The prevalence of helicobacter antibodies increases with age, and in many developed countries, is highest in people born before 1940. Data on very old subjects are, however, limited. In this study we wanted to determine whether the age-related increase in the seroprevalence of H. pylori infection continues even in the oldest age group alive in Finland, the centenarians.

Methods. Sera from 173 subjects (93% of all centenarians alive in Finland in 1991) were available for the present study. IgG and IgA antibodies against H. pylori were determined by an in-house enzyme immunoassay. To estimate the influence of atrophic gastritis on the prevalence of helicobacter antibodies, serum pepsinogen I (PG I) concentrations and parietal cell antibodies (PCAs) were measured by an enzyme immunoassay and indirect immunofluorescence, respectively.

Results. The prevalence of helicobacter antibodies in Finnish centenarians was 66%. Low PG I values (<28 µg/l) were found in 36% and positive PCAs in 16% of the subjects studied. The prevalence of PCAs was especially high (50%) in H. pylori-negative subjects with low PG I values, suggesting severe gastric atrophy.

Conclusions. The age-related increase in H. pylori seroprevalence did not continue in the oldest age group alive in Finland. This may be explained partly by a relatively high frequency of atrophic gastritis (as suggested by low PG I values) in H. pylori-negative centenarians, but other factors—such as selective H. pylori-related mortality—may also have contributed to the fairly low seroprevalence (66%) observed.

Helicobacter Antibodies in Finnish Centenarians

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Background. The prevalence of helicobacter antibodies increases with age and, in many developed countries, is highest in people born before 1940. Data on very old subjects are, however, limited. In this study we wanted to determine whether the age-related increase in the seroprevalence of H. pylori infection continues even in the oldest age group alive in Finland, the centenarians.

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Conclusions. The age-related increase in H. pylori seroprevalence did not continue in the oldest age group alive in Finland. This may be explained partly by a relatively high frequency of atrophic gastritis (as suggested by low PG I values) in H. pylori-negative centenarians, but other factors—such as selective H. pylori-related mortality—may also have contributed to the fairly low seroprevalence (66%) observed.

H. pylori causes active chronic gastritis and is an important risk factor for peptic ulcer disease (1) and gastric cancer (2). The association between gastric malignancy and H. pylori infection is at least partly explained by the natural progression of chronic gastritis into atrophy, a well-known risk factor for gastric cancer (3,4). The prevalence of helicobacter antibodies increases with age, and this increase seems to reflect a cohort effect due to acquisition of infection in childhood (5). In developing countries the infection is acquired early, and usually more than 80% of the adult population is infected (6). In the majority of developed countries, the highest prevalence rate (50%-60%) dates to people born before 1940; after this peak there is often a lower prevalence in the oldest age groups (6). Data on very old subjects are, however, limited.

In Finland, the prevalence of helicobacter antibodies mainly parallels that of other developed countries. In Finnish blood donors from the Helsinki area, the seroprevalence increased from 10% in subjects 18 to 25 years of age to 60% in those aged 56 to 65 (7). A similar trend was observed in a recent study in the population of Vammala (5). Further age-related increase up to 69% was demonstrated in a Helsinki aging study comprising people 75 to 85 years of age (8). The aim of this study was to determine whether the age-dependent increase in frequency of helicobacter antibodies extends to the oldest age group alive in Finland, or if there is a similar drop in the seroprevalence as seen in many other countries in persons who are even a couple of decades younger. To estimate the influence of atrophic gastritis on the prevalence of helicobacter antibodies in centenarians, serum pepsinogen I (PG I) concentrations and parietal cell antibodies (PCAs) were also measured.

Materials and Methods

Subjects.—All Finns older than 100 years participated in a Finnish health evaluation study in 1991 (9). Sera from 173 subjects (93% of the study population of which 83% were women) were available for the present study. The mean age of the subjects studied was 101 years and 1 month. None of the centenarians had ever received an antihelicobacter treatment, and there were no gastric cancers diagnosed (information acquired from the Finnish Cancer Register). Nine subjects had a history of peptic ulcer disease (four gastric and two duodenal ulcers; the location of three ulcers was not known). In addition to these H. pylori-related gastrointestinal diseases, the medical history of the study population included possible heart disease, vitamin B12-deficiency anemia, cerebrovascular disease, eczema, rheumatoid arthritis, and hip prosthesis. The use of antimicrobial agents, acid suppressors, and nonsteroidal anti-inflammatory drugs (NSAIDs) at the time of clinical assessment was recorded. The centenarians were divided into four social classes on the basis of their occupation at the time of retirement. The educational level was classified into four grades as well, and the place of birth and the place of residence in 1991 were recorded.
Serology.—Immunoglobulins G (IgG) and A (IgA) antibodies against \textit{H. pylori} were determined by an in-house enzyme immunoassay (10). The antigens applied were an acid glycine extract (7) and a sonicate (11) prepared from \textit{H. pylori} strain 11637. The test samples were applied in duplicate wells, in threefold dilutions when needed. The reference sera were placed on each microtiter plate along with test samples and were used for the determination of the cutoff level. Separate reference pools were used for IgG and IgA. The absorbance values at 405 nm were recorded with a Titertek Multiskan analyzer (Eflab Oy, Helsinki, Finland) and converted to reciprocals of the end-point titers. The lower limits of raised titers were 700 for IgG and 70 for IgA. With these limits, the sensitivity and specificity of the tests were 94% and 93% for IgG and 73% and 95% for IgA, as determined in a separate series of 544 patients (using culture and histology as reference methods) during the same period as this study.

\textbf{Pepsinogen I measurements.}—The PGI concentration was determined by an enzyme immunoassay (Gastroset PG I, Orion Diagnostica, Espoo, Finland) performed according to the manufacturer's instructions. Briefly, serum samples along with prediluted calibrators (pure PG I antigen in phosphate buffer) and controls (lyophilized preparations of pure PG I in human serum) were pipetted into the wells coated with monoclonal antibody against human PG I. Assay buffer was added and plates were incubated at room temperature on a shaker for 30 minutes before they were washed. An enzyme-labeled monoclonal antibody against human PG I (anti-PG I-HRP-conjugate) was added. The plates were incubated for 30 minutes and washed before a prediluted substrate solution was pipetted into each well. The reaction was stopped after incubation of 30 minutes with 2 M \text{H}_2\text{SO}_4, and plates were shaken for 1–2 minutes. The absorbance values at 450 nm were recorded with a Titertek Multiskan analyzer and converted to PG I concentrations. The reference range of PG I values for both sexes was 28–158 \text{ug/l}, according to the manufacturer.

\textbf{Parietal cell antibodies.}—PCAs were detected by an indirect immunofluorescence technique (12) using polyvalent (rabbit antihuman IgG, IgM, and IgA antibodies (Dako, Glostrup, Denmark) conjugated with fluorescein isothiocyanate. Unfixed, frozen (5 \text{m} \text{m} \text{m} thick) sections of mouse and rat stomach were applied as substrates. Rat kidney and mouse liver sections were used as controls to exclude staining due to mitochondrial antibodies. For screening, the investigated sera were diluted 1:10 in phosphate-buffered saline (PBS, pH 7.2). Serum samples with positive PCA reactions were retested at fivefold dilutions, and sera reacting with parietal cells at a dilution of $\geq 1:50$ were considered positive.

\textbf{Statistical methods.}—The statistical significances were calculated by the chi-square test or the Fisher's exact test, when appropriate.

\textbf{RESULTS}

Elevated IgG antibodies were found in 59% and IgA antibodies in 48% of the subjects by the acid glycine extract test. Elevated IgA antibodies without elevated IgG antibodies were recorded in 5% of persons studied, and a further increase in the prevalence of helicobacter antibodies to 66% was reached by using the data obtained with the sonicate in the IgG test. The prevalence of antibodies was 67% in women and 62% in men, a statistically nonsignificant difference (Table 1).

PG I concentration was measured from 170 serum samples. Concentrations <28 \text{pg/l} were interpreted as low and indicative of atrophic gastritis. The median PG I concentration in the whole study population was 44.7 \text{pg/l} (44.8 \text{pg/l} in \textit{H. pylori}-positive, and 43.5 \text{pg/l} in \textit{H. pylori}-negative subjects). The total prevalence of low PG I values in these centenarians was 36%; 44% (12 of 27) in men and 35% (50 of 143) in women (difference statistically nonsignificant). The overall distribution of PG I values was quite similar in both the \textit{H. pylori}-positive and \textit{H. pylori}-negative groups, as was the proportion of low PG I values (37% or 42 of 112 and 34% or 20 of 58, respectively; see Figure 1).

PCAs were found in 26 of 163 (16%) samples and were more common in \textit{H. pylori}-positive (15 of 56, 27%) than in \textit{H. pylori}-negative (11 of 107, 10%) subjects ($p = .006, \chi^2$ test). The prevalence of PCAs was higher in those with low (<28 \text{pg/l}) PG I concentrations, and this difference was statistically significant ($p = .003, \chi^2$ test) in \textit{H. pylori}-negative subjects (Figure 2).

There were no differences in \textit{H. pylori}-positive and -negative subjects with respect to demographic and medical aspects studied.

| Table 1. Prevalence of Elevated \textit{H. pylori} Antibodies (IgG and IgA) in Finnish Centenarians |
|-----------------|-----------------|-----------------|
| IgG or IgA       | n (%)           | n (%)           | n (%)           |
| Men             | 17 (59)         | 9 (31)          | 18 (62)         |
| Women           | 89 (62)         | 74 (51)         | 97 (67)         |
| Total           | 106 (61)        | 83 (48)         | 115 (66)        |

Note: IgG $\geq 70\%$; IgA $\geq 70\%$.

Figure 1. Distribution of pepsinogen I (PG I) concentrations in \textit{H. pylori}-positive (HP+) and \textit{H. pylori}-negative (HP-) subjects. *Concentrations <28 \text{pg/l} regarded as low.

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In this study, low PG I concentrations (<28 μg/l) were demonstrated in about one third of the subjects regardless of their helicobacter status. PCA were positive in 16% of sera studied, and 61% (16 of 26) of these PCA-positive centenarians also had lowered PG I concentrations. Half of the *H. pylori*-negative subjects with low PG I values had PCAs suggesting severe corpus atrophy. If one assumes that low PG I and/or occurrence of PCA is indicative of severe gastric atrophy, resulting eventually in the disappearance of *H. pylori* infection and helicobacter antibodies, the "true," pre-atrophic prevalence of helicobacter infection in this birth cohort might have been somewhere between 72% and 78%.

Some authors have reported reduced sensitivity of *H. pylori* serology in subjects older than 60–70 years of age (19,20,21). If true, the lower sensitivity of *H. pylori* serology could lead to falsely decreased seroprevalence in our very old subjects, as well. The results presented in literature are, however, conflicting, and sensitivities between 90% [histology as a reference method; (22)] and 96% [histology and/or culture as a reference method; (8)] have also been reported. In the latter study, the age of the study population varied between 75 and 85 years, and the serology was carried out in our laboratory. Furthermore, in a recent study we demonstrated that in elderly men with atrophic gastritis, serology was a more sensitive marker of ongoing infection than invasive methods (urea breath test and histology) used (23). Because the same (sensitive) serological method was also applied in the present study, we believe that underestimation of seroprevalence has not occurred in the centenarians studied.

This study on a unique population of centenarians was possible because of the well-compiled statistics provided in Finland; few countries are able to screen out centenarians reliably from the rest of the population. The subjects comprised 93% of all centenarians in Finland. The proportion of each birth cohort that survives to this very old age is small and, thus, quite selected. In this selected population, the mortality related to helicobacter-associated diseases (e.g., peptic ulcer disease, gastric cancer) and to risk factors of *H. pylori* infection (e.g., poor hygiene, poverty) may also have contributed to the relatively "low" prevalence of helicobacter antibodies observed.

As *H. pylori* infection is mainly acquired in childhood, it is only natural that childhood living conditions (e.g., social class, household crowding, number of siblings) greatly influence the risk for infection (24,25). Our knowledge of the social parameters of the centenarians studied was limited, however, and restricted only to adulthood. Thus, it was not surprising that socio-economic conditions and educational level at the time of retirement were not related to helicobacter status.

Although well documented in younger age groups, the association between helicobacter infection and (histologically verified) peptic ulcer disease may not be quite as clear in the older subjects (21). In our study group, 9 out of 173 centenarians had a history of peptic ulcer disease, but this history was not correlated with the presence of *H. pylori* infection. The information about the actual timing of peptic ulceration, diagnostic method(s) used, or the present status of stomach and duodenum at the time of clinical investigation were not available to us. No relation was found between the *H. pylori* status and use of antimicrobial agents, NSAIDs, or acid suppressors at the time of clinical assessment. Similar lack of association has been re-
recently reported in elderly inpatients and institutionalized people by Neri and colleagues (13).

In conclusion, the age-related increase of *H. pylori* seroprevalence did not continue in the oldest age group alive in Finland. This lack of increase may partly be explained by a relatively high frequency of atrophic gastritis (as indicated by low PG I concentration and/or presence of PCAs) in *H. pylori*-negative centenarians, but other factors, such as selective mortality, also may have contributed to the fairly low seroprevalence (66%) observed.

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