INFECTION WITH *HELICOBACTER PYLORI*

1. Exposure Data

1.1 Structure and biology of *Helicobacter pylori*

1.1.1 *Taxonomy*

The presence of spiral-shaped bacteria on human gastric mucosa was first recognized nearly one hundred years ago (Pel, 1899). These bacteria were isolated for the first time in 1982, in cultures of endoscopic biopsy specimens from patients with gastritis and peptic ulceration (Marshall, 1983; Warren, 1983). For phenotypic reasons, such as spiral shape, motility, growth under microaerophilic conditions and isolation from the alimentary tract, the organism was classified as a member of the genus *Campylobacter* and was called *Campylobacter pyloridis* (Marshall *et al.*, 1987), and then *C. pylori* (Marshall & Goodwin, 1987). It became clear, however, that *C. pylori* differed significantly from other members of the genus with respect to cellular fatty acids, lack of a methylated menaquinone, antimicrobial susceptibility and ribosomal ribonucleic acid sequences.

In 1989, a new genus, *Helicobacter*, was proposed, and *C. pylori* was renamed *Helicobacter pylori* (Goodwin *et al.*, 1989). The genus now includes a variety of 'gastric' and 'non-gastric' *Helicobacter* species. Classification of bacteria into the new genus was based mainly on a homology greater than 90% of the nucleotide sequence in the 16S ribosomal RNA molecule (Lee & O'Rourke, 1993). The gastric *Helicobacter* spp. are *H. pylori*, *H. mustelae* (ferrets; Fox *et al.*, 1986, 1988), *H. felis* (cats and dogs; Lee *et al.*, 1988, 1990, 1992), *H. nemestrinae* (macaque monkeys; Bronsdon *et al.*, 1991) and *H. acinonyx* (cheetahs; Eaton *et al.*, 1991a). One non-gastric *Helicobacter* sp. is *H. hepaticus* (mouse liver and intestine; Fox *et al.*, 1994). An additional spiral bacterium commonly found in the stomachs of cats, dogs and pigs and infrequently in those of humans, which has not yet been cultured and is known provisionally as '*Gastrospirillum hominis*' or '*H. heilmannii*', has been proposed for addition to the genus on the basis of morphological and RNA similarities (Solnick *et al.*, 1993).

1.1.2 *Biology*

(a) Morphology; ultrastructural features

H. pylori is a spiral or slightly curved gram-negative rod with two to six characteristic unipolar flagella. The bacterium has bluntly rounded ends and measures 2.5–4.0 μm in length and 0.5–1.0 μm in width. The cell wall is smooth and may be coated with a prominent

glycocalyx with a thickness up to 40 nm (Goodwin *et al.*, 1989); it is covered with ring-like subunits with a diameter of 12–15 nm. Occasionally, bacteria may contain bacteriophages. The flagella measure 2.5 μm in length and around 30 nm in thickness and have a distinctive terminal bulb (Goodwin & Worsley, 1993). Each flagellum consists of a central filament enveloped by a flagellar sheath. The filament consists mainly of a polymer of a 53-kDa [80 base-pair] flagellin protein (Geis *et al.*, 1989, 1993); it ends proximally in a basal body, which is associated with the cytoplasmic membrane. The sheath is formed by a lipid bilayer, which extends as a direct continuation from the bacterial outer membrane (Geis *et al.*, 1993). The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility (Hazell *et al.*, 1986; Suerbaum *et al.*, 1993). *H. pylori* may change from its normal morphological appearence into a range of coccoidal forms, especially *in vitro* after prolonged culture or after antibiotic treatment. It is not certain whether the coccoidal forms can resume the spiral, multiplying form. The viability of coccoidal organisms has been proven by means of acridine orange staining, bromodeoxyuridine incorporation and urease activity (Goodwin & Worsley, 1993; Nilius *et al.*, 1993).

(*b*) *DNA content; genome and plasmids*

The DNA of different *H. pylori* strains contains 34–38 mol % guanine and cytosine (Goodwin & Worsley, 1993). The genome varies in size from 1.6 to 1.73 megabases (Taylor *et al.*, 1992). About 35–50% of *H. pylori* strains contain plasmids, which have not been associated with any biological characteristic of the bacteria (Majewski & Goodwin, 1988; Penfold *et al.*, 1988; Simor *et al.*, 1990).

A number of specific genes have been cloned, including two structural urease genes which encode the subunits of the urease enzyme (Labigne *et al.*, 1991), two flagellin genes, called *fla*A (Leying *et al.*, 1992) and *fla*B (Suerbaum *et al.*, 1993), a cytotoxin production-associated gene, the *cag*A gene (Tummuru *et al.*, 1993), the cytotoxic *vac*A gene (Cover *et al.*, 1994) and a heat-shock protein encoding gene (Macchia *et al.*, 1993).

(*c*) *Growth conditions*

H. pylori can be cultured in both solid and liquid media. Basal solid media, such as Colombia blood agar base and brain–heart infusion agar supplemented with serum or charcoal, yield good results (Dent & McNulty, 1988; Goodwin & Worsley, 1993). Brain–heart infusion (or brucella) broth supplemented with charcoal, serum or cyclodextrins can also be used (Olivieri *et al.*, 1993). Microaerophilic culture conditions are essential, with optimal oxygen concentrations between 2 and 8%. Addition of extra carbon dioxide or 1–5% whole blood or serum may stimulate culture yields. Bacteria of the genus *Helicobacter* do not catabolize carbohydrates (Mégraud *et al.*, 1985; Goodwin & Worsley, 1993), but *H. pylori* can use glucose via the pentose phosphate pathway (Mendz *et al.*, 1993). Maximal growth occurs at 37 °C and neutral pH (Goodwin & Worsley, 1993). The bacterium is sensitive to almost all antibiotics *in vitro*, with the exception of nalidixic acid, trimethoprim, sulfonamides and vancomycin (Goodwin *et al.*, 1989; Goodwin & Worsley, 1993). Section 1.5 provides further information about the efficacy of antibiotics *in vivo*.

(*d*) *Enzymatic activity*

H. pylori is characterized by strong urease activity, with a Michaelis constant of 0.48 mmol/L for urea (Goodwin & Worsley, 1993). The hexameric enzyme has a relative molecular mass of about 600 kDa [909 base pairs] and is composed of six monomers, each with two protein subunits of 66 and 31 kDa [100 and 47 base pairs]. It is active at pH 4.0–10.0 and has an isoelectric point of 5.93 (Evans et al., 1992; Goodwin & Worsley, 1993; Mobley & Foxall, 1994). Of the total protein production of the bacterium, 6% consists of urease (Hu & Mobley, 1990). The urease molecule is associated with a 62-kDa [94-base-pair] heat-shock protein, the function of which has not been fully elucidated (Evans *et al.*, 1992).

H. pylori is oxidase-positive and produces large amounts of catalase (Goodwin *et al.*, 1989) and superoxide dismutase (Spiegelhalder *et al.*, 1993). The tetrameric catalase, with subunits of 50 kDa [76 base pairs], has an isoelectric point of 9.0–9.3. *H. pylori* also produces phospholipase A2 and C, γ-glutamyltranspeptidase, DNase, both acid and alkaline phosphatase, a mucus-degrading glycosulfatase (Mégraud *et al.*, 1985; Freland & Drugeon, 1988; Slomiany *et al.*, 1992; Ottlecz *et al.*, 1993), alcohol dehydrogenase (Salmela *et al.*, 1993) and leucine aminopeptidase (Mégraud *et al.*, 1985). It has significant alcohol dehydrogenase activity at both low and high concentrations of ethanol (Salmela *et al.*, 1993; Salaspuro, 1994). Hippurate hydrolysis and nitrate reduction do not occur (Goodwin & Worsley, 1993), nor does *H. pylori* contain indole or produce hydrogen sulfide (Mégraud *et al.*, 1985).

1.1.3 *Agent–host relationship*

(*a*) *Host and target tissues*

Natural infection with *H. pylori* has been demonstrated only in humans and in nonhuman primates. Oral challenge under laboratory conditions may lead to colonization in *Macaca* species, gnotobiotic piglets and dogs (Fox *et al.*, 1991). The reasons for this narrow host range are unknown but may be related to specific binding capacities for human mucosal antigens (Husson *et al.*, 1993). In infected humans, *H. pylori* specifically colonizes the gastric mucosa, as it is uniquely adapted to survive the acidic environment. Within the stomach, infection is usually greatest in the antrum (Dixon, 1991); colonization densities in the acid-producing corpus region of the stomach are lower. For unknown reasons, antral colonization may decrease and corpus colonization may increase under conditions of lower acid output (Louw *et al.*, 1993). Microscopically, the bacterium can usually be observed within the surface mucus layer, both on the surface epithelium and within the pits. Under the electron microscope, it is usually observed close to intercellular junctions of mucus-secreting cells (Hazell *et al.*, 1986; Caselli *et al.*, 1989). It is not found in areas of intestinal metaplasia (Correa *et al.*, 1989). Epithelial cell invasion is very rare (Caselli *et al.*, 1989). The specific affinity of *H. pylori* for gastric epithelium is exemplified by the occasional demonstration of these bacteria on metaplastic gastric mucosa in the oesophagus (Paull & Yardley, 1988), in the duodenum, in Meckel's diverticulum or in the rectum (Offerhaus *et al.*, 1990; Kestemberg *et al.*, 1993).

Interest in possible routes of transmission (see section 1.3) has focused research on the presence of *H. pylori* in the mouth and faeces of infected individuals. Although *H. pylori* has been detected in both dental plaque and faeces (Thomas *et al.*, 1992; Nguyen *et al.*, 1993), a limited number of successful isolations have been made, the number of cases studied is small,

and occasionally the cultured bacteria have been incompletely identified. The bacterium has been found only in the gastrointestinal tract.

(*b*) *Immune response of infected individuals*

The presence of *H. pylori* on the gastric mucosa elicits an inflammatory response in all infected individuals. This response is characterized by inflammatory cells in the mucosa (see sections 1.4 and 4.1) and by local and systemic humoral immune responses. The specific immunoglobulin (Ig)A response, both locally and systemically, consists mainly of the IgA1 subclass (van der Est *et al.*, 1992). The systemic IgG response involves all four subclasses. Different subclass responses have been noted in gastritis patients with and without duodenal ulcer; it is unknown whether this difference is related to the host or to the bacterial strain (Bontkes *et al.*, 1992). The IgG response diminishes within 6–12 months after the infection has been eradicated with antibiotics (Kosunen *et al.*, 1992). It also appears to diminish after histological disappearance of *H. pylori* due to the development of gastric mucosal atrophy, which is unfavourable to colonization; however, only retrospective evidence is available to substantiate this claim (Crabtree *et al.*, 1993a), and long-term follow-up studies have not yet been carried out (Kuipers *et al.*, 1994a).

(*c*) *Colonization factors*

A variety of factors play a role in the establishment and maintenance of *H. pylori* colonization in the strongly acidic stomach. Motility makes possible rapid transit through the acidic lumen and penetration into the viscous epithelial mucus layer, which protects against acid contact. The unipolar flagella are essential for this motility: aflagellated mutants have been shown to be immobile (Suerbaum *et al.*, 1993). Adherence to the gastric epithelium is the next important factor for virulence. Microscopic research has shown adherence to epithelial pedestals (Caselli *et al.*, 1989), and several investigators have shown specific binding capacities for both extracellular matrix proteins and cellular antigens (Borén *et al.*, 1993; Moran *et al.*, 1993). Binding to Lewis^b blood group antigens has been reported (Borén *et al.*, 1993).

The production of enzymes, especially urease, is a third factor of importance in *Helicobacter* colonization. In laboratory experiments, a mutant strain of *H. pylori* with only very weak urease activity was unable to colonize gnotobiotic piglets (Eaton *et al.*, 1991b). Urease inhibition does not, however, eradicate an established infection. *In vitro*, urease-positive bacteria do not survive at pH 1.5 in the absence of urea but can survive when urea is added (Marshall *et al.*, 1990; Ferrero & Lee, 1991). These observations led to the hypothesis that the potent urease is required to establish new infections; however, once the bacteria have reached a protected niche deep within the mucus layer, protection is no longer necessary and urease may be needed only for delivery of nitrogen.

(*d*) *Pathogenic mechanisms*

In the interaction between *H. pylori* and the gastric mucosa, a number of factors have been claimed to play a role in the chronic inflammatory reaction and epithelial cell damage which, in some cases, lead to overt clinical disease (see section 1.4). Firstly, the bacterium secretes several enzymes that can alter the integrity of both the mucus and epithelial cells. It

produces a glycosulfatase that causes loss of mucus viscosity and a diminished capacity to retard hydrogen ion diffusion (Slomiany *et al.*, 1992); mucus secretion is also diminished (Micots *et al.*, 1993). Ammonia produced by the potent urease enzyme is directly toxic to gastric epithelial cells both *in vivo* and *in vitro* (Mégraud *et al.*, 1992; Tsujii *et al.*, 1992). The phospholipase activity of the bacterium (Daw *et al.*, 1993) can cause degradation of membrane phospholipids, and its alcohol dehydrogenase activity leads to production of the toxic acetaldehyde in the presence of ethanol (Salmela *et al.*, 1993). The clinical importance of the latter finding is unknown.

Helicobacter also produce a variety of substances that may damage the infected host. Shedding of bacterial surface proteins in close proximity to the mucosa may have a chemotactic action on leukocytes (Mai *et al.*, 1992). About 50–60% of *H. pylori* strains can produce a cytotoxic protein that causes vacuolization of cultured epithelial cells (Cover *et al.*, 1990; Fox *et al.*, 1992).

1.2 Methods for detection of infection

1.2.1 *Methods based on gastric biopsy specimens*

Specimens collected before treatment from both the antrum and the corpus with standard forceps can be cultured after placing them in either saline (analysis within 4 h) or transport medium (analysis after up to 24 h) or freezing them at –70 °C or in liquid nitrogen (delayed analysis).

(*a*) *Rapid urease test*

The urease in *H. pylori* breaks down urea into carbon dioxide and ammonia; as ammonia raises the pH, a positive reaction can be read on a pH indicator within a few minutes (Langenberg *et al.*, 1984). Urease tests are agar-based, designed for use in hospital and give results in less than 1 h; their sensitivity has been reported to be 80–98% and their specificity close to 100% (Marshall *et al.*, 1987). Clinical experience indicates, however, that this test may not be specific enough to test the success of treatment. A reading at 24 h increases the sensitivity but decreases the specificity.

(*b*) *Histological examination*

Sections, which must include the superficial and foveolar epithelium, are fixed in formaldehyde or Bouin solution. They can be stained with the standard haematoxylin–eosin stain (Taylor *et al.*, 1987), also used in grading gastritis, but most researchers favour the modified Giemsa stain because better contrast with the background is obtained (Gray *et al.*, 1986). *H. pylori* is best seen under oil immersion. A positive result is expressed semi-quantitatively according to the histological subclassification of the Sydney system (see pp. 207–208) (Price, 1991)

The sensitivity and specificity of histological examination for detecting *H. pylori* depend on the observer's experience. Specificity can be impaired by the presence of other spiral bacteria or coccoidal bacteria, and interpretation may be difficult when only a small number of bacteria are present. Histological methods are best for detecting the non-culturable *Helicobacter*, *H. heilmannii* (Heilmann & Borchard, 1991).

(*c*) *Bacteriological tests*

Smears are prepared by scraping a biopsy specimen with the mucus side against the slide. Gram staining allows observation of curved and spiral gram-negative bacteria. This is a quick, simple and inexpensive test with a sensitivity of about 80% (Montgomery *et al.*, 1987).

Culture is the best means of identifying most infectious agents, because the presence of even one bacterium in the specimen can result in the growth of colonies, allowing precise identification of the organism. For optimal recovery of *H. pylori*, biopsy specimens should be ground, and fresh media containing blood, preferably of human origin, should be used (Westblom *et al.*, 1991). 2,3,5-Triphenyltetrazolium chloride can be included in the medium in order to detect early *H. pylori* colonies (Queiroz *et al.*, 1987). Both selective and non-selective media should be inoculated (Tee *et al.*, 1991), and the culture should be incubated in a microaerobic atmosphere at 37 °C for up to 10 days.

H. pylori colonies are identified by microscopic examination and biochemical tests (see above). Antimicrobial susceptibility tests and molecular fingerprinting can be undertaken in cultures. Since acquired resistance has been noted to four groups of agents used to eradicate *H. pylori*—nitroimidazoles, macrolides, fluoroquinolones and rifamycins, resistance—must be monitored in clinical trials (Glupczynski *et al.*, 1991).

(*d*) *Polymerase chain reaction*

The primers used for detection of *H. pylori* by the polymerase chain reaction (PCR) correspond to genes that encode urease (Labigne-Roussel *et al.*, 1989), 16S ribosomal RNA (Ho *et al.*, 1991), a specific 26-kDa [40-base-pair] protein (Hammar *et al.*, 1992) and an uncharacterized 1.9-kilobase-pair fragment of chromosomal DNA (Valentine *et al.*, 1991). No one pair of primers has proved to be superior to another, but the use of two pairs of primers from different genes may increase specificity. PCR can be used to detect specific genes of pathogenic relevance, such as the *cag*A gene (Figura & Crabtree, 1994).

1.2.2 *Methods based on gastric juice samples*

The techniques used for gastric biopsy specimens can also be used for gastric juice samples. PCR is equally reliable for gastric juice and biopsy specimens (Westblom *et al.*, 1993a). Culture is less sensitive when performed with gastric juice, probably because viable *H. pylori* are lost during prolonged contact with acid (Freland & Drugeon, 1988).

1.2.3 *Methods based on faecal specimens*

Techniques based on faecal specimens are still in an early stage of development. *H. pylori* has been cultured from faeces of infants in the Gambia (Thomas *et al.*, 1992) and has been detected by PCR in faeces (Mapstone *et al.*, 1993), although faecal inhibitors of the reaction remain a problem.

1.2.4 *Methods based on dental plaque and saliva samples*

H. pylori has also been cultured from dental plaque (Krajden *et al.*, 1989) and saliva (Ferguson *et al.*, 1993). Use of PCR has been reported, but these techniques cannot be used as diagnostic methods.

1.2.5 *Methods based on blood samples*

The systemic immune response present in 98% of infected individuals (Glupczynski *et al.*, 1992) can be used for the serological diagnosis of infection (Dooley *et al.*, 1989). Cross-reactions to *C. jejuni* may occur (Newell, 1987). After infection, IgG antibodies are detected within a few weeks. Where it has been validated, the sensitivity and specificity of an enzyme-linked immunosorbent assay (ELISA) with IgG are greater than 90%. Ideally, such tests should be standardized in the population under study; however, it may sometimes be difficult to identify a sufficient number of uninfected people as controls. When *H. pylori* has been eradicated, titres decrease consistently after six months (Kosunen *et al.*, 1992). Immunoblotting allows the detection of a *H. pylori*-specific 120–128-kDa [182–194-base-pair] cytotoxin-associated protein, the *cag*A gene product (Crabtree *et al.*, 1991; Tummuru *et al.*, 1993).

1.2.6 *Urea breath test*

Urea can be hydrolysed by the strong urease of *H. pylori*. In the urea breath test, urea labelled with $^{13}CO_2$ is absorbed and subsequently eliminated in the breath. Breath samples are collected before and 30 min after absorption of labelled urea and analysed by mass spectrometry (Graham *et al.*, 1987). A European protocol has been proposed for this test (Logan *et al.*, 1991). Similar tests involve the use of ^{14}C-urea, as $^{14}CO_2$ can be measured easily with a scintillation counter, but some concern has been expressed over the use of a radioactive isotope. Low-dose tests are being developed to overcome this problem (Bell *et al.*, 1987).

1.3 Epidemiology of infection

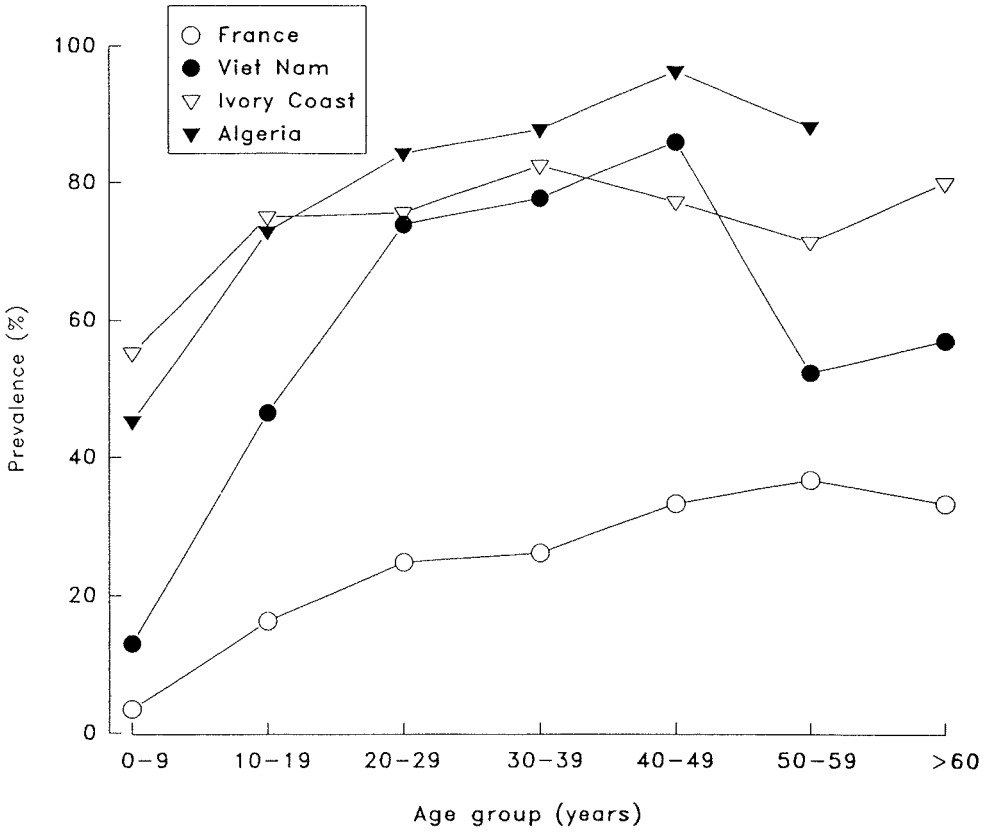
H. pylori infection is long-standing and only rarely resolves spontaneously; it may occasionally be influenced by concomitant antimicrobiological treatment. Thus, it is the prevalence of this infection rather than its incidence that is usually estimated in epidemiological studies (Langenberg *et al.*, 1988; Kuipers *et al.*, 1993a).

1.3.1 *Prevalence*

The prevalence of *H. pylori* infection has been estimated in all the continents on the basis of the results of serological tests on populations such as blood donors, individuals presenting themselves to health centres and volunteers recruited in different ways.

In developing countries, the prevalence of infection increases rapidly during childhood and adulthood and is usually 80–90%. The prevalence is substantially lower in developed countries, especially in childhood (see section 1.3.2). These findings are illustrated in a study in which the same ELISA technique was used in subjects from four countries with different geographical and socioeconomic status (Mégraud *et al.*, 1989) (Figure 1). Similar results were reported in the EuroGast study, in which defined populations of two age groups, 25–34 and 55–64 years, from 17 geographical areas, mainly European, were studied by the same protocol (EuroGast Study Group, 1993a,b; Table 1).

Figure 1. Distribution of seropositivity for *Helicobacter pylori* immunoglobulin G antibodies by age and country of origin



From Mégraud *et al.* (1989)

1.3.2 *Risk factors for infection*

The difference in prevalence between developed and developing countries seems to be linked to socioeconomic factors rather than to ethnicity. In most developed countries, the poorest people also have the highest prevalence. A low level of education and poor housing conditions have been associated with infection (Al Moagel *et al.*, 1990; Fiedorek *et al.*, 1991; Sitas *et al.*, 1991; EuroGast Study Group, 1993a).

No difference in seroprevalence has been found between men and women (Mégraud *et al.*, 1989), and no consistent association has been found with smoking or drinking habits (EuroGast Study Group, 1993a) or any particular diet (Hansson *et al.*, 1993a; Palli *et al.*, 1993). One study in Japan showed a positive association between eating salty food and infection with *H. pylori* (Tsugane *et al.*, 1994). No sexual transmission has been observed (Polish *et al.*, 1991). In cross-sectional studies, an association is always observed between prevalence of infection and age (see Figure 1 and Table 1). Two mechanisms may contribute to this age pattern of prevalence: an age effect, i.e. the progressive acquisition of infection throughout adult life (Graham *et al.*, 1991), and a cohort effect, i.e. a progressive reduction of the rate of infection early in life of people in successive birth cohorts. The extent to which

Table 1. Prevalence of seropositivity to *Helicobacter pylori* in 17 populations

Country	Centre	*H. pylori* seropositivity (%)				
		25–34 years		55–64 years		Total sample
		Male	Female	Male	Female	
Algeria	Algiers	42	44	49	69	200
Belgium	Ghent	20	17	60	47	208
Denmark	Copenhagen	23	5	34	27	157
Germany	Augsburg	14	22	57	65	187
	Deggendorf	40	40	74	76	198
	Mosbach	24	33	65	75	158
Greece	Crete	53	54	80	70	229
Iceland	S. Region	31	40	56	62	206
Italy	Florence	17	14	38	57	205
Japan	Miyagi	55	64	88	87	186
	Yokote	70	54	90	80	200
Poland	Adamowka	69	70	79	93	171
Portugal	Gaia	57	57	73	65	132
Slovenia	Ljubljana	51	27	71	70	201
United Kingdom	Oxford	8	8	49	42	158
	Stoke	27	10	49	41	200
USA	Minneapolis-St Paul	13	16	36	32	198

From EuroGast Study Group (1993b)
NA, not available

these two effects contribute to the cross-sectional association of age with prevalence of infection may vary between populations. The following observations indicate the relative importance of a cohort effect:

- Infection is more strongly correlated to risk factors present during childhood (crowding, size of the family, sharing a bed) than to current risk factors (Mendall *et al.*, 1992; Mitchell *et al.*, 1992; Whitaker *et al.*, 1993; Webb *et al.*, 1994).
- Among adults in developed countries, new cases of infection are uncommon (Mégraud *et al.*, 1989; Rautelin *et al.*, 1990).
- Crude rates of seroconversion from negative to positive have been estimated to be around 0.3–0.5% per year (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1993a); recurrence of infection after eradication therapy may reflect recrudescence of the treated infection rather than true reinfection.
- The cohort effect has been demonstrated in a cohort in the USA (Parsonnet *et al.*, 1992) and in a cohort in the Netherlands (Kuipers *et al.*, 1993a) as well as in a study performed in the United Kingdom. In the last study, sera collected from the same area in 1969, 1979 and 1989, tested for *H. pylori* antibodies by immunoblot and plotted by age group showed that at a given age the prevalence had decreased over the two decades (26% per decade) (Banatvala *et al.*, 1993).

In some populations, a decrease in seroprevalence has been observed in older people. This finding has been attributed to the disappearance of *H. pylori* from the gastric mucosa (loss of *H. pylori* infection) when atrophy develops as a result of long-standing gastritis. Such loss has been observed in some populations (Karnes *et al.*, 1991; Kuipers *et al.*, 1994b) but not in another (Guarner *et al.*, 1993). Furthermore, it is still unclear whether a gradual decrease in *H. pylori* colonization also leads to negative seroconversion. Negative seroconversion was claimed in one retrospective study (Crabtree *et al.*, 1993a) but not in two prospective studies (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1994a).

The prevalence of infection is consistently higher in institutionalized children than in control groups from the surrounding area (Berkowitz & Lee, 1987; Pérez-Pérez *et al.*, 1990).

For a long time, the stomach was thought to be sterile, and precautions such as the use of gloves were not taken in performing endoscopies. A higher prevalence of *H. pylori* infection has now been found among gastroenterologists who perform endoscopies than among other physicians or dentists (Mitchell *et al.*, 1989). In countries with a high prevalence of infection, endoscopists have, nevertheless, a lower prevalence than the general population, probably due to the fact that they come from the middle and upper classes (Matysiak-Budnik *et al.*, 1994).

1.3.3 *Routes of transmission*

Reservoirs of *H. pylori* are the digestive tracts of humans and some primates. Transmission from reservoirs is considered to be person-to-person. This assumption is supported by the finding of clustering of similar strains within families, as shown by molecular fingerprinting (Bamford *et al.*, 1993) and by the consistent demonstration of close interpersonal contact as a risk factor for infection. The *H. pylori* status of mothers of *H. pylori*-positive children is significantly different from that of mothers of *H. pylori*-negative children, indicating that the intimate contact between mother and child could be a cause of transmission (Drumm *et al.*, 1990). Transmission can exist between couples: 68% of spouses of *H. pylori*-infected people were infected, whereas 9% of spouses of uninfected people were infected (Malaty *et al.*, 1991). In another study, the association disappeared in a multiple logistic regression analysis (Pérez-Pérez *et al.*, 1991). Two modes of transmission have been proposed: oral–oral and faecal–oral transmission.

(*a*) *Evidence for faecal–oral transmission*

H. pylori is eliminated in faeces after turnover of the gastric mucosa. It has been detected by PCR (Mapstone *et al.*, 1993) and by culture (Thomas *et al.*, 1992). Consumption of raw vegetables fertilized with human faeces was found to be a risk factor for infection in Santiago, Chile (Hopkins *et al.*, 1993), and consumption of municipal water was found to be a risk factor in children in Lima, Peru (Klein *et al.*, 1991). *H. pylori* has been detected by PCR in sewage water in Peru (Westblom *et al.*, 1993b).

(*b*) *Evidence for oral–oral transmission*

H. pylori has been detected in the oral cavity (Mapstone *et al.*, 1993) and in the saliva of one person (Ferguson *et al.*, 1993). Several claims have been made of the detection of *H. pylori* by PCR in dental plaque (Krajden *et al.*, 1989; Majmudar *et al.*, 1990). When

gnotobiotic puppies infected with *H. felis* were put together with uninfected litter-mates in a germ-free isolator, with continual oral–oral contact, the agent was transmitted. Transmission did not occur between germ-free mice, which are coprophageous, under the same conditions (Lee *et al.*, 1991).

1.4 Clinical disease in humans (other than cancer)

1.4.1 *Gastritis*

H. pylori is a major cause of gastritis. This inference is based on the following observations: (i) ingestion of *H. pylori* led to acute gastritis in a small number of case studies (Marshall *et al.*, 1985a; Morris & Nicholson, 1987; Sobala *et al.*, 1991); (ii) *Helicobacter* colonization of the stomach is virtually always accompanied by inflammation of the mucosa (Dixon, 1991); (iii) *H. pylori* infection can be detected in more than 85% of patients with inflammation of the gastric mucosa (Dooley *et al.*, 1989); and (iv) this inflammation disappears completely within two to three years after eradication of the infection (Rauws *et al.*, 1988; Genta *et al.*, 1993a).

The infection disappears only as a result of antibiotic therapy, after the development of unfavourable gastric conditions such as mucosal atrophy or after partial gastrectomy with bile reflux (Karnes *et al.*, 1991; Kuipers *et al.*, 1993a). 'Spontaneous' clearance of infection is very rare and may in fact be due to unreported use of antibiotics (Kuipers *et al.*, 1993a). In some infected individuals, endoscopic signs of gastritis can be found. The gastritis affects predominantly the antrum (Tytgat *et al.*, 1993), although corpus involvement is observed histologically in most infected individuals (see also section 4).

1.4.2 *Duodenal ulcer disease*

H. pylori infection is the most significant risk factor for duodenal ulcer disease. After exclusion of a small subset of cases of duodenal ulcer with specific etiology, such as use of non-steroidal anti-inflammatory drugs, Crohn's disease or ischaemia, the remaining cases are caused by *H. pylori* (Mégraud & Lamouliatte, 1992). The main arguments for a causal relationship between *H. pylori* infection and duodenal ulcer disease are that the infection is seen to precede the disease (Sipponen *et al.*, 1990) and that the disease disappears after treatment of the infection. While ulcers have been shown in many studies to relapse within 12 months after symptomatic treatment in 50–100% of patients (Tytgat *et al.*, 1993), eradication of *H. pylori* almost totally prevents ulcer recurrence (Marshall *et al.*, 1988; Graham *et al.*, 1992; Tytgat *et al.*, 1993). It has been estimated that up to 10% of infected people will develop duodenal ulcer during life (Tytgat *et al.*, 1993).

1.4.3 *Gastric ulcer disease*

H. pylori infection is present in approximately 70% of patients with gastric ulcers (Labenz & Börsch, 1994). A variety of noxious agents such as non-steroidal anti-inflammatory drugs and bile reflux are risk factors for the development of gastric ulcers. After exclusion of patients with those risk factors, the bacterium is present in more than 95% of the remaining cases. Eradication of the infection significantly prevents ulcer recurrence (Graham *et al.*, 1992; Labenz & Börsch, 1994).

1.4.4 *Hypertrophic protein-losing gastritis*

Hypertrophic protein-losing gastritis is a rare clinical disorder characterized by chronic gastritis with giant folds, gastric protein loss and hypoalbuminaemia. The etiology of this disorder is unknown. Significant clinical improvement was seen after *H. pylori* eradication therapy in two studies (Lepore *et al.*, 1988; Meuwissen *et al.*, 1992).

1.4.5 *Childhood diseases*

In children in developing countries, *H. pylori* infection has been associated with chronic diarrhoea and malnutrition (Sullivan *et al.*, 1990). In developed countries, it has also been associated with chronic abdominal pain and growth retardation.

1.5 Treatment and control

1.5.1 *Antibiotics and acid suppressive therapy*

Since the introduction of H2-blockers and proton pump inhibitors, *H. pylori*-related disorders have been treated with moderate success (Susi *et al.*, 1994). The effects of acid suppressive medication on *H. pylori*-related gastritis have not been examined adequately; however, such medication does not cure the infection (Kuipers *et al.*, 1993b). The bacterium is sensitive to a wide range of antibiotics *in vitro*, but most are unsuccessful *in vivo*. Three strategies have been chosen to overcome this problem: (i) combination of multiple synergistic antibiotic drugs; (ii) prolongation of drug administration; and (iii) combination of antibiotics with acid suppressors. A large number of clinical trials have been carried out to find an effective treatment regimen. The current preference is for therapy lasting 14 days with either two antibiotics combined with a bismuth preparation or with one to two antibiotics combined with an acid inhibitor, usually omeprazole (Labenz *et al.*, 1993). With these regimens, eradication has been achieved in 60–95% of cases, depending upon the prevalence of antibiotic-resistant strains and patient compliance.

1.5.2 *Vaccination*

H. pylori infection is always accompanied by local and systemic immune responses, with no clearance of infection (Bontkes *et al.*, 1992). It is thus unclear whether immunization can prevent new infections. Successful oral immunization of mice with a sonicated preparation of *H. felis* plus adjuvant (cholera toxin) has been achieved (Chen *et al.*, 1993).

2. Studies of Cancer in Humans

2.1 Descriptive studies

2.1.1 *Geographical correlations*

(*a*) *Gastric carcinoma*

Table 2 lists eight studies in which the prevalences of *H. pylori* infection were compared in geographical regions with different gastric cancer rates. The presence of infection was

Table 2. Geographical correlation studies of the prevalence of *Helicobacter pylori* infection and incidence or mortality rates for gastric cancer

Country	Populations	Total number of people surveyed	Gastric cancer		*H. pylori* infection		Results of comparison	Reference
			Period	Range of occurrence (rate)	Period	Range of prevalence (%)		
Colombia	Gastrointestinal patients, aged 15–84; 1 low-risk, 1 high-risk city	78	1972–81	Incidence, 26–150/100 000	NR	63–93	$p = 0.01$	Correa *et al.* (1990a)
Costa Rica	Healthy individuals, aged 7–20; 1 low-risk, 1 high-risk rural area	282	1984–88	Incidence, 20–49/100 000	NR	66–72	$p > 0.05$	Sierra *et al.* (1992)
Italy	Population sample, aged 35–74; 3 high-risk, 2 low-risk areas	930	1975–77	Mortality, 3–43/100 000	1985–88	44–45	$p > 0.05$	Buiatti *et al.* (1989a); Palli *et al.* (1993)
China	Gastrointestinal patients, aged 17–72; 1 low-risk, 1 medium-risk, 1 high-risk area	690	1985–87	CM, 8–60/ 100 000	NR	13–63[a]	$p < 0.01$	Lin *et al.* (1989)
Japan	Blood donors, aged 16–64; 4 prefectures	1815	1982–87	SM, 48–136 (M), 40–117 (F)	NR	50–60 (M) 41–60 (F)	[$r = 0.01$, $p > 0.05$ (M) $r = -0.57$, $p > 0.05$ (F)]	Fukao *et al.* (1993)
Japan	Population sample, aged 40–49, men, 5 areas	624	1985–89	CM, 0–74, 2.2–5.7%	NR	63–86	$r = 0.75$ [$p = 0.14$]	Tsugane *et al.* (1993)
China	Population sample, men aged 35–64; 46 rural counties	1882	1973–75	CM, 0–64, 0.3–6.9%	1983	28–96	$r = 0.34$[c] [$p = 0.02$]	Forman *et al.* (1990)
13 countries	Population sample, aged 25–34 and 55–64; 17 areas or cities (16 with data on mortality, 11 with data on incidence)	3194	Early–mid-1980s	CI, 0–74, 0.9–9.9% (M) 0.3–4.0% (F) CM, 0–74, 0.6–5.7% (M) 0.2–2.1% (F)	NR	8–70 (25–34 years) 31–87 (55–64 years)	$\beta = 1.79$ ($p = 0.002$) (M) $\beta = 2.68$ ($p = 0.001$) (I)	EuroGast Study Group (1993b)

NR, not reported; CM, cumulative mortality; SM, standardized mortality; CI, cumulative incidence; (M), males; (F), females

[a]Based on gastric biopsy

[b]Other cancer sites also studied

[c]$r = 0.40$ after adjustment for within-county variability

determined in most studies by ELISA for IgG antibodies to *H. pylori* in serum. In all studies, infection rates were compared with cancer rates in contemporaneous time periods, although a more appropriate comparison would be between infection prevalence rates and cancer rates several years or even decades later. Such a comparison would reflect the time sequence involved if there were a causal relationship between infection and cancer.

Four of the studies were comparisons of regions of high and low risk for gastric cancer within a single country; two showed a significant difference between *H. pylori* prevalence rates, with an increase in the high-risk region (in Colombia, Correa *et al.*, 1990a; and in China, Lin *et al.*, 1989), while the other two showed no significant difference between the two regions (in Italy, Palli *et al.*, 1993; and in Costa Rica, Sierra *et al.*, 1992). Two studies from Japan (Fukao *et al.*, 1993; Tsugane *et al.*, 1993) compared populations within five and four areas, respectively; neither showed a significant association between *H. pylori* seropositivity and gastric cancer mortality.

Forman *et al.* (1990) examined the prevalence of *H. pylori* IgG antibodies in 1882 residents of 46 rural counties in China and compared them with the gastric cancer mortality rates in the same counties. The correlation between *H. pylori* antibody prevalence rate and gastric cancer mortality rate was 0.34 ($p = 0.02$). The significant positive correlation remained after adjustment for dietary factors associated with risk for gastric cancer (Kneller *et al.*, 1992).

The EuroGast Study Group (1993b) examined the seroprevalence of *H. pylori* IgG antibodies in 3194 randomly selected subjects resident in 17 centres in 13 countries, chosen to reflect the global range in gastric cancer incidence. In regression analyses, in which the two sexes were combined, there were significant relationships between the prevalence of *H. pylori* antibodies and both log-transformed gastric cancer cumulative mortality ($p = 0.002$) and incidence ($p = 0.001$) rates. Exclusion of the regions with highest and lowest mortality rates (Japan and the USA, respectively) reduced the strength of the relationship with mortality from gastric cancer to a nonsignificant ($\beta = 0.62; p = 0.3$) level (Forman *et al.*, 1993).

It has been noted (Holcombe, 1992) in Nigeria and other African countries (e.g. Sudan, Uganda and Zimbabwe) that gastric cancer rates are relatively low (< 2–3% of all malignant tumours) despite a very high prevalence of *H. pylori* infection. The populations of other developing countries with low incidence rates of gastric cancer, but for which no estimates of the prevalence of infection are available, include Kuwaitis, non-Jews in Israel, Malays in Singapore and those of Ahmedabad, Bangalore, Madras and Bombay in India. Gastric cancer incidence rates in the three population-based cancer registries in Africa (Sétif, Algeria; Bamako, Mali; and the Gambia) range from 3.9 to 19.4 per 100 000 in males and from 1.5 to 10.3 per 100 000 in females (Parkin *et al.*, 1992). These rates are substantially below those in high-risk regions of the world (e.g. Costa Rica: 46.9 in males and 21.3 in females) and are comparable to the rates in US blacks (12.4 in males and 5.6 in females) and in England and Wales (16.9 in males and 6.8 in females).

(*b*) *Gastric lymphoma*

Doglioni *et al.* (1992) compared the incidence of primary gastric lymphoma, determined from endoscopy clinic records, in an area of northeastern Italy with that in three communities

in the United Kingdom. In the Italian city of Feltre, the estimated incidence rate for gastric lymphomas was 66/100 000 per five years for the period 1986–90 (37 cases). In three districts in the United Kingdom, the comparable rates were 6/100 000 (six cases), 4/100 000 (seven cases) and 6/100 000 (20 cases). The *H. pylori* infection rate of all patients undergoing endoscopic biopsy was 87% in Feltre in 1991 and 50–60% in the United Kingdom. [The Working Group noted that this was a hospital-based study with no information about the referral patterns to the local endoscopy units. There is, therefore, uncertainty about the denominator populations used in this study.]

(c) *Other cancers*

In the study of Forman *et al.* (1990) (see above), correlation coefficients were calculated for associations between *H. pylori* IgG antibody prevalence and mortality rates from cancers at 12 sites other than the stomach. None was significant. The correlation with lymphoma (all types) was 0.32 and of borderline significance.

2.1.2 *Time trends*

Gastric cancer incidence and mortality rates have been declining rapidly in nearly all developed countries for several years. There are few data for developing countries, but the same trend has generally been observed (Coleman *et al.*, 1993). Secular trends in the prevalence of *H. pylori* infection have not been investigated extensively, but the one serological study that has been conducted in the United Kingdom (Banatvala *et al.*, 1993) indicated that the prevalence has decreased in recent decades. If *H. pylori* is acquired predominantly in childhood (see section 1.3.2), then data on age prevalence (section 1.3.1) can be interpreted as indicating a declining prevalence rate over much of the 20th century. This is also consistent with observed secular trends in duodenal ulcer disease in the USA (Sonnenberg, 1993), the United Kingdom (Susser & Stein, 1962) and Europe (La Vecchia *et al.*, 1993), a disease strongly associated with *H. pylori* infection. Data from Japan (Blaser, 1993) indicate that mortality from gastric cancer in that country has decreased over the past 50–80 years, an effect consistent with a secular decrease in exposure to an environmental agent. The prevalence of gastric cancer of the cardia, in contrast to that of more distal sites within the stomach, has been shown to be increasing in a number of populations (Powell & McConkey, 1990; Blot *et al.*, 1991; Hansson *et al.*, 1993b). Gastric cancer of the cardia has been shown in some studies (Talley *et al.*, 1991a; Hansson *et al.*, 1993b) not to be associated with *H. pylori* infection (see sections 2.3 and 2.4).

2.1.3 *Socioeconomic trends*

Gastric cancer has been shown consistently in several countries to be commoner in poorer socioeconomic groups (Howson *et al.*, 1986; Buiatti *et al.*, 1989b; Logan, 1982). The same association has been observed consistently for *H. pylori* infection (see section 1.3.2).

2.2 Case series

2.2.1 *Gastric carcinoma*

The presence of *H. pylori* infection has been determined in numerous series of gastric cancer patients, usually by histological examination of biopsy and/or gastrectomy samples

but also by microbiological culture; in some studies, serological tests were used to determine the presence of specific IgG antibodies to *H. pylori*. A number of studies were designed specifically to estimate the prevalence of *H. pylori* infection in gastric cancer patients; the majority, however, were broader surveys of patients with upper gastrointestinal disease and included a small subgroup of patients with gastric cancer. In the latter studies, it is unclear whether adequate mucosa was available to evaluate the presence of *H. pylori*; there was also frequently a subgroup of patients who had dyspeptic symptoms but no lesions in their stomachs and who were used as a control series. In a few studies, the control series were healthy volunteers who had undergone endoscopy. Serologically based studies in which data from matched case and control series were available are summarized in sections 2.3 and 2.4.

Table 3 lists the 11 largest case series. The percentage of gastric cancer patients who had *H. pylori* infection varied from 43 to 83%. Particular interest has focused on the Laurén histological classification of gastric adenocarcinoma into cancers of the intestinal (glandular) type and cancers of the diffuse type (Laurén, 1965). It has been reported that the incidence of the former varies between populations whereas that of the latter remains relatively constant (Laurén, 1965; Muñoz *et al.*, 1968; Muñoz & Asvall, 1971; Correa *et al.*, 1973). Environmental exposures are thought to be more important in the etiology of intestinal-type than of diffuse-type cancers (Howson *et al.*, 1986). Table 4 lists eight series in which the cancer cases were classified into intestinal and diffuse histological categories. In some of these studies, an increased prevalence of *H. pylori* infection was seen in association with intestinal-type cancers (Parsonnet *et al.*, 1991a; Tatsuta *et al.*, 1993), but this difference was not observed consistently.

2.2.2 *Gastric lymphoma*

Wotherspoon *et al.* (1991) examined 110 patients in the United Kingdom with gastric B-cell mucosa-associated lymphoid tissue lymphomas, a subset of primary gastric lymphomas. In this group, 101/110 patients (92%) had histological evidence of *H. pylori* infection.

A total of 205 surgical specimens containing primary malignant B-cell lymphomas were investigated in Germany. *H. pylori* colonization was found in 175/178 (98%) cases in which the mucosa some distance from the tumour could be evaluated (Stolte *et al.*, 1994).

2.3 Cohort studies

2.3.1 *Gastric carcinoma*

Four prospective studies have been reported in which the relationship between *H. pylori* infection and the subsequent risk of gastric cancer has been assessed. All were case–control comparisons nested in prospective cohort studies in which blood samples had been taken from cancer-free individuals and stored. Specific antibodies to *H. pylori* were then measured in blood samples from individuals who subsequently developed gastric cancer, and the proportion of individuals with antibodies was compared with that in a matched control group.

Table 3. Prevalence of *Helicobacter pylori* in series of gastric cancer cases

Country	Gastric cancer cases			Method of assessment	Comments	Reference
	No.	*H. pylori* infection				
		No.	%			
Europe						
Italy	44	26	59	Histology	22/44 cases were 'early' gastric cancer, 17 (77%) positive	Caruso & Fucci (1990) (letter)
Italy	277	216	78	Histology	137/167 (82%) early gastric cancers positive; 79/110 (72%) advanced gastric cancers positive	Fiocca *et al.* (1993)
Netherlands	91	54	59	Histology		Loffeld *et al.* (1990)
Turkey	46	34	74	Histology		Buruk *et al.* (1993)
United Kingdom	136	67	49	Histology		Armstrong *et al.* (1991) (letter)
United Kingdom	224	96	43	Histology		Clarkson & West (1993)
North America						
USA	59	40	68	Histology		Parsonnet *et al.* (1991a)
South America						
Brazil	40	33	83	Histology	18/19 (94%) cases positive by histology and culture	Nogueira *et al.* (1993)
Asia						
Japan	94	66	70	Serology		Takahashi *et al.* (1993)
Japan	41	24	59	Culture	All tumours were 'early' gastric cancers	Tatsuta *et al.* (1993)
Singapore	137	103	75	Histology		Wee *et al.* (1992)

Table 4. Prevalence of *Helicobacter pylori* infection in gastric cancer case series by histological type (Laurén classification)

Country	Histological classification[a]						Reference
	Intestinal			Diffuse			
	Total no.	*H. pylori* infection		Total no.	*H. pylori* infection		
		No.	%		No.	%	
Europe							
Italy	166	119	72	79	71	90	Fiocca *et al.* (1993)
Netherlands	80	48	60	11	5	45	Loffeld *et al.* (1990)
Turkey	26	23	88	20	11	55	Buruk *et al.* (1993)
United Kingdom	120	56	47	69	24	35	Clarkson & West (1993)
North America							
USA	37	33	89	22	7	32	Parsonnet *et al.* (1991a)
South America							
Brazil	31	24	77	5	5	100	Nogueira *et al.* (1993)
Asia							
Japan[b]	24	19	79	17	5	29	Tatsuta *et al.* (1993)
Singapore	87	64	74	50	39	78	Wee *et al.* (1992)

[a]Method of assessment shown in Table 3.
[b]In this study the terms 'differentiated early gastric cancer' and 'undifferentiated early gastric cancer' were used for intestinal and diffuse, respectively.

Forman *et al.* (1991) compared 29 gastric cancer patients with 116 age-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Steer *et al.*, 1987) with a reported sensitivity of 93% and a specificity of 96% (Talley *et al.*, 1991b). The subjects were all men taking part in one of two cohort studies: in one study, 20 179 men, aged 35–64 and living in south-east England, provided blood between 1975 and 1982 during a health check-up. In the other study, 2512 men, aged 45–59 and living in Caerphilly, Wales, provided blood between 1979 and 1982 as part of a population study of cardiovascular disease. Cancers or deaths among cohort participants were notified to the study organizers routinely; 23 men with gastric cancer were identified from the first cohort and six from the second. Cancers were diagnosed between 1980 and 1989, with a mean interval between blood sampling and diagnosis of six years (range, four months to 13 years seven months). The mean age of the cancer patients was 54 years (range, 41–63 years) at blood sampling and 60 years (range, 47–76 years) at diagnosis. Four controls were selected for each case by

matching on cohort, date of birth (within one year), date of blood sampling (within one year) and number of freeze–thaw cycles the blood sample had undergone. Twenty of the 29 (69%) gastric cancer patients and 54 of the 116 (47%) controls had antibodies to *H. pylori*, resulting in a matched odds ratio of 2.8 (95% confidence interval [CI], 1.0–8.0). Stratifying the cases and corresponding controls into those diagnosed within five years of blood sampling and those diagnosed five or more years after sampling did not result in a significant difference in the resulting odds ratios. No information was available on site of cancer within the stomach or on histological subtype.

Parsonnet *et al.* (1991b) compared 109 gastric patients with 109 age-, sex- and race-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Evans *et al.*, 1989) with a reported sensitivity of 91% and a specificity of 98%. The subjects were taking part in a cohort study in which 128 992 participants living in California, USA, provided blood between 1964 and 1969 during a health check-up. A total of 246 gastric cancer registrations and/or hospitalizations for gastric cancer among cohort participants were notified to the study organizers routinely, and 200 of these were randomly selected. Availability of blood samples resulted in final inclusion of 186 patients with gastric cancer. Cancers were diagnosed between 1964 and 1989, with a mean interval between blood sampling and diagnosis of 14.2 years (range, 1–24 years). One control was selected for each case by matching on age at blood sampling (within one year), sex, race, date of blood sampling (within 0.5 year) and site of the health check-up. Of the 186 patients, 109 had histologically confirmed adenocarcinoma of the stomach; of these, 92 (84%) had antibodies to *H. pylori*, as did 66 of the 109 (61%) controls, resulting in a matched odds ratio of 3.6 (95% CI, 1.8–7.3). When the cases and controls were stratified by sex, the odds ratio for women was nonsignificantly higher than that for men; when they were stratified by race, the odds ratio for blacks was nonsignificantly higher than that for whites. Eighty-one patients had the intestinal type of adenocarcinoma (Laurén classification), and 67 (83%) of these were seropositive (odds ratio, 3.1; 95% CI, 1.5–6.6); 28 patients had a diffuse type, and 25 (89%) of these were seropositive (odds ratio, 8.0; 95% CI, 1.0–64). Four patients had an adenocarcinoma at a site in the cardia; one was seropositive, as was one of the four matched controls. An additional 27 patients had adenocarcinoma of the gastroesophageal junction (not included in the main analyses above); of these, 17 (63%) were seropositive, as were 19 (70%) controls (odds ratio, 0.8; 95% CI, 0.3–2.1).

Nomura *et al.* (1991) compared 109 patients with gastric carcinoma with 109 age-matched controls for the presence of IgG antibodies to *H. pylori* using a commercial ELISA. The subjects were all men taking part in a cohort study in which 7498 Japanese–Americans living in Oahu, Hawaii, USA, provided blood between 1967 and 1970 as part of a population study of heart disease. A total of 137 gastric cancer registrations and/or hospital discharges for gastric cancer among cohort participants were notified to the study organizers routinely, all with histologically confirmed gastric cancer. As insufficient serum was available from 26 men and the results of the ELISA were indeterminate for two, a total of 109 were included in the study. Cancers were diagnosed between 1968 and 1989, with a mean interval between blood sampling and diagnosis of 13 years (standard deviation, five years). The mean age of the cancer patients at recruitment was 59 years. One control was selected for each case by matching for age at recruitment and date of blood collection. Excluded from the control

series were men who had had a gastrectomy before blood sampling or who had had a diagnosis of peptic ulcer at any time. The exclusion criteria reduced the pool of available controls by 13%. [The Working Group noted that the exclusion criteria would be likely to reduce the prevalence of *H. pylori* infection in the control group and, hence, bias the estimated odds ratio upwards.] Also excluded were men with cardiovascular disease or any other type of cancer diagnosed at any time. These exclusions reduced the control pool by 33%. Controls had to be alive when the cancer cases with which they were matched were diagnosed. Of the 109 gastric cancer patients, 103 (94%) had antibodies to *H. pylori*, as did 83 of the 109 (76%) controls, resulting in a matched odds ratio of 6.0 (95% CI, 2.1–17). Stratification of the cases into three groups (26, 40 and 43 pairs) on the basis of time between blood sampling and cancer diagnosis resulted in odds ratios of 1.5 (95% CI, 0.3–9.0) for less than 10 years, 6.0 (1.3–27) for 10–14 years and indeterminate (1.7–97) for 15 years or more. Stratification into two birth cohorts resulted in odds ratios of 3.0 (0.8–11) for those born in 1900–09 and 15 (2.0–114) for those born in 1910–19. Eighty-one patients had an intestinal type of carcinoma, and 75 (93%) of these were seropositive (odds ratio, 4.5; 95% CI, 1.5–13); 23 patients had a diffuse type, and all were seropositive (odds ratio, indeterminate; 1.1–64). Five patients had cancer at the cardia, and two were seropositive; after exclusion of these patients, the overall odds ratio was 12 (95% CI, 2.8–51). In this study, a trend was observed ($p = 0.0009$) of an increasing odds ratio with an increase in the quantitative antibody level. [The Working Group estimated that, had the exclusion criteria relating to controls with a history of gastrectomy or peptic ulcer not been used, the prevalence of *H. pylori* infection in the controls would have been increased by approximately 4% and the overall odds ratio would have been decreased by about 20%, i.e. from 6.0 to 4.8.]

In a combined analysis of the three nested case–control studies described above, Forman *et al.* (1994) showed that, overall, 215/247 (87%) gastric cancer patients and 203/334 (61%) controls were seropositive for IgG antibodies to *H. pylori*, resulting in a matched odds ratio of 3.8 (95% CI, 2.3–6.2). When these results were stratified by time between sample collection and cancer diagnosis into four periods—fewer than five years, 5–9 years, 10–14 years and 15 years or more—there was a significant trend ($p = 0.049$) towards an increased odds ratio with increasing time interval. The odds ratio changed from 2.1 (95% CI, 0.6–8.7) to 2.3 (0.9–6.5), 4.4 (1.8–13) and 8.7 (2.7–45) over the four periods, respectively. There were 20/25 (80%), 37/46 (80%), 70/78 (90%) and 88/98 (90%) seropositive cases and 34/58 (59%), 46/85 (54%), 58/93 (62%) and 65/98 (66%) seropositive controls in the four strata, respectively. This trend was interpreted by the authors as indicating that false-negative assessments of *H. pylori* status may have occurred more frequently among cancer cases than among matched controls, especially among those diagnosed soon after providing blood. False-negative assessments were believed to derive from the precancerous conditions, severe atrophic gastritis and intestinal metaplasia, from loss of *H. pylori* colonization and loss of seropositivity.

Lin *et al.* (1993a [abstract]) compared 29 gastric cancer patients in Taiwan, China, with 220 controls matched by age, sex and area of residence, for the presence of *H. pylori* IgG antibodies by an ELISA. The subjects were participants in a cohort study in which 9777 people in Taiwan had provided blood since 1984. The mean interval between blood sampling and diagnosis of cancer was 3.1 years. Sixty-nine percent of the gastric cancer patients and

59% of the controls were seropositive for antibodies to *H. pylori*, resulting in an odds ratio of 1.6 (95% CI, 0.68–2.6).

The four prospective studies are summarized in Table 5.

2.3.2 *Gastric lymphoma*

Parsonnet *et al.* (1994) compared 33 patients with gastric non-Hodgkin's lymphoma with 134 age- and sex-matched controls for the presence of *H. pylori* IgG antibodies using an ELISA with a reported sensitivity of 96% and a specificity of 76% for active gastric infection. The subjects were taking part in one of two cohort studies, one in California, USA, described above (Parsonnet *et al.*, 1991b), and the other of 170 000 participants living in Norway who provided blood between 1973 and 1991 during blood donation and health screening programmes. Cancer registrations between 1973 and 1990 among the Norwegian cohort were notified to the study organizers. Twenty gastric lymphomas were identified from the US cohort and 13 from the Norwegian cohort, with median intervals between blood sampling and diagnosis of 14 and 13 years, respectively. The median ages of the lymphoma patients at diagnosis were 66 and 55 years, and 40 and 69% patients in the two cohorts were men, respectively. Four cancer-free controls were selected for each case and matched on cohort, date of birth, age (five-year groups in the USA; within six months in Norway), sex, date and location of blood collection and ethnic group (only in the USA). Twenty-eight of the 33 (85%) gastric lymphoma patients were seropositive for antibodies to *H. pylori*, as were 74 of the 134 (55%) controls, resulting in a matched odds ratio of 6.3 (95% CI, 2.0–20). There was no significant difference between odds ratios when the cases and corresponding controls were stratified on the basis of cohort, sex, age at diagnosis (< 65 or ≥ 65 years) or time between blood sampling and diagnosis (< 14 or ≥ 14 years). In a separate analysis of 31 patients with non-gastric non-Hodgkin's lymphoma and 61 matched controls, 20 patients (65%) and 36 controls (59%) were seropositive, resulting in a matched odds ratio of 1.2 (95% CI, 0.5–3.0).

2.4 Case–control studies

2.4.1 *Gastric carcinoma*

Nine case–control studies have been carried out in which serological assessment of infection was done retrospectively in cancer patients after diagnosis.

Talley *et al.* (1991a) compared 69 patients with gastric adenocarcinoma with 252 controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Pérez-Pérez *et al.*, 1988) with a reported sensitivity of 96% and a specificity of 94% (Talley *et al.*, 1991b). The cases of cancer had been confirmed histologically and diagnosed between 1982 and 1989 at a single hospital in Minnesota, USA. The median age of the patients was 63 years (25th and 75th percentiles, 56.5 and 71 years), and 52% were men. The controls comprised 76 asymptomatic volunteers with no history of gastrointestinal disease and 176 patients who were treated between 1976 and 1989 at the same hospital as the cancer patients for a variety of non-malignant conditions: 67 for benign musculoskeletal problems, 52 for benign oesophageal disease and 57 for benign lung diseases. The median age of the controls

Table 5. *Helicobacter pylori* seroprevalence rates in gastric cancer patients and matched controls: prospective studies

Country	Cohort	Cases			Controls				Odds ratio[a]	95% CI	Mean follow-up (years)	Reference
		No.	*H. pylori* infection		No.	Matching	*H. pylori* infection					
			No.	%			No.	%				
United Kingdom	English undergoing health check-up; Welsh heart disease study Men	29	20	69	116	Cohort, date of birth, date of blood sampling, no. of freeze–thaw cycles	54	47	2.8	1.0–8.0	6	Forman *et al.* (1991)
USA (California)	Men and women undergoing health check-up	109	92	84	109	Age, sex, rate, date of blood sampling, place of health check-up	66	61	3.6	1.8–7.3	14	Parsonnet *et al.* (1991b)
USA (Hawaii)	Japanese–Americans; heart disease study Men	109	103	94	109	Age, date of blood sampling	83	76	6.0	2.1–17	13	Nomura *et al.* (1991)
China (Taiwan)	General population Men and women	29	20	69	220	Age, sex, residence	130	59	1.6	0.68–2.6	3	Lin et al. (1993a) [abstract]

CI, confidence interval
[a]From matched analysis

was 61 years (25th and 75th percentiles, 54 and 67 years), and 50% were men. Of the 69 gastric cancer patients, 36 (52%) had antibodies to *H. pylori*, as did 96 (38%) of the controls. The odds ratio, after adjustment for age and sex, was 1.6 (99% CI, 0.79–3.4). Adjustment for length of storage of the blood samples had no substantial effect on the results. The odds ratio for gastric cancers at sites other than the cardia ($n = 37$) was 2.7 (99% CI, 1.0–7.1), while that for cancers at sites in the cardia ($n = 32$) was 0.94 (99% CI, 0.34–2.6). For the intestinal type of gastric cancer, according to the Lauren classification ($n = 32$), the odds ratio was 1.9 (99% CI, 0.67–5.1), while for cancers of the diffuse histological type ($n = 22$) it was 2.5 (99% CI, 0.73–8.2). After the cancers of the cardia had been excluded, the odds ratios were 4.6 (99% CI, 0.78–27) for the intestinal type ($n = 13$) and 2.3 (99% CI, 0.63–8.1) for the diffuse type ($n = 19$). There were five additional groups of patients in this study. The proportions with antibodies to *H. pylori* were 44% of nine with benign gastric lesions, 89% of nine with gastric cancers other than adenocarcinoma, 51% of 80 with colorectal cancer, 49% of 41 with oesophageal cancer and 56% of 79 with lung cancer. In comparisons with the cancer-free control group, as used in the study of gastric adenocarcinoma, the odds ratios, after adjustment for age and sex, were 1.5 (99% CI, 0.23–9.1) for benign gastric neoplasms, 13 (99% CI, 0.77–203) for other gastric cancers, 1.8 (99% CI, 0.86–3.4) for colorectal cancer, 1.4 (99% CI, 0.58–3.4) for oesophageal cancer and 1.8 (99% CI, 0.91–3.6) for lung cancer.

Sipponen *et al.* (1992) compared 54 patients with gastric adenocarcinoma with 84 controls for the presence of IgG, IgA and IgM antibodies to *H. pylori* using a previously described ELISA (Kosunen *et al.*, 1989). The cases of gastric cancer were confirmed histologically and occurred in a consecutive series of patients diagnosed in 1988 and 1989 at a single hospital in Finland. Patients with cancers of the region of the cardia were excluded, as were patients who had previously undergone gastric surgery. The mean age of the patients who were included was 65 years (SD, 16 years), and 48% were men. The controls were 35 patients with cancers at gastrointestinal sites other than the stomach (6 in the oesophagus, 7 in the pancreas and 22 in the colon) and 48 patients with cancers at sites other than the gastrointestinal tract. The mean ages of these two groups of controls were 65 years (SD, 12 years) and 66 years (SD, 12 years), respectively, and 57 and 71%, respectively, were men. IgG antibodies to *H. pylori* were found in 38/54 (70%) of the gastric cancer patients and 43/84 (51%) of the patients with other cancers. [The unadjusted odds ratio was calculated by the Working Group to be 2.3 (95% CI, 1.0–5.0).] IgA antibodies to *H. pylori* were found in 76% of the gastric cancer patients and 58% of the controls [the unadjusted odds ratio was 2.3 (95% CI, 1.1–4.8]; IgM antibodies were found in 6% of the cases and 5% of the controls. When the gastric cancer patients were stratified into three age groups, IgG antibodies were found in 8/10 (80%) aged 30–49 years, 13/19 (68%) of those aged 50–69 years and 17/25 (68%) of those aged 70 years or more. For the patients with other cancers, the respective proportions were 5/9 (56%), 22/38 (58%) and 16/37 (43%), resulting in odds ratios for the three strata of [3.2 (95% CI, 0.3–45.4)], [1.6 (0.4–6.2)] and [2.8 (0.9–9.4)], respectively. Thirty-one gastric cancer patients had tumours of the intestinal type, and 22 (71%) of them were seropositive; 21 gastric cancer patients had tumours of the diffuse type, and 15 (71%) of them were seropositive.

Kang and Chung (1992) compared 28 patients with gastric adenocarcinoma in the Republic of Korea with 30 age- and sex-matched controls for the presence of IgG antibodies

to *H. pylori*, using a commercial ELISA kit. The gastric cancer patients had all undergone resection, had histological confirmation of their disease and had been diagnosed in 1991. The mean age of the cases was 50 years (range, 29–67 years), and 66% were men. Controls were hospital patients with a variety of diagnoses other than gastrointestinal disease and included nine patients with non-gastrointestinal cancer. The mean age of the controls was 52 years (range, 28–69 years), and 67% were men. Twenty-five (89%) of the gastric cancer patients had antibodies to *H. pylori*, as did 20 (67%) of the control patients. A matched analysis resulted in an odds ratio of 4.2 (95% CI, 1.0–17). Ten of the patients had intestinal-type cancers, and eight (80%) of these were seropositive; 18 patients had diffuse-type cancers, and 17 (94%) of these were seropositive. All nine gastric cancer patients who had 'early gastric cancer' were seropositive; of the 19 who had advanced cancer, 16 (84%) were seropositive.

Hansson *et al.* (1993a) compared 112 gastric adenocarcinoma patients with 103 controls for the presence of IgG antibodies to *H. pylori* using a commercial ELISA kit with a reported sensitivity of 98.7% and a specificity of 100% (Evans *et al.*, 1989). The cases were confirmed histologically and occurred in a consecutive series of patients diagnosed between 1989 and 1991 at eight hospitals in central and northern Sweden. Patients over 79 years of age and with advanced disease (20% of study base) were excluded, as were patients who refused (3%) or were unable (14%) to give blood. The mean age of the gastric cancer patients was 67 years, and 63% were men. Controls were patients admitted to the same hospitals with a variety of non-gastrointestinal diseases, who were frequency matched to the cases by 10-year age group, sex and hospital. The mean age of the controls was 67 years, and 66% were men. Antibodies to *H. pylori* were found in 90/112 (80%) of the gastric cancer patients and 63/103 (61%) of the controls (odds ratio, 2.6; 95% CI, 1.4–5.0). When the analysis was stratified into three age groups, the odds ratios were 9.3 (1.4–101) for patients aged less than 60 years, 4.3 (1.3–15) for those 60–69 years and 1.2 (0.44–3.0) for those aged 70 or more. The interaction between age and *H. pylori* seropositivity was significant. There was a higher odds ratio in men than in women, but the effect was of borderline significance. The multivariate odds ratio for *H. pylori* seropositivity, estimated in a multiple regression model with adjustment for occupation, diet, smoking and alcohol consumption (multivariate odds ratio, 2.7; 95% CI, 1.3–5.8) showed little difference from the univariate odds ratio. Of patients with gastric cancers at sites other than the cardia, 77/93 (83%) were seropositive (odds ratio, 3.1; 1.5–6.3), while 13/19 (68%) patients with cancers of the cardia were seropositive (1.4; 0.44-4.8). Of patients with intestinal-type gastric cancer, 60/75 (80%) were seropositive (2.5; 1.2–5.4), while 22/28 (79%) of patients with diffuse-type cancer were seropositive (2.3; 0.82–7.6).

Blaser *et al.* (1993) compared 29 gastric adenocarcinoma patients with 58 age- (within one year) and sex-matched controls for the presence of IgG antibodies to *H. pylori*, using a previously described ELISA (Pérez-Pérez *et al.*, 1988) with a reported sensitivity of 96% and a specificity of 94% (Talley *et al.*, 1991b). The cases were confirmed histologically and had been diagnosed between 1990 and 1992 in one city, Ichikawa, in Japan. The median age of patients was 63 years (range, 46–82 years), and 62% were men. Controls were out-patients attending the same hospital as the gastric cancer patients for a variety of illnesses, excluding 'known stomach disease' and chronic liver disease. Twenty-four of the 29 (83%) gastric

cancer patients and 39/58 (67%) controls had antibodies to *H. pylori* (matched odds ratio, 2.1; 95% CI, 0.72–6.4). Exclusion of the three gastric cancer patients with cancers of the cardia and the corresponding controls, justified because of the previously identified specificity of association with cancer other than of the cardia (Nomura *et al.*, 1991; Talley *et al.*, 1991a), resulted in an odds ratio of 2.8 (95% CI, 0.82–9.6) for the patients with cancers at sites other than the cardia. Exclusion of non-cardia gastric cancer patients aged 70 years or over (and corresponding controls), justified because of the previously identified reduced association in the elderly (Nomura *et al.*, 1991), resulted in an odds ratio of 6.0 (95% CI, 1.1–34). Comparisons of cases on the basis of stage or severity of pathological lesions were reported not to affect the odds ratio. [The Working Group noted that the exclusion of patients with known stomach disease from the control group would be likely to reduce the prevalence of *H. pylori* infection in the group and, hence, bias the estimated odds ratio upwards.]

Lin *et al.* (1993b,c) compared 148 gastric adenocarcinoma patients with two series of controls (n = 92 and 823) for the presence of IgG antibodies to *H. pylori*, using a commercial ELISA kit with a reported sensitivity of 96% and a specificity of 93%. The cases were confirmed histologically and occurred in a consecutive series of patients diagnosed in 1992 at a single hospital in Taiwan, China. The mean age was 59 years (range, 24–87 years), and 61% were men. The first control series were part of a group of asymptomatic subjects who had had an endoscopic examination with negative results during a routine health check in 1992. Their mean age was 52 years (range, 22–77 years), and 59% were men. The second control series were randomly selected from household registry files in one precinct and three townships in Taiwan. The subjects included people of all ages, from < 10 years to ≥ 70 years, and 50% were men. [The Working Group noted that the two reports of the study had slightly different numbers of cases: 148 (Lin *et al.*, 1993b) and 143 (Lin *et al.*, 1993c). In the results reported below, the larger number was used, except where stated. The Working Group also noted that the selection of controls for the first series, excluding volunteers who did not have endoscopically normal stomachs, would be likely to reduce the estimated prevalence of *H. pylori* infection in the control group and, hence, bias the estimated odds ratio upwards.] Ninety-two of the 148 (62%) gastric cancer patients and 57/92 (62%) controls in the first series had antibodies to *H. pylori* (age- and sex-adjusted odds ratio, 1.0; 95% CI, 0.59–1.8), as did 448/823 (54%) controls in the second series [unadjusted odds ratio, 1.4 (95% CI, 1.0–2.0); after exclusion of controls from the second series who were aged less than 20 years, 347/527 (65%) were seropositive, giving a calculated unadjusted odds ratio of 0.85 (95% CI, 0.58–1.2)]. Among subjects below the age of 60 years, 44/64 (69%) of gastric cancer cases, 40/66 (61%) of the first series of controls and 280/436 (64%) of the second series of controls (20–59 years) were seropositive; among those 60 years of age or more, 48/84 (57%) of the cancer patients, 17/26 (65%) of the first series of controls and 67/91 (74%) of the second series of controls were seropositive. Twenty-six of the cancer patients had their tumour in the region of the cardia, and 17 of these (65%) were seropositive; 114 cancer patients had their tumour in regions other than the cardia, and 71 of these (62%) were seropositive. Of the 52 patients who had cancers of the intestinal type, 31 (60%) were seropositive, whereas of 96 patients with cancers of the diffuse type, 61 (64%) were seropositive. Of 26 'early' gastric

cancer patients, 16 (62%) were seropositive, and of 122 patients with advanced cancers, 76 (62%) were seropositive.

Kuipers *et al.* (1993c) compared 116 gastric adenocarcinoma patients with 116 age- and sex-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Peña *et al.*, 1989). The cases were confirmed histologically; the patients were resident in the Netherlands and had a median age of 67 years (range, 23–92 years); 56% were men. Controls were subjects undergoing upper gastrointestinal investigations, excluding those with endoscopic and histological abnormalities such as peptic ulcer, atrophic gastritis and intestinal metaplasia. Antibodies to *H. pylori* were found in 89/116 (77%) gastric cancer patients and 92/116 (79%) controls [resulting in an unadjusted and unmatched odds ratio of 0.86 (95% CI, 0.44–1.7)]. Stratification into five age groups (< 50, 50–59, 60–69, 70–79 and > 79 years) did not significantly change the odds ratios for gastric cancer within any strata [figures not available]. Of the 67 gastric cancer patients who had tumours of the intestinal type, 51 (76%) were seropositive; of the 36 patients with tumours of the diffuse type, 28 (78%) were seropositive. [The Working Group noted that, despite the exclusions from the control series, the use of symptomatic gastrointestinal disease patients would be likely to increase the estimated prevalence of *H. pylori* infection among the controls and, hence, bias the odds ratio downwards.]

Estevens *et al.* (1993) compared 80 gastric adenocarcinoma patients with 80 age- and sex-matched controls for the presence of IgG antibodies to *H. pylori* using an ELISA developed in their laboratory on the basis of a previously described assay (Evans *et al.*, 1989). The cases were confirmed histologically and occurred in a consecutive series diagnosed in 1990–91 at a single hospital in Lisbon, Portugal. The mean age was 66 years (SD, 11.9 years), and 58% were men. Controls were blood donors and hospital out-patients attending trauma and orthopaedic clinics. Antibodies to *H. pylori* were found in 56/80 (70%) gastric cancer patients and 65/80 (82%) controls, resulting in an odds ratio of [0.54 (95% CI, 0.24–1.2)]. Of the gastric cancer patients with tumours of the cardia, 67% were seropositive; of the patients with tumours at other sites, 70% were seropositive. Of the patients with tumours of the intestinal type, 64% were seropositive, whereas of those with tumours of the diffuse type, 50% were seropositive.

Archimandritis *et al.* (1993) compared 47 gastric adenocarcinoma patients with 50 controls, matched for age, sex, socioeconomic status and area of residence. The presence of IgG antibodies to *H. pylori* was assessed using a commercial ELISA kit. The cases were confirmed histologically; patients with tumours of the cardia were excluded. Patients were from all over Greece, their mean age was 62 years (SD, 12.6 years) and 62% were men. Controls were healthy people from all over Greece with 'no evidence of peptic ulcer or non-ulcer dyspepsia'; their mean age was 62 years (SD, 14.1 years), and 54% were men. Of the 47 gastric cancer patients, 34 (72%) were seropositive for *H. pylori* antibodies, as were 34/50 (68%) controls (odds ratio, 1.2; 95% CI, 0.51–3.0). When the analysis was stratified by age, the odds ratio for subjects aged < 60 years was 1.5 (0.42–5.0) and that for subjects > 60 was 0.87 (0.23–3.3). Of the 31 gastric cancer patients with tumours of the intestinal type, 22 (71%) were seropositive (1.2; 0.43–3.1); of nine patients with tumours of the diffuse type, seven (78%) were seropositive (0.83; 0.13–5.3). [The Working Group noted that the information provided about control selection was inadequate to allow a judgement about the

adequacy of the control group. The exclusion of controls with peptic ulcer or non-ulcer dyspepsia would be likely to reduce the prevalence of *H. pylori* infection in the control group and, hence, bias the esimated odds ratio upwards.]

The studies are summarized in Table 6.

2.4.2 *Other cancers*

No case–control studies of cancers other than gastric cancer have been reported, although the study of Talley *et al.* (1991a) (see above) compared patients with lung, oesophageal and large bowel cancers.

2.5 Intervention studies

Wotherspoon *et al.* (1993) gave *H. pylori* eradication therapy to six patients (three men, aged 37, 76 and 42, and three women, aged 75, 60 and 57) with histological and molecular genetic evidence of primary gastric low-grade B-cell mucosa-associated lymphoid tissue lymphoma with concomitant *H. pylori* infection. *H. pylori* was eradicated in all six patients, and repeated biopsies, 4–10 months after eradication, in five patients showed no evidence of lymphoma.

Stolte *et al.* (1994a) treated 16 patients with low-grade mucosa-associated lymphoid tissue lymphomas, *H. pylori* infection and gastritis with *H. pylori* eradication therapy. The patients were followed up with repeated endoscopic biopsies 3–12 months after treatment; 12 patients showed regression of the lymphoma. In six of the 12, sparse residual lymphoma tissue was found.

The gastric lymphomas that respond to *H. pylori* eradication therapy, the well-differentiated mucosa-assocated lymphoid tissue lymphomas, were previously called 'pseudolymphomas'. They are known to remain localized for many years before invading other tissues.

3. Studies of Cancer in Experimental animals

3.1 Infection with *Helicobacter pylori* alone

No data were available to the Working Group.

3.2 Infection with *Helicobacter pylori* in combination with administration of known carcinogens

Rat: A total of 90 male Wistar WKY/Std rats, eight weeks of age, received 50 mg/L *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MMNG) in the drinking-water for 40 weeks. One group of 30 rats received MNNG alone; a second group of 30 rats was given MNNG plus oral intubations of 0.2 ml brucella broth three times a week for the 40 weeks; the third group of 30 rats received MNNG and brucella broth containing 10^6–10^8 colony-forming units/ml of culture of fresh isolates of *H. pylori* three times a week for 40 weeks, since permanent

Table 6. Seroprevalence for *Helicobacter pylori* in gastric cancer patients and matched controls: retrospective studies

Country	Cases			Controls			Odds ratio[a]	95% CI	Controls	Reference
	No.	*H. pylori* infection		No.	*H. pylori* infection					
		No.	%		No.	%				
USA	69	36	52	252	96	38	1.6	[0.9–2.8]	Volunteers (76), hospital patients except cancer (176)	Talley *et al.* (1991a)
Finland	54	38	70	84	43	51	[2.3	1.0–5.0]	Cancer patients except gastric	Sipponen *et al.* (1992)
Republic of Korea	28	25	89	30	20	67	4.2	1.0–17	Hospital patients	Kang & Chung (1992)
Sweden	112	90	80	103	63	61	2.6	1.4–5.0	Hospital patients	Hansson *et al.* (1993a)
Japan	29	24	83	58	39	67	2.1	0.72–6.4	Hospital out-patients	Blaser *et al.* (1993)
China (Taiwan)	148	92	62	92	57	62	1.0	0.59–1.8	Health check-up participants	Lin *et al.* (1993b)
Netherlands	116	89	77	116	92	79	[0.86]	[0.44–1.7]	Gastroenterology patients except ulcer, gastritis	Kuipers *et al.* (1993c)
Portugal	80	56	70	80	65	81	[0.54]	[0.24–1.2]	Blood donors, hospital out-patients	Estevens *et al.* (1993)
Greece	47	34	72	50	34	68	1.2	0.51–3.0	Healthy people	Archimandritis *et al.* (1993)

CI, confidence interval

[a]From primary analysis reported in paper, using all cases of gastric cancer

colonization of the rat gastric mucosa by the *H. pylori* is not achieved. All rats survived 35 or more weeks. After the 40 weeks of treatment, the two control groups had very similar numbers of gastroduodenal tumours (adenomatous polyps, adenocarcinomas and carcinomas): 7/30 of those given MNNG alone and 6/30 of those given MNNG plus brucella broth; a slight reduction in the number of gastroduodenal tumours was seen in the group given MNNG plus the living cultures of *H. pylori* (4/30). No difference in the incidence of gastritis was seen among the three groups (Kawaura *et al.*, 1991). [The Working Group noted that exposure to *H. pylori* was intermittent in this model, thus unlike the conditions of human exposure.]

3.3 Infection with other *Helicobacter* species

Mouse: In a study reported as an abstract (Enno *et al.*, 1994), 260 specific pathogen-free BALB/c mice were infected with *H. felis*. Groups of 20 mice were killed at 2–3-month intervals up to 26 months. Up to 18 months after infection, minimal gastritis was observed; however, at 22–26 months after infection, 51/80 *H. felis*-infected animals and 4/48 uninfected controls had large lymphoid aggregates in the cardia. Lymphoepithelial lesions that were not seen in control animals and which, according to the authors, are similar to those observed in association with human gastric low-grade B-cell lymphomas, were observed in 27/80 infected animals.

3.4 Infection with other *Helicobacter* species in combination with administration of known carcinogens

Ferret: A group of nine female ferrets (*Mustela putorius furo*), four to five months of age, ovariohysterectomized and naturally infected with *H. mustelae*, received single oral doses of 50 mg/kg bw MNNG in 3 ml of olive oil. One additional four-month-old ferret received 100 mg/kg bw MNNG, and five controls animals received olive oil only. Mucosal punch biopsies were obtained by endoscopy from the same region of the stomach at 6–12-month intervals; no adenocarcinoma was seen in the limited samples taken. Seven of the nine ferrets dosed with 50 mg/kg bw MNNG were killed between 51 and 55 months after treatment; one other ferret died, and one was killed at 25 months. At necropsy, two ferrets had pyloric ulcers and two had obvious nodules on the mucosal surface of the pylorus. The single ferret that received 100 mg/kg bw and was killed at 29 months had clinical gastrointestinal disease. It had a grossly thickened pyloric area with a 1-cm ulcer at the pyloric–duodenal junction. Histopathological examination of all the stomachs revealed that all ferrets, control and treated, had marked chronic gastritis with the major characteristics of multifocal atrophic gastritis. One or more foci of neoplasia were seen in 9 of the 10 MNNG-treated ferrets. Two had well-defined invasive adenocarcinomas, and four had multiple independent primary adenocarcinomas. The neoplasms were concentrated in the pyloric antrum at the transition zone between the corpus and antral mucosa. Metastasis to regional lymph nodes was observed in one animal. The five control animals were killed 47–67 months after dosing with olive oil; two that were killed had chronic renal failure, while the other three were asymptomatic when they were killed. No gross lesion was seen in the stomachs of the control ferrets; the only histopathological change observed was mild to moderate gastritis in the

antrum with small foci of gland loss. Adenocarcinomas were not observed in the stomachs of hundreds of untreated laboratory ferrets examined at routine necropsy (Fox *et al.*, 1993a). [The Working Group noted that the study did not include a group uninfected with *H. mustelae* but given MNNG.]

4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

Cross-sectional and longitudinal observations in human populations indicate that a series of alterations of the gastric mucosa precede gastric carcinoma (Siurala *et al.*, 1985; Correa *et al.*, 1990b; Kuipers *et al.*, 1994a): They follow a sequential presentation of chronic nonatrophic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia. Atrophy (loss of gastric glands) is a pivotal change in the precancerous process. It radically alters the gastric microenvironment by reducing acid secretion, elevating the gastric luminal pH and resulting in an overgrowth of anaerobic bacteria. Many such bacteria produce reductases which act on nitrate molecules (from food and other sources) and result in elevated concentrations of NO_2^- in the gastric lumen. Dietary factors that are important in the progression of the precancerous process include high salt (NaCl) intake and low consumption of fresh fruits and vegetables (Nomura *et al.*, 1982; Fontham *et al.*, 1986; Buiatti *et al.*, 1989b, 1990; Chen *et al.*, 1990; Forman, 1991).

4.1.1 *Humans*

The anatomical substratum resulting from *H. pylori* infection is chronic gastritis. Although the association between the bacterium and gastritis was recognized only in 1983 (Warren, 1983; Marshall, 1983), the pathological manifestations of chronic gastritis and several nosological entities of gastritis had been described previously.

(a) Specific lesions

Colonies of *H. pylori* are characteristically located extracellularly in the mucus layer immediately adjacent to the gastric surface epithelium. They are prominently concentrated in front of the intercellular junctions of the epithelial cells. Most bacteria float freely within the mucus layer; a few adhere to pedestals formed by the epithelial cytoplasmic membrane. They may sometimes penetrate the intercellular spaces and, rarely, the ductules of the parietal cells (Chen *et al.*, 1986; Fiocca *et al.*, 1987; Hessey *et al.*, 1990).

H. pylori infection is associated with degenerative changes in the cytoplasm of the surface epithelial cells, identified on haematoxylin–eosin staining as loss of the superficial portion of the cytoplasm, resulting in microerosions of the surface epithelium (Chan *et al.*, 1991). Under the electron microscope, partial loss and stunting of the microvilli and numerous intracellular phagolysosomes may be seen (Chen *et al.*, 1986; Fiocca *et al.*, 1987; Hessey *et al.*, 1990).

H. pylori infection results in infiltration of leukocytes into the gastric mucosa. The most abundant are B lymphocytes, which occupy the lamina propria and may lead to formation of

lymphoid follicles (Genta *et al.*, 1993a,b). Polymorphonuclear neutrophils, although less abundant than lymphocytes, are common in *H. pylori* infections. They are seen in the lamina propria, in the space between the epithelial cells and in the gastric lumen; they typically aggregate in the neck area of the gastric glands. Other inflammatory cells identified in *H. pylori*-infected mucosa are plasma cells, T lymphocytes, macrophages and eosinophils (Marshall *et al.*, 1985b; Dixon *et al.*, 1988; Wyatt & Rathbone, 1988; Genta *et al.*, 1993b).

The lesions associated with acute (new) infection are similar to those described above, except that the polymorphonuclear infiltrate is prominent and precedes the lymphocytic infiltrate (Marshall *et al.*, 1985a; Morris & Nicholson, 1987; Graham *et al.*, 1988).

(*b*) *Nosological entities*

In early stages of *H. pylori* infection, gastritis is nonatrophic. Later, it leads to gland loss (atrophic gastritis) and is frequently followed by intestinal metaplasia.

Atrophic gastritis in patients with the pernicious anaemia syndrome diffusely involves the oxyntic mucosa while sparing the antrum. This gastritis is called type A or autoimmune (Strickland & Mackay, 1973; Correa, 1980). In populations at low risk for pernicious anaemia, atrophic gastritis is multifocal and involves both the antrum and the corpus. This gastritis is called type B (Strickland & McKay, 1973) or multifocal atrophic (Lambert, 1972; Correa, 1980). The two entities coexist in a few patients, leading to the denomination type AB gastritis (Glass & Pitchumoni, 1975). A frequent form of nonatrophic gastritis is located predominantly in the antrum, with mild or no involvement of the oxyntic mucosa. Such lesions have been called diffuse antral (Correa, 1988), interstitial (Cheli *et al.*, 1980), pre-atrophic (Cheli & Testing, 1993) or hypertrophic gastritis (Schindler, 1969). This type of gastritis is seen most frequently in conjunction with duodenal ulcer, while multifocal atrophic gastritis is associated particularly with gastric ulcer or gastric carcinoma (Schindler, 1969; Lambert, 1972).

Once the prominent role of *H. pylori* in chronic gastritis had been recognized, a grading of gastritis, the Sydney system, was designed (Price, 1991), which is intended to include microscopic, gastroscopic and etiological factors, including *H. pylori* infection. The system allows the grading of inflammatory and atrophic changes in the corpus and antrum on a semiquantitative scale of 0–3. The name 'pangastritis' is proposed for lesions covering both the antrum and the corpus, which can be atrophic or nonatrophic (Sipponen *et al.*, 1991).

H. pylori infection has a prominent role in diffuse antral (nonatrophic) gastritis and in multifocal atrophic gastritis (Siurala *et al.*, 1985). It has no role in corpus-limited (type A or autoimmune) atrophic gastritis, or in other specific forms of gastritis such as those associated with bile reflux or use of nonsteroidal anti-inflammatory drugs, known as 'reflux', 'reactive' or 'chemical irritational' gastritis (Dixon *et al.*, 1988; Flejou *et al.*, 1989), or in 'lymphocytic gastritis' (Haot *et al.*, 1986).

H. pylori infection has also been associated with other, less frequent types of gastritis, such as that characterized by prominent hyperplastic foveola, also called 'hypertrophic' or 'focal foveolar' hyperplasia (Stolte *et al.*, 1994b).

(c) *Epidemiology of chronic gastritis*

H. pylori infection is very prevalent in some populations of low socioeconomic status (Holcombe, 1992; Sierra *et al.*, 1992). In a few, gastric biopsy specimens and pepsinogen levels indicate that the gastritis is not of the atrophic type (Sierra *et al.*, 1992; Shousha *et al.*, 1993). In populations at high risk for gastric cancer, atrophic forms of gastritis predominate. Atrophic gastritis associated with the pernicious anaemic syndrome, not usually related to *H. pylori* infection, is strongly related to genetic susceptibility and affects mainly populations of northern European extraction. In other populations at high risk for gastric cancer, such as those of the Andean regions of Latin America, those of China and Japan, and US blacks, atrophic gastritis is multifocal and linked in part to dietary factors (Fontham *et al.*, 1986; Nomura *et al.*, 1982).

People of each sex are equally affected, and the prevalence of gastritis is highly age-dependent. Nonatrophic gastritis is more frequent in people under the age of 50, whereas atrophic gastritis and intestinal metaplasia are more frequent among people over that age (Siurala *et al.*, 1985). In samples from 500 blood donors in Finland, the prevalences of both *H. pylori* antibodies (IgG class in particular, but also IgA and IgM) and gastritis were shown to increase with age (Kosunen *et al.*, 1989).

(d) *Relation of infection to gastritis*

The first demonstration of an association between *H. pylori* infection and human disease was the result of two experiments in which *H. pylori* organisms were ingested voluntarily. Acute gastritis was seen in biopsy specimens from both subjects (Marshall *et al.*, 1985a; Momms & Nicholson, 1987), and one of the volunteers developed chronic gastritis. An epidemic of hypochlorhydric gastritis (epidemic achlorhydria) described in 1979 was later shown to be due to transmission of *H. pylori* infection via endoscopy. Acute granulocytic gastritis, lasting some weeks, developed into chronic gastritis within 74 days to two years in these cases (Ramsey *et al.*, 1979; Graham *et al.*, 1988). Successful treatment of *H. pylori* infection leads to healing of gastritis (Rauws *et al.*, 1988; Valle *et al.*, 1991; Kosunen *et al.*, 1992; Genta *et al.*, 1993a).

A positive relationship exists between *H. pylori* infection and gastritis, i.e. with regard to the degree of mucosal inflammation by mononuclear inflammatory cells, polymorphonuclear neutrophils and eosinophils, particularly in the antrum (Stolte *et al.*, 1990; Satoh *et al.*, 1991; McGovern *et al.*, 1991; Stolte *et al.*, 1994b). Specific cytotoxic strains are shown to enhance the inflammatory response, and their occurrence differs between populations.

In a random sample of gastric biopsy specimens from the antrum, corpus or both in Finland, up to 91% of people with nonatrophic (superficial) gastritis, up to 41% with advanced atrophic gastritis but none with normal stomachs or severe atrophic gastritis of the autoimmune (type A, or corpus-limited) type contained *H. pylori* (Siurala *et al.*, 1988). In a subset of patients with advanced atrophic gastritis, the estimated prevalence of *H. pylori* infection was higher when assessed by both serological and histological methods than when it was assessed by histology alone (Karnes *et al.*, 1991). In populations at high risk for gastric cancer, such as in Colombia, the prevalence of *H. pylori* is close to 100% (Correa *et al.*, 1989).

(*e*) *Atrophic gastritis and intestinal metaplasia*

In a 3–16-year (average, 5.1 years) follow-up study (7290 person-years) of people in Narino, Colombia, the rate of transition from normal histological appearance or superficial gastritis to atrophic gastritis or more advanced lesions was 3.3% per year, corresponding to 1.7% for atrophic gastritis, 0.9% for intestinal metaplasia and 0.7% for dysplasia (Correa *et al.*, 1990b).

Mathematical modelling of cross-sectional data on gastritis in Finland and Estonia indicated a slow, stepwise transition from nonatrophic gastritis to atrophic gastritis over time (Kekki & Villako, 1981; Kekki *et al.*, 1983). The fractional transition rate from the pool of nonatrophic to the pool of atrophic gastritis was estimated to be 2.1–2.6% per year for people aged 25–75 (Kekki & Villako, 1981; Villako *et al.*, 1982).

In an 11.5-year (range, 10–13) follow-up of 113 patients with and without *H. pylori* gastritis in the Netherlands, significant progression of nonatrophic gastritis to atrophic gastritis was demonstrated endoscopically (Kuipers *et al.*, 1994a). Fifteen of 56 (27%) patients with *H. pylori* infection and nonatrophic gastritis developed atrophic gastritis, whereas only two of 49 (4%) patients without *H. pylori* infection, all of whom had normal gastric mucosa at the beginning of follow-up, developed the atrophic stage. The difference was significant ($p < 0.001$).

An endoscopic follow-up of 377 subjects in Finland for 30–34 years (Ihamäki *et al.*, 1985) revealed that progression of atrophic gastritis occurs in the gastric corpus and regression may occur in the antrum in the long term.

Since nonatrophic gastritis involves predominantly the antrum and multifocal atrophic gastritis compromises to a large degree both the antrum and the corpus, it is important to study the dynamics of involvement of *H. pylori* in different regions of the stomach. The location and severity of gastritis vary in different disease manifestations of *H. pylori* infection. Thus, inflammation in duodenal ulcer patients is generally restricted to the antrum, while in those with gastric ulcer and gastric cancer the gastritis is more widely distributed in the corpus of the stomach (Stolte *et al.*, 1990). Observations on patients with different acid outputs may be relevant. In patients given the anti-acid secretory drug omeprazole, gastritis in the antrum is reduced, while inflammation in the corpus increases, i.e. pangastritis is observed (Solcia *et al.*, 1994). In a study on the long-term effects of omeprazole in 91 patients, only 1% had atrophic gastritis at the beginning of therapy, but on follow-up (mean, 48 months; range, 36–64 months), 25% had atrophic gastritis (Klinkenberg-Knol *et al.*, 1994). Other studies of prolonged omeprazole treatment show lower rates of transition to atrophic gastritis (Lambert *et al.*, 1993).

Studies conducted before identification of *H. pylori* also showed changes in the distribution and intensity of gastritis after acid suppression. After vagotomy, a surgical procedure to reduce acid output in duodenal ulcer patients, a marked increase in both the extent and severity of proximal gastritis was seen, but the distal gastritis remained unchanged (Meikhle *et al.*, 1976).

The development of atrophic gastritis depends on factors in addition to *H. pylori* infection (Correa, 1992; Fukao *et al.*, 1993). Genetic susceptibility to atrophic gastritis was seen in segregation analysis in Narino, Colombia, suggesting that expression of a single

autosomal recessive gene, with age-dependent penetrance, is involved (Bonney *et al.*, 1986; see also section 1.1.2(*b*)).

(*f*) *Atrophic gastritis and gastric cancer*

In a meta-analysis of six independent follow-up studies (Varis, 1983), 58 cases of gastric cancer (severe corpus-limited atrophic gastritis) were recorded among 843 patients with pernicious anaemia who were followed up for 7.8–15 years (mean, 11 years; 8990 person-years), providing an estimate of 0.6% for the annual cancer risk and suggesting that the occurrence of cancer is approximately five times higher among patients with severe atrophic corpus gastritis than in the population at large. An 11–14-year follow-up of three population samples in Finland (over 800 people) with and without gastritis indicated that the risk for developing gastric cancer was two to three times higher than that expected in people who had advanced atrophic gastritis. All 10 patients with gastric malignancy had had gastritis at the beginning of follow-up, and none without it developed advanced disease (Ihamäki *et al.*, 1991).

Estimates of cancer risk in association with multifocal atrophic gastritis have been based on the results of case–control studies (Sipponen *et al.*, 1985, 1994a), which suggest that the age- and sex-adjusted relative risk for gastric cancer is increased by up to 18 fold. The risk rises to 90 fold in patients with severe pangastric atrophy (Sipponen *et al.*, 1985).

The risk for gastric cancer and, in particular, intestinal-type gastric cancer, is increased in the presence of intestinal metaplasia and atrophic gastritis (Correa, 1992; Sipponen *et al.*, 1992), especially if the intestinal metaplasia is of type III (Jass & Filipe, 1980), also called the colonic or incomplete type (Jass & Filipe, 1979; Jass, 1980; Sipponen *et al.*, 1980). Precancerous lesions of various types and nature (polyps, dysplasia) have been shown to be associated with atrophic gastritis and intestinal metaplasia (Laxén *et al.*, 1983; Correa *et al.*, 1990b). The risk for gastric cancer associated with different types of intestinal metaplasia was investigated in a cohort of 1525 Slovenian patients. The standardized incidence ratio for stomach cancer was 2.2. When type I metaplasia was used as the reference category, the risk was 2.1 for type II and 4.6 for type III (Filipe *et al.*, 1994).

There is some evidence of a relationship between the occurrence of intestinal metaplasia and atrophic gastritis and tumours at the same anatomical site in the stomach (Sipponen *et al.*, 1983).

(*g*) *Nonatrophic gastritis and gastric cancer*

In a case–control study, the age- and sex-adjusted risk for gastric cancer was slightly but significantly increased (two to three fold) in patients with nonatrophic gastritis over that in subjects with normal, uninfected stomachs (Sipponen *et al.*, 1994).

(*h*) *Mucosal-associated lymphoid tissue*

B-Cell lymphoid follicles and aggregates resembling intestinal Peyer's patches appearing mainly in the gastric antrum and small curvature of the stomach are a characteristic feature of *H. pylori*-related gastritis; they represent acquired mucosa-associated lymphoid tissue in the stomach (Isaacson, 1992). These follicles do not occur in uninfected subjects or in special forms of gastritis (Stolte & Eidt, 1989; Genta *et al.*, 1993a), whereas they

have been reported to occur in 27–100% of cases with *H. pylori*-related gastritis (Genta *et al.*, 1993a). Their prevalence increases with the degree of inflammatory reaction (Stolte & Eidt, 1989). Treatment of *H. pylori* infection results in a slow decrease (but not the disappearance) of lymphoid follicles within 12 months (Genta *et al.*, 1993b).

In the most comprehensive study, which was designed to determine the frequency and distribution of gastric lymphoid follicles in *H. pylori* infection, mapped gastric biopsy specimens were obtained from 20 normal, uninfected volunteers, 25 asymptomatic volunteers with *H. pylori* infection and no ulcer disease, 21 duodenal ulcer patients, and 16 patients with gastric ulcer. None of the uninfected patients had lymphoid follicles, while all subjects infected with *H. pylori* had follicles. Eradication of the organism with antimicrobial agents resulted in a slow decrease in the prevalence of follicles (Genta *et al.*, 1993a).

4.1.2 *Experimental systems*

Investigation of animals infected with different *Helicobacter* species provides the opportunity to confirm the role of these bacteria in chronic gastritis and also to demonstrate the progression of chronic gastritis to atrophic gastritis.

(*a*) *Non-human primates*

Many studies have shown that a number of primate species are colonized with bacteria similar to *H. pylori*. In a closed colony of rhesus monkeys (*Macaca mulatta*), chronic gastritis was found in 8 of 11 animals surveyed, and inflammation was correlated with the presence of *H. pylori*-like bacteria (Baskerville & Newell, 1988). The inflammatory infiltrate was primarily mononuclear, and the lamina propria was heavily infiltrated by lymphocytes, plasma cells and histiocytes. Large lymphoid follicles occurred in most stomachs. Polymorphonuclear leukocytes were rarely seen. When intense cellular infiltration was present in the body of the stomach, atrophy of glands containing parietal and chief cells was observed.

Examination of another rhesus monkey colony revealed marked abnormalities in a number of animals (Euler *et al.*, 1990). There was a noticeable mixed mononuclear cell inflammatory response in 14/35 animals examined. *H. pylori* was cultured from 12/35 animals. A strong correlation was seen with gastritis: inflammation occurred in 83% of infected animals and in only 17% of uninfected animals. When two groups of five uninfected monkeys without gastritis at the time of screening were inoculated experimentally with either human or monkey isolates of *H. pylori*, the human strain did not colonize the animals, but all of them became infected with the monkey isolate and all had gastritis by 28 days after inoculation.

Dubois *et al.* (1991) found *H. pylori*-like bacteria in 8 of 29 colony-bred rhesus monkeys, and all had gastritis; however, of 14/29 infected with '*H. heilmanni*', only two had gastritis. Uninfected animals had no gastritis.

The Japanese monkey (*Macaca fuscata*) has also been used as an experimental model (Shuto *et al.*, 1993). Of 12 animals inoculated with a human isolate of *H. pylori*, seven became infected and inflammation characterized by polymorphonuclear leukocytes and monocytes was observed. *H. pylori*-associated gastritis persisted in two animals followed for more than 18 months.

(*b*) *Gnotobiotic piglets*

Krakowka *et al.* (1987) were able to infect 17 gnotobiotic domestic Yorkshire piglets with a human isolate of *H. pylori*. Histopathological lesions indicative of chronic active gastritis were seen in all infected piglets. A neutrophilic response was present for two weeks but then resolved, and the gastritis consisted primarily of mononuclear cells and prominent lymphoid follicles. As piglets can be maintained in the gnotobiotic state for only six weeks, the progression of gastritis could not be assessed.

In a further study, seven pigs were immunized with 10^9 *H. pylori* in incomplete Freund's adjuvant in two doses given subcutaneously seven days apart; these pigs and eight un-immunized control pigs were then infected with a human strain of *H. pylori*. The gastritis was much more severe in the previously immunized than in the unimmunized piglets. Neutrophilic infiltrates and neutrophilic gland abscesses were seen in the immunized but not in the unimmunized piglets (Eaton & Krakowka, 1992).

(*c*) *Dogs*

H. pylori has been shown to infect gnotobiotic, germ-free beagle puppies, and significant chronic gastritis was induced in all infected animals (Radin *et al.*, 1990). A more intense gastritis was induced when the pups were inoculated with pure cultures of *H. felis*, an organism commonly seen in dogs (Lee *et al.*, 1992). All infected dogs showed extensive mononuclear inflammation, with the appearance of large lymphoid aggregates. As the animals were kept for only 30 days after infection, no progression of gastritis was observed.

(*d*) *Cats*

When kittens were infected with either *H. acinonyx*, a species of *Helicobacter* isolated from a group of cheetahs with gastritis, or '*H. heilmannii*', a *Helicobacter*-like bacterium found in the same groups of cheetahs, both organisms colonized the feline stomachs and induced a mild lymphofollicular gastritis, which did not change over 11 months (Eaton *et al.*, 1993).

A closed colony of cats bred by a commercial vendor was shown to be infected by *H. pylori*. The bacterium colonized primarily the antrum and induced antral gastritis (Handt *et al.*, 1994).

(*e*) *Ferrets*

In a study in which 11 adult ferrets were extensively examined (Fox *et al.*, 1990), *H. mustelae* was present in all animals, and a diffuse antral gastritis similar to that seen in humans infected with *H. pylori* was observed. In some animals, the changes observed in the proximal antrum and the transitional zone appeared to be similar to the early stages of multifocal atrophic gastritis in humans.

(*f*) *Rodents*

Rodents have not been shown convincingly to become colonized with *H. pylori*; however, the feline *Helicobacter*, *H. felis*, readily colonizes both rats and mice for the life of the animal (Lee *et al.*, 1993). When four-week-old female Swiss-Webster, isolator-reared, axenic mice were given viable *H. felis* orally (Lee *et al.*, 1990), 18/20 mice became infected. The first

evidence of gastritis was seen two weeks after inoculation and was mainly neutrophilic; by four weeks, the severity of inflammation had increased and there were more lymphocytes. By eight weeks, all mice had a relatively diffuse active chronic gastritis, with a cell infiltrate composed of approximately equal numbers of mononuclear and polymorphonuclear leukocytes, with lymphocytes and neutrophils as the predominant cell types. Small lymphoid nodules had formed in the submucosa, and small aggregates of lymphocytes in the subglandular area displaced or compressed mucosal glands. In a more extensive study, the course of gastritis was followed up to 50 weeks after infection (Fox *et al.*, 1993b). Between 20 and 50 weeks, the gastritis became more chronic, although microabscesses were seen in some animals even at this late stage. A similar study in rats showed the induction of chronic gastritis that was less florid than that in the mice (Fox *et al.*, 1991).

The only long-term animal study that allows assessment of the severity of gastritis over the life of infected animals is one in conventional Quackenbush Swiss mice (Lee *et al.*, 1993). A total of 221 seven-week-old female mice were infected with either a living culture of *H. felis* or a gastric homogenate from mice infected with '*H. heilmannii*'. The severity of gastritis was assessed in mice killed at regular intervals for up to 72 weeks. All infected mice showed a slowly progressive chronic gastritis, with increasing numbers of infiltrating mononuclear cells and polymorphonuclear leukocytes. After a year and a half, the inflammatory reaction was so severe that atrophic changes were seen in both the antral and fundic mucosa. Control animals initially showed no inflammatory changes; however, as the animals aged, the gastric mucosa of some animals became infected with a bacterium, *H. muridarum*, that normally inhabits the small and large bowel of the rodent. The presence of this bacterium was also associated with gastritis and atrophic changes.

A severe, long-term gastritis was shown in mice infected for more than six months with an '*H. heilmannii*', *Helicobacter*-like organism originating from a cheetah that had gastritis (Eaton *et al.*, 1993). The infected mice had grossly evident gastric mucosal hypertrophy at sacrifice, with severe lymphoplasmacytic inflammation, lymphoid follicles and microscopic ulcers.

Mice infected with another animal *Helicobacter*, '*Gastrospirillum suis*' from pigs, also developed gastritis (Moura *et al.*, 1993). Some degree of glandular destruction in the oxyntic mucosa due to an inflammatory reaction involving granulocytes and mononuclear cells was described.

4.2 Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1 *Humans*

H. pylori may act in the development of gastric cancer by a number of possible mechanisms: (i) an increase in the rate of epithelial cell proliferation; (ii) damage to mucus secretion and the cytoplasm of foveolar cells; (iii) facilitation of the synthesis and delivery of carcinogens at the site, especially *N*-nitroso compounds; (iv) inhibition of the local effect of antioxidants, especially L-ascorbic acid; and (v) induction of mutations and other molecular lesions, either directly or through the release of active oxygen species and $NO^{\bullet}$ by polymorphonuclear cells and macrophages attracted by the bacteria.

(*a*) *Increased cell replication*

Atrophic gastritis increases the rate of proliferation of the gastric epithelium (Lipkin *et al.*, 1985), as measured by tritiated thymidine incorporation. This effect was found to be associated with *H. pylori* infection (Buset *et al.*, 1992; Cahill *et al.*, 1993; Fischbach *et al.*, 1993) in patients with multifocal atrophic gastritis and infected with *H. pylori*. Gastric biopsy specimens taken from patients before and after therapy for *H. pylori* infection were immunostained with antibodies against the proliferating cell nuclear antigen (Brenes *et al.*, 1993). In patients who cleared the infection, the labelling index was reduced from 19.95 to 14.12 ($p < 0.001$), close to the normal index of 13.05. Patients who did not clear the infection showed no reduction in labelling index (18.9 before and 17.9 after treatment). Hyperproliferation of the gastric epithelium thus appears to be caused by *H. pylori* infection.

Both cell proliferation and ploidy have been assessed on the basis of the nucleolar organizer regions. The number of regions is increased in the gastric epithelium of patients infected with *H. pylori*, but after successful treatment the region count is rapidly reduced to normal levels (Correa *et al.*, 1994).

(*b*) *Alteration of the mucus barrier*

This mechanism is presumed to be important because the gastric microenvironment of atrophic gastritis patients contains concentrations of NO_2^- and nitrogen-containing species that can produce carcinogens but may be separated from the target cell by a normal mucus barrier. The gastric epithelium is thus protected from the acid environment in the gastric lumen by complex mucus glycoproteins. *H. pylori* organisms produce proteases and lipases which degrade the mucus gel, causing loss of hydrophobicity (Goggin *et al.*, 1992; Go *et al.*, 1993) and viscosity, which induces breaks in the continuity of the mucus layer (Sidebotham *et al.*, 1991; Slomiany & Slomiany, 1992). This change is followed by increased production of prostaglandin E2 (Oderda *et al.*, 1993). The damage to the mucus is also associated with bile reflux, a common finding in *H. pylori*-associated gastritis (Sobala *et al.*, 1991).

(*c*) *Facilitation of synthesis of carcinogens* in situ

There is an extensive literature on the possible generation of *N*-nitroso compounds by overgrowing bacteria in the stomachs of patients with atrophic gastritis (Hill, 1986; Correa, 1992). Substrates involved in this process may be nitrogen-containing compounds in foods, which can react with nitrite to produce carcinogenic and mutagenic *N*-nitroso compounds. Examples include indole substances in fava beans (Yang *et al.*, 1984) and Chinese cabbage (Wakabayashi *et al.*, 1985), which are frequently consumed by inhabitants in areas of high risk for stomach cancer. In Costa Rican schoolchildren, *N*-nitrosoproline excretion after proline intake, measured as a marker of endogenous nitrosation, was slightly higher (about 1.5-fold) in an area of high gastric cancer risk than in a low-risk area (Sierra *et al.*, 1993), although *H. pylori* infection is very prevalent (around 70%) in both high- and low-risk areas. These results indicate either that *H. pylori* infection is not causally related to nitrosation or that nitrosation is selectively inhibited in the low-risk area. As *H. pylori* infection is also prevalent in other areas of low risk for stomach cancer, such as in Africa, other environmental, social and genetic factors appear to be involved in the etiology of gastric cancer (Holcombe, 1992).

H. pylori contains alcohol dehydrogenase but not aldehyde dehydrogenases. The bacterium can thus produce acetaldehyde from even low (0.1%) concentrations of ethanol (Salaspuro, 1994). Acetaldehyde is a highly reactive, toxic substance which has been classified as possibly carcinogenic to humans by an IARC working group (IARC, 1987).

(*d*) *Decreased levels of* L-*ascorbic acid*

Infection with *H. pylori* interferes with the normal capacity of the gastric mucosa to concentrate ascorbic acid. This conclusion is inferred from the fact that uninfected patients have a higher concentration of ascorbic acid in the gastric juice than infected patients (Sobala *et al.*, 1989; Rood *et al.*, 1994); furthermore, previously infected patients can concentrate ascorbic acid at near normal levels after successful antimicrobial therapy (Sobala *et al.*, 1993; Ruiz *et al.*, 1994).

(*e*) *Induction of mutations*

H. pylori has no direct mutagenic activity, and reports of differences in the mutagenicity of gastric juice from patients with and without gastritis are equivocal (Montes *et al.*, 1979; Morris *et al.*, 1984; O'Connor *et al.*, 1984; Farinati *et al.*, 1989). Investigations of alterations in the *p53* gene in 10 gastric adenomas and one carcinoma, however, showed that three of the adenomas contained *p53* mutations (Tohdo et al., 1993). In a study of samples obtained by gastrectomy from 12 patients with gastric cancer in Italy, mutations of the *p53* gene were found in 3/12 normal areas of the stomach, 4/8 areas of metaplasia, 8/12 areas of dysplasia and 9/12 of the carcinomas. In five of seven of the samples that were analysed further, the mutations were shown to be GC→AT transitions in exons 5–8 (Shiao *et al.*, 1994). Amplification of the C-*erb*B.2 gene is related to invasion and nodal involvement. Differential expression of the ras oncoprotein in diffuse-type and in poorly differentiated intestinal-type gastric carcinomas implies that there are two distinct subtypes of gastric carcinoma (Tehara, 1993).

It has been proposed that the activation of polymorphonuclear leukocytes that occurs in the gastritis induced by *H. pylori* could result in the production of oxygen and nitrogen radicals (e.g. hydroxy radicals, nitric oxide), which induce DNA damage (Wink *et al.*, 1991; Nguyen *et al.*, 1992). Davies *et al.* (1994) reported that gastric biopsy specimens from *H. pylori*-infected subjects show more production of reactive oxygen metabolites than specimens from uninfected individuals. An inducible form of nitric oxide synthetase was detected immunohistochemically in epithelial cells of the stomach infected with *H. pylori* in subjects with chronic atrophic gastritis (Pignatelli *et al.*, 1994). [The Working Group noted the inadequate reporting of the data.]

(*f*) *Cytotoxin and cytotoxin-associated protein*

Only one, variable property of *H. pylori* has been shown to be correlated with the severity of disease. It is the vacuolating cytotoxin, first described by Leunk *et al.* (1988), who showed that broth-culture filtrates induced intracellular vacuolation in seven of nine mammalian tissue culture cell lines tested. This toxin was later found to be an 87-kD protein with partial homology with the internal sequences of ion channel proteins (Cover & Blaser, 1992).

Bacterial culture filtrates from a cytotoxin-producing strain of *H. pylori* were incubated with cell cultures. After 16 h, cells were harvested and the Na^+/K^+-ATPase activity was

measured. An immediate reduction in enzyme activity was observed. Filtrates of a non-cytotoxin-producing strain did not inhibit enzyme activity (Ricci *et al.*, 1993).

Soon after identification of the cytotoxin, it was shown that a greater proportion of strains isolated from duodenal ulcer patients were toxigenic (66.6%) than strains isolated from asymptomatic patients (30.1%) (Figura *et al.*, 1989). Also, patients with duodenal ulcer were more likely to have antibodies that neutralize the activity of the toxin in their serum than asymptomatic patients (Pereira Lage *et al.*, 1993).

The sera from all six gastric carcinoma patients and three of five sera from peptic ulcer patients showed neutralizing activity to the cytotoxin, and 21 of 22 stored sera from gastric cancer patients also showed neutralizing activity (Hirai *et al.*, 1994). [The Working Group noted the small number of sera in the first part of this study and the lack of control sera from non-cancer patients in the retrospective study.]

In a study of 30 *H. pylori* patients, 47% of the infecting strains were toxin producers. Cytotoxin production *in vitro* was shown to be associated with increased antral mucosal polymorphonuclear leukocyte infiltration (Cover *et al.*, 1993). Sixty-nine percent (18/26) of strains of *H. pylori* isolated from patients with diffuse antral gastritis and 89% (70/79) of strains isolated from patients with chronic atrophic gastritis were toxin producers ($p = 0.043$) (Fox *et al.*, 1992).

Antibodies against an *H. pylori* 120-kDa protein were found in gastric biopsy specimens from patients infected with *H. pylori*. The presence of the antibody was correlated strongly with the presence of peptic ulcer and severe gastritis (Crabtree *et al.*, 1991). This very immunogenic protein is expressed in association with the vacuolating toxin (Crabtree *et al.*, 1992), and the antigen has been named cagA (cytotoxin-associated protein); its gene (*cagA*) has been sequenced. Clinical isolates that do not produce the antigen do not have the gene and are unable to produce an active vacuolating cytotoxin (Covacci *et al.*, 1993). An ELISA for the 120-kDa protein on sera has allowed investigations of the sera of *H. pylori*-infected patients (Crabtree *et al.*, 1992).

Crabtree *et al.* (1993a) examined the systemic IgG response to *H. pylori* in 70 gastric cancer patients; 79% were seropositive by ELISA for *H. pylori* infection. Of these ELISA-positive sera, 91% recognized the *H. pylori* 120-kDa cagA protein by western blotting, significantly more than a control group of 47 ELISA-positive patients with non-ulcer dyspepsia (72%).

Cytotoxic strains that express the cagA antigen of *H. pylori* have also been shown to induce rapid secretion of significantly more IL-8 in gastric epithelial cell lines than non-cytotoxic strains (Crabtree *et al.*, 1994). IL-8 has been shown to be expressed *in vivo* in *H. pylori*-infected people and is known to be a potent neutrophil chemotactic and activating factor (Crabtree *et al.*, 1993b; Noach *et al.*, 1994). Increased IL-8 production has also been seen in neoplastic tissue. In a further study using immunofluorescence techniques to locate IL-8 in cryosections of gastric and duodenal biopsies and resected gastric tumour tissue samples, it was found in the epithelium of histologically normal gastric mucosa, with particularly strong expression in the surface cells. Gastric epithelial IL-8 expression was increased in chronic *H. pylori*-associated gastritis, and expression of IL-8 within the lumina propria was evident. Gastric carcinoma cells also expressed IL-8 (Crabtree *et al.*, 1994).

4.2.2 *Experimental systems*

Molecular lesions, cell changes and other precancerous markers have not been measured directly in experimental animals, but *Helicobacter*-induced changes have been mimicked and the effects measured. Thus, Tsujii *et al.* (1993) administered ammonia to rats in the drinking-water for three days a week for one, two, four and eight weeks at a concentration (0.01%) that was considered to be equivalent to that of gastric juice in *H. pylori*-infected people (reported to be 0.015%, as compared with < 0.005% in uninfected people) (Triebling *et al.*, 1991; Neithercut *et al.*, 1993). Controls were given tap-water alone. After four to eight weeks, the mucosal thickness of the antrum but not of the body of the stomach was decreased. Epithelial cell migration rates, measured by incorporation of 5-bromo-2′-deoxyuridine (BrdU), were significantly increased, particularly in the antrum. The BrdU-labelling index was also significantly increased in all ammonia-treated groups. The proliferative zone in the antrum was significantly enlarged as mucosal atrophy developed, whereas in the corpus mucosa enlargement of the proliferative zone occurred despite the absence of mucosal atrophy.

In an investigation by the same group of the possible role of ammonia as a promoter, 85 male Sprague-Dawley rats, five weeks of age, received MNNG at 83 mg/L in the drinking-water for 24 weeks. Forty treated animals were then given tap-water, and 40 were given 0.01% ammonia in the drinking-water. Animals were kept for a further 24 weeks. All rats were killed when moribund or at 48 weeks after the commencement of MNNG treatment. A significantly higher proportion of the rats given MNNG followed by ammonia developed gastric adenocarcinomas (26/37) than those given MNNG followed by tap-water (12/39; $p < 0.01$) (Tsujii *et al.*, 1992).

Administration into the stomachs of mice of a sonicated sample of a cytotoxin-producing strain of *H. pylori* induced epithelial vacuolation and limited infiltration of mononuclear cells into the lamina propra. A sonicated sample of a non-toxin-producing strain did not cause epithelial lesions. The *H. pylori* cytotoxin gene has been cloned into *E. coli*, where a protein was synthesized as a 140-kDa precursor that is processed to a 94-kDa fully active toxin. Oral administration of this recombinant toxin to the mice induced vacuolation but not cell infiltration (Telford *et al.*, 1994).

Consistent with the observation of changes in the location of gastritis with reduced gastric acidity, *H. felis*, which is normally restricted to the antrum, appears in the body of the stomach of rodents given the acid-suppressive drug, omeprazole. Groups of specific pathogen-free BALB/c mice were colonized with *H. felis* and given omeprazole or no treatment for one month; one month after cessation of treatment, *H. felis* was seen in all areas of the stomach in the omeprazole-treated group but only in the cardia and antrum in the controls (Danon *et al.*, 1994). A similar result was obtained in omeprazole-treated *H. felis*-infected rats (Mellgard *et al.*, 1994). [The Working Group noted the incomplete reporting of the data.]

A recently identified bacterium, *H. hepaticus*, was first isolated in association with hepatocellular tumours in mice. Mice infected with this bacterium developed liver lesions, but tumour development has not yet been seen because of the short duration of the experiments reported (Ward *et al.*, 1994).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Helicobacter are spiral, flagellated, gram-negative bacteria that colonize the gastrointestinal tract of human beings and animals. *H. pylori* is restricted to human gastric mucosa and can infect some other primates. *H. pylori* strains are genetically heterogeneous, and this attribute is useful in studies of transmission. *H. pylori* can be cultured, is sensitive to most antibiotics *in vitro* and is characterized by very strong urease activity.

Colonization of the gastric mucosa and subsequent development of gastritis are dependent on bacterial factors, including motility, potent urease activity and specific adherence to gastric epithelium.

H. pylori can be detected in gastric biopsy specimens and indirectly by serology and analysis of breath after ingestion of labelled urea. Standard histological and bacteriological techniques, the polymerase chain reaction and indirect tests are highly sensitive. The rapid urease test on biopsy specimens is practical but less sensitive. Epidemiological studies currently involve use of serological tests and mainly commercially available enzyme-linked immunosorbent assay kits.

H. pylori occurs worldwide and causes a chronic infection which rarely resolves spontaneously. Its prevalence is highest in developing countries and increases rapidly during the first two decades of life, such that 80–90% of the population may be infected by early adulthood. In most developed countries, the prevalence of infection is substantially lower at all ages, and especially in childhood. The prevalence increases gradually throughout life up to the age of 70–80 years. The prevalence in both developed and developing countries is higher among people in lower socioeconomic classes and may be associated with crowding in childhood. A progressive reduction in the rate of infection early in life of people in successive birth cohorts has been observed in developed countries. Transmission occurs from one person to another; both oral–oral and oral–faecal routes have been postulated.

H. pylori causes gastritis in all infected people. This is accompanied by a specific, systemic immunoglobulin G response. Nevertheless, many such infections are asymptomatic. In some people, the infection gives rise to duodenal or gastric ulceration. The infection can be eradicated successfuly with several regimens in which different drugs are combined. Eradication of *H. pylori* resolves gastritis, prevents recurrence of peptic ulcer disease and leads to a significant decline in immunoglobulin response within six months.

5.2 Human carcinogenicity data

Six studies in which estimates of prevalence of infection by *H. pylori* were related to estimates of concurrent or earlier incidence of or mortality from cancer of the stomach in five or fewer populations show no consistent association between these variables. Significantly positive geographical correlations were observed, however, in two larger studies in which the ranges of cancer incidence and mortality were much wider: one in 46 rural populations in China and the other in 17 populations in Europe, Japan and the USA. The populations of

certain developing countries, including many in Africa and some in Asia, have low rates of gastric cancer; the prevalence of *H. pylori* infection has been studied in some of these populations and is known to be high.

The association between prior seropositivity for *H. pylori* and subsequent gastric cancer has been evaluated in three cohort studies, yielding 29–109 cases of gastric cancer. Significant positive associations were observed in all three, with estimated relative risks, based on case–control analyses within the cohorts, varying from 2.8 to 6.0. In a pooled analysis of the three studies, the relative risk was 3.8, which was significant, and there was a significant trend towards increasing estimated relative risks with increasing length of follow-up. In these cohort studies, potential confounding by dietary and other factors that have previously been associated with gastric cancer was not assessed. The extent to which such factors could have contributed to the association between gastric cancer and infection with *H. pylori* is difficult to estimate in view of the imprecision of assessments of past dietary habits.

Nine retrospective case–control studies have addressed the association between seroprevalence for *H. pylori* infection and incidence of gastric cancer. The estimated relative risks for gastric cancer were elevated in six studies, ranging from 1.2 to 4.2, and were significant in three studies. In a number of studies, the control series may not have been representative of the population that gave rise to the cases, either because of the method of sampling (e.g. subjects requiring gastrointestinal investigation) or because of exclusions on the basis of a history of gastric symptoms or disease.

When appropriate stratifications of the results of the prospective and retrospective studies were reported, the association between infection with *H. pylori* and gastric cancer was stronger in younger patients and for cancers at sites other than the cardia. The association was similarly strong for the intestinal and diffuse histological types of cancer.

The association between *H. pylori* infection and gastric lymphoma has been investigated in some studies. In two series of 110 and 178 patients with gastric B-cell mucosa-associated lymphoid tissue lymphomas, 92 and 98%, respectively, had histological evidence of *H. pylori* infection. In two studies of treatment, five of six patients and 12 of 16 patients showed tumour regression after therapy to eradicate *H. pylori*. Thirty-three cases of gastric non-Hodgkin's lymphoma were observed in a cohort study of patients with *H. pylori* infection in the USA and Norway, giving a significant estimated relative risk of 6.3.

5.3 Animal carcinogenicity data

No adequate study on *H. pylori* was available.

5.4 Other relevant data

The gastric precancerous process is characterized by sequential lesions of the gastric mucosa, namely chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia. This constellation of lesions occurs in one major type of gastric adenocarcinoma, the intestinal type, the prevalence of which has been declining in developed countries. The other major type is diffuse carcinoma, which is becoming relatively more frequent in those countries and is associated with chronic nonatrophic gastritis.

H. pylori is the main cause of most types of chronic gastritis. This statement is supported by the observation that gastritis developed after voluntary ingestion of bacterial cultures, the consistent association between infection with the bacterium and gastritis throughout the world and the disappearance of gastritis after successful treatment of the infection.

Three independent cohort studies have shown the progression of gastritis from the non-atrophic to the atrophic form. Epidemiological studies of atrophic gastritis have also shown an association with dietary factors, especially excessive salt intake and inadequate consumption of fresh fruits and vegetables.

The bacteria are present in the human gastric stomach as extracellular colonies in the gastric mucus. In most patients, some bacteria adhere to the epithelial cells. Atrophic gastritis induced by *H. pylori* results in overgrowth of other bacteria.

Several *Helicobacter* species induce gastritis in many domestic and experimental animals. Infection with *H. felis* induced chronic gastritis followed by atrophy in mice.

The mechanisms by which *H. pylori* may increase the risk for gastric cancer are unknown. The bacterium has been shown to increase cell replication in the gastric mucosa. Some strains of *H. pylori* which induce inflammation of the gastric mucosa produce cytotoxin. Cytotoxin-associated strains are predominant in both gastric cancer patients and patients with both duodenal ulcer and atrophic gastritis. A protein associated with cytotoxin-positive *H. pylori* strains (cagA) induces expression of interleukin 8 in gastric mucosa, which appears to be correlated with degree of inflammation.

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Helicobacter pylori*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of infection with *Helicobacter pylori*.

Overall evaluation[2]

Infection with *Helicobacter pylori* is carcinogenic to humans (Group 1).

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[1]For definition of the italicized terms, see Preamble, pp. 30–34.
[2]Dr T. Shirai disassociated himself from the overall evaluation.

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