Gastric and duodenal mucosa in 'healthy' individuals

An endoscopic and histopathological study of 50 volunteers

J. KREUNING¹, F. T. BOSMAN², G. KUIPER¹, A. M. v.d. WAL², AND J. LINDEMAN²

From the Department of Gastroenterology¹ and the Department of Pathology², University Medical Centre, Wassenaarseweg 62, Leiden, The Netherlands

SUMMARY The results of histological and immunohistochemical examination of gastric and duodenal biopsy specimens from 50 volunteers without a clinical history of gastrointestinal disease are reported. Multiple specimens of tissue from standard sites in the stomach and duodenum were carefully orientated, and serially sectioned for examination by light microscopy and for immunohistochemical characterisation of plasma cells within the lamina propria.

The antrum and fundus were normal in 32 of the 50 subjects but the other 18 showed histopathological evidence of gastritis in either the antrum or fundus. The latter appeared to be age-related.

There was considerable variation in the appearance of the surface epithelium of the duodenum within as well as among individual subjects. Superficial gastric metaplasia in one or more biopsy specimens from the duodenal bulb was found in 64% of individuals. Histopathological examination of the duodenum revealed signs of chronic inflammation in 12% of the subjects. In two individuals there was active inflammation but in only one of these was the diagnosis made on endoscopic appearances. Histological criteria important for the diagnosis of duodenitis are discussed.

The number of plasma cells in different biopsy specimens from subjects not showing histological signs of inflammation was variable. The ratio IgA:IgG:IgM producing plasma cells was remarkably constant from subject to subject as well as from specimen to specimen.

The advent of fiberoptic endoscopy in recent years has enormously extended the diagnostic possibilities in gastroenterology. Endoscopic appearances may be valuable in diagnosis but more accurate and detailed information results from histological examination of mucosal biopsy specimens. In several papers (Whitehead, 1973; Wolff, 1974; Chaput *et al.*, 1974; Whitehead *et al.*, 1975; Cheli and Aste, 1976) the significance of histological examination of biopsy specimens has been stressed and classification schemes for different types of gastritis and duodenitis have been proposed. Little attention has so far been paid to the histological spectrum of gastric and duodenal mucosa that may be found in individuals without a clinical history of gastrointestinal disease (Siurala *et al.*, 1968; Whitehead, 1973; Korn and

Foroozan, 1974). In biopsy specimens where histological examination reveals slight abnormalities it is important to know whether changes are correlated with specific complaints or are merely a variation of normal appearances. Variation of 'normal' gastric and duodenal mucosa taken in multiple biopsies has not been extensively studied. In order to establish diagnostic criteria for borderline lesions, for example, in chronic simple gastritis and duodenitis, knowledge of the variation in appearance of normal mucosa is of the utmost importance. Similarly, the diagnostic significance of changes in the density of cellular infiltrate in the lamina propria and in relative numbers of different immunoglobulin-producing plasma cells can be established only if variation of these values in normal mucosa is known. For this reason gastric and duodenal biopsy specimens taken from multiple standardised sites in 50 'healthy' volunteers have been studied.

Material and methods

TISSUE SPECIMENS

Fifty healthy volunteers, 31 of whom were members of the hospital staff, were examined. Their ages ranged from 20 to 58 years with an average of 33 years (Fig. 1). There were 23 women and 27 men. Volunteers with any clinical history of gastrointestinal disease were excluded. In all individuals endoscopy was performed with an Olympus GIF-K 30° forward-oblique-viewing fibrescope. Biopsy specimens were obtained from seven standard sites in the stomach and also from six standard sites in the duodenum (Fig. 2). The specimens were immediately examined with a stereomicroscope to judge mucosal thickness and surface structure. Some specimens were weighed.

TISSUE PROCESSING

Specimens were fixed in formol sublimate for six hours at room temperature (Bosman *et al.*, 1977). They were processed routinely in an autotechnicon, embedded after careful orientation in paraplast and sectioned at 4 μ . Multiple sections were mounted on each of three slides and stained with haematoxylin and eosin. Further adjacent sections were stained by James' reticulin method and the periodic-acid Schiff method.

Fig. 1 Age and sex distribution of volunteers

Fig. 2 Standard biopsy sites within the stomach and duodenum:

- 1 prepyloric
- 2 minor curvature just above the angulus
- 3 major curvature opposite to 2
- 4 high up the minor curvature, just below the cardia
- 5 antrum
- 6 middle of corpus, posterior wall
- 7 fundus
- I pars descendens next to papilla
- II pars descendens opposite to I
- III superior curvature of the bulb
- IV inferior curvature of the bulb
- V anterior wall of the bulb
- VI posterior wall of the bulb

IMMUNOHISTOCHEMISTRY

Immunoperoxidase staining for IgA, IgG, IgM, IgD, IgE, and κ and λ light chain immunoglobulin-producing cells was performed as described by Bosman *et al.* (1977). The sections were deparaffinised and rehydrated in phosphate buffered saline at pH 7.4. Sections were given three washes of five minutes each in phosphate buffered saline and then incubated in antiserum for 30 minutes. The following commercially available antisera were used: rabbit anti-human IgA, IgM, IgG, and IgE (Behring) and anti-human κ and λ light chain and IgD (Nordic). The method of Sternberger (1974) was used as a control for the first and second layers of the immunological reagents. As a final control the sera were

Table 1 Grading of density of infiltration in the lamina propria

Grade I	Few scattered mononuclear cells, mainly localised between the crypts
II	Fair number of mononuclear cells more diffusely distributed through the lamina propria
III	Large number of mononuclear cells, often in combination with short and broad villi
IV	Very heavy infiltrate with short and broad villi or absence of villi
Lymphoid follicles were excluded.	

tested on bone marrow cell preparations from IgA, IgG, IgM, IgD, and Bence-Jones κ and λ myelomas as recommended by Hijmans *et al.* (1969). The second layer consisted of a goat anti-rabbit γ -globulin conjugated to horseradish peroxidase (Sigma Chemicals C, type 6) using the method of Avrameas and Ternynck (1971). Peroxidase staining was performed with diaminobenzidine using the method of Graham and Karnovsky (1966). Sections were counterstained with haematoxylin.

Biopsy specimens taken from 12 randomly selected individuals with no histopathological changes were used for quantitative evaluation of numbers of different immunoglobulin-bearing plasma cells using a modification (Vermeer *et al.*, 1977) of the method described by Skinner and Whitehead (1974).

HISTOPATHOLOGY

All gastric biopsy specimens were classified according to the criteria of Whitehead (1973). All duodenal biopsy specimens were evaluated with regard to (1) shape of villi, (2) inflammatory infiltration and superficial gastric metaplasia in surface epithelium, (3) depth of crypts, depletion or hyperplasia of Paneth cells, and mitotic index in crypts, (4) localisation of Brunner's glands above or below the muscularis mucosae, (5) cellular infiltrate in the lamina propria (see Table 1), and (6) fibrosis of the lamina propria.

Results

STOMACH (Fig. 3)

Most volunteers showed no abnormality in the stomach on endoscopic examination. In two subjects, however, the suspicion of severe atrophic gastritis was confirmed on histological examination. All biopsy specimens were of adequate size, the mean weight being 7.2 mg (range 6.0-10.4 mg). Normal gastric mucosa was found in 64% of the subjects whereas in the other 36%, that is, 18 individuals, there was evidence of gastritis. In two cases this was restricted to the antrum. In the others the inflammation was present in both antrum and fundus. The

findings are summarised in Table 2. The age and sex distribution of those subjects showing gastritis is given in Table 3.

DUODENUM (Fig. 4)

Endoscopy revealed a distorted bulb with mucosal erosions in one instance. In this case biopsies showed degeneration and regeneration of surface epithelium and infiltration by neutrophil polymorphonuclear leucocytes. In another subject nodules were found on the posterior wall of the bulb, and histology revealed these to be areas of fundic-type mucosa with parietal and chief cells. Almost all biopsy specimens were of adequate size in that they included submucosa and more than four villi per section. The shape of the villi in the bulb was variable, some appearing finger-like and others leaf-like or ridge-like.

Table 4 shows the grading of the density of cellular infiltrate in the lamina propria. In only two biopsy specimens was migration of neutrophil polymorphonuclear leucocytes into the surface epithelium seen. Table 5 shows the relative frequency of histological changes found in duodenal tissue. In six patients a combination of histological changes was present. It is noteworthy that in 16 cases gastric metaplasia was present in one duodenal specimen, in nine it was seen in two, in four in three, and in three subjects it was present in all biopsy specimens. Gastric metaplasia was most frequent in the bulb, and only on one occasion was it noticed in the descending part of the duodenum. Brunner's glands were found above the muscularis mucosae in 48 subjects. In only 17 subjects were they present in the descending part of the duodenum. In four patients abnormal duodenal mucosa occurred simultaneously with gastritis.

IMMUNOHISTOCHEMISTRY

The number of plasma cells per unit area in the lamina propria in 12 subjects with no histopathological changes in any of the biopsy specimens is given in Fig. 5. The numbers vary considerably from person to person but, in general, the duodenum showed the highest and the fundus the lowest counts. The absolute numbers of IgG, IgM, and IgA producing plasma cells showed considerable inter-individual variation. The relative numbers exhibited a constant pattern in all specimens in all individuals (Fig. 6). The average ratio IgA:IgG:IgM was 10:2.4:1.1.

Discussion

STOMACH

In 50 apparently healthy volunteers histological evidence of inflammation was found in gastric

Fig. 3 (a) Normal fundus mucosa ($\times 50$). (b) Superficial fundus gastritis ($\times 75$). (c) Normal pyloric mucosa ($\times 125$). (d) Antrum gastritis ($\times 125$).

*Gastric and duodenal mucosa in 'healthy' individuals***Table 2** *Type of gastritis found in 18 subjects*

Diagnosis	No. of persons
Superficial antrum gastritis with superficial fundus gastritis	1 (1)
Atrophic antrum gastritis (AAG) with normal fundus mucosa	2
AAG with superficial fundus gastritis	1 (1)
AAG with fundus gastritis with slight atrophy	8 (5)
AAG with fundus gastritis with moderate atrophy	4 (2)
AAG with fundus gastritis with severe atrophy	2 (1)
Total	18

The numbers of cases with active gastritis are given in parentheses

Table 3 *Age and sex distribution of individuals with histological evidence of gastritis*

Age (yr)	Female	Male
20-29	2	2
30-39	2	2 (1)
40-49	6 (3)	2 (1)
50-59	1 (1)	1

The numbers of cases with moderate to severe atrophy are given in parentheses.

biopsy specimens in 18 individuals. Histological signs are not always paralleled by symptoms. Atrophic gastritis appeared to be an age-related process, the majority of subjects with moderate to severe forms of the condition being over 40 years. The severity of gastritis also increased with age. These findings are in agreement with those reported elsewhere by Siurala *et al.* (1968) and Wolff (1974). These authors also concluded that women over the age of 50 years were affected more frequently than men, and the findings in the present investigation support this contention. It is noticeable that gastritis is more frequent and more severe in the distal part of the stomach, which is a pattern also found in the gastric mucosa in patients with duodenal ulcer (Meikle *et al.*, 1976). An increased number of lymphocytes and plasma cells in the lamina propria may be the only histological sign of inflammation in the gastric mucosa but it should be borne in mind that in normal healthy individuals there is a fairly considerable variation in the number of plasma cells within the lamina propria.

DUODENUM

Direct vision endoscopic biopsies of duodenal mucosa yield sufficient material for histological examination provided that specimens are adequately orientated and serially sectioned. The shape of duodenal villi varied considerably in the biopsy specimens reported here and also in those reported

by Korn and Foroozan (1974). Unless changes in villous shape are of an extreme nature they should not be used as a criterion for the diagnosis of duodenitis.

In the present investigation the density of cellular infiltrate in the lamina propria was classified according to visual impression. Reliable cell counting is difficult to accomplish as considerable variations exist from specimen to specimen and also within a given biopsy specimen from villus to villus and indeed from section to section of the same villus. Grading according to visual impression appeared to be reasonably reproducible and this is in agreement with the findings of Beck *et al.* (1965). Owing to sampling difficulties it was not possible to use the density of cellular infiltrate in the lamina propria as a single indication of inflammation but it had to be employed in combination with other abnormalities such as increased numbers of plasma cells, extension of the infiltrate between Brunner's glands, and changes in the surface epithelium. Because of sampling variation duodenal disease cannot be excluded on a single normal biopsy. Lesions are often focal and found in only one or two of the four specimens of tissue taken from the bulb. Perera *et al.* (1975) regard superficial gastric metaplasia as a lesion of questionable significance. The frequent occurrence of this phenomenon in our material in specimens which showed no other signs of inflammation indicates, contrary to the opinion of Whitehead *et al.* (1975), that it cannot be considered a finding indicative of duodenitis. Superficial gastric metaplasia has a focal distribution, and in only three instances was it found in all four specimens from the duodenal bulb. Heterotopic gastric mucosa in the duodenum, which has been reported to occur more frequently in duodenal ulceration (Hoedemaeker, 1970), was encountered on only one occasion.

In no less than 96% of the biopsies from the duodenal bulb Brunner's glands occurred both above and below the muscularis mucosae. This is much more frequent than the incidence reported by Korn and Foroozan, probably due to the greater number of biopsies taken from each individual in the present series. In the descending part of the duodenum the presence of Brunner's glands was found in 34% of cases and this should be regarded as a normal phenomenon not related to duodenal disease. Slightly increased cellularity of the lamina propria, referred to as 'minimal inflammatory change' by Perera *et al.* (1975), pseudostratification of enterocytes, and slight variations in the shape of enterocytes are phenomena which are readily influenced by the orientation and thickness of sections and therefore need very cautious interpretation. Without other criteria of inflammation these phenomena should be regarded as variations of normal. Based on our

Fig. 4 (a) Chronic duodenitis ($\times 50$). (b) Villus with pseudostratification of surface epithelium and infiltrate grade III ($\times 125$). (c) Extension of infiltrate between Brunner's glands ($\times 300$). (d) Superficial gastric metaplasia ($\times 300$).

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Table 4 Histological grading of cellular infiltrate in duodenal biopsies

Grade	No. of subjects
I	23
II	20
II-III	3
III	4
IV	0
Total	50

Table 5 Histological findings in duodenal biopsy specimens

Characteristic	No. of subjects
Superficial gastric metaplasia	32
Fibrosis	5
Degeneration and/or regeneration of surface epithelium	4
Polymorphs in the lamina propria	4
Infiltrate grade III	4
Extension of infiltrate between Brunner's glands	6

results we propose the following classification of non-specific duodenitis.

(i) Chronic duodenitis

Grade III to IV cellularity of lamina propria (see Table 1)

Extension of infiltrate between Brunner's glands

Slight degeneration or regeneration of surface epithelium

Fibrosis of lamina propria

(ii) Silent chronic duodenitis

As in (i) but with

Grade I or II cellularity of lamina propria (see Table 1)

Absence of surface epithelial changes

(iii) Active chronic duodenitis

As in (i) but with

Marked degeneration or regeneration of surface epithelium

Invasion of epithelium by neutrophil polymorphonuclear leucocytes.

Evaluation of duodenal biopsies in patients with suspected duodenal disease will have to prove the value of this classification.

The association between gastritis and duodenitis is still debatable. In peptic ulcer of the duodenum, antral gastritis is quite frequent (Meikle *et al.*, 1976). Cheli and Aste (1976) reported the simultaneous occurrence of gastritis and duodenitis but rejected a causal relationship between the two conditions. The data presented here do not allow a firm conclusion to be drawn.

In all biopsy specimens from the 12 individuals selected for plasma cell counting a constant relationship was found between the number of IgA, IgG, and IgM producing cells regardless of the absolute number, which showed a considerable variation. Søltoft (1969) reported a differing ratio from ours in plasma cells counted in jejunal mucosa. This difference could well be due to the difference in the type of mucosa studied. It is important, in comparing such

Fig. 5 Plasma cell numbers in 12 individuals without histological signs of gastric or duodenal disease.

Fig. 6 Relative numbers of IgA, IgG, and IgM producing plasma cells expressed as percentages of total plasma cell counts. Bars indicate standard deviation.

differences, to take note of variation of plasma cell counts within and between individuals if only a limited number of sections from a single biopsy is studied. The diagnostic value of changes in relative numbers of plasma cell types in gastric and duodenal disease remains to be investigated.

The present study reveals that histological evidence of severe gastritis or of severe duodenitis may exist without clinical symptoms. It also shows that in duodenal mucosa a slight increase in cellularity of the lamina propria, variation in villous shape and size, gastric metaplasia, and the occurrence of Brunner's glands above the muscularis mucosae may all be regarded as within normal limits.

Mrs E. M. de Groot-van der Hoeven typed the manuscript; Mr K. van der Ham prepared the photographs; and Mr G. Flippo prepared the graphs.

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