Gastrin cells and fasting serum gastrin levels in duodenal ulcer patients

A quantitative study based on multiple biopsy specimens

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SUMMARY The number of gastrin-producing cells in biopsy specimens from the gastric and duodenal mucosae in 19 duodenal ulcer patients was quantitated using a morphometric method. The level of immunoreactive gastrin in a sample of fasting serum obtained at the time of the biopsy was measured by radioimmunoassay. The results show no significant difference when compared with those from a group of normal control volunteers. Moreover, there was no correlation between the numbers of gastrin-producing cells and the fasting serum gastrin level in either controls or duodenal ulcer patients.

It is widely believed that hypersecretion of gastric hydrochloric acid is a factor involved in the aetiology of some duodenal ulcers. Although some controversy remains, most authors believe that the level of fasting serum gastrin in patients with duodenal ulcers is not significantly higher than that of the normal population (Trudeau and McGuigan, 1970, 1971; Korman *et al.*, 1971; Ganguli and Hunter, 1972; McGuigan and Trudeau, 1973; Stadil and Rehfeld, 1973), the main source of gastrin being the gastrin-producing cells in the stomach. A number of published reports suggest that in some duodenal ulcer patients there is hyperplasia of gastrin-producing cells in the pyloric antrum (Solcia *et al.*, 1970; Polak *et al.*, 1972; Cowley *et al.*, 1973; Ganguli *et al.*, 1974). Only a few of these reports are based on reliable, objective, quantitative methods, and none has used multiple biopsy specimens obtained with the fiberoptic endoscope. Our paper presents the results of a quantitative study of gastrin-producing cells in the stomach of 19 patients suffering from duodenal ulcer who had multiple gastric biopsies.

Material and methods

Nineteen patients diagnosed as having duodenal ulceration were studied. All gave a typical clinical history of abdominal pain and dyspepsia. Each had a barium meal examination and gastroduodenoscopy on at least one occasion. An ulcer crater was

demonstrated by the radiologist or seen at endoscopy in all patients. There were 15 males and four females with a mean age of 38.4 ± 13 years, ranging from 16 to 62 years.

A group of 15 young adults with no recognisable disease of the gastrointestinal tract acted as controls. Seven of them were healthy volunteers; the remaining eight were examined by endoscopy and biopsy for a variety of clinical reasons, and all were found to be normal. The results of the investigations performed in this group of controls can be found elsewhere (Piris, 1975; Piris and Whitehead, 1975). There were seven males and eight females, with ages ranging from 17 to 53 (mean 30.4 ± 9.5) years.

Endoscopy was performed in both groups after an overnight fast. At the same time a sample of blood was taken for measurement of serum gastrin levels by radioimmunoassay. After careful examination of the complete gastric and duodenal mucosae, biopsies were obtained from the duodenum (duodenal cap and proximal descending duodenum) and from five standard sites in the stomach: (1) the pyloric antrum on the lesser curvature between the pyloric ring and the incisura angularis; (2) at about the middle of the lesser curve; (3) opposite to this on the greater curve; (4) high on the lesser curve at about 3 cm from the cardia; and (5) the fundus. In patients with duodenal ulcers, biopsies were also taken from the ulcer edge.

DEMONSTRATION OF GASTRIN CELLS

Gastrin-producing cells (G-cells) were demonstrated in biopsy specimens obtained using an immuno-

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peroxidase method with antisera raised in rabbits against synthetic human gastrin I (ICI), as described earlier (Piris and Whitehead, 1974). Briefly, specimens were formalin-fixed and embedded in paraffin; 6μ sections were then exposed to anti-gastrin serum (diluted 1:10 with phosphate buffer saline (PBS), pH 7.2 for 60 minutes, and after washing in PBS the sections were exposed to swine anti-rabbit IgG labelled with peroxidase. The bound peroxidase was developed by treating with diaminobenzidine tetrahydrochloride containing hydrogen peroxide (Nakane and Pierce, 1967). The specificity of the reactions shown in Figs 2, 3, and 4 was established by preabsorbing the antisera with synthetic human gastrin I; normal rabbit serum did not produce any staining of gastrin cells. In the final preparation, the gastrin-producing cells stain dark brown, and background tissues, whose structural features are well shown, appear a light green.

QUANTITATION OF G-CELLS

The morphometric method for the quantitation of gastrin-producing cells related to the volume of epithelium, which was described in a previous paper (Piris and Whitehead, 1975), was slightly modified by the addition of lines to the grid. This was done for the purpose of measuring the length of the muscularis mucosae in accordance with the principle developed by Short (1950). It assumes that if a series of lines of a constant length are randomly cast on a cut surface of a composite object, the number of times that the lines 'cut' the linear component to be measured is proportional to its total length in the section. A modification of this principle to estimate volume-surface ratios was used by Dunnill and Whitehead (1972), who obtained an index relating the surface to volume ratio of the mucosa of small intestinal biopsy specimens. Weibel (1963) described a template for the dual purpose of point counting

Fig. 1 Morphometric grid with diagrammatic representation of antral mucosa superimposed (mm = muscularis mucosae).

Fig. 2 Antral mucosa with gastrin cells in the superficial zone. There is a marked degree of chronic atrophic gastritis ($\times 120$).

and linear intercepts estimation. The grid designed for the present study was derived from that of Weibel; a diagrammatic representation of the grid cast on the tissue section is shown in Figure 1. Sections were projected onto the grid, and the total number of G-cells in the entire grid square was counted. The grid points falling on the epithelium and lamina propria were counted separately. An estimate of the length of the muscularis mucosae was made by counting the number of times the horizontal and vertical lines intercepted it (see below). As the section is projected on the grid in a random fashion, the muscularis mucosae can in fact occupy every possible position, and 'cuts' usually occur with the lines in both directions.

SERUM GASTRIN IMMUNOREACTIVITY

This was measured in the sera of 14 fasting patients by radioimmunoassay using a Gastrin Immutope Kit (Squibb & Son Inc), which employs an antibody to human gastrin I.

Results

INFLAMMATION AND ATROPHY

Conventional light microscopy in haematoxylin and eosin, periodic acid Schiff (PAS), and reticulin preparations revealed that the biopsy specimens from the middle of the lesser curvature in seven duodenal ulcer patients consisted of simple mucosecretory

glands of the pyloric type. Another four patients showed a transitional type of mucosa (Whitehead, 1973). In the control group, on the other hand, tissue from these sites always showed body mucosa.

Chronic gastritis was present in specimens obtained from the pyloric antrum in all patients; in some there was also glandular atrophy. Chronic gastritis was noted in all biopsy specimens from the middle of the lesser curvature that contained pyloric or transitional type mucosa. Specimens from the fundal area had a normal appearance or showed mild superficial gastritis.

GASTRIN-PRODUCING CELLS

G-cells were present in all antral biopsy specimens from ulcer patients. They were easily and clearly demonstrated by the peroxidase method, their cytoplasm appearing densely stained and fully granulated, thus contrasting with the cells in the control cases. In general, G-cells were in the usual location, that is, the middle third of the pyloric mucosa, but there were many exceptions, particularly when the degree of chronic atrophic gastritis was marked. In those cases, the remaining glandular tubules were displaced by inflammatory infiltrate to the upper part of the mucosa, and gastrin cells were found at this site (Fig. 2). Another important difference was found in specimens displaying transitional type mucosa on the middle of the lesser curvature; these contained numerous G-cells, in

Fig. 3 Transitional type mucosa from the middle of the lesser curvature. Numerous gastrin cells are seen ($\times 460$).

Fig. 4 Gastrin cells in duodenal Brunner's glands ($\times 560$).

contrast with those of control cases in which they were rarely present (Fig. 3).

Biopsy specimens obtained from remaining sites within the stomach showed a body type of mucosa with no gastrin cells.

In duodenal biopsy specimens there was no difference in distribution of G-cells between controls and patients. At this site G-cells are found in small numbers in gland crypts and occasionally in Brunner's glands (Fig. 4).

QUANTITATION OF GASTRIN CELLS

The results of the quantitative study are given in Tables 1 and 2. Owing to atrophy and inflammation in the antrum of patients with duodenal ulceration, there was considerable variation in the relative volume proportions occupied by the epithelial component and lamina propria in these biopsy specimens. This could be objectively quantified by means of the counts of points falling on epithelium (E) and on lamina propria (LP), which are respect-

Table 1 *Control group*

No.	Sex	Age		E	LP	I	G	G/I index	SG
1	M	25		658	417	262	186	0.71	42
2	F	27		1178	978	270	417	1.54	57
3	F	34		547	436	118	195	1.65	33
4	M	34		699	388	229	202	0.88	55
5	F	20		650	382	280	192	0.69	43
6	F	22		649	375	221	120	0.54	68
7	M	30		933	656	255	184	0.72	69
8	F	17		737	536	227	278	1.23	95
9	M	43	A	946	630	290	267	0.92	—
			B				<6		
10	M	28	A	1026	612	324	238	0.74	63
			B				<20		
11	M	32	A	835	766	265	228	0.86	—
			B				<4		
12	F	53		968	572	260	307	1.18	54
13	F	30		1062	670	309	438	1.42	—
14	M	21		1712	886	522	470	0.90	55
15	F	38		714	475	228	86	0.38	—

E = Points falling on epithelium; LP = Points falling on lamina propria; I = Intercepts with muscularis mucosae; G = Number of gastrin cells; SG = Serum gastrin (pg/ml); A = Biopsy antrum; B = Biopsy middle of lesser curve.

Table 2 *Duodenal ulcer group*

No.	Sex	Age		E	LP	I	G	G/I	G/I index	SG
1	M	38		812	537	229	113	0.49	0.49	60
2	M	30		576	468	112	120	1.07	1.07	24
3	F	34	A	584	632	206	279	1.36	1.36	76
			B				<10			
4	M	34	A	531	611	192	43	0.22		
			B	529	619	176	100	0.57	0.79	132
5	M	26	A	874	866	191	485	2.54		
			B	295	234	74	82	1.11	3.65	—
6	M	24		324	816	142	140	0.99	0.99	—
7	F	38		598	717	174	281	1.62	1.62	105
8	M	40	A	567	459	138	131	0.95		
			B	514	445	113	101	0.89	1.84	30
9	M	53	A	423	397	113	160	1.42		
			B	378	367	86	166	1.93	3.35	90
10	M	44		685	711	147	66	0.45	0.45	—
11	M	28	A	634	788	224	148	0.66		
			B	300	449	95	83	0.87	1.53	—
12	M	24	A	662	465	120	112	0.93	0.93	—
			B				10-15			
13	M	44		514	479	127	101	0.79	0.79	85
14	M	37	A	425	275	84	257	3.06		
			B	330	158	116	95	0.82	3.88	75
15	F	34		401	534	139	175	1.26	1.26	95
16	M	30		426	565	165	191	1.16	1.16	60.5
17	M	16		392	406	135	126	0.93	0.93	93
18	F	46	A	663	602	226	197	0.87	0.87	75.5
			B				<10			
19	M	62		976	1051	346	643	1.86	1.86	58.5

See footnote to Table 1

ively proportional to the areas occupied by these two components (Weibel, 1963; Piris and Whitehead, 1975). In addition, it was found that the thickness of the complete mucosa—from muscularis mucosae to surface epithelium—was not comparable for all patients. This total mucosal volume is proportional to the figure obtained by adding the points falling on epithelium to those falling on the lamina propria (that is, E + LP). For the above reasons it is clear that a comparison of the number of gastrin cells per unit volume of epithelium between control subjects and patients with duodenal ulceration is not valid since numbers of G-cells and/or the volume of epithelium could change and thus alter the ratio. Therefore it was necessary to introduce the measurement of a fixed parameter, which is not altered by the disease. The length of the muscularis mucosae fulfils this criterion. For this reason the number of gastrin cells per unit length of muscularis mucosae (G/I) was chosen as the best means of comparing the results in both groups.

An additional difficulty encountered was the presence of significant numbers of gastrin cells in specimens from the middle of the lesser curve in some duodenal ulcer patients. This was not encountered in the control group.

For the purpose of comparing the G-cell density in these patients and the controls, the ratio G/I (gastrin cells per unit length of muscularis mucosae) obtained in the antral specimen (A) and in the tissue from the middle of the lesser curve (B) were added together. The result, which is no longer mathematically a ratio, has been called the *G/I index*. In those cases in which G-cells were found only in the antral biopsy specimen (A) and in the control cases, the G/I index is identical with the ratio of gastrin cells to intercepts with the muscularis mucosae obtained in the antral specimen (Fig. 5).

STATISTICAL ANALYSIS OF DATA

This was done using non-parametric statistical tests since it could not be assumed that the numbers of gastrin cells in both groups—controls and duodenal ulcer subjects—were distributed in accordance with the normal distribution. In fact the variance-ratio, or F-test, demonstrated the inequality of the corresponding variances for the two populations. The Mann-Whitney U test—a ranking test—was used to compare the G-cell index of the 19 patients having duodenal ulceration with those of the 15 controls. The value of U obtained was 88, which is not significant at the 5% level.

SERUM GASTRIN

Serum gastrin levels were not available in five cases. The mean serum gastrin level in 14 patients with

Fig. 5 Ratios of G-cells to intercepts with muscularis mucosae (G/I) for the specimens obtained from the antrum (A) and lesser curve (B) and the two sites combined (A + B) in duodenal ulcer patients and controls. Using the Mann-Whitney U test, the corresponding values of U are: (A) $U = 113$; (B) $U = 97$, (A + B) $U = 88$, which are not significant at the 5% level.

duodenal ulcer was 75.61 pg/ml with a standard deviation of 28.50. There was no significant difference between these values and those of the control group (11 patients, mean 57.64 pg/ml, SD 16.60).

SERUM GASTRIN AND G-CELL NUMBERS

There was no correlation ($r = 0.031$) between fasting serum gastrin levels and the G-cell index for the 14 patients for whom these two sets of data were available. The presence of gastrin cells in the middle of the lesser curve was not associated with an increased fasting serum gastrin level.

Structural changes of antral mucosa

It has been said that the antral biopsy specimens from the duodenal ulcer patients showed a degree of chronic gastritis and atrophy. Since the inflammatory infiltrate is concentrated in the lamina propria of the mucosa, the proportion of volume occupied by it should be larger than that of the non-inflamed cases such as the controls. An accurate measurement of the volume of lamina propria can be obtained by relating the number of points falling on the lamina (LP) to the intercepts (I) with the muscularis mucosae. The mean ratio LP/I for the control group (Table 1) was 2.25 ± 0.69 . The mean ratio for the 19 patients with duodenal ulcer was $3.65 \pm$

0.80. The difference in the ratios of the two groups as measured by the Mann-Whitney U test gives a value of U of 26, which is significant at the 0.2% level. In order to test whether or not the degree of inflammation in the antrum did have an effect on the number of gastrin cells, a correlation test between the ratio LP/I and the G/I index was carried out. The correlation coefficient for the 19 duodenal ulcer patients was 0.07, which is not significant.

Discussion

It appears from the data presented above that the density of gastrin cells in patients with duodenal ulcer is not significantly higher than that of normal controls. This is so when the two groups are compared as a whole but in the case of duodenal ulcer patients the density of gastrin cells in the antrum shows a wider range, from normal to a moderate (up to twofold) increase.

It is also worth noting that in some of the duodenal ulcer patients—six out of 19—considerable numbers of gastrin cells were found in the metaplastic mucosa of the lesser curve; in spite of this, when the numbers of G-cells of the antrum and of the lesser curve are independently compared in the two groups, no significant difference emerges.

It has been shown by Stave and Brandtzaeg (1976) that the density of gastrin cells is greatest around the pylorus and decreases proximally in the stomach. An exception to this pattern was, however, found in some of our patients in whom there was a severe degree of antritis, and in them a higher density of gastrin cells is found (cases 4, 9, and 11) in the specimen obtained from the middle of the lesser curve.

It is also of interest that the gastrin cells in the duodenal ulcer patients were more intensely stained than those of the controls; this indicates that, in the fasting state, the cells contain more gastrin in their cytoplasm and may provide an explanation for the increased response to gastrin-releasing stimuli found in duodenal ulcer disease.

We did not find in our duodenal ulcer patients two populations, one with normal or lower numbers of gastrin cells, the other with much larger numbers of gastrin cells, as described by Ganguli *et al.* (1974), even when the numbers of gastrin cells were related to the intercepts with the muscularis mucosae, which gives a ratio independent of the presence and degree of inflammation and/or atrophy in the antrum.

More recently, a report of the number of gastrin cells in the antrum of patients with duodenal ulcer (Creutzfeldt *et al.*, 1976) has appeared, based on the results of counting gastrin cells in single biopsy specimens from the pyloric antrum or duodenal

mucosa. In addition, counting was limited to the mid-zone of the antral mucosa, which is the area where most gastrin cells are to be found in normal human stomach, but, as illustrated in Fig. 2, not in all cases where there is inflammation and atrophy. In their investigation the size of the area used for counting antral G-cells (0.35×0.23 mm) and the number of fields counted (at least 10) were seemingly chosen in an arbitrary manner. It must be pointed out that, in the presence of a marked inflammatory infiltrate, the volume occupied by the lamina propria will be expanded, and that therefore within one of their 'counting areas' fewer glands are included and consequently possibly fewer gastrin cells. If, for instance, an increase in the number of gastrin cells within each gland has taken place, this could be missed by counting a smaller number of glands. From the quantitative data presented here it is clear that marked differences in relative volume of epithelium and of lamina propria occur in the presence of inflammation. Using their method, Creutzfeldt *et al.* (1976) could not detect any significant differences in the number of G-cells of patients with duodenal ulcer and of controls. Our study paradoxically agrees with their conclusions but not with the methods whereby such conclusions were reached. It is also interesting to find that the number of gastrin cells appears to be independent of the degree of inflammation of the antrum, as indicated by poor correlation between ratios of volume lamina propria to length of muscularis mucosae and G-cells per length of muscularis mucosae.

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