LETTERS TO THE EDITOR

Serum pepsinogens in blood transfusion donors

EDITOR,—We read with great interest the paper by Veenendaal *et al* in *Gut*.¹ In this study the authors showed that blood donors positive for *Helicobacter pylori* antibodies revealed higher serum pepsinogen A and C levels than their seronegative counterparts. In addition, the authors plotted the measured IgA and IgG absorbance index against age and showed a weak but significant correlation between the height of the absorbance index and rising age. The authors suggested that this increase could be caused by progression of chronic superficial gastritis.

We cannot confirm these findings and do not agree with this conclusion. In an earlier study we showed that the positive/negative (P/N) ratio (identical to Veenendaal's absorbance index) in patients with non-ulcer dyspepsia (NUD) and proved active helicobacter associated gastritis was significantly higher than in healthy seropositive blood donors² (mean 3.63 (1.24) range 1.06–6.45 *v* mean 2.32 (0.47) range 1.51–3.43 respectively, *p*<0.001). Although considerable overlap was present in the lower ranges, it was concluded that high P/N ratios occur with active inflammation and that lower P/N ratios can reflect a serological scar of past infection as well. It was also shown that inflammation with polymorphonuclear cells invading the mucosa causes a higher P/N ratio, hence antibody response, compared with a milder degree of inflammation.³ In the Table

TABLE Mean P/N ratio and standard deviation (SD) in patients with proved helicobacter gastritis and healthy seropositive blood donors

H Pylori gastritis		Blood donors	
Age (yr)	Number	Mean P/N ratio (SD)	Mean P/N ratio (SD)
21–30	7	3.34 (0.93)	2.14 (0.43)
31–40	8	2.90 (1.24)	2.24 (0.41)
41–50	8	3.75 (0.97)	2.35 (0.49)
51–60	12	3.71 (0.92)	2.46 (0.48)
61–70	11	4.37 (1.25)	2.23 (0.62)

and Figure the mean P/N ratio and the standard deviation of the healthy seropositive blood donors and the patients with NUD are plotted against age. It is clear that the findings of Veenendaal *et al* cannot be confirmed as no increase in P/N ratio with rising age is seen in the healthy blood donors. On the other hand, a slight increase in antibody response is seen among the patients with non-ulcer dyspepsia. Comparison of age cohorts 21–30 and 31–40 with age cohort 61–70 revealed a *p* value of 0.08 and 0.02 respectively (Student's *t* test); the other cohorts showed no differences in the height of the P/N ratio. In our opinion, this does not indicate that the antibody titre in an individual patient increases with rising age. Longitudinal serological follow up has not been done to our knowledge. The only data reported in previous studies show a decrease in antibody

Figure: Data from the table shown graphically.

titre after therapeutic intervention aimed at suppression or eradication of *H pylori*, and an increase in the incidence of recrudescence or reinfection. From the paper of Veenendaal *et al* it cannot be concluded that the antibody titre from an infection with *H pylori* acquired at an early age rises when the patients get older.

The statement that chronic active gastritis becomes worse with rising age can be considered correct if the development of intestinal metaplasia and glandular atrophy are considered a part of the deterioration of gastric mucosal histology. As reported in previous studies, however, the role of *H pylori* during the course diminishes and it is more logical to expect that the antibody response in the individual patient does not rise during the course of helicobacter gastritis. Serological follow up in gastritis patients is necessary to learn more of the natural history of the antibody response.

R J L F LOFFELD
Department of Internal Medicine,
Ziekenhuis De Heel Zaandam,
E E STOBBERINGH
Department of Microbiology
J W ARENDS
Department of Pathology,
University Hospital,
Maastricht,
The Netherlands

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Reply

EDITOR,—We thank Loffeld *et al* for their interest in our paper which showed that the age related increase in serum pepsinogen A and C occurring in healthy populations, is caused by *Helicobacter pylori* infection.¹ As Loffeld *et al* do not mention data regarding Pepsinogen A and C, further comparisons between their patient group and ours on this point are thus unfortunately not possible. In addition, we also noted an age related increase in *H pylori* antibodies expressed as an IgA and IgG absorbance index which gives a semiquantitative serological result. In their letter, Loffeld *et al* do not mention data regarding a correlation

analysis. They do state, however, that the positive/negative (P/N) ratio in patients with non-ulcer dyspepsia increases weakly with rising age (comparison of age cohorts 21–30 and 31–40 with age cohort 61–70 showed a *p* value of 0.08 and 0.02 (Student's *t* test)).

In Dr Loffeld's group of seropositive blood transfusion donors, a slight increase of the P/N ratio with rising age was also seen (comparison of age cohorts 21–30 and 31–40 with age cohort 51–60 gave a *p* value of 0.06 and 0.04), when the unrepresentative age cohort 61–70 with only seven donors (maximal age for blood donation 65 years) was disregarded. Loffeld *et al* also found that a higher serological P/N ratio is related with more active inflammation. We feel that these findings support rather than dispute our suggestion previously mentioned.

We do agree, however, with Loffeld *et al* that only long term longitudinal serological follow up together with histological examination of repeated gastric biopsy specimens can provide certain proof for our suggestion.

R A VEENENDAAL
I BIEMOND
Department of Gastroenterology-Hepatology,
University Hospital Leiden,
Leiden,
The Netherlands
A S PENA
Department of Gastroenterology,
Free University Hospital,
Amsterdam,
The Netherlands

- 1 Veenendaal RA, Biemond I, Peña AS, van Duijn W, Kreuning J, Lamers CBHW. Influence of age and *Helicobacter pylori* infection on serum pepsinogens in healthy blood transfusion donors. *Gut* 1992; 33: 452–5.

Faecal pH and colon cancer

EDITOR,—I would like to comment on the excellent letter¹ from Johannesburg reporting on the low faecal pH levels in African subjects and their possible connection with the low incidence of colorectal cancer.

My own experience in India supports the concept of a possible relation between faecal pH and the incidence of large bowel cancer.^{2,3} A group of 60 patients with colorectal cancer, principally south Indians, had a median faecal pH of 8.6–9.0, compared with a median pH of 6.1–6.5 in 120 matched healthy Indian subjects. The large differences in pH values between patients with colorectal cancer and the control group appear to be dependent on diet. The former ate non-masticatory meals of boiled refined rice, which were low in dietary fibre, and low in fermented milk products. The latter ate high fibre meals of thick whole wheat chapatties, vegetable and legume curries, and their diets were also high in fermented milk products such as yoghurt, yoghurt drink, white cheese, and ghee – made from fermented milk and rich in short chain fatty acids. Fibre ferments in the gut lumen liberating large quantities of acetic acid (precursor of short chain fatty acids). This augments the H⁺ ion in the case of the controls.⁴

S L MALHOTRA
27 East 37th Street, Apt 4,
New York,
NY 10016 USA

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Gastric cancer mucins as clinical markers

EDITOR,—We read with interest the article by Hakkinen *et al*¹ reporting the development of a new monoclonal antibody against two epitopes of mucin which they had precipitated from the gastric juice of a patient with gastric carcinoma. The epitopes were expressed in fetal tissue and most cases of adult gastric carcinoma tested.

We have detected a glycoprotein fragment of molecular size 55–65 kilodaltons cofractionating with mucin in the gastric mucus (not gastric juice) of 30 patients with gastric carcinoma.^{2–4} We also found it in 30 patients with gastric ulcers in common with Hakkinen *et al*.¹ We did not detect it in the mucus from the stomachs of 16 normal subjects (eight at necropsy, eight transplant donor). In our study crude mucus scrapings were used and mucins were extracted from the gel in the presence of proteolytic inhibitors and isolated by density gradient ultracentrifugation in caesium chloride, twice for 48 hours each at 105 000 *g*. Mucins were then characterised by Sepharose 2B and 4B gel filtration and polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE).

Mucins eluted as a carbohydrate positive peak mainly in the included volume of a

Sepharose 2B gel filtration column suggesting that they were degraded; a protein (Lowry) positive peak eluted in the near total volume of this volume under a carbohydrate (PAS) positive shoulder. Sepharose 4B gel chromatography, on the other hand, showed that this degradation of the mucins was most extensive in the cancer group, followed, in order, by ulcer and control groups. This finding was confirmed on a 4–20% gradient gel stained with PAS.

SDS-PAGE analysis of the protein positive peak in the near total volume of the Sepharose 2B column showed a glycosylated fragment of Mr 55–65 kilodaltons which was not dissimilar in size to the epitopes found by Hakkinen *et al*¹ with their immunoblotting experiments; this appeared in mucins from the cancer and ulcer groups. We also note that Hakkinen *et al*¹ showed a protein positive band at molecular weight approximately 67 kilodaltons, which they have not elaborated upon (Fig 2). We have also detected this band and found it to be albumin by western blot analysis using a human albumin antibody. The use of different gel stains showed the albumin to be clearly distinguishable from the carbohydrate positive fragment.⁴

This albumin carbohydrate complex obviously resisted dissociation in 3.5 M CsCl, but could be separated partially from the mucin in sodium dodecyl sulphate at 100°C for one to two minutes, or by gel filtration. Treatment of mucin with 0.2 M 2-mercaptoethanol did not produce this albumin carbohydrate complex from the control groups nor did it increase its

amount in the diseased states. It has also been found in the gastric juice of a patient with cancer but was absent in the juice of patients of other groups.

We are continuing our work with gastric juice rather than mucus scrapings which are more difficult to obtain; we hope to harvest this albumin carbohydrate complex in sufficient quantities and, like Hakkinen *et al*,¹ to raise an antibody against it and investigate its potential as a marker for premalignancy in a high risk group.

A MALL
H McLEOD
D DENT
R HICKMAN
*Department of Surgery and
MRC Liver Research Centre,
University of Cape Town Medical School,
Observatory 7925,
Republic of South Africa*

This work is supported by the National Cancer Association and Medical Research Council of South Africa.

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