gastric antrum and body of the 100 alcohol misusers: 13% had normal antral histology and 22% normal body histology. The occurrence of \emph{H. pylori} associated gastritis was 31% in the antrum and 29% in the body. Acute gastritis was equal at 3% in both regions but reactive gastritis was higher in the antrum than body (7% vs 3% N.S.). In contrast intestinal metaplasia (IM) and/or atrophy was commoner in the body than antrum (7% vs 4% N.S.). The commonest histological abnormality was chronic gastritis, which occurred in the antrum in 41% of individuals and 35% in the body. No significant differences between sites for individual histological abnormalities were detected.
The overall concordance between sites, meaning the same histological diagnosis was made for the antrum as body, was 71%. One patient had *H. heilmannii* gastritis.

**Figure 4.9A: Histological Changes in the Gastric Antrum of 100 Alcohol Misusers**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
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<td>Normal</td>
<td>41%</td>
</tr>
<tr>
<td>Acute gastritis</td>
<td>31%</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>4%</td>
</tr>
<tr>
<td>Reactive gastritis</td>
<td>1%</td>
</tr>
<tr>
<td>H. pylori IM/atrophy</td>
<td>13%</td>
</tr>
<tr>
<td>H. heilmannii</td>
<td>3%</td>
</tr>
</tbody>
</table>

**Figure 4.9B: Histological Changes in the Gastric Body of 100 Alcohol Misusers**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>35%</td>
</tr>
<tr>
<td>Acute gastritis</td>
<td>29%</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>7%</td>
</tr>
<tr>
<td>Reactive gastritis</td>
<td>3%</td>
</tr>
<tr>
<td>H. pylori</td>
<td>22%</td>
</tr>
<tr>
<td>IM/atrophy</td>
<td>3%</td>
</tr>
<tr>
<td>H. heilmannii</td>
<td>3%</td>
</tr>
</tbody>
</table>
4.7.7 Gastric Inflammation, Alcohol Misuse and \textit{H. pylori} and Dyspepsia

The mean gastric inflammatory score for the 100 alcohol misusers was 1.07. The mean gastric inflammatory score in the alcohol misusers who were \textit{H. pylori} positive was 2.37 and for \textit{H. pylori} negative alcohol misusers 0.411. This difference was highly significant (p<0.0001).

Following classification of the 100 alcohol misusers into \textit{H. pylori} positive and negative individuals, no relation between inflammatory scores (> median or <median) and either current smoking, dyspepsia score (>median or <median), consumption of less than or more than median daily or lifetime ethanol existed. Although recent usage of antibiotics was associated with a trend towards \textit{H. pylori} negative status (p=0.08), there was no relationship between any individual drug and inflammatory score. Significantly higher inflammatory scores were observed in \textit{H. pylori} negative individuals, abstinent for more than 7 days prior to biopsy when compared with less than 7 days abstinence (p=0.007). A similar significant relationship between inflammation and abstinence was not however observed for either longer periods of abstinence (30 day cut off) or in the \textit{H. pylori} positive group.

Congestion and oedema was noted in 31 patients in body mucosa and 23 patients in antral mucosa (Figure 4.10)
Figure 4.10: Antral and Body Congestion and Oedema

4.7.8 Antral Endoscopy and Histology

In 81% (n=81) of all the alcohol-misusing patients, the antrum was endoscopically normal. In this group, 12.4% (n=10) also had normal histology. In the remaining patients, 34.6% (n=28) had *H. pylori* associated gastritis, 38.3% (n=31) chronic gastritis, 7.4% (n=6) reactive gastritis, 3.7% (n=3) acute gastritis and 1.2% (n=1) had *H. heilmannii* gastritis (Figure 4.11).

In 19% of all alcohol-misusing patients (n=19) endoscopic antral gastritis was diagnosed. Of these 15.8% (n=3) had no detectable abnormality on biopsy and in one patient (5.3%) histology was not available. In the remaining 89.9% (n=15) patients with antral gastritis at endoscopy, histological abnormality was present. This included *H. pylori* associated gastritis in 21.1% (n=3), chronic gastritis in 52.6% (n=10) and reactive gastritis in 5.3% (n=2) (Figure 4.11).

None of the observed differences in histology between endoscopically normal antrum and endoscopic antral gastritis were significant. Similarly, analysis of histological diagnosis after initial endoscopic designation and then by *H. pylori* status did not show any significant difference between groups apart from the expected *H. pylori* difference between positive and negative *H. pylori* patients.
Figure 4.11 Antral Histological Change After Endoscopic Classification

4.7.9 Body Endoscopy and Histology
18% (n=18) of all patients had either body and/or generalised gastritis at endoscopy. Of these, 44.4% (n=8) had normal histology. In the remaining patients with gastritis and an abnormality on histology, 50% had *H. pylori* associated gastritis (n=5), 40% (n=4) chronic gastritis and 10% (n=1) reactive gastritis (see Figure 4.12).

In the 82% (n=82) of patients with a normal gastric body at endoscopy, 18.3% (n=15) had normal histology. In 2.4% (n=2) data was incomplete. In the remaining patients with histological abnormality, 41.5% (n=34) had chronic gastritis, 30.5% (n=25) *H. pylori* as the predominant histological abnormalities. These histological changes are summarised in Figure 4.12.
4.7.10 Gastric Inflammatory Scores and Endoscopic Findings

55% of all patients had a normal gastric appearance at endoscopy. Of these, 50.9% (n=28) had no inflammation using combined inflammatory scores and 45.4% (n=25) had any inflammation. Incomplete data was available for 3.6% (n=2). In the 45% of patients who had any abnormality in the stomach at endoscopy, 68.9% (n=31) had no inflammation and 28.9% (n=13) had any inflammation. Incomplete data was available for 2.2% (n=1). These results were not significant (p=0.08).

33% of all alcohol misusers (n=33) were *H.pylori* positive and of these, 63.6% (n=21) had a normal gastric appearance at endoscopy. In this group of patients, 85.7% (n=18) had any inflammation and 9.5% (n=2) no inflammation. Incomplete data existed for the remaining 4.76% (n=1). In the 38.2% (n=12) of patients who were *H.pylori* positive but who had any abnormality in the stomach at endoscopy, 84.6% had any inflammation and 7.7% (n=1) had no inflammation or incomplete data. These differences were not significant.

67% of all alcohol misusers (n=67) were *H.pylori* negative and of these, 50.7% (n=34) had a normal gastric appearance at endoscopy. In this group of patients, 14.7% (n=5) had any inflammation and 82.3% (n=28) no inflammation. Incomplete data existed for the remaining 2.9% (n=1). In the 48.0% (n=32) of patients who were *H.pylori* negative but who had any...
abnormality in the stomach at endoscopy, 9.3% (n=3) had any inflammation and 90.6% (n=29) had no inflammation. These differences were not significant.

4.7.11 Prevalence of Duodenal Pathology in 100 Unselected Alcohol Misusers
Normal duodenal mucosa in D1 was present in 73.9% and in D2, 71.4% (Figure 4.13 A and B). The prevalence of chronic duodenitis was 22.7% in D1 and 28.6% in D2 (N.S.). No active duodenitis or gastric metaplasia was observed in D2. The prevalence of active duodenitis in the first part of the duodenum was 1.1% and gastric metaplasia 2.3% (N.S.) (Figure 4.13A and B).
Chapter 4: *H. pylori* and Upper GI pathology

Figure 4.13A: D1 Duodenal Pathology in Alcohol Misuse

![D1 pathology chart]

Figure 4.13B: D2 Duodenal Pathology in Alcohol Misuse

![D2 pathology chart]
4.7.12 Duodenal Endoscopy and Histology

For comparisons between endoscopy and histology in the duodenum, the results from D1 and D2 were combined in view of the much higher rates of normal histology previously noted (section 4.7.11). In the 81 alcohol misusers (81%) who had a normal D1 and D2 endoscopy, histological data from both D1 and D2 was not available in 14 patients. 85.1% (n=57) of the patients for whom data was available, had normal endoscopy appearances of D1 and D2 and also normal histology. 19% (n=19) patients had duodenitis at endoscopy. In this group, 31.6% (n=6) had chronic duodenitis on histology and 68.4% (n=13) had normal histology. This confirms the accuracy of endoscopy appearances and subsequent histology in the duodenum.

After classification by *H. pylori* status, 30.7% (n=8) of *H. pylori* positive patients had duodenitis in either D1 or D2 and 31.6% (n=19) of *H. pylori* negative patients had histological gastritis.

4.8 Results: 50 Pairs of Age and Sex Matched Alcohol Misusers with a Control Group

4.8.1 Symptoms, *H. pylori* status and Endoscopic Findings

The indications for endoscopy in the 50 age and sex matched patients are presented in Table 4.3 and the endoscopy findings in Table 4.4.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Patients % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspepsia</td>
<td>62% (31)</td>
</tr>
<tr>
<td>Ulcer follow- up</td>
<td>14% (7)</td>
</tr>
<tr>
<td>Other*</td>
<td>24% (12)</td>
</tr>
</tbody>
</table>

* dysphagia, anemia or melaena

Table 4.3: Indications for Endoscopy in 50 age and sex-matched patients
Chapter 4: *H. pylori* and Upper GI pathology

### Upper GI Region

<table>
<thead>
<tr>
<th>Endoscopic Diagnosis</th>
<th>Total % (n)</th>
<th><em>H. pylori</em> Positive % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oesophagus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>58% (29)</td>
<td>41% (12)</td>
</tr>
<tr>
<td>Hiatus hernia</td>
<td>14% (7)</td>
<td>57% (4)</td>
</tr>
<tr>
<td>Oesophagitis</td>
<td>22% (11)</td>
<td>46% (5)</td>
</tr>
<tr>
<td>Polyp</td>
<td>2% (1)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>ulcer</td>
<td>4% (2)</td>
<td>100% (2)</td>
</tr>
<tr>
<td><strong>Stomach</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>46% (23)</td>
<td>43% (10)</td>
</tr>
<tr>
<td>Antral Gastritis</td>
<td>18% (9)</td>
<td>56% (5)</td>
</tr>
<tr>
<td>Body gastritis</td>
<td>6% (3)</td>
<td>100% (3)</td>
</tr>
<tr>
<td>Generalised gastritis</td>
<td>24% (12)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Gastric erosions</td>
<td>4% (2)</td>
<td>100% (2)</td>
</tr>
<tr>
<td>atrophy</td>
<td>2% (1)</td>
<td>0% (0)</td>
</tr>
<tr>
<td><strong>Duodenum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>74% (37)</td>
<td>46% (17)</td>
</tr>
<tr>
<td>Duodenitis</td>
<td>16% (8)</td>
<td>63% (5)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>10% (5)</td>
<td>80% (4)</td>
</tr>
</tbody>
</table>

Table 4.4: Endoscopy Findings in 50 age and sex matched patients

#### 4.8.2 Gastric Pathology by Site for 50 Matched Pairs

The histological findings from antral and body biopsies for the 50 pairs of age and sex matched patients, are summarised in Figure 4.14. The prevalence of intestinal metaplasia was significantly lower in the antrum of the alcohol misusers when compared with controls (6% alcohol vs 22% control; p=0.021). A lower prevalence of *H. pylori* was observed in the antrum of the alcohol misuse patients when compared with control but the difference was not significant (36% vs 46%; p=0.22). Similarly the prevalence of *H. pylori* in the gastric body was also lower in the alcohol misusers than control but the difference was not significant (32% alcohol vs 42% control; p=0.3). There was no significant difference between the alcohol misusers and control patients for any other histological feature in either the antrum or body of the stomach (See Figure 4.14).
Figure 4.14: Antrum and Body Biopsies in 50 Age and Sex Matched Pairs

*Intestinal metaplasia/atrophy: control vs alcohol misuse p=0.021

The activity (neutrophil score) by gastric site are presented in figure 4.15. Higher activity scores were observed in control patients when compared with alcohol misusers in both antrum and body. When regional scores were combined, the overall activity scores were significantly lower in controls than in alcohol misusers (p=0.0014: Figure 4.16).
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Figure 4.15: Gastric Activity Scores by Region

![Figure 4.15: Gastric Activity Scores by Region](image)

Figure 4.16: Overall Gastritis Activity Scores

![Figure 4.16: Overall Gastritis Activity Scores](image)

Difference gastric activity scores control vs alcohol misuse p=0.00014
4.9 Discussion

4.9.1 Alcohol, *H.pylori* and Dyspepsia.

These results show that dyspepsia occurs in 64% of alcohol misusers and 24% of this group have severe dyspepsia, which is not related to *H.pylori* infection. Dyspepsia is recognised in association with *H.pylori* infection in the otherwise normal population, but, in this alcohol misusing population, only 33% of patients were *H.pylori* positive. Further analysis revealed no association between either prevalence or severity of dyspepsia and *H.pylori* status.

4.9.2 Alcohol and Other Irritants as a Primary Cause of Dyspepsia.

*H.pylori* infection was not an attributable cause of the high prevalence of dyspepsia in alcohol misusers and therefore alternatives need consideration. The next putative irritant is excess alcohol consumption. However there was no relationship between dyspepsia score and either the amount of alcohol consumed or the abstinence period prior to assessment of dyspepsia. The majority of dyspepsia assessments were however, performed acutely on admission and possibly too early to detect any potential beneficial effects of abstinence related to symptoms.

Similarly there was no correlation between dyspepsia scores in the alcohol-misusing patients and other alleged factors in dyspepsia that include smoking and drug usage. This lack of association between putative factors for dyspepsia including alcohol consumption, abstinence period, smoking and drug usage were observed both in the overall population and after analysis by *H.pylori* status.

4.9.3 Alcohol and Gastritis

Overall, combined active and chronic inflammatory histology scores were significantly higher in alcohol misusing patients who were *H.pylori* positive than those without infection. Whilst this may be expected in view of the association between *H.pylori* and chronic active gastritis, it is in variance with the results of the study by Roberts *et al* (1972), previously discussed (section 4.4.1), which suggested that gastric inflammation increased as alcohol consumption also increased. It is however difficult to make a direct comparison between our results and those of Roberts *et al* for several reasons. These include the use of a single biopsy from an unspecified site, lack of *H.pylori* status and difficulty in interpretation of alcohol consumption data in the study by Roberts *et al* (1972).

We are therefore left with the conclusion that although dyspepsia is both common and often severe in alcohol misusing patients, there is no relationship between symptoms and *H.pylori* status in this group. Indeed *H.pylori* was only present in 33% of the alcohol misusers studied.
Chapter 4: H. pylori and Upper GI pathology

Similarly, no association between symptoms and either gastric inflammatory score, alcohol consumption, period of abstinence or drug usage prior to assessment could be determined. This is in spite of a highly significant increase in gastric inflammatory scores observed in H. pylori positive alcohol misusers. It appears therefore that despite increases in inflammation seen in the minority third of alcohol misusers who are also H. pylori positive, no correlation with symptoms exists. An attractive, albeit speculative explanation for this observation, may be that the effects of alcohol misuse on gastric motility have a much more profound effect on dyspeptic symptoms than any contribution from mucosal inflammation whatever the cause (Paton 1998).

4.9.4 Alcohol and Reactive Gastritis
An interesting feature in our data is that reactive or chemical gastritis in alcohol misusers was less prevalent than in the control group of patients in a general gastroenterological clinic referred for endoscopy. This was unexpected as the view of the Sydney System predicts that reactive gastritis is associated with chemical irritants such as alcohol. However, on return to the original literature it has been shown that there is a significant association between reactive gastritis and bile reflux following surgery and NSAID use, but not with alcohol consumption of >30 units (240g)/week (Sobala et al. 1990, 1993). Although the latter individuals are classified as heavy drinkers in the original paper, the majority of the alcohol misusers in this study typically consumed more than 30 units daily and therefore might not be considered strictly comparable. We can therefore conclude that the association between alcohol consumption as a classical exogenous factor and histological [reactive] gastritis may indeed be based more on observation than experimentally determined fact (Roberts 1972).

4.9.5 Alcohol and Intestinal Metaplasia
Alcohol misuse is associated with a significantly lower prevalence of intestinal metaplasia and atrophy in the antrum. No significant difference between alcohol misusers and control was however observed in the gastric body. This observation is difficult to explain. As previously discussed, intestinal metaplasia is part of the inflammation-metaplasia-dysplasia spectrum. It is believed to be the result of constant regeneration of the mucosa in response to noxious damage (Sobala et al. 1993). Since we have not demonstrated a clear association between inflammatory scores and alcohol misuse per se perhaps the lack of relationship with intestinal metaplasia is easier to understand. Speculatively, if alcohol misuse did lead to a reduced prevalence and/or viability of H. pylori, then a source of inflammatory drive in the long term might be reduced. This would in turn be associated with less response to this noxious stimulus and less intestinal metaplasia. Alcohol then has a protective effect against H. pylori damage in the stomach.
4.9.6 Alcohol and Duodenal Pathology

In the duodenum, there was no relationship between duodenal pathology and dyspeptic symptoms in the alcohol misusers. Similarly there was no relationship between \( H. pylori \) status and symptoms of dyspepsia. Analogous to gastric mucosa, there was no significant relationship between dyspepsia scores and duodenal inflammation in either the first or second part of the duodenum. Unlike the gastric mucosa however, the prevalence of normal duodenal histology was high with 81.0% of alcohol misusers having normal D1 histology. In particular, there was no increase in the prevalence of gastric metaplasia in D1 in alcohol misusers.

4.10 Summary and Conclusions

Alcohol misuse is associated with a high prevalence of dyspeptic symptoms, which may be severe. These symptoms do not show any clear relationship with \( H. pylori \) status, endoscopic appearances, gastric pathology or inflammatory scores. Whilst \( H. pylori \) positive alcohol misusers do have higher inflammatory scores than \( H. pylori \) negative alcohol misusers, there is no relationship between \( H. pylori \) status and histological changes in the gastric or duodenal mucosa. In particular, predictions of the Sydney system that alcohol misuse is associated with chemical gastritis were not observed. It is speculated that a cycle of \( H. pylori \) clearing due to alcohol led symptoms may account for this observation.

A trend, but non-significant, towards a lower prevalence of \( H. pylori \) in alcohol misusers when compared with age and sex matched controls was observed. This may demonstrate indirect evidence to support a hypothesis that alcoholic beverage consumption some way interferes with the natural history of \( H. pylori \) infection. Data presented in this chapter argues against a clear relationship between \( H. pylori \), alcohol misuse and mucosal change as a potential mechanism for a lower \( H. pylori \) prevalence in alcohol misusers. We are therefore left with the alternative hypothesis, previously proposed, that alcoholic beverages may influence the natural history of \( H. pylori \) infection by a hitherto undetermined toxic effect. This toxic effect of alcoholic beverages on \( H. pylori \) will be studied in the next chapter.

Since the controls are not truly normal but had dyspepsia, the prevalence of \( H. pylori \) in this group is likely to be higher than the otherwise normal population therefore increasing the likelihood of a significant difference.

The potential of alcoholic beverages to suppress mucosal damage by \( H. pylori \) may explain the reduced prevalence of intestinal metaplasia in active alcohol misusers when compared with controls.
CHAPTER 5: THE EFFECTS OF ALCOHOLIC BEVERAGES ON THE MOTILITY AND SURVIVAL OF HELICOBACTER PYLORI IN VITRO.

5.1 Introduction

5.1.1 Congener Effects of Alcoholic Beverages

A recurring theme in previous chapters has been the possibility that alcohol consumption, especially if taken in excess, alters the natural history of H. pylori infection: In chapter 2 a complex interaction between lifetime alcohol dose, extent of liver injury and H. pylori seroprevalence was examined. In chapter 3, evidence was presented suggesting that diagnostic tests reliant upon viable H. pylori bacteria lose sensitivity and specificity in the context of alcohol misuse. It was suggested that this might, in turn, reflect inhibition of H. pylori due to high levels of alcohol exposure. When comparison of H. pylori prevalence between alcohol misusing patients and controls was made, in the previous chapter, a trend, albeit non-significant, to lower H. pylori prevalence was observed in the alcohol misusing patients. This apparent difference in turn, could not be attributed to any discernible changes in gastric mucosa.

One basic assumption made in this analysis, and in most other published studies, has been that the putative factor influencing H. pylori natural history and hence prevalence of infection, is the ethanol contained in the alcoholic beverages. Intuitively, this appears plausible, in view of the widespread use of ethanol as an antiseptic. Little thought, however, has been given to other components, or congeners, of the beverages consumed; any observed effect of alcoholic beverages on H. pylori may, in fact, be due to a ‘congenler effect’ rather than ethanol per se. Similarly, congeners may exert this effect either independently or additionally to ethanol. If congeners do have independent effects on H. pylori in terms of survival then prevalence rates, at least in alcohol misusers, might reflect the type of beverage habitually consumed.

5.1.2 What are Congeners?

The term ‘congener’ refers to any chemical found in a beverage. Usually, however, the term is restricted to the non-alcohol and non-aqueous components of alcoholic beverages. The majority of work on the chemical nature and biological effects of congeners has concentrated on studies of wine and has been extensively reviewed by Soleas et al (1997). A number of reasons explain why wine is the preferred beverage when studying the biological effects of alcoholic beverages and these include:
5.1.3 The History of Congeners

The importance of wine and vineyards is first recorded in the bible when Noah established a vineyard after surviving the ‘Great Flood’ (Genesis; Chapter IX, Verse 20-24: Figure 5.1).

High concentrations of tartaric acid have been detected in jars found in Hajji Firutz, Tepe, Iran, dating from between 5400 and 5000 BC (McGovern et al 1996). Tartaric acid is found both in grapes and in the resin of the terebinth tree. The latter was widely used in ancient times as a preservative for wine. It is therefore likely to represent the earliest use of an added congener as an anti-bacterial agent (McGovern et al 1996).

In the seventeenth century, wines began to take on a more modern characteristic; the use of sulphur in barrels both as a preservative and to improve quality was widespread (Soleas et al 1997). At the same time, the practice of ‘fortification’ of wine with added alcohol was being developed. Again, the principle reason for adding additional alcohol was to help preserve the wine for long sea voyages with the increased intoxicating potential perceived as a bonus (http://www.taylorreserve.com/about/history). It is now known that increasing alcohol concentration favours the fermenting yeast *Saccharomyces cerevisiae* in preference to other microorganisms (Soleas et al 1997). Integral to the process of ‘fortification’ is the use of ‘barrel-aging’; now appreciated as a method of improving taste, it was originally developed as part of the preserving process. Wine ethanol removes tanins and other congeners from the barrel wood which in turn act as preservatives (http://www.winespectator.com/port).
'And Noah began to be an husband man and he planted a vineyard. And he drank of the wine, and was drunken. And he was uncovered within his tent. And Ham...saw the nakedness of his father and went backwards and covered the nakedness. And Noah awoke from his wine and knew what his younger son had done unto him'.

Genesis Chapter IX; verse 20-24

Photo courtesy of HM Gordon
By the eighteenth century, the potential health benefits of wine congeners was starting to be recognised: In 1721 four condemned criminals were recruited to dig graves during ‘The Plague’ in Marseilles. The grave-diggers were apparently immune to the disease and this was attributed to consumption of *vinaigre des quatre voleurs* (‘four thieves’ vinegar’) - a mixture of red wine and garlic. Still available in France today, it is promoted as demonstrating the health benefits of garlic although the ‘true’ anti-bacterial agent is more likely to be due to the red wine (Block 2000).

5.1.4 The Chemical Constituents of Wine

The principle alcohol in wine is ethanol. Small quantities of methanol can also be detected, although wine has the lowest concentration of all fermented beverages (Gnekow *et al.* 1976; Lee *et al.* 1979). Complex higher alcohols have also been detected in trace amounts in wine and include 1-propanol, 2-methyl-1-propanol; 2-methyl-1-butanol and 3-methyl-1-butanol (Rapp *et al.* 1986).

The acidic components of wine are classified by volatility; acetic acid is the commonest volatile acid and it can be removed by distillation. Non-volatile acids are carboxylic acids and include tartaric, malic, succinic, oxalic, fumaric and citric acids. These ‘non-distillable’ acids are the major contributors to acidity and give wine a typical pH of 3 (Soleas *et al.* 1997; Sheth *et al.* 1988). This puts wine at the more acidic end of the beverage spectrum when compared with beer (pH 3.8), spirits (pH 4.4) or tap-water (pH 6.2). But, in pH terms, wine is similar to sour mix (non-alcoholic lemonade) with a pH of 3.1 and more alkaline than Pepsi™ Cola at pH 2.4 (Sheth *et al.* 1988).

The phenolics are a large and complex group of chemicals found in wine. For the vineyard, phenolics are the most important component to any wine as they are responsible for colour, smell and taste. Indeed, it is only the phenolic component that differentiates between red and white wine on a gross composition basis (Table 5.1). Changes in grape variety and processing technique all produce changes in the quality of wine produced. These changes, in turn, are due to alterations in the phenolic components as assessed by HPLC (Blanco *et al.* 1998).

Chemically phenols are cyclic benzene compounds with one or more hydroxyl groups attached to the ring. Nonflavonoid phenols have a single ring structure whereas flavonoids are comprised of two phenol rings linked by a pyran, or oxygen containing, carbon ring structure. (Figure 5.2)
Chapter 5: Effects of Alcoholic Beverages on *H. pylori* in vitro

The primary nonflavonoids in wine are benzoic acid, benzaldehyde and cinnamic acid. Their overall concentration is however low unless the wine has been aged in oak barrels; under these circumstances, the concentration of hydroxybenzoic acid derivatives increases as these are the primary tanins released from the oak wood (Soleas *et al.* 1997).

The commonest flavonoids in wine are the flavonols and flavan-3-ols (stilbenes) (Figure 5.2). The commonest flavanol in wine is quercetin, whereas the flavan-3-ol group contains several important compounds including flavan-3-ol itself (catechin) and *trans*-3,5,4'-trihydroxystilbene (resveratrol). In red wine, significant amounts of the flavonoid group of anthrocyanins are also present contributing to the characteristic colour. All flavonoids can exist in wine either freely or esterified to sugars as glycosides or to nonflavonoids as acyl derivatives. Polymerisation of individual flavan-3-ol units, with or without pre-esterification, is the principle ‘tanin’ in wine that has not been barrel aged (Soleas *et al.* 1997).

The near ubiquitous occurrence of complex polyphenols in animal and vegetable matter means the majority are detectable in fermented alcoholic beverages. The flavan-3-ols are however found in a narrower range of spermatophytes including peanuts, pine and vines (Soleas *et al.* 1997). Flavan-3-ols are therefore found in wine at concentrations 10-100 times that of any other beverage (Abu-Amsha *et al.* 1996; Sato *et al.* 1997) with the method of production being a critical determinant of final concentration (Celotti *et al.* 1996). This is of some practical importance to the vineyard as the flavan-3-ol, resveratrol (*trans*-3,5,4'-trihydroxystilbene), is believed to be the principle natural ‘mould-resistance’ agent (Celotti *et al.* 1996). Other complex polyphenols are also generally found in higher concentration in wines than other alcoholic beverages (Abu-Amsha *et al.* 1996; Fulcrand *et al.* 1998).

5.1.5 The *in vivo* and *ex vivo* effects of congeners

The flavan-3-ol, resveratrol (*trans*-3,5,4'-trihydroxystilbene), has been proposed as the ‘active’ polyphenol in wine to explain the ‘Mediterranean paradox’ of reduced cardiovascular mortality despite high levels of other risk factors usually associated with increased mortality (Lugasi *et al.* 1997; Rodriguez *et al.* 1996). Resveratrol has been shown to have anti-oxidant properties (Soleas *et al.* 1997) as well as effects on platelet aggregation in *ex vivo* biological systems (Bertelli *et al.* 1995). This apparent anti-platelet aggregation effect of resveratrol has also been tested in healthy volunteers using fruit juices, grape juices and wines. The results are however conflicting; Pace-Asciak (1996) could not demonstrate any differences in *ex vivo* platelet aggregation in healthy volunteers who had been consuming wine, grape juice or resveratrol fortified grape juice. The author concluded that any beneficial effect on platelet
function is due to ethanol in wine rather than congener. In contrast, using a randomised, cross over design and a different *ex-vivo* platelet aggregation assay, Keevil *et al.* (2000) demonstrated a 77% reduction in platelet aggregation after healthy volunteers consumed grape juice but not ‘citrus’ juices. The authors attributed these differences to the three-fold higher polyphenol content of grape juice when compared to citrus juices. The conclusion that platelet function is affected by beverage congener has been further supported by an animal model demonstrating reduced platelet aggregation in wine fed rats when compared with laboratory ethanol fed rats at the same ethanol concentration as wine (Ruf *et al.* 1995). The authors were not, however, able to identify the active congener(s) involved.

5.1.6 The *in vitro* anti-bacterial effects of alcoholic beverages

The importance of wine as an anti-bacterial agent in *vitro* was recognised as early as 1945 (Scherb 1945; Gimel 1948). Masquelier and Jensen (1952) extended these earlier studies and showed that several enteric bacteria including *Salmonella* spp and *Escherichia* spp when cultured *in vitro* at a concentration of 100 bacteria/ml, were killed within 15 minutes of exposure to red Bordeaux wine. The work can be criticised for not testing a simultaneous laboratory ethanol control culture. But, the authors did manage to identify that the active congener(s) was contained in a solvent extractable fraction. This fraction was most likely rich in polyphenols as their organic structure would favour dissolution in solvent.

In 1985 Dickens *et al.* investigated the hypothesis that travelers to developing countries should avoid ice cubes in drinks as the enteric bacteria responsible for food poisoning could survive freezing: First, the enteric bacteria *Shigella flexneri*, *Shigella sonnei*, *Escherichia coli* and *Salmonella typhi* were frozen into conventional ice cubes. The ice cubes were then allowed to melt in several alcoholic and non-alcoholic beverages including tap water, ‘cola,’ tequila, neat and 50% diluted Scotch whiskey. The authors found that all of the test organisms could be partially recovered even after 24 hours freezing and dissolving the ice cubes in neat whisky or tequila. As expected, bacterial survival was less in the higher alcohol content drinks than non-alcoholic drinks. The authors conclude that contaminated water, if used in ice cubes, could be a potential source of enteric bacterial infection and that ‘high proof’ alcoholic drinks did not provide adequate anti-bacterial protection. The authors did not however study either *H. pylori* or wine.

A broadly similar study, that did test wine, was performed by Sheth *et al.* (1988): Again, enteric bacteria were exposed to differing beverages but without prior freezing into ice cubes. The enteric bacteria tested included *Salmonella typhimurium*, *Shigella sonnei* and *Escherichia*...
coli. 50ml aliquots of beverage were inoculated with $1 \times 10^8$ CFU (colony forming units)/ml of bacteria. These were incubated under standard conditions. At timed intervals up to 48 hours, aliquots of beverage exposed bacteria were plated onto agar to assess survival in terms of log counts/ml. The beverages tested included, Pepsi™ cola, 'sour mix', beer, wine (colour not stated), skim milk and tap water. All three bacteria tested, exhibited similar survival curves after beverage exposure; A decline of approximately two log cycles was observed after 24 hours incubation and no viable bacteria could be recovered after 48 hours incubation. These results were obtained with all beverages tested with the striking exception of wine exposed bacteria; following wine incubation, bacterial viability was reduced to zero after four hours incubation. The authors speculate that the reduced survival after wine incubation was due to a combination of the ethanol at 10% and pH. The average pH of the wine incubated broth over 3 days was 3.0, which although lower than the pH of 6.2 for water or 6.8 for milk, was very similar to the pH of either sour mix or cola at 3.1 and 3.2 respectively. It is therefore hard to accept pH as an important factor in determining bacterial survival in vitro unless it is assumed to be synergistic with ethanol. It would not be possible to evaluate this further however because no 'ethanol only' bacterial culture was tested. The authors also did not discuss a possible 'congener-effect' as an alternative explanation for their findings with wine.

The most comprehensive study to date on the survival of enteric organisms in alcoholic beverages was by Weisse et al (1995). The main strengths of this study were the inclusion of both red and white wine as well as a laboratory ethanol control. Eighteen hour cultures of *Escherichia coli*, *Salmonella enteritidis* and *Shigella sonnei* were suspended in sterilised tap water to a density of $10^7$ colony forming units/ml. An aliquot of this suspension was then added to test solution. Beverages tested included Californian white Chardonnay (11% alcohol), Portugese red Table wine (9%), laboratory ethanol and tequila (both diluted to 10% with sterilised water). After timed exposure to beverage, cultures were plated onto agar plates and incubated for 24 hours. Bacterial survival was then assessed as number of colonies per plate.

The most striking feature of the results of Weisse et al (1995) was that laboratory ethanol was equivalent to sterilised tap water in terms of bactericidal activity on all organisms tested. Tequila, reduced survival after 120 minutes incubation, by approximately one log cycle for *E.coli*, two cycles for *S.enteritidis* and four cycles for *S.sonnei*. By contrast, both red and white wine reduced bacterial survival of all species to zero after only 20 minutes incubation. In order to evaluate this wine effect further, serial dilution experiments of red and white wine were performed; wine was diluted by either 1:1 or 1:2 and added to *E.coli* culture. A profound
effect on *E.coli* survival, even after further dilution was still observed, with white wine proving to be more effective than red.

5.1.7 The Congener Hypothesis

The above literature helps support a ‘congener-hypothesis’ to explain the mode of action of alcoholic beverages on biological systems. While not dismissing the role of ethanol *per se*, to account for the various study observations, an important additional role of congener has been emphasised. Whether this congener-effect is independent, independent and additive or independent and synergistic to the effect of ethanol alone is not clear.

Several studies have demonstrated anti-enteric bacterial effects of alcoholic beverages *in vitro*. None, however, studied *H.pylori*. If the findings in enteric bacteria could be repeated with *H.pylori*, then a biologically plausible hypothesis to explain prevalence in terms of beverage consumed could be advanced.
Chapter 5: Effects of Alcoholic Beverages on *H. pylori in vitro*

<table>
<thead>
<tr>
<th>Component</th>
<th>Red wine</th>
<th>White wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Other volatiles</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Extract</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Sugars</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Pectins</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Acids</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Ash</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Phenols</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>Amino acids</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Fats</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamins etc</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5.1: Estimates of Typical Gross Composition (% weight) of Wines
[modified from Singleton (1982)]
Figure 5.2: Chemical Structures of Common Polyphenols in Alcoholic Beverages.

<table>
<thead>
<tr>
<th>General Type</th>
<th>General Structure</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Nonflavonoids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td><img src="image" alt="Benzoic acid" /></td>
<td>benzoic acid</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td><img src="image" alt="Benzaldehyde" /></td>
<td>benzaldehyde</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td><img src="image" alt="Cinnamic acid" /></td>
<td>coumaric acid</td>
</tr>
<tr>
<td>Cinamaldehyde</td>
<td><img src="image" alt="Cinamaldehyde" /></td>
<td>comiferaldehyde</td>
</tr>
<tr>
<td>Tyrosol</td>
<td><img src="image" alt="Tyrosol" /></td>
<td>tyrosol</td>
</tr>
</tbody>
</table>

B) Flavonoids:

- Flavonols
  - Quercetin

- Anthocyanins
  - Cyanin

- Flavan-3-ols
  - Catechin
  - Epicatechin

[From Soleas et al (1997)]
5.2 Aims
To investigate the hypothesis that alcoholic beverages have differential effects on the in vitro motility and survival of H. pylori in vitro due to differences in beverage congeners.

5.3 General Experimental Outline
H. pylori were cultured under strictly controlled conditions and suspended in broth during the logarithmic phase of their growth—a time when maximum motility is observed. Aliquots of laboratory ethanol (Analar®) and of various conventionally and organically produced alcoholic and non-alcoholic beverages were diluted to the same ethanol concentration, where applicable, and then added to separate H. pylori broth culture. Separate aliquots of the beverage or laboratory ethanol-exposed broth suspensions were removed at timed intervals up to 3 hours and used for:

- Assessment of H. pylori motility using a Hobson Bac-Tracker.
- Re-incubation in standard broth for 24 hours in order to assess survival.

Cultures to which no beverage had been added served as controls.

5.3.1 Preparation of H. pylori
H. pylori was prepared using the same protocol for all experiments: The H. pylori was cultured in brain heart infusion broth (BHI) with newborn calf serum and antibiotic supplements of nalidixic acid (20mg/l), amphotericin B (2mg/l) and vancomycin (3mg/l). Stock cultures of H. pylori were stored frozen at -20°C until needed. Prior to use, the cultures were removed from the freezer and thawed at room temperature and then incubated in an atmosphere of 8% CO₂ in air at 37°C for 48 hours. 10μl of this culture, in suspension, was then plated onto agar (see below, section 5.3.6) and incubated for a further 24 hours. Bacteria from this plate culture were then re-inoculated into fresh broth and re-incubated as above. This technique ensures purity of culture of the bacterial suspension when used for motility studies and also ensures that at 24 hours maximum motility is observed (Mandelstam et al 1996). After 24 hours of growth, an aliquot of broth culture was taken and diluted with phosphate citrate buffer at pH 7, to give a final bacterial concentration of 10^6-9 bacteria/ml. The concentration of bacteria was confirmed using a spectrophotometer (Cecil Instruments); 0.4-0.5 optical density at 550 nm UV light corresponds to the intended bacterial concentration.

5.3.2 Motility Assessment and the Hobson BacTracker
The Hobson BacTracker (Hobson Tracking Systems, Sheffield, UK) allows precise and objective measurements of bacterial motility to be made in vitro. The operating principles and
practical use of the Hobson BacTracker have been extensively reviewed by Karim et al (1998) and the following is a summary of their work:

Prior to development of the Hobson BacTracker, all methods of assessing bacterial motility in vitro relied on direct observation. Inevitably these techniques were crude and prone to subjective bias: The earliest technique used involved timing bacterial movements with a stopwatch under direct vision. The distance covered by an individual bacterium is measured using a planimeter grid. Knowing time and distance, a mean velocity can be calculated. With video recordings of bacterial motility, this analysis can be performed more accurately, but it is still semi-quantitative (Munoz et al. 1993).

The Hobson BacTracker, in contrast, allows the movement of multiple bacteria to be tracked simultaneously in real time. The tracker comprises a phase contrast video microscope interfaced to a video recorder and microprocessor (Figure 5.3). The software of the BacTracker allows a maximum of 120 bacteria to be continuously and simultaneously tracked in real time. It does this by first identifying moving parts of the image and separating them from static parts. The moving object can be then have a coloured blob assigned to it. This coloured blob superimposed on the video image of the moving bacteria allows a visual check to be made on the tracking process by the operator. The ‘field of view’ of the video microscope is limited in size to approximately 10 μM². Therefore, although 120 bacteria are initially tracked, the majority move out of this field over the next second and are effectively lost from view. The tracker only records a complete movement event from stationary to stationary again that occurs within its’ field of view. Bacteria that move out of the field of view are not counted. As the majority of tracked bacteria are lost, it can take several minutes to accrue 50 or more complete movement events even when the tracker is able to follow much larger numbers at any given moment. The tracker can be programmed to accrue any number of complete movements, but 100 is both convenient and provides superior statistical power.

5.3.3 Microscopy of H. pylori for the BacTracker

The liquid culture to be observed is drawn into rectangular capillary microslides of internal diameter 100μM (Camlab. VD/5010 050 Cambridge, UK) Direct observation is made under phase contrast microscopy at 40X magnification on a stage slide heater at 37°C (MS100 Linkam Scientific Instruments, Surrey, UK). Both ends of the slide are sealed with vinyl plastic putty. This seals the contents and also prevents an oxygen gradient occurring along the length of the tube from the oxygen rich unsealed end to the oxygen poor sealed end. With both ends sealed, uniform oxygenation is ensured and the H. pylori are distributed uniformly along
the microslide as no one area offers optimal microaerophillic conditions. The microslide is first assessed by direct microscopy to confirm visible moving bacteria before switching over to the digital camera of the Bac-Tracker.

5.3.4 The motility of H. pylori.

H. pylori motility occurs in bursts typically following a curved trajectory. The bacterium then stops its forward progress and 'tumbles' around its axis while only making forward progress by Brownian motion. After re-aligning itself, it starts on a new path by active movement. By this constant and repeating pattern of stopping, tumbling and then re-starting the bacterium is able to follow preferential gradients of oxygen and nutrition needed for its survival. Although a teleological argument, significant survival benefit must be obtained from motility because of the high level of energy expenditure needed (Armitage 1992).

All aspects of H. pylori motility can be measured using the BacTracker and every part of the movement cycle is given a specific designation (Figure 5.4): A stop is defined as when the forward movement of the bacteria in any direction is equal to formalin killed bacteria showing Brownian motion only. A run is the distance between two stops. The tracker can measure both the distance and speed of the run. The speed in a straight line from one stop point to the next is defined as the straight-line velocity. As the typical motility pattern of H. pylori from one stop point to the next follows a curved arc, the speed over this arc, or curvilinear velocity, is a better description of movement than the straight-line velocity. A track is made up of several runs and is the path travelled by the bacterium from point of first detection to it leaving the microscope field. Over the length of the track both curvilinear and straight-line distance and speed can be recorded.

5.3.5 Statistical Analysis of Motility

The software of the Bac-Tracker is able to provide frequency histograms of any motility parameter measured. For H. pylori, curvilinear velocity is of most physiological relevance and interest; the Bac-Tracker presents the frequency of individual run curvilinear velocities as a histogram with 6.3μM/sec intervals (Figure 5.5). These divisions are chosen as curvilinear velocities below 6.3μM/sec represent Brownian motion (Karim et al 1999). The frequency distributions of H. pylori curvilinear velocity under control and experimental conditions were analysed using simple descriptive statistics ('Astute'R add-on statistical package for Microsoft Excel® (1995), University of Leeds U.K.) All curvilinear velocity distributions were found to be non-Gaussian in nature as previously described (Karim et al 1999). The median (range) is therefore the most appropriate measure of central tendency. The non-parametric, Mann-
Whitney U test was used for comparison between control and experimental groups and to determine statistical significance.

5.3.6 Survival Studies.
After timed intervals, 10 μL aliquots of beverage exposed bacterial suspension were inoculated onto plates of Columbia agar (BBL) containing 7% horse blood (TSC, Buckingham, UK) made selective for *H. pylori* growth by using antibiotic supplements of 20mg/l nalidixic acid, 2 mg/l amphotericin B and 3mg/l vancomycin. Plates were incubated at 37°C in the same microaerophillic environment used for cultures using gas packs (Campypak, BBL, Maryland USA) in gas jars (Oxoid, Basingstoke, UK).

Survival was assessed at 24 hours by counting the size and number of colonies present on the plate. A semi-quantitative scoring system was used where 3+ represented maximum confluent growth (equal to control); 2+, less than confluence; 1+, minimal growth and 0+, zero growth.

5.3.7 Ethanol Content of Beverages
Commercially obtained beverages were used for all experiments (Table 5.2). All beverages sold in the UK must, by law, state the percentage of alcohol by volume (%ABV). The absolute amount of alcohol taking into account its specific gravity can then be obtained from the published formula (Ash et al. 1993).

\[
% \text{ ABV} \times 0.78 = \text{g ethanol/100ml}
\]

5.3.8 Calculation of Beverage and Culture Dilutions
Stock solution of broth bacterial culture was added to beverage in an Eppendorf tube. The tube was then immediately inverted to ensure adequate mixing. The final volume (V2) of beverage, bacteria and broth was 1ml. The desired final concentration of ethanol (C2) in broth is predetermined. Knowing the initial concentration of ethanol in the beverage (C1) it is possible to calculate the volume of beverage (V1) needed to produce a final volume of 1ml at the defined final ethanol concentration, from the formula:

\[
C_1 \times V_1 = C_2 \times V_2
\]

Subtracting V1 from V2 (1ml – volume of beverage to be added) gives the volume of bacterial stock solution that needs to be added to the volume of beverage producing a final volume of 1ml at the specified ethanol concentration.
Chapter 5: Effects of Alcoholic Beverages on *H. pylori* in *vitro*

<table>
<thead>
<tr>
<th>Beverage Class</th>
<th>Experimental name</th>
<th>Full Name</th>
<th>ABV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirits</td>
<td>Whiskey</td>
<td>Paddy Old Irish, Cork, Distilleries, Eire</td>
<td>43.0%</td>
</tr>
<tr>
<td>Spirits</td>
<td>Mint</td>
<td>Crem de Menthe, Victor Fauconnier, France</td>
<td>15.0%</td>
</tr>
<tr>
<td>Spirits</td>
<td>Cognac</td>
<td>Cognac Brandy VSOP, France (for Londis PLC)</td>
<td>36.0%</td>
</tr>
<tr>
<td>Spirits</td>
<td>Gin</td>
<td>Gordons, London, UK</td>
<td>37.5%</td>
</tr>
<tr>
<td>Wine</td>
<td>Red-Hardy (conventional)</td>
<td>Hardy’s Stamp, Shiraz-Cabernet, Australia</td>
<td>12.5%</td>
</tr>
<tr>
<td>Wine</td>
<td>White-Hardy (conventional)</td>
<td>Hardy’s Stamp, Chenin-Blanc, Australia</td>
<td>11.5%</td>
</tr>
<tr>
<td>Wine</td>
<td>Rose</td>
<td>Rose d’Anjou, Angivine, France</td>
<td>8.0%</td>
</tr>
<tr>
<td>Wine</td>
<td>Tinto</td>
<td>Encanto Tinto, La Coruna, Spain</td>
<td>12.0%</td>
</tr>
<tr>
<td>Wine</td>
<td>Organic-Red</td>
<td>Volcanic Hills, Kekfrankos, Hungary</td>
<td>12.0%</td>
</tr>
<tr>
<td>Wine</td>
<td>Organic-White</td>
<td>Soave, Agricola Bettili, Italy</td>
<td>11.5%</td>
</tr>
<tr>
<td>Sherry</td>
<td>Sherry</td>
<td>Harvey’s, Bristol Cream, UK</td>
<td>17.5%</td>
</tr>
<tr>
<td>Port</td>
<td>Port</td>
<td>Safeway Finest Port, Portugal (bottled UK)</td>
<td>16.5%</td>
</tr>
<tr>
<td>Rice-Wine</td>
<td>Sake</td>
<td>Choya Sake, Osaka, Japan</td>
<td>13.5%</td>
</tr>
<tr>
<td>Lager</td>
<td>Lager (conventional)</td>
<td>Carlsberg Special Brew, Denmark</td>
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</tr>
<tr>
<td>Lager</td>
<td>Lager (organic)</td>
<td>Bucher Oko Bier, Bavaria, Germany</td>
<td>5.2%</td>
</tr>
<tr>
<td>Beer</td>
<td>Beer (organic)</td>
<td>Organic Best Ale, Samuel Smith, Tadcaster, UK</td>
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</tr>
<tr>
<td>Grape Juice</td>
<td>Grape-red</td>
<td>Red Grape Juice, Safeway PLC, UK</td>
<td>0%</td>
</tr>
<tr>
<td>Grape Juice</td>
<td>Grape-white</td>
<td>White Grape Juice, Safeway PLC, UK</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Alcohol by Volume*

Table 5.2: Experimental Beverages
5.3.9 Calculation of Final Ethanol Concentration

The maximum ethanol concentration that can be tested for all beverages is 0.05g ethanol/ml. If it is assumed, theoretically, that the volume of stock broth culture to be added to beverage is zero, then the ethanol concentration that the *H. pylori* are exposed to is equal to the concentration in neat beverage with no dilution from stock culture. The ethanol concentration of undiluted whiskey, at 40% ABV, is 0.312 g ethanol/ml (40% × 0.78 = 31.2 g ethanol/100 ml). This could be conveniently diluted to approximately one third of the final volume with stock bacterial culture, to produce a final ethanol concentration of 0.1 g ethanol/ml. But, undiluted wine at 8% ABV only has an ethanol content of 0.062 g ethanol/ml (8% × 0.78 = 6.24 g ethanol/100 ml). It would therefore be impossible to produce a solution containing 0.1 g ethanol/ml unless the wine was first ‘fortified’ with exogenous ethanol. Since the *H. pylori* have to be added in a volume of broth, albeit small, 0.05 g ethanol/ml represents a maximum convenient final ethanol concentration achievable with all beverages.

5.3.10 Physiological Relevance of Ethanol Concentrations

A final ethanol concentration of 0.05 g ethanol/ml has physiological relevance if the following assumptions are made:

- Beverage is consumed in the fasting state.
- Gastric juice volume is 20 ml (Greenfield *et al.* 1996; Webster *et al.* 1996).
- Immediate and homogeneous mixing occurs between gastric juice and beverage.

Under these circumstances, a standard measure 75 ml glass of 8% ABV wine will be diluted to a final ethanol concentration of approximately 0.05 g ethanol/ml gastric juice. For stronger wines at 11% ABV the final ethanol concentration achieved in gastric juice using the same assumptions, is 0.07 g ethanol/ml.

Spirits and ‘fortified’ beverages will produce higher intra-gastric ethanol concentrations than wine: Using the same assumptions for gastric juice volume and a standard measure of approximately 30 ml of whiskey at 43% ABV, final intra-gastric ethanol concentration is approximately 0.2 g/ml gastric juice.

For the recidivist alcoholic an estimate of intra-gastric ethanol concentration can be made if it is assumed that 1 L of spirits is consumed in three equal doses of 333 ml during the day. Under these conditions, gastric juice volume provides proportionally less dilution than when smaller volumes are consumed. On these assumptions, final intra-gastric ethanol concentration is approximately 0.3 g/ml gastric juice.
Chapter 5: Effects of Alcoholic Beverages on *H.pylori* in vitro

It can therefore be seen that there is an approximate six-fold variation in intra-gastric ethanol concentration between the 'normal' drinker consuming wine to produce a final intra-gastric ethanol concentration of 0.05 g ethanol/ml, through to the recidivist spirit drinker at 0.3 g ethanol/ml of gastric juice.

Ethanol is absorbed *in vivo* by simple diffusion across the gastrointestinal mucosa with no evidence of any active transport (see section 1.3.1). During this passive movement of ethanol through the gut wall the *H.pylori*, within the mucosa must be exposed to the diffusing ethanol. It is therefore a reasonable assumption that gastric *H.pylori* will be exposed to gastric ethanol concentrations in the range of 0.05-0.3 g ethanol/ml depending on beverage type, volume consumed and drinking pattern. To what extent gastric mucus is able to 'protect' the bacterium against ethanol *in vivo* is unknown but contact with ethanol at 0.05g ethanol/ml would appear to be at the lower end of expectation based on the above discussion.
Chapter 5: Effects of Alcoholic Beverages on *H. pylori in vitro*

Figure 5.3: The Hobson Bac-Tracker

The Hobson Bac-Tracker apparatus consists of a conventional microscope with digital camera attachment (foreground). The microscope slide plate is heated to 37°C using a slide heater. Direct observation of bacterial movement in real time is possible using the right hand monitor screen. This allows confirmation of the computer tracking process as each tracked bacterium has an individual ‘blob’ superimposed on its path by the Bac-Tracker software. The digital images of movement tracks from the microscope camera are processed using a personal computer with Bac-Tracker software. Near instantaneous analysis of movement is possible and displayed on the left hand monitor screen. A typical frequency distribution of bacterial velocity can be seen on this monitor screen.
Figure 5.4: Bacterial Movement Analysis with the Bac-Tracker

The left hand panel (A) shows several trail draws of moving, single, *H. pylori* bacteria as followed by the Hobson Bac-Tracker. Some bacteria remain in the field of view and their movement analysed, whereas others leave the field of view and are not analysed as discussed in the text.

The right hand panel (B) is a diagramatic representation of the movement of a single *H. pylori* bacterium moving from left to right: 3 stops are shown (short black arrow). This is when the bacterium stops, re-orientates and then starts moving in a new direction. A run is the distance between two stops (long arrow). The whole distance covered in the field of view is designated a track (dotted line).

[From Karim *et al* 1999]

Figure 5.5: Frequency Histogram of *H. pylori* Motility *in vitro*

Typical frequency histogram of control *H. pylori* curvilinear velocities as produced by the Hobson Bac-Tracker software. 6.3μM divisions are used as velocities below 6.3μM/sec. are due to Brownian motion.
5.4 Experiment 1

5.4.1 Introduction
A body of literature exists that describes the in vitro motility of *H. pylori* (section 5.3.4). Literature also exists describing the in vitro survival of enteric organisms, excluding *H. pylori*, after incubation in alcoholic beverages (section 5.1.6). To date, however, no studies have investigated the effect of alcoholic beverages on either motility or survival of *H. pylori* in vitro.

In the absence of any previous work in this area, experiment 1 served as a pilot study to identify if any potential interaction between *H. pylori* and alcoholic beverages existed and to help formulate a more formalised protocol for future experiments.

5.4.2 Aim
To examine the in vitro motility of *H. pylori* incubated with Whiskey.

5.4.3 Methods
A 10µl aliquot of stock *H. pylori* (J187 Cag A+) broth prepared for motility assessment as previously described (section 5.3.1), was mixed, in an Eppendorf tube, with an equal volume of neat Whiskey (Table 5.2). The tube was immediately inverted to ensure mixing of broth culture and whiskey to produce a final ethanol concentration in the broth of 0.16g ethanol/ml. For control, 10 µl of stock broth was mixed with an equal volume of phosphate buffered saline. The Eppendorf tubes were incubated at 37°C. After 15 minutes incubation, assessments of bacterial motility were made as described in section 5.3.3. The Bac-Tracker was set to measure 100 curvilinear velocity tracks and to display the results as a frequency histogram with 6.3µM/sec intervals as previously described (section 5.3.5).

5.4.4 Statistical Analysis
Statistical analysis was performed as outlined in section 5.3.5: The frequency distributions of curvilinear velocity for both control and Whiskey exposed bacteria were confirmed to be non-Gaussian using simple descriptive statistics. Results are therefore expressed as median (range) curvilinear velocity. Comparison between distributions, for statistical significance, was performed using the Mann-Whitney U test.

5.4.5 Results
After 15 minutes, the median (range) curvilinear velocity for control broth was 37.67 (18.9-69.3) µM/sec. (Figure 5.6). Following exposure to Whiskey for the same time period, the
median curvilinear velocity was 12.6 (6.3-18.9) μM/sec, or equivalent to 33.5% of the median control value. These differences were highly significant (p<0.0001). After Whiskey exposure, 22% of all measured curvilinear velocities were at 6.3 μM/sec-equal to Brownian motion. The maximum recorded curvilinear velocity after whiskey exposure was 18.9 μM/sec-equivalent to the lowest measured control velocity (Figure 5.6).

After 30 minutes incubation, only curvilinear velocities equivalent to Brownian movement could be detected in the Whiskey exposed culture (<6.3 μM/sec). For control, the observed median curvilinear velocity of 37.33 (18.9-69.3) μM/sec was similar to that observed at 15 minutes. (Differences between 30 minute control vs 15 minute control N.S. and between 30 minutes Whiskey exposure p<0.0001).

**Figure 5.6: H.pylori Curvilinear Velocity in Whiskey at 0.016g ethanol/ml.**

5.4.6 Discussion and Conclusions

Exposure of *H.pylori* broth culture to whiskey at 0.16g ethanol/ml caused a profound reduction in motility within 15 minutes of exposure. By 30 minutes, motility was equivalent to Brownian motion.

A final ethanol concentration of 0.16g/ml for the pilot study was chosen partly for convenience as it is a 50% dilution and also because it is at the upper end of final intra-gastric ethanol concentration obtained under normal consumption conditions using the assumptions
outlined in section 5.3.10. The ethanol concentration is however approximately three times the 0.05g/ml gastric juice achieved in vivo with wine using these same assumptions.

While demonstrating a profound effect on the in vitro motility of H.pylori following Whiskey exposure, a number of further questions arise including:
Is the loss of motility observed temporary or permanent?
Does loss of motility equate with loss of bacterial viability?
What is the effect of other ethanol concentrations on motility and survival?
Is the observed effect due to ethanol per se or a unique effect of a Whiskey congener(s) either working alone or synergistically with ethanol?
Do other alcoholic beverages also contain motility inhibiting congeners?

These questions will be addressed in the next experiments.

5.5 Experiment 2

5.5.1 Introduction
Experiment 1 (section 5.4) demonstrated a profound reduction in H.pylori motility following exposure to Whiskey at an ethanol concentration of 0.16g ethanol/ml. Limitations of this experiment included lack of assessment of bacterial viability and the use of only one test beverage.

5.5.2 Aim
To investigate the effect of different alcoholic beverages, at defined ethanol concentrations, on the motility and survival of H.pylori in vitro.

5.5.3 Methods
A fresh broth culture of identical strain H.pylori (J187 Cag A+) as used in previous experiments, was prepared as outlined in section 5.3.1. An appropriate volume of broth culture was added to beverage in an Eppendorf tube (section 5.3.8). The tube was immediately inverted to ensure adequate mixing and incubated at 37°C. At timed intervals, two aliquots were removed from the beverage-exposed broth: The first, a standard capillary sample for assessment of bacterial motility as described in section 5.3.4 and the second, a 10 μL aliquot to be plated onto agar plates for assessment of bacterial viability. The agar plates were incubated anaerobically, for 48 hours and then scored as described in section 5.3.6.
Chapter 5: Effects of Alcoholic Beverages on \textit{H. pylori} \textit{in vitro}

The Bac-Tracker was set to measure 100 curvilinear velocities and to calculate the median for that beverage and ethanol concentration. Three time points of 15, 30 and 120 minutes exposure were chosen for both ethanol concentrations tested.

During a single experimental session, a maximum of four beverages at two chosen ethanol concentrations, in addition to a control were tested: For the lower ethanol concentration, the four beverages were added in appropriate volumes of broth and clock 1 started. The process was then repeated 5 minutes later for the higher ethanol concentration and the second clock started. Motility assessments have to be performed in sequence. But, with two operators, these measurements can be performed in a matter of minutes. The timing error introduced as a result of this sequential processing is therefore minimal and further reduced by running different ethanol concentrations in a ‘staggered’ sequence. For every experimental session a completely fresh \textit{H. pylori} broth preparation was prepared for ethanol exposure and simultaneous control.

The beverages tested are detailed in Table 5.2 and included Whiskey, Crème de Menthe, Lager and four wines including two varieties of red (Hardy and Tinto), rose and white wine (Hardy). All beverages were tested at two ethanol concentrations of 0.01 and 0.05g ethanol/ml: As previously discussed, (section 5.3.9), 0.05g ethanol/ml is the maximum achievable concentration for all beverages unless exogenous ethanol is added. The ethanol concentration of 0.01g ethanol/ml was an arbitrary choice and using the assumptions in section 5.3.10, is likely to be less than the intra-gastric ethanol concentration that can be expected \textit{in vivo}.

5.5.4 Presentation of Results

Results are expressed as median (range) of curvilinear velocities. For comparison between control and beverage groups the Mann-Whitney U test was used (section 5.3.5). At the higher ethanol concentration tested, significant differences between control and beverage-exposed curvilinear velocity were observed. In this case, median beverage curvilinear velocity was expressed as a percentage of median control curvilinear velocity for the same time point. This automatically corrects for any variation in median control velocity at any particular time point and allows more meaningful comparison between different experimental sessions.

5.5.5 Results

The median (range) of curvilinear velocities for \textit{H. pylori} in control media ranged from 40.0 (18.9-69.3) $\mu$M/sec at 15 minutes to 42.5 (18.9-69.3) $\mu$M/sec at 120 minutes. The equivalent
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Median values for curvilinear velocity following incubation in laboratory ethanol at 0.01 g ethanol/ml were 36.8 (18.9-69.3) μM/sec at 15 minutes and 40.7 (18.9-69.3) μM/sec at 120 minutes (Figure 5.7).

The median curvilinear velocities for the other beverages tested at 0.01 g ethanol/ml are also shown in Figure 5.7: Following exposure to Hardy’s red wine at 0.01 g ethanol/ml, median curvilinear velocity declined from 41.1 μM/sec at 15 minutes to 32.7 μM/sec at 120 minutes. This decline was however non-significant (Mann Whitney U, p>0.05). Similarly, no significant difference in curvilinear velocity between other beverage exposed bacteria at 0.01 g ethanol/ml and control was observed at any time point tested (Figure 5.7).

At 0.01 g ethanol/ml incubation no apparent effect on bacterial survival was observed: Colony counts were equal to control (3+) for all beverages and time points including laboratory ethanol (Table 5.3).

After incubation with laboratory ethanol at 0.05 g ethanol/ml, median curvilinear velocity of *H. pylori* declined from a control value of 40.2 at 15 and 30 minutes to 28.3 at the same time points (Figure 5.8). This represents a decline in motility to 70.4% of control. By 120 minutes incubation, curvilinear velocity had declined further to 11 μM/sec or 25.9% of control values (Figure 5.8). These differences were highly significant (p<0.01).

The two spirits tested at 0.05 g ethanol/ml also produced a stepwise decline in curvilinear velocity with increasing incubation time: By 120 minutes incubation, median curvilinear velocity for *H. pylori* motility was 16.7% of control for Whiskey and 23.8% for Crème de Menthe (Figure 5.9). These differences from control were significant (p<0.001).

After 15 minutes incubation with lager and both red wines, *H. pylori* median curvilinear velocity declined to 20.6% of control. At all subsequent time points however, motility was equivalent to Brownian motion (14.8% of control values). For rose and white wine, a reduction in median curvilinear velocity equivalent to Brownian movement was observed within 15 minutes of beverage exposure (Figure 5.9).

Bacterial survival following beverage exposure at 0.05 g ethanol/ml is shown in Table 5.3: Incubation of *H. pylori* with laboratory ethanol, Whiskey and Crème de Menthe at 0.05 g ethanol/ml, produced no apparent effect on bacterial survival at all time points measured up to 120 minutes. Initially a similar pattern was observed with lager and all wines, with survival...
equal to control up to the 30 minutes time point. By 120 minutes, however, reduced growth was seen in the lager-incubated culture and no viable *H.pylori* could be recovered from any of the wine incubated cultures (Table 5.3)

**Figure 5.7:** *H.pylori* Motility Following Incubation in Beverages at 0.01g ethanol/ml

**Figure 5.8:** *H.pylori* Motility Following Incubation in Beverage at 0.05g ethanol/ml
Figure 5.9: *H. pylori* Motility (% control) Beverage at 0.05g ethanol/ml

<table>
<thead>
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<th>Time of Incubation (min)</th>
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<th>30</th>
<th>120</th>
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<td>3+</td>
<td>3+</td>
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<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Hardy’s red wine</td>
<td>3+</td>
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<td>0+</td>
</tr>
<tr>
<td>Tinto red wine</td>
<td>3+</td>
<td>3+</td>
<td>0+</td>
</tr>
<tr>
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<td>3+</td>
<td>0+</td>
</tr>
<tr>
<td>Hardy’s white wine</td>
<td>3+</td>
<td>3+</td>
<td>0+</td>
</tr>
</tbody>
</table>

3+: normal growth  
2+: reduced growth  
1+: scanty growth  
0+: no growth

Table 5.3: Bacterial Colony Growth Following Incubation at 0.05g ethanol/ml
5.5.6 Discussion and Conclusions

Incubation of \( H. pylori \) with laboratory ethanol and a range of beverages at 0.01g ethanol/ml had no effect either on bacterial motility or viability in vitro. As discussed in section 5.3.10, 0.01g ethanol/ml represents only 20% of the minimum expected in vivo intra-gastric ethanol concentration following ‘normal’ consumption. It is therefore not surprising that this lower ethanol concentration had little effect in vitro since \( H. pylori \) is clearly able to survive in the stomach of individuals who habitually consume ethanol at much higher levels.

At 0.05g ethanol/ml, laboratory ethanol produced a modest reduction in motility but no effect on bacterial viability. Spirits and liqueurs had a more marked effect on motility, but again no effect on viability. Lager and wines at the same ethanol concentration had a profound effect on motility both in terms of absolute decline and speed of onset of this observed effect. A modest loss of viability was observed after incubation with lager for a prolonged period. Over the same time period, however, wine incubation caused loss of all bacterial viability.

A ‘congener-hypothesis’ might help to explain these experimental findings: Ethanol alone causes a modest inhibition of \( H. pylori \) motility in vitro but does not affect viability. With the addition of ‘spirit congeners’ an additive effect at reducing bacterial motility can be achieved. This ‘additive effect’ of congener on motility does not have any apparent effect on viability.

The congeners in lager and wines are more effective at inhibiting bacterial motility than either spirits or laboratory ethanol. Indeed, these beverages appear to be ‘turning-off’ the motility apparatus because after only very short incubation times no motility other than Brownian motion can be detected. The loss of motility is not due to bacterial death because only wines after 120 minutes incubation are able to cause complete loss of bacterial viability. It is therefore possible to conclude that congeners in wine are more effective at inhibiting \( H. pylori \) motility in vitro than either lager or spirits. In addition, wine congeners have a more profound effect on bacterial survival than congeners found in other beverages.

The mechanism by which wine congeners inhibit both \( H. pylori \) in vitro motility and viability may be relatively independent and additive to any ethanol effect or inter-dependent and critically important on ethanol. \( H. pylori \) is known to contain alcohol dehydrogenase (see section 1.8) and although a teleological argument, it has been suggested that the bacterium acquired this enzyme during evolution to help metabolise toxic acetaldehyde. In the presence of ethanol, the reaction would reverse with the production of acetaldehyde and produce potentially damaging effects on motility and survival as acetaldehyde concentrations rose. It could therefore be argued that if \( H. pylori \) is able to metabolise ethanol from one beverage...
differently than from another, this might, in turn, influence motility and survival. Beverage congeners could potentially influence this process by acting either as ‘inhibitors’ or catalysts to the bacterial alcohol dehydrogenase. The profound effects observed with wine congeners might therefore be due to a catalytic effect on *H.pylori* alcohol dehydrogenase causing toxic accumulation of acetaldehyde.

The hypothesis that beverage congener may catalyse metabolism of ethanol to toxic acetaldehyde can be reversed: If congener catalyses a reaction that allows removal, as opposed to accumulation, of toxic chemicals, then ‘non-wine’ beverage congeners may be showing less profound effects on *H.pylori* through this mechanism.

As neither ethanol nor acetaldehyde concentrations were measured at the end of the experiment it is not possible to directly refute either hypothesis for the role of congener in affecting *H.pylori* motility. If however congener alone, without any ethanol being present, was able to produce similar effects to that observed with alcoholic beverage then support would be given to the hypothesis that the congener effect in beverage was additive to any effect observed with ethanol alone. This will be examined in the next experiment.

5.6 Experiment 3

5.6.1 Introduction

Experiment 2 demonstrated differences in *H.pylori* survival and motility following exposure to different beverage at the same ethanol concentration. While demonstrating a ‘congener effect’ in different beverages, it is possible that one of the ‘congeners’ may in fact be a recognised bactericidal agent or biocide used in the production process. In practice, it would be difficult to obtain exact production process methods of all beverages tested especially as these may be commercially sensitive. Organic production methods are however strictly defined: For example, for a beverage to be classed as ‘organic’ conventional spraying with biocides or other agents is not permitted. Other restrictions also apply and the following is a summary of organic wine cultivation practices produced by the Organic Wine Company (http://www.ecowine.com/):

- A US Federal definition of ‘organic wine’ is currently pending.
- An official body must recognise the vineyard as ‘organic’
- No chemical fertiliser, weed killer, insecticide or biocides are allowed.
- The need for cultured yeast is eliminated as the farming practice allows soil-based yeast to survive.
• Physical treatments like filtering are kept to a minimum
• Use of sulphur dioxide as an anti-oxidant preservative is strictly limited in its use. As a consequence, most organic wines are virtually sulphite free.

Similar principles also apply to the production of organic lager and beer although an exact standard and definition, like organic wine, is currently only pending. With organically produced beverages therefore one can be confident that all ‘congeners’ are an integral component of the beverage itself rather than biocides added as part of the production process.

One of the deficiencies of experiment 2 is that although ‘pure’ ethanol was tested, the equivalent ‘pure-congener’ was not. To obtain ‘pure-congener’ would be difficult technically as the distillation process to remove the ethanol from conventional beverage would also tend to remove other potentially important volatile congeners. As a matter of common experience commercially available ‘de-alcoholised’ wines and beers have a different taste to conventional beverage and this must, in part, reflect removal of congeners as well as ethanol during the production process. As already discussed (Section 5.1.4), taste is critically dependent on congener content.

A potential method to test wine congener without ethanol being present, is to use non-alcoholic grape juice. This does not take into account any change that may occur in congeners during the production process, but it is convenient. The equivalent ‘congener-only’ beverage does not however exist for beer and lager.

If an effect on motility and survival of H.pylori can be demonstrated in the absence of any ethanol or potentially added biocide, then our ‘congener-hypothesis’ would be further strengthened.

5.6.2 Aims
To investigate the effect on H.pylori motility and survival in vitro following exposure to: (1) organically produced beverages and (2) non-alcoholic grape juice.

5.6.3 Methods
The same strain of H.pylori (cag A+ J187) was used as in all previous experiments. This eliminated any possibility of inter-strain variability influencing results. H.pylori cultures were prepared as before (Section 5.3.1) and were in the logarithmic phase of growth at the time of assessment. Beverage was diluted in the Eppendorf tube with broth culture as previously
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described to produce a final volume of 1ml and a final ethanol concentration of 0.05g ethanol/ml. For non-alcoholic grape juice, the same volume was used as for the equivalent wine. At timed intervals, a motility assessment was performed and a 10µL aliquot plated onto agar for assessment of bacterial viability as previously described (section 5.3.6).

In any one experimental session up to 5 beverages and one ‘nil-added’ control were assessed. For every experimental session a fresh culture was prepared, and while a new non-alcohol containing control was used for every session, for experimental purposes, laboratory ethanol was classed as a ‘beverage’ and therefore not re-assessed every time.

The Bac-Tracker was set to measure 100 curvilinear velocity tracks and present the data as a histogram. As previously discussed, median curvilinear velocity for the 100 tracks was expressed as a percentage of the median of the 100 control tracks for that experimental session. The median curvilinear velocity for beverage-exposed bacteria was expressed as a percentage of control median velocity at the same time point.

The beverages tested are given in full in Table 5.2 and included:

- Conventional lager.
- Organic lager
- Organic beer
- Conventional red wine
- Conventional white wine
- Organic red wine
- Organic white wine
- Conventional red grape juice
- Conventional white grape juice

5.6.4 Results

Within 15 minutes of exposure to conventional lager, *H. pylori* median curvilinear velocity was reduced to 20.6% of control value. By 30 minutes, median curvilinear velocity was 15.7% of control—a value equal to Brownian motion (Figure 5.10). In contrast, even after 120 minutes incubation, the maximum observed decline in median curvilinear velocity following exposure to either organic lager or beer was 83.3% of control values. Laboratory ethanol produced a decline in curvilinear velocity that was intermediate between organic beers and conventional
lager; median curvilinear velocity was 70.4% of control for the first 30 minutes, declining to 25.9% of control at 120 minutes (Figure 5.10).

Incubation of \textit{H. pylori} with conventional wines reduced median curvilinear velocity at 15 minutes to 20.4% and 16.4% of control for red and white wine respectively. By 30 minutes incubation, median curvilinear velocity was equal to Brownian motion (15.7%) for both conventional wines (Figure 5.11).

Following incubation with organic wine, median curvilinear velocities at 15 minutes were 25.9% and 54.5% of control values for red and white wine respectively. The median curvilinear velocities declined further with increasing length of exposure, so that by 120 minutes, median curvilinear velocities for both organic wines tested was 20.8% of control.

Following 15 minutes incubation with non-alcoholic grape juice, \textit{H. pylori} median curvilinear velocities were 46.5% and 43.8% of control for red and white grape juice respectively. After 120 minutes incubation, motility was similar for both grape juices, with a median curvilinear velocity of approximately 21.9% of control (Figure 5.11).

The \textit{H. pylori} survival after beverage exposure is summarised in Table 5.4. Survival following exposure to all lagers and beer, despite the observed differences in motility, was equal to control for all time points tested. Similarly, incubation with laboratory ethanol and both grape juices had no effect on bacterial viability.

After 120 minutes exposure to conventional red and white wine, no viable bacteria could be recovered. At the same time point, a scanty growth of viable bacteria could be recovered after incubation with organic red wine (1+) and reduced growth (2+) following incubation with organic white wine.
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**Figure 5.10:** Different Beers and *H. pylori* Motility in vitro (0.05g ethanol/ml)

**Figure 5.11:** Wines and Grape Juice on *H. pylori* Motility in vitro (0.05g ethanol/ml)
Chapter 5: Effects of Alcoholic Beverages on *H. pylori in vitro*

<table>
<thead>
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<th>Time of Incubation (min)</th>
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<th>30</th>
<th>120</th>
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<tr>
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<td>Organic white wine</td>
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<td>2+</td>
</tr>
</tbody>
</table>

3+: normal growth  
2+: reduced growth  
1+: scanty growth   
0+: no growth

**Table 5.4: Bacterial Growth Following Beverage Incubation (0.05g ethanol/ml)**

**5.6.5 Discussion**

Organic lager and beer produced a small reduction in curvilinear velocity of *H. pylori in vitro* but no effect on subsequent bacterial viability. The observed reduction in motility was less than that observed with laboratory ethanol alone. An interpretation of this result could be that organic beer and lager provide an additional 'congener' that supports motility and so offsets the inhibitory effects of ethanol alone. This might be a catalyst to the alcohol dehydrogenase enzyme as previously discussed (section 5.5.6) or an additional energy source similar to the 'sugars' utilised by the yeast as part of the brewing process. The striking difference in curvilinear velocity observed between organic beer and lager and conventional lager suggests that the differences in production methods between the two is an important factor in determining *H. pylori* motility. While organic production methods might provide a 'motility-supporting congener', a more plausible explanation for the observed differences is that conventional brewing adds a 'motility-inhibiting congener'. This is likely to be a biocide or preservative agent.
Despite the profound effect on bacterial motility following exposure to conventional lager, no effect on viability was observed. Therefore, the mechanism involved in affecting motility, does not apparently influence viability. It does however suggest that the mechanism of motility inhibition observed with some beverages, most notably conventional lager, is to switch off the motility apparatus of *H. pylori* rather than cause actual bacterial death.

Conventional red and white wines promptly reduced bacterial motility to Brownian motion over a similar time course as conventional lager. The loss of motility observed with conventional wine was, unlike conventional lager, associated with bacterial non-viability. It is therefore possible to speculate that the ‘motility-inhibiting’ congener found in conventional wine may be different from that found in conventional lager. This congener, may work through a different mechanism to that observed with conventional lager; rather that just switching off motility, a more direct cell toxicity may be involved. Another possibility would be a ‘two-hit’ hypothesis whereby ethanol inhibits and weakens the bacteria or a specific sub-population that is in turn further killed by congener.

Organically produced wines also inhibited *H. pylori* motility *in vitro*. The reduction was not however as much as that observed with conventional wines and was less marked with organic white than red wine. Bacterial survival mirrored these changes in motility with organic red wine reducing viability more than organic white wine. These differences are best explained by a ‘congener-hypothesis’ with red wine having different congeners from white wine. As discussed in section 5.1.4, the only significant difference between red and white wine is the polyphenol content. It is therefore possible to speculate that the active congener affecting *H. pylori* motility *in vitro* is contained within the polyphenol fraction.

Another interesting feature of organic wines is that unlike conventional wines, motility and viability are never completely lost even after prolonged exposure up to 120 minutes. An interpretation of this observation could be that organic wine lacks a congener(s) found in non-organic wine. The additional effect on motility and survival seen after exposure to conventional wines, like exposure to non-organic lager, is best explained by additional use of biocides during production.

A good line of evidence to support a congener hypothesis for explaining motility and survival of *H. pylori* comes from the grape juice data: With zero ethanol present, an effect on motility could be observed that was intermediate between organic red and organic white wine. The reduction in motility was more than that observed with laboratory ethanol alone. This helps
support not only a congener hypothesis but also the concept that congener may be more important at inhibiting bacterial motility than ethanol itself.

A criticism of the grape juice data is that the amount added to culture was equal in volume to the equivalent wine. This is an arbitrary amount and could potentially mean, if grape juice is particularly 'congener rich,' that the bacteria were exposed to a relative excess of congener. The other criticism already discussed in section 5.7.1, is that grape juice is not a true 'wine-congener equivalent'.

If congeners are important factors in influencing \textit{H.pylori} motility and survival \textit{in vitro} then it should be possible to observe further differential effects using beverages produced with different production methods. In particular, if grape based congeners are of critical importance in determining motility and survival, as suggested by this experiment, then non-grape based beverages would be expected to produce differing results. This will be further investigated in the next experiment.

\textbf{5.7 Experiment 4}

\textbf{5.7.1 Introduction}

Experiments 2 and 3 demonstrated that \textit{H.pylori} motility and survival after beverage exposure was critically dependent on the non-ethanol congener. If congeners do have a role in influencing motility and survival of \textit{H.pylori in vitro}, then beverages that are particularly 'congener-rich' might be expected to have a more profound effect on the bacterium than other less 'congener-rich' beverages.

Following a similar line of argument, beverages that do not use grapes as a basis for fermentation can be expected to contain different congeners than those found in wines and wine-based beverages. If beverages produced from non-grape sources demonstrate different effects on motility and survival of \textit{H.pylori in vitro} then a 'congener hypothesis' would be further strengthened.

The 'congener rich' beverages are wine based but undergo barrel ageing as part of the production process. The blender plays a critical role in controlling the maturation and the final quality of the beverage is dependent on its relative ethanol to congener content. As previously discussed (section 5.1.3), ethanol extracts tanins from the barrel wood to give the distinctive flavour required.
Spanish sherry is matured in oak barrels that are placed outside to allow a baking process known as the ‘Tressler’ method. The sherry is fortified by the addition of brandy and the practice of topping off older wines with the more recently produced sherry is known as the ‘solaria’ system. As a consequence, no ‘vintage’ sherry exists and the quality is consistent on a year on basis (www.taylorreserve.com/about/history.htm)

Port is also ‘fortified’ but the production method differs from sherry in several ways including:
- The oak barrels used for production are smaller and kept inside under controlled conditions.
- ‘Neutral spirit’ ethanol is used as a fortification agent rather than brandy.
Variations in taste and quality are highly variable and depend on the time spent in the barrel as well as time further maturing in the bottle. Generally, higher quality ports remain in the barrel longer, sometimes for many decades before filtering and bottling. Late bottled vintage port, as the name suggests, is bottled after many years and is immediately ready to drink. Tawny port is matured partly in the barrel and then further in the bottle giving it different taste characteristics.

Cognac, like port and sherry, is wine based. The increased ethanol content of Cognac is achieved by distillation in contrast to ethanol addition in the ‘fortified’ beverages. The distillation process produces a beverage with different qualitative properties than either wine or fortified wine and this must, in turn, relate to a difference in congener content.

All beverages discussed above, while being different in production methods are still based on grapes as the essential raw material. In contrast, Gin uses cotton berries and sake uses rice as a basic fermenting medium. These fundamentally different raw materials can be expected to produce beverages with different congeners.

5.7.2 Aim
To investigate if alcoholic beverages produced by different methods and containing different congeners influence motility and survival of \( H. pylori \) in vitro.

5.7.3 Methods
The same strain of \( H. pylori \) (cag A+ J187) as used in previous experiments was used to eliminate any potential ‘strain-variation’ effect. The \( H. pylori \) were cultured as previously described (section 5.3.1) until in the logarithmic part of the growth curve and suitable for  

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motility studies. Beverage was diluted in the Eppendorf tube with broth culture as in experiments 2 and 3 to produce a final volume of 1ml at an ethanol concentration of 0.05g ethanol/ml. At timed intervals, a motility assessment was performed and a 10µL aliquot plated onto agar for assessment of bacterial viability as previously described (section 5.3.6). Up to 5 beverages and one ‘nil-added’ control was assessed in any one experimental session. A fresh broth was used for every experimental session.

The Bac-Tracker was set to measure 100 curvilinear velocity tracks and present the data as a histogram. The median curvilinear velocity for the 100 tracks was expressed as a percentage of the median of the 100 control tracks for that experimental session.

The beverages tested are detailed in Table 5.2 and included sherry, port, cognac, gin and sake.

5.7.4 Results

Both sherry and cognac reduced *H. pylori* motility to Brownian motion within 120 minutes of incubation (Figure 5.12). There was also a marked reduction in median curvilinear velocity at the first 30 minutes time point of 62.5% of control for cognac and 37.5% for sherry.

After incubation with port, there was a reduction of median curvilinear velocity to 83.0% of control at 30 minutes. At 120 minutes, unlike sherry and cognac, motility was still observed at 47.5% of control values. By 180 minutes motility could still be detected and was 34.8% of control.

The effect on *H. pylori* motility following exposure to the non-grape based beverages was less marked than that seen with cognac and fortified wines: A decline in median curvilinear velocity was observed from 95.5% of control at 30 minutes to 43.5% of control at 180 minutes for sake. Similar values for gin were 75.5% and 53.0% respectively (Figure 5.12).

Only incubation with sherry caused a reduction in bacterial survival with 1+ survival after 120 minutes exposure and no viable bacteria recoverable at 180 minutes (Table 5.5). For all other beverages tested, bacterial survival was equal to control values at all time points.
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Figure 5.12: Different Production Methods and *H. pylori* Motility in vitro (0.05g ethanol/ml)

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<tr>
<td>Cognac</td>
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<tr>
<td>Gin</td>
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</tr>
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<td>Sake</td>
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</tbody>
</table>

3+: normal growth
2+: reduced growth
1+: scanty growth
0+: no growth

Table 5.5: Bacterial Growth with Different Production Method Beverages (0.05g ethanol/ml)
5.7.5 Discussion

Sherry and cognac produced a broadly similar inhibition of \textit{H.pylori} motility \textit{in vitro}, with sherry having a more marked initial onset of motility inhibition. This result might be anticipated as the two beverages are closely related with cognac being used to ‘fortify’ sherry. But, sherry caused loss of motility with quicker onset than cognac and also lead to loss of bacterial viability—a phenomenon not seen with cognac. The effect of sherry on \textit{H.pylori} motility and viability is similar to the effects observed with wine and grape juice observed in experiment 3. It is therefore interesting to note that sherry is ‘closest’ to the original wine used in its production. As already discussed ‘vintage’ sherry does not exist, and so the wine and grape content is fairly ‘young.’ In contrast, cognac is both distilled and ‘matured’ for long periods with inevitably a change in congener composition.

The theory that inhibiting congeners may be lost with the maturing process is further supported by the results obtained with port: This beverage is essentially red wine to which ethanol has been added in the form of ‘neutral spirit.’ If it is assumed that ‘neutral spirit’ is similar in purity to laboratory ethanol, then port might be expected to have effects on motility and survival similar to red wine. In fact the observed effects on motility and survival were less than that observed with red wine in previous experiments or sherry in this experiment. This could be interpreted as the addition of ‘neutral spirit ethanol’ dilutes the original wine congeners making the beverage ‘ethanol-rich’ but ‘congener-depleted.’ With the experiments controlling for ethanol concentration, rather than ‘congener-concentration’ a relative loss of congener when compared to other beverages could be expected with ethanol ‘fortified’ wines. Since ‘total-congener’ concentration was not measured it is not possible to prove this hypothesis either way.

With the non-grape based spirits of sake and gin, declines in motility were less than that observed with grape-based beverages. In addition no effect on bacterial survival was observed. This gives indirect support to the previous observation that it is the congeners in grape based beverages with or without ethanol that produce the most profound effect on \textit{H.pylori} \textit{in vitro}. It is interesting to note that rice based sake had the least effect on \textit{H.pylori} \textit{in vitro} of all beverages tested. With the apparent lack of ‘effective-congeners’ one can not resist citing the claim that sake rarely produces hangover if taken in excess, presumably due to its’ low congener content (http:/www.sake.com/sakeprod/).

This experiment has shown that the closer beverages are in production to grape based wine the more profound the effect on \textit{H.pylori} motility and survival \textit{in vitro}. Some discrepancy has however arisen between effect on motility and survival with both sherry and cognac
producing profound effects on bacterial motility but only sherry affecting survival. It is possible that sherry contains both a motility inhibiting congener and a survival inhibiting congener not found in cognac or port. Alternatively, the relatively crude assessment of bacterial survival could be missing subtle difference in survival between beverages. This will be investigated in the next experiment.

5.8 Experiment 5

5.8.1 Introduction
Experiments 2-4 provided evidence that congeners, especially of grape origin, were important in influencing \textit{H. pylori} motility \textit{in vitro}. A correlation between loss of motility and subsequent bacterial survival was however not always seen with all beverages tested. This could be explained by the hypothesis that motility and viability are affected through different mechanisms. Thus, differences in beverage congener would be expected to have differential effects on these two separate mechanisms. An alternative explanation could however be methodological; all previous experiments have relied on colony counting as a means of assessing bacterial viability following beverage exposure. While convenient, this method is semi-quantitative and subjective. Subtle differences in bacterial survival could as a consequence be potentially missed.

5.8.2 Aim
To assess \textit{H. pylori} survival quantitatively following exposure to different alcoholic beverages used in previous experiments.

5.8.3 Methods
The same strain of \textit{H. pylori} (J187 cagA+) as used in previous experiments was used for quantitative survival studies. Broth culture, in the logarithmic phase of growth as previously described (section 5.3.1), was mixed with alcoholic beverage in an Eppendorf tube. The final ethanol concentration was 0.05g ethanol/ml in a final volume of 1ml.

At timed intervals, a 10μL aliquot of alcohol exposed bacteria was transferred to an Eppendorf tube containing 1ml of broth. This effectively stops further beverage exposure. A 10μL aliquot of this new culture was then transferred to fresh broth culture and incubated for 48 hours anaerobically as described in section 5.3.6. A sample of this broth was then assessed for bacterial numbers by direct microscopy and plasmacytometer grid. From these counts an estimate of bacterial numbers/ml could be estimated.
Results are expressed as absolute bacteria/ml and also as a percentage of control at the same time point.

5.8.4 Results

Bacterial counts following beverage exposure at 0.05 g ethanol/ml are shown in Figures 5.13-5.15. The doubling time for *H.pylori* culture under control conditions was approximately 30 minutes—a phenomenon observed at each time point of study. After ethanol incubation, bacterial counts initially increased in a similar pattern to control (Figure 5.13). But, the actual increase in bacterial numbers was only 47.4% of that observed for control at 45 minutes and 78.3% of control at 60 minutes incubation. By 120 minutes, ethanol incubated *H.pylori*, had declined to 21.3% of the equivalent control value (Figure 5.15).

Following incubation with the non-grape based beverages of lager, whiskey and gin, a broadly similar pattern of bacterial survival to laboratory ethanol was observed (Figures 5.13 and 5.15): Again, bacterial numbers increased up to the 60 minute time-point albeit to lesser values than either control or ethanol incubated bacteria. By 120 minutes incubation, bacterial numbers had declined to 3.6-5.4% of control values depending on beverage (Figure 5.15).

Figure 5.14 shows bacterial survival data following incubation with grape based beverages including grape juice, cognac, sherry and wines. Note how the actual bacterial counts are orders of magnitude less than the values observed with other beverages. With grape juices, rather than a doubling of bacterial counts at each time point, a decline was observed so that by 120 minutes incubation bacterial survival was 3% of control for red grape juice and 0.3% for white grape juice (Figure 5.15). Cognac demonstrated a similar pattern of survival; only 18.3% of control values were recoverable at 60 minutes and 5.4% at 120 minutes. Sherry demonstrated a 6.6% of control viability at 30 minutes incubation, but at time points beyond this, viability was less than 1% of control (Figure 5.15).

The effects following incubation with wine were again striking; within 30 minutes incubation bacterial counts were $0.08 \times 10^5$/ml with red wine and $0.038 \times 10^5$/ml with white (Figure 5.14). These values are equivalent to 0.2 and 0.1% of control values respectively (Figure 5.15). After 120 minutes exposure only 100 bacteria/ml could be recovered from white wine and 20 bacteria/ml from red wine. These values are approximately five orders of magnitude less than control for the same time point, four orders of magnitude less than laboratory ethanol and 2-3 orders of magnitude less than spirits or the equivalent grape juice.
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**Figure 5.13: Bacterial Counts After Non-Grape Beverage (0.05g ethanol/ml)**

![Bar chart showing bacterial counts after non-grape beverages.](chart1)

**Figure 5.14: Bacterial Counts After Grape Beverage (0.05g ethanol/ml)**

![Bar chart showing bacterial counts after grape beverages.](chart2)
Chapter 5: Effects of Alcoholic Beverages on *H. pylori* *in vitro*

Figure 5.15: Bacterial Counts After Beverage Exposure (%control: 0.05g ethanol/ml)

5.8.5 Discussion

Control *H. pylori* in broth culture grows exponentially under these experimental conditions with a doubling time of approximately 30 minutes. After incubation with ethanol, the bacterial population is still able to increase for the first 60 minutes but at a slower rate and with fewer viable bacteria than observed with control. By 120 minutes incubation, bacterial numbers have declined, in contrast to the increase observed in control. A possible explanation for this result is that ethanol is selecting a particular population of *H. pylori* which can withstand ethanol incubation. This however, comes at a metabolic cost, so that by 120 minutes decline in bacterial numbers is observed. It is tempting to speculate that this ‘ethanol-resistant’ population has high levels of alcohol dehydrogenase and so is able to metabolise ethanol but at the expense of eventual acetaldehyde accumulation and cell toxicity.

The striking effect seen on *H. pylori* viability following incubation with wines is strong evidence to support the ‘congener-hypothesis’ as the ethanol concentration was the same as for ethanol alone and yet virtually no viable *H. pylori* could be recovered. As previously discussed (sections 5.7.1 and 5.7.5), sherry is very close in characteristic to wine. It is therefore not surprising that it too causes profound loss of *H. pylori* viability as it is basically a blend of wines of various ages.
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The observation that grape juice caused loss of *H.pylori* viability *in vitro* that was intermediate between laboratory ethanol and wine is again evidence to support a ‘congener-hypothesis’ for mode of action of beverages on *H.pylori*. It is however not possible to combine grape-juice and laboratory ethanol data together to estimate the relative contribution of each to affecting bacterial viability in wine for the reasons discussed in section 5.6.1 and 5.6.5.

The results of bacterial survival with lager suggest that beverage is selecting two distinct populations of *H.pylori*. When first incubated, a population of bacteria is killed by beverage that then allows a sub-population to grow rapidly. This rapid growth initially even exceeds control until metabolic toxicity or deficiency occurs and the population rapidly declines. A similar explanation can be forwarded to explain the ‘boom and bust’ following whiskey exposure.

Incubation with gin allows the *H.pylori* population to initially grow faster than control, implying that gin congeners are able to ‘offset’ the deleterious effects of ethanol. The rapid increase in bacterial numbers again causes the population to rapidly exceed its metabolic resources and subsequently collapse.

5.9 Experiment 6

5.9.1 Introduction

All previous experiments used the same strain of *H.pylori* (J187cagA+) prepared in an identical fashion for each experiment as outlined in section 5.3.1. While ensuring consistency from one experiment to the next, it raises the possibility that the observed effects with different beverages may be a unique effect of the *H.pylori* strain tested rather than a more ‘general’ effect. It was therefore necessary to test several different strains of *H.pylori*, incubated in beverage to determine if any inter-strain variability existed.

5.9.2 Aim

To determine if changes observed in the *in vitro* motility of *H.pylori* following alcoholic beverage incubation, are strain specific or a more general phenomenon.

5.9.3 Methods

Seven strains of *H.pylori* were chosen for testing. All were CagA+ and had been isolated from both clinical isolates and reference cultures. Each was given a laboratory number code and prepared identically as outlined in section 5.3.1. Each strain was tested against a
representative panel of beverages including laboratory ethanol, Whiskey, lager, rose, red and white wines. In a single experimental session of two hours, a single strain of *H. pylori* was tested against all beverages at an ethanol concentration of 0.05g ethanol/ml as well as control. The experiments were conducted identically to experiment 2 with the exception of an aliquot taken for survival plating.

The Bac-Tracker was set to record 100 curvilinear velocity tracks and express the result as median velocity. This in turn was expressed as a percentage of simultaneous control of the same strain, median velocity for the same time point as discussed in section 5.6.4. The percentage median curvilinear velocities for each strain, for each beverage, were then combined to give a mean % curvilinear velocity (+/-2SEM).

5.9.4 Results

The mean curvilinear velocities for the seven strains tested are shown in Figure 5.16. Mean curvilinear velocity decreased with increasing length of exposure to ethanol so that by 120 minutes incubation mean (+/-2SEM) curvilinear velocity was 22.48 (+/-1.88) % of control. Whiskey also showed a decline in mean curvilinear velocity for all strains tested with a decline to 23.6 (+/-1.67) % of control.

Figure 5.16: Curvilinear Velocity for 7 Strains of *H pylori in vitro* (0.05g ethanol/ml)

Results expressed as mean of 7 strains +/- 2XSEM
Chapter 5: Effects of Alcoholic Beverages on \textit{H.pylori} in vitro

5.9.5 Conclusions
The effects on curvilinear velocity of \textit{H.pylori} in vitro following beverage exposure are similar, regardless of strain of bacteria used, under the experimental conditions used.

5.10 Summary and Conclusions
In this chapter, experimental work has been presented that demonstrates differential effects of alcoholic beverages on the motility and survival of \textit{H.pylori} in vitro. In all experiments, the beverage ethanol concentration was carefully controlled. Therefore, the differences between beverages must, at least in part, be due to non-ethanol congener(s).

Grape based beverages have the most profound effect on \textit{H.pylori} in vitro: Incubation with wine caused rapid loss of motility and ultimately bacterial death. This effect appears to be independent of vintage, as it was observed with several red and white wines. Similarly, method of production in terms of 'conventional' versus 'organic' does not alter this phenomenon. It is therefore likely to represent a true effect of congener(s), as opposed to an additional substance(s), such as a preservative or biocide, added during production.

Other beverages that use wine as a starting medium also show effects on motility and survival of \textit{H.pylori} in vitro. These effects are not as profound as with wine alone, and presumably are due to alterations that occur in congener(s) during different production processes. In general terms, however, the closer the beverage is to the original wine in the production process the more profound the effect on \textit{in vitro} motility and survival. This is best illustrated by sherry which showed marked effects on motility and survival of \textit{H.pylori} in vitro. Sherry is nearest in the production process to the original wine as it is a blend of ‘older’ and ‘younger’ wines. No vintage sherry exists because the barrel ageing which is such an integral part of other wine based beverages such as port, does not occur. Presumably, there is less opportunity for the active congener(s) to be altered during sherry production.

Non-alcoholic grape juice is able to inhibit \textit{H.pylori} motility to a level intermediate between wine and laboratory ethanol. This is strong evidence to support the importance of congeners, as opposed to ethanol, in beverages to account for the observed effects. Grape juice is however only a ‘surrogate’ marker for wine congener(s), as these would be expected to change during the fermentation process.
Chapter 5: Effects of Alcoholic Beverages on *H. pylori* in vitro

Beverages that are non-wine based, including spirits such as Whiskey and gin but also rice-based sake, show much less effect on *H. pylori* motility and survival *in vitro*. This is consistent with the hypothesis that wine contains the most active congener(s) against *H. pylori*.

Further support for a 'congener-hypothesis' comes from the observation that ordinary lager causes a profound loss of *H. pylori* motility but very little effect on survival. Since lager is not wine based, this initially appears to be at odds with a hypothesis that it is wine congeners that have the most effect on *H. pylori*. If however, organic beer or lager is used, very little effect is seen either on motility or survival. The most likely explanation for these observed effects, is that conventional brewing processes add a preservative or other biocide that affects motility which is not added during the more rigorous production of organic beers and lagers.

5.11 Future Directions

The inevitable question that follows from this experimental work is 'what is the congener(s) in wine that affects *H. pylori* in vitro motility and survival?' Based on the discussion in section 5.1.4, it is most likely to be a complex polyphenol such as the flavan-3-ol, resveratrol (trans-3,5,4'-trihydroxystilbene). The reasons for this include:

- Flavan-3-ols are found in wine at concentrations 10-100 times that of other beverages (Abu-Amsha *et al.* 1996; Sato *et al.* 1997).
- Resveratrol may be the 'mould resistant agent' (Celotti *et al.* 1996).
- Resveratrol has been shown to have several different biological effects and may be the active agent to explain the 'Mediterranean paradox' (see section 4.1.5)

I am grateful to Dr Graham Taylor at Imperial College Medical School for performing HPLC of red wine as presented in figure 5.17: With identification of the individual fraction peaks, future work will involve testing these against *H. pylori* in vitro with a view to identifying the active congener involved.
Figure 5.17: HPLC of Red Wine

(Courtesy of Dr Graham Taylor, Imperial College London, Hammersmith Hospital Campus)
6.1 Summary

A literature review, presented in the introduction, demonstrated that the prevalence of peptic ulcer disease in patients with liver disease was up to three times that of the general population. The exact aetiology was however, far from clear. At least 80% of peptic ulcer disease, in the otherwise normal population, is caused by \textit{H.pylori}. Therefore, a starting point to examine the aetiology of peptic ulcer disease in patients with liver disease was to examine the \textit{H.pylori} prevalence in this patient population. The literature was however contradictory and difficult to interpret. Problems of interpretation included, reliance on a single diagnostic test for \textit{H.pylori}, absence of age stratification and poor characterisation of liver injury. Comparisons of \textit{H.pylori} prevalence between patients with liver injury and the otherwise normal population were often thwarted by use of inappropriate controls; frequently ‘control’ patients had dyspepsia which can not be considered appropriate because of the known associations between this symptom and \textit{H.pylori}. A further problem of interpretation occurred with alcohol history data in patients with liver disease in general, and alcoholic related liver injury in particular; this was either rudimentary or non-existent. Lack of alcohol data posed a particular problem of interpretation because excellent epidemiological studies have suggested a lower \textit{H.pylori} prevalence in healthy volunteers consuming higher amounts of alcohol. This effect was also, at least in part, beverage dependent. It could therefore be hypothesised that alcohol misusers might show an exaggeration of this phenomenon due to both their increased consumption and narrower repertoire of beverage consumed. Significant changes in the natural history of \textit{H.pylori} infection in alcohol misusers, with varying levels of liver injury, might therefore be anticipated. But, with data available from the current literature, this hypothesis could not be adequately explored. It was however possible to conclude that the higher prevalence of peptic ulceration observed in patients with liver disease was not clearly associated with any increase in \textit{H.pylori} prevalence in this patient population.

A hypothesis that alcohol might influence the natural history of \textit{H.pylori} infection in alcohol using and misusing patients is intuitively attractive in view of the widespread use of alcohol as an antiseptic. A potential mechanism for this can also be proposed because \textit{H.pylori} is known to contain alcohol dehydrogenase (ADH). Although the teleological nature of the argument is acknowledged, it is likely that the bacterium developed ADH during evolution to metabolise highly toxic acetaldehyde to less toxic, and energy providing, ethanol. In the presence of
excess alcohol, toxic acetaldehyde would accumulate and might be expected to cause bacterial inhibition and/or death.

The hypothesis that alcohol might inhibit *H. pylori* in vivo can be further elaborated by the observation that gastric mucosa also contains ADH. The contribution gastric ADH plays in the overall first-pass metabolism of alcohol is controversial. But, when alcohol is consumed, especially in excess, acetaldehyde would be expected to increase as it is produced by both gastric and *H. pylori* ADH. This might, in turn, be expected to show increasing toxicity against *H. pylori*.

The 'direct-toxicity' hypothesis of alcohol on *H. pylori*, as described above, only partially fits known experimental facts. Of particular interest is the observation that gastric mucosal ADH falls in the presence of active *H. pylori* infection. It is easy to appreciate that *H. pylori* gastritis, by damaging parietal cells, would cause a secondary loss of gastric ADH activity. But, this would in turn, be expected to cause reduced levels of acetaldehyde production in the presence of alcohol and consequently a less toxic effect on *H. pylori*. It can therefore be seen that gastric mucosal change may be just as critical in determining outcome of *H. pylori* infection in the context of alcohol use and misuse as the amount of alcohol consumed.

In chapter 2, some of these issues were addressed: *H. pylori* seroprevalence was assessed in four carefully characterised populations in terms of age, drinking habits and extent of liver injury. In healthy volunteers and hospital patients, *H. pylori* seroprevalence increased with increasing age-a phenomenon noted in numerous other published studies. The behaviour of *H. pylori* seroprevalence in patients with liver disease, however, behaved in a unique fashion hitherto not described. In this patient population, the average *H. pylori* seroprevalence declined by 5%/year of increasing age. This decline in *H. pylori* seroprevalence was observed in patients with both alcoholic and non-alcoholic liver disease. The decline in *H. pylori* seroprevalence with age was, in turn, made up of two distinct components dependent on liver injury.

In minimal change, non-alcoholic liver injury, *H. pylori* seroprevalence increased by 3%/year of increasing age. But, seroprevalence of *H. pylori* in non-alcoholic cirrhosis declined by approximately 20%/year, to give the overall decline in *H. pylori* seroprevalence of 5%/year of increasing age. It is suggested that patients with minimal liver injury behave most like the normal population because their disease is generally mild and as a consequence *H. pylori* prevalence increases with age. The decline in *H. pylori* seroprevalence observed in patients with non-alcoholic cirrhosis is harder to explain and several theories are discussed in the text.
including immunological change and/or development of atrophic gastritis with bacterial clearing.

In the patients with alcohol related liver injury, \textit{H.pylori} seroprevalence again declined by 5%/year of increasing age. But, the \textit{H.pylori} seroprevalence in the alcoholic cirrhotic patients remained constant at approximately 35%. The overall decline in alcohol related \textit{H.pylori} seroprevalence occurs due to a 10%/year decline in seroprevalence seen in the minimal liver injury group. It is difficult to propose a single unifying hypothesis to explain all of these findings. But, a decline in \textit{H.pylori} seroprevalence in alcohol misusing patients with minimal liver injury is consistent with the hypothesis that alcohol might in some way clear the organism, as discussed above, and account for the change in natural history of infection seen in these individuals.

To examine a hypothesis that alcohol misuse might influence the natural history of \textit{H.pylori} infection \textit{in vivo}, it is necessary to establish the sensitivity and specificity of the different diagnostic tests used for diagnosis. In chapter 3, data from 100 alcohol misusers who were recruited sequentially and prospectively, is presented. These patients had their \textit{H.pylori} status assessed using several conventional techniques. Particular attention was paid to extent of liver injury and 92% of patients had this characterised by liver biopsy, with 83% having minimal liver injury and the remainder significant liver injury. A full demographic, medical, social and alcohol history was also obtained.

The overall \textit{H.pylori} prevalence in this patient population using a gold-standard technique combining histology and conventional tests was 46.0%. Using histology alone 33.0%, serology 43.6%, $^{13}$C-urea breath test 66.7% and urease testing 25.5%. Reasons for the significantly lower \textit{H.pylori} prevalence as assessed by urease testing are discussed in the text and include the hypothesis that alcohol may be causing direct inhibition of the \textit{H.pylori} bacterium as urease testing is dependent on viable \textit{H.pylori}.

Using the gold standard for \textit{H.pylori} diagnosis, the sensitivity (specificity) of different diagnostic tests for \textit{H.pylori} in this patient population were as follows: histology alone 71.7% (100%), serology 64.4% (75.5%), urease 52.2% (98.2%) and $^{13}$C-urea breath-testing 92.9% (90.0%). Maximum sensitivity of 85.7% (100.0%) was achieved with a combination of serology and $^{13}$C-urea breath-testing. But there were some methodological difficulties with $^{13}$C-urea breath-testing which are discussed in the text.
Chapter 6: Summary, Conclusions and Future Directions

The prevalence, by age of *H. pylori* in this patient population, as assessed by the gold-standard was 56.3% (<40yr), 40.5% (41-50), 52.2% (51-60) and 66.6% (61+). No significant difference in prevalence between decades was observed. The lack of an age-related increase in *H. pylori* prevalence is again a new finding and again emphasises the unique nature of this population. A discussion about the relative contribution of direct alcohol toxicity against *H. pylori* vs mucosal injury leading to bacterial clearing to account for this result is presented.

Chapter 4 examined the relationship between endoscopic appearance and symptoms in the same series of 100 prospectively assessed alcohol misusers. Symptoms occurred in 64% of individuals and in 24% were classed ‘severe’. Despite the frequency of symptoms, no relationship between *H. pylori* status, daily alcohol intake, smoking or drugs could be determined.

Endoscopic abnormalities were also common and occurred in 60% of patients. But, no correlation with either symptom scores or *H. pylori* status could be determined. Similarly, even apparent ‘classical’ associations between antral gastritis and *H. pylori* presence were not seen with antral gastritis occurring in 17.6% of the *H. pylori* positive patients and 19.7% in the *H. pylori* negative patients.

It was therefore not possible to find any apparent relationship between symptoms in alcohol misusers and either findings at endoscopy or correlation with any usual risk factors for *H. pylori* infection including *H. pylori* status.

Assessment of histological changes were also made using the modified Sydney system: 13% of alcohol misusers had normal antral histology and 22% normal body histology. The occurrence of *H. pylori* associated gastritis was 31% in the antrum and 29% in the body. Acute gastritis was equal at 3% in both regions but reactive gastritis was higher in the antrum than body (7% vs 3% N.S.). In contrast intestinal metaplasia (IM) and/or atrophy was commoner in the body than antrum (7% vs 4% N.S.). The commonest histological abnormality was chronic gastritis, which occurred in the antrum in 41% of individuals and 35% in the body. No significant differences between sites for individual histological abnormalities were detected. The overall concordance between sites, meaning the same histological diagnosis was made for the antrum as body, was 71%. One patient had *H. heilmannii* gastritis.

The mean gastric inflammatory score for the 100 alcohol misusers was 1.07. The mean gastric inflammatory score in the alcohol misusers who were *H. pylori* positive was 2.37 and for *H. pylori* negative alcohol misusers 0.411. This difference was highly significant (p<0.0001).
Histological findings in the alcohol misusers were also compared with 50 age and sex matched non-alcoholic controls: The prevalence of *H. pylori* in the antrum of the alcohol misusers was non-significantly lower than control (36% vs 46%; p=0.22) as well as in the body (32% vs 42%; p=0.3). A significantly lower prevalence of chemical gastritis and antral intestinal metaplasia was observed in the alcohol misusers when compared with controls. These results were the opposite of that expected from the Sydney system. A hypothesis is proposed that if alcohol misuse was associated with *H. pylori* inhibition *in vivo* then the source of chronic gastric inflammation would be obtunded and the consequences of chronic inflammation-reactive gastritis and intestinal metaplasia consequently reduced.

In chapter 5 the hypothesis that alcoholic beverages might directly inhibit *H. pylori* was investigated *in vitro*. Following *in vitro* incubation of *H. pylori* with alcoholic beverage at the same ethanol concentration, it was demonstrated that different beverages produced differential effects on both *H. pylori* motility and survival. All wines, regardless of production method, produced both a profound and rapid loss of *H. pylori* motility and bacterial death. Lager also caused loss of *H. pylori* motility but with minimal, if any, influence on survival. This effect was attenuated when organically produced lager and beer were tested implying that conventional lager might contain a biocide and/or preservative. On the basis of these observations a ‘congener-hypothesis’ was proposed: This proposes that alcoholic beverages affect *H. pylori* *in vitro* motility and survival in a manner critically dependent not on the ethanol content but the non-ethanol component(s) or congeners.

To provide support for a congener-hypothesis for the action of alcoholic beverages on *H. pylori* *in vitro*, it was observed that as beverages moved further away from grape in the production process, the less the observed effect on *H. pylori* motility and survival. For example, sherry that is a blend of older and young wine had an effect on *H. pylori* motility near to the effect observed with wine alone. In contrast, non-grape based alcoholic beverages in general, and sake in particular, had the least effect on *H. pylori* motility and survival *in vitro*.

Some of the strongest evidence for the importance of congeners in affecting *H. pylori* motility *in vitro* comes from experiments with non-alcoholic grape juice: This caused inhibition of *H. pylori* motility *in vitro* to an extent that was intermediate between wine and laboratory ethanol. It was not however possible to calculate the relative contribution of congener vs ethanol in having this effect on *H. pylori* *in vitro*. This was because grape juice is only a surrogate marker for wine congener as it does not undergo the same fermentation process as
congeners in wine nor was it possible to ensure congener concentration equivalence in experiments as only ethanol was corrected for.

6.2 The 'Congener-Hypothesis'
A speculative schema, as a working hypothesis for future work, is presented in Figure 6.1: The second division emphasises the importance of considering both ethanol and congener in studying the effects of alcoholic beverage consumption on both the upper gastrointestinal mucosa and liver. The importance of congener origin is emphasised in the third division; here it is hypothesised that the in vitro effects of congener observed in chapter 5 can be applied directly in vivo. Habitual consumption of grape based alcoholic beverage causes maximum inhibition of H. pylori and may clear or at least inhibit, the infection. Alternatively, non-grape based congeners have very little inhibitory effect on H. pylori and our in vitro data presented in chapter 5, found Sake (rice-wine) the least effective of the beverages tested.

Data presented in Chapter 4 showed that the expected increase in reactive (chemical) gastritis in alcohol misusers predicted by the Sydney system did not occur and was, in fact, less than control. Coupled with the observation of less intestinal metaplasia in alcohol misusers, which is a marker of chronic inflammation, is indirect evidence to support beverage inhibition of H. pylori. This effect on gastric inflammation dependent on congener inhibition of H. pylori is presented in division five of figure 6.1. Prolonged gastritis due to H. pylori may be a factor in the development of gastric carcinoma. It is hard to resist at least making the observation that the highest incidence of gastric cancer World wide is in Japan. Of all the beverages tested in chapter 5, Sake, the Japanese national drink, had the least potent congener(s) against H. pylori.

Despite H. pylori containing ADH, its presence in gastric mucosa reduces the overall gastric ADH level due to the development of H. pylori related gastritis and loss of gastric parietal cell ADH. As a consequence, grape-based beverage congener(s), by inhibiting H. pylori and reducing gastritis may lead to preservation of gastric ADH. This is presented in division six of figure 6.1. The effects on gastric ADH can, in turn, be expected to influence the first-pass metabolism of the ethanol component in the beverage. Intact gastric ADH may reduce peak ethanol blood concentrations by initial gastric metabolism. With lower peak ethanol concentration, and a complex interaction with genetic factors, it can be speculated that ethanol related liver injury might be limited. This is presented in the final division. It is therefore possible that the ‘old wives tail’ anecdotally favoured by many alcohol misusers, that avoiding certain beverages, such as spirits, protects them from liver injury, may have some basis in fact. Based on the hypothesis outlined here, avoidance of non-grape based beverages may indeed be of value.
Figure 6.1: A Speculative Schema of the ‘Congener-Hypothesis’

- Alcoholic beverage
- Ethanol
- Congener
  - Grape
    - Maximum Hp inhibition
    - Less Hp gastritis
    - Higher gastric ADH
    - Lower blood ethanol
    - Genetic factors
    - Less liver injury
  - Non-grape
    - Minimal Hp inhibition
    - More Hp gastritis
    - Lower gastric ADH
    - Higher blood ethanol
    - Genetic factors
    - More liver injury
6.3 Future Directions

6.3.1 Population Studies

This work has shown the importance in future studies that examine *H. pylori* prevalence in patients with liver disease to carefully control for degree of liver injury, age, control population and alcohol history. This work was not able to identify a clear beverage dependent effect on *H. pylori* prevalence in the group of alcohol misusers studied. On the basis of a 'congener-hypothesis' this reflected the small number of alcohol misusers consuming wine. A study that specifically compared wine drinkers with other beverage consuming groups would help examine this problem in more detail.

Another question that arises from this work is that if we accept that alcoholic beverage consumption might influence the natural history of *H. pylori* infection, then is there an optimal time for this interaction to occur? A longitudinal study examining loss of *H. pylori* with time would address this issue. The congener hypothesis predicts that *H. pylori* positive wine drinkers would show faster rates of spontaneous *H. pylori* clearance than non-wine drinkers. In addition, gastric inflammatory scores might be expected to be lower in wine drinkers when compared with non-wine drinkers because of the enhanced *H. pylori* inhibition seen with wine and discussed above.

6.3.2 Volunteer Studies

The inhibition of *H. pylori* motility and survival produced by wine incubation *in vitro* can also be indirectly examined in healthy volunteers. It can be hypothesised that the curve and peak of the C¹³ urea breath test would be reduced in *H. pylori* positive individuals after wine consumption but that this phenomenon would be less after consumption of equivalent pure ethanol.

A second stage to this study would be to look at ethanol kinetics both before and after *H. pylori* eradication. If volunteers drank wine for several weeks prior to the breath test, the difference between pre-*H. pylori* eradication alcohol kinetics and post-*H. pylori* eradication alcohol kinetics, might be less than that observed in a similar group consuming just the ethanol equivalent. prior to testing. The congener hypothesis predicts that those *H. pylori* positive volunteers drinking wine in the initial stages would have more marked inhibition of *H. pylori* than the ethanol equivalent group. As a consequence, the hypothesis predicts that the wine drinkers would have less gastritis and higher levels of gastric ADH. The difference in ethanol kinetics before and after *H. pylori* eradication would be predicted to be less than that observed in the volunteers consuming the ethanol only beverage.
This study could be extended to alcohol misusers as it might be expected that any effect would be exaggerated in this patient population because of their increased consumption. The study is however, unlikely to be attempted because of the ethical problems of encouraging alcohol misusers to drink.

6.3.3 Ex-vivo Studies
The congener hypothesis can also be examined by an ex-vivo study: Biopsies for urease testing are frequently taken during routine endoscopic practice. The congener hypothesis predicts that immersion of the biopsy sample in wine prior to placing it into the well of a commercial CLO\textsuperscript{R} test would inhibit the \textit{H.pylori} so that a false negative colour change would be observed. Other beverages and laboratory ethanol are predicted to have less or no effect on \textit{H.pylori} and so a true positive test would be obtained.

6.3.4 Clinico-Pathological Studies
The congener hypothesis as discussed above, predicts that beverage consumed influences \textit{H.pylori} which in turn causes gastritis. Future work will involve staining gastric biopsies for gastric ADH; this technique will allow comparison between \textit{H.pylori} positive and negative biopsies to directly examine the hypothesis that gastritis due to \textit{H.pylori} causes a loss of gastric ADH as a secondary phenomenon.

6.3.5 Laboratory Studies
Chapter 5 presented data demonstrating that wine congener(s) was more effective against \textit{H.pylori} motility and survival than other beverage congener(s). The obvious question that follows from this is what is the congener(s) involved? At the time of writing, initial HPLC separation of wine congeners has been performed and is presented figure 5.17. These individual fractions will then be tested in the \textit{in vitro} assay system to help identify the active wine congener against \textit{H.pylori}. 


Bibliography


Bibliography


Bibilography


Bibliography


Bibliography


Bibliography


Bibliography


APPENDIX 1: DATA COLLECTION PROTOCOL SUMMARY

1. SUMMARY
   Patient Hospital Number
   Patient Name
   Patient Study Number
   Diagnosis of Alcoholic Liver disease
   Pugh’s Score  Pugh’s Grade  HE Status: normal/ subclinical/overt
   Hp infection  Positive/Negative

2. DEMOGRAPHY
   Address/tel /D.O.B /sex/ occupation (now/best)/ethnicity

3. HISTORY OF LIVER DISEASE
   Additional risk factors to alcoholic liver disease: HBV/HCV/PBC/PSC/Autoimmune/Other
   Liver Biopsy Done: Yes/No. Date of Biopsy/ Tick when report obtained
   Events of P.S.E. in last year: 0/ 1/2/ >2
   Fluid Retention (ascites / SOA):
   On Diuretics:
   SBP No/Yes:
   Sepsis summary (SBP or systemic):

4. ALCOHOL HISTORY
   Drinks Alcohol: No/Yes Units/week
   If actively abusing alcohol or alcoholic cirrhosis:
   First alcoholic drink age/ daily drink age/ abuse age/ favourite drink/ last drank / stopped drinking /date stopped
   CAGE /4
   Detox in past: I/P or O/P
   Ep/withdrawal/collapse/LOC

5. SOCIAL HISTORY
   Current Smoker: No/Yes ..../day X ...years X 0.05= pack years
   Ex smoker: No/Yes Time since stopped (Years). Pack years
   Caffeine: Tea/coffee cups per day

6. PREVIOUS HELICOBACTER STATUS
   If known, record PREVIOUS Hp status Positive/Negative/Date tested/Diagnosed by

7. UPPER GASTROINTESTINAL HISTORY
   • Dyspepsia History (score 1-3 )
     Epigastric pain/odynophagia/heartburn/dysphagia/acidreflux/weight loss/sucking sensation/nausea and vomiting/eructation/other
   • Bleeding History
     Ever bled/ Patient has varices?: date diagnosed/ever bled/injected/banded/wedge
     • Endoscopic summary

8. OTHER SIGNIFICANT ILLNESSES
   Has patient ever had diabetes/ MI/ angina/ heart failure/asthma / COAD/ other

9. NEUROPSYCHIATRIC HISTORY
   Overt encephalopathy/subclinical encephalopathy/ normal/ unknown

10. DRUG HISTORY
    CURRENT/STOPPED/ TOTAL DAILY dose
    Ppi/H2RA/Cisapride/metaclopramide/Denol/Pylorid/Propranolol/Aspirin/OtherNSAIDs
    Misoprostol/Lactulose/Lactitol/Antibiotics-which/Antacids-which/ Other medication

11. PHYSICAL EXAMINATION
    General
    BP/pulse/heart/chest/
    Abdominal
    Flap/foetor/ascites/S.O.A./Duputren's/palmar erythema/gynaecomastia/spider naevi
    Blood tests
    Bilirubin/ P.T./albumin/ammonia/ EEG/ Pugh's score/ PSE index/ Child grade

12. H. PYLORI SCREENING (test and date)
    Serology/breath test/endoscopy-CLO and histology
ADDENDUM

Typographical corrections:
p24 predominantly
p26 decade
p28 necessity, predictable
p30 vegetable
p31 *Pseudomonas aeruginosa*
p34 *ex-vivo*
p40 oesophageal
p48 in to
p51 almost
p55 prevalence
p65 non-Gaussian
p68 35% remove ‘but check figure’
p81 remove ‘no’
p83 haematoxylin
p97 Fig 3.5B (not A)
p100 study
p104 Table 3.3 (not 3.1)
p112 remove ‘X40’
p113 MALToma
p115 X 1200
p122 was
p124 was
p127 was
p136 remove ‘a’
p140 notable
p143 vegetable detectable
p144 extractable *in vitro*
p144 travellers
p159 0.016
p174 suitable
p176 cognac
p177 its
p184 versus
p185 inevitable
p187 (insert) ‘of’
p190 (insert) ‘itself’
Examiners Questions:
p20: Shearman 1989 cited at www.ebando.co.uk. Where is the reference?
p21: Overall mortality constant at 1%...not true see Rockall
‘Of 2071 patients with peptic ulcer presenting with acute haemorrhage, 251 (12%) had an operative
intervention with a mortality of 24%. In the non-operative group mortality was 10%. The operative
intervention rate increased with risk score, ranging from 0% in the lowest risk categories to 38% in the
highest. Much of the discrepancy between operative and non-operative mortality was explainable by
case mix; however, for high-risk cases mortality was significantly higher in the operated group’.
gastrointestinal haemorrhage. Steering Group for the National Audit of Acute Upper Gastrointestinal
p23 a) 20-30%—explain b) how do these figures compare with an empty stomach?
Following ingestion of alcoholic beverage on an empty stomach, measurable peak blood ethanol levels
are reached earlier after ingestion of sherry at approximately 20% ABV than Whiskey at 40% ABV.
The higher ethanol concentration in whiskey delays gastric emptying. This in turn delays the passage of
ethanol from stomach to duodenum where significant quantities of ethanol are absorbed. Since
absorption of duodenal ethanol is by passive diffusion, the prandial state will critically affect the speed
of absorption.
p24 is this baseline or induced ADH activity?
Baseline
p25 a) in rats b) was the Seitz et al (1993) study longitudinal or cross-sectional? c) is gastric ADH
polymorphic? d) are there racial differences in ADH activity? e) Is gastric ADH measured in the
same way in all studies?
a) yes, rats
b) cross-sectional
c) yes, at least four isoenzymes of ADH have been described of which sigma ADH is the predominant
Experimental Research 14, 946-950
d) Gastric sigma ADH is reduced or absent in the Japanese. This is associated with reduced first pass
Research 14, 946-950
e) No different assay methods are used
p27 a) Morgan and Ritson—original reference preferable b) does it matter whether 5 times alcohol
consumption is due to dependence (misuse) or just lifestyle?
a) It is based on a review and consensus statement about alcohol use and misuse from several sources
including The Royal Colleges of Psychiatrists (1986), General Practitioners (1986) and Physicians
(1987)
b) No, liver injury is dependent on lifetime total ethanol dose and genetic factors. Although binge
drinking of the same number of units of alcohol may be associated with increased liver injury.
p30 low or moderate alcohol associated with binge drinking?
Binge drinking
p32 bed sharing and outside toilet
Bed sharing is a marker of crowded living conditions in childhood with increased risk of acquiring
H. pylori infection by person to person spread. Whether use of an outside toilet is simply a marker of
poorer socioeconomic circumstances or helps facilitate direct H. pylori infection is unknown.
p36 a) how does urease change colour? b) should it be a gold-standard?
a) The basis of the urease test is urea and the pH sensitive indicator phenol red. When a gastric biopsy,
containing H. pylori, is placed in this solution or the well of a commercial urease test such as a CLO®
test, the H. pylori urease metabolises the urea to CO₂ and ammonia. The ammonia immediately
dissolves in the aqueous phase to produce ammonium. This reaction, in turn, changes the pH of the test
solution alkaline. The phenol red detects this pH change and turns from yellow to red, indicating a
positive result.
b) Urease testing should not be the ‘Gold-Standard’ for H. pylori testing as false negative results can
occur following recent drug ingestion including PPI and antibiotic usage.
p39 clarify the point you are trying to make about viability?
Urease testing, as discussed above, is dependent on H. pylori urease activity. It is assumed that viable
bacteria are needed to continually produce urease in order for the organism to survive the acid
environment of the stomach. Killed bacteria would therefore be expected to produce a negative urease test but positive histology and serology. It helps to introduce the theory, put forward in chapter 3, that a direct alcohol toxicity effect on *H. pylori* might account for the low sensitivity of urease testing observed in alcohol misusers.

**p40** a) NSAIDs do not work through a direct irritant effect, this is far to simplistic  b) the role of Hp in DU/GU pathogenesis is only explored superficially here

a) The role of NSAIDs in the pathogenesis of peptic ulceration is complex. Interference with prostaglandin metabolism, in turn, disrupting the gastric mucosal/epithelial barrier is the preferred theory.

b) Agreed

**p43** This section is good

Thank-you. Rothenbacher has asked for a pre-publication copy of the papers that will accompany Chapter 5 as the data presented in this chapter, in our opinion, provide a biologically plausible explanation for the epidemiological observations made by Brenner and Rothenbacher as discussed in the text.

**p61** a) verification of history and alcohol b) size of groups

a) Alcohol histories were obtained by direct subject questioning. In group 4, the alcohol misusers, collateral verification was obtained from family and friends where possible.

b) The size of the groups was dictated by available stored sera.

**p66** Are there more sophisticated statistical tests that could be used for comparing age related trends?

No, the advice given by our statistician was due to the large numbers of subjects being studied fitting age as a continuous variable in a logistic regression model was the most appropriate way to analyze age-related trends.

**p70** Is this surprising—how come?

A relationship between higher levels of alcohol misuse and more advanced liver injury might be anticipated but was not observed. It can be speculated that this was due to unspecified genetic differences.

**p76** a) serology may be positive 5 years after *Hp* eradication b) are the older alcoholics ‘survivors’ because they started with a higher social class c) why is social class and ethnic mix not discussed as potential confounders?

a) Agreed. But loss of seropositivity following *H. pylori* eradication has been observed at only 6 months following successful eradication and no longitudinal studies on *H. pylori* seropositivity in liver disease have been performed.

b) Social class data was not available for this study and this deficiency is addressed in the prospective study in chapter 3.

c) This data was not available for analysis although the majority of patients were of Celtic origin (White English, Irish or Welsh)

aData proforma to appendix

This is a large document. A summary is included as an addendum.

**p83** Why was equivocal assumed negative?

Patients with an equivocal serological result had antibodies to *H. pylori* but only at low titre. This could be due to previous infection, poor immune response or assay failure to name a few possible explanations. In routine clinical practice, these equivocal results would be reported as negative.

**p86** what was the social class at birth?

This data was not collected

**p90** a) The high UBT positivity in 21 patients may be a small number artefact. b) What is the correlation with other tests? C) How were they selected?

Agreed and also had a high selection bias as discussed in section 3.12 (p102)

**p91** table 3.5 paired means both positive

‘Paired tests’ means both diagnostic tests had to be positive for the patient to be classified *H. pylori* positive.

**p93** a) antibiotic use prior to endoscopy ‘not significant’ = type 2 error b) why is urease positivity less than serology positivity (also p 103 discussion)?

a) The numbers of patients who had received antibiotics prior to study was small and so the possibility of a type 2 error is high

b) The preferred hypothesis is a direct inhibitory effect of alcoholic beverage on *H. pylori* which could be expected to reduce urease activity but not serological response

**p105** perhaps the alcoholics in chapter 2 are an odd population? NB socio-economic class were not investigated

Yes they could be although I would still favour an alcohol effect.
Does *H. pylori* cause dyspepsia?
Not by itself, but the pathology associated with colonization such as antral gastritis is.

Section 4.2 could be in the introduction
Yes it could. But while a detailed discussion of the Sydney system was essential prior to presentation of the data in this chapter, an earlier detailed discussion would, I feel, make the introduction too long and lead to argument dilution for the role of alcohol on the natural history of *H. pylori* infection.

Previous NSAID or other drug use
This and other data were collected as outlined in section 3.6.4.

Was congestion and oedema suggestive of PHG and did it correlate with liver biopsy?
No there was no correlation. But any patient who had overt endoscopic PHG or varices was automatically excluded from the study on safety grounds. This selection bias explains why in the 92 patients who underwent liver biopsy (Table 3.1) only 20 were classified as having significant liver injury (Table 3.3).

Why are the data not presented in parallel for comparison?
The control patients to provide 50 pairs of age and sex matched pairs were recruited separately. They therefore can only be regarded as control for histological comparisons.