Mucosal gastrin concentration, molecular forms of gastrin, number and ultrastructure of G-cells in patients with duodenal ulcer¹

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SUMMARY The mean antral immunoreactive gastrin (IRG) concentration of 38 duodenal ulcer (DU) patients was significantly higher ($35.9 \pm 5.2 \mu\text{g/g}$) than that of 21 controls ($15.9 \pm 2.6 \mu\text{g/g}$). Also the mean IRG concentration in the proximal duodenal mucosa of 15 DU patients ($3.2 \pm 0.8 \mu\text{g/g}$) was higher (but not significantly) than that of 10 controls ($1.8 \pm 0.5 \mu\text{g/g}$). The number of G-cells in the antral mucosa of 58 DU patients and in the duodenal mucosa of 29 DU patients was not larger than that of controls. The distribution of immunoreactivity in gastrin components has been investigated in the antral and duodenal mucosa of six DU patients and six controls. In the antral mucosa the mean percentage of G-17 was 93.3% in DU patients and 92.0% in controls. G-34 amounted to 4.0% in DU patients and to 5.0% in controls. The G-34 percentage in the duodenal mucosa was higher (however not significantly) in the DU patients than in the controls (50.1% versus 35.8%). Ultrastructurally, the antral G-cells of DU patients had a significantly lower density index of their secretory granules suggesting higher functional activity. It is concluded that the exaggerated serum IRG response of DU patients to different stimuli is not a consequence of an increased G-cell mass.

In duodenal ulcer (DU) patients there is a significantly higher increase in serum immunoreactive gastrin (IRG) after a protein rich meal (Korman *et al.*, 1971; McGuigan and Trudeau, 1973; Stern and Walsh, 1973; Mayer *et al.*, 1974), insulin hypoglycaemia (cf. Stadil, 1974), or sham feeding (Mayer *et al.*, 1974) than in healthy controls. The exaggerated IRG response of DU patients may result from: an increased number of antral and/or duodenal G-cells, an increased antral and/or duodenal gastrin content or an increased release of higher-molecular-weight-gastrin-components characterized by a longer half-life.

The gastrin concentration of antral mucosa has been investigated using bioassay (Blair *et al.*, 1962; Emås *et al.*, 1971) and radioimmunoassay (Creutzfeldt *et al.*, 1974; Hughes and Hernandez, 1975; Malmström and Stadil, 1975). The results were contradictory. Few data have been published on the number of G-cells in the antral mucosa. Also these

results were contradictory (cf. Creutzfeldt *et al.*, 1975a).

This report describes the IRG concentration, the molecular forms of IRG, and the number and ultrastructural appearance of G-cells in the antral and duodenal bulb mucosa of patients with duodenal ulcer and in healthy controls. Preliminary data have been reported at recent symposia (Creutzfeldt *et al.*, 1974; Arnold *et al.*, 1974; Creutzfeldt *et al.*, 1975b).

Methods

SUBJECTS

Gastric and/or duodenal mucosal samples were investigated from 63 patients with duodenal ulcer (DU) and 23 control subjects. The mean age of the DU patients (57 males and six females) was 39 years (range 21-68) and of the control subjects (13 males and 10 females) 28 years (range 19-58). All DU patients had a characteristic history with relapsing ulcer disease. The diagnosis was made by radiographs and gastroduodenoscopy or during operation.

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The control subjects consisted of three groups: (1) 13 volunteers (eight males, five females) without known diseases of the gastrointestinal tract; (2) five patients (two males, three females) with dyspeptic complaints; (3) five patients (three males, two females) undergoing duodenopancreatectomy (Whipple's procedure) because of an insulinoma (two cases) or chronic pancreatitis (three cases).

All control subjects had normal basal and penta-gastrin-stimulated gastric secretion. Group 2 and 3 had normal radiographs of the stomach and the duodenal bulb and normal findings with respect to stomach and duodenum at endoscopy or laparotomy. The volunteers were not subjected to barium meal investigation but underwent gastroduodenoscopy. They were medical students and technicians who were fully informed about the object and the risk of the investigation. All endoscopies and tissue sampling were performed after intravenous injection of diazepam with a prograde ACMI fibroscope by the same investigator. As the results in these three groups did not differ, they have been pooled in one control group. Simultaneous biopsies of antral and duodenal mucosa both for immunohistology and gastrin extraction were not done in all subjects. Therefore, the respective number of cases is noted in the Tables and Figures.

TISSUE SAMPLING

Specimens of antral or duodenal mucosa were obtained either by forceps biopsy during gastroscopy or during operation. Antral mucosa was excised from 1 to 3 cm proximal to the pylorus. Duodenal mucosa was excised from the duodenal bulb. The samples were either fixed immediately for histological or ultrastructural investigation or stored in a deep-freeze (-30°C) until assay and weighed before homogenization. The average weight of the forceps biopsies was as follows: antral mucosa $6.1 \text{ mg} \pm 0.4$ (range 2.4–12.3 mg); duodenal mucosa $4.3 \text{ mg} \pm 0.5$ (range 0.8–6.7 mg). The average weight of the surgical biopsies: antral mucosa $235.2 \text{ mg} \pm 68.9$ (range 58.9–871.2 mg); duodenal mucosa $152.8 \text{ mg} \pm 36.7$ (range 37.0–321.1 mg).

ESTIMATION OF MUCOSAL IRG CONCENTRATION

For extraction, the samples were weighed, quickly thawed in a water bath at 27°C and homogenized using an Ultraturrax homogenizer in 0.01 M phosphate buffer, pH 7.5, supplemented with 0.15 M sodium chloride and 0.03 M sodium azide. The homogenate was placed in a water bath at 100°C for 10 minutes and centrifuged. The IRG level was determined by radioimmunoassay in the supernatant in a series of dilutions from 1:100 to 1:100 000.

Sensitivity and specificity of the radioimmunoassay was described in detail elsewhere (Mayer *et al.*, 1974). Antiserum 118/2/3, which was used for all estimations, had three times lower affinity towards human G-34 than towards equimolar amounts of G-17. It did not cross-react with highly purified CCK-PZ (a gift from Dr V. Mutt, Stockholm) in concentrations up to 50 ng/ml. The recovery of synthetic gastrin added to tissue samples was above 90%.

Fractionation of total IRG

Antral and duodenal bulb mucosa extracts were chromatographed upon Sephadex G-50 fine columns ($1 \times 100 \text{ cm}$) in 0.01 M phosphate buffer, pH 7.5, supplemented with 0.15 M sodium chloride and 0.03 M sodium azide. The column flow rate was 18 ml per hour. Successive 1.0 ml fractions were collected and assayed for immunoreactive gastrin content. The column was calibrated with natural human big (G-34) and little (G-17) gastrins (kindly provided by Professor R. A. Gregory, Liverpool). G-34 eluted from 120–135 ml and G-17 from 150–165 ml from the column. The elution positions of G-45 (component I) and G-13 (component IV) were calculated from published column profiles (Rehfeld and Stadil, 1973; Rehfeld *et al.*, 1974). The sensitivity of the column was 1 ng for G-17 and G-34. The column was loaded with 5–10 ng equivalent IRG.

IMMUNOHISTOLOGY

Tissue samples were fixed in Bouin's fluid and embedded in paraffin. Sections 5μ thick, cut vertically to the surface, were deparaffinized and stained for gastrin as described previously (Creutzfeldt *et al.*, 1971). For evaluating the number of G-cells the stained sections were examined with a Zeiss-Photo-Microscope II. The size of the area used for counting antral G-cells ($0.35 \times 0.23 \text{ mm}$) encompasses the midzone of the antral mucosa, where antral G-cells are predominantly situated in man (Fig. 1). The size of the area used for counting G-cells in the duodenal bulb mucosa was 6.6 times larger ($0.89 \times 0.60 \text{ mm}$) and encompasses the whole region between surface of mucosa and lamina muscularis propria, because duodenal G-cells are not concentrated in a certain zone (Fig. 2). Only sections revealing the whole area between surface and lamina muscularis propria were evaluated. At least 10 subsequent areas from three different sections of antral and duodenal bulb mucosa were counted. In the case of very small mucosal specimens more than three sections were evaluated. The mean number of antral and duodenal G-cells counted in each case was called the G-cells/area.

Fig. 1 The size of the area used for counting antral G-cells (0.35×0.23 mm) encompasses the midzone of the antral mucosa. The number of G-cells found in this area is expressed as 'G-cells/area'. Bouin fixation. Paraffin embedding. Incubation with 1/50 diluted antigastrin serum; after washing, incubation with 1/50 diluted peroxidase labelled anti-rabbit- γ -globulin from sheep. $\times 400$.

Fig. 2 The size of the area used for counting G-cells in the duodenal bulb mucosa (0.89×0.60 mm) encompasses the region between surface of mucosa and lamina muscularis propria. In contrast with antral G-cells, duodenal G-cells are sparse. (Technique as in Fig. 1.) $\times 160$.

Fig. 3 Calculation of G-cell granule density indices in two different G-cells (a) and (b). A full granule (F) scores 4, intermediate-full (IF) 3, intermediate-empty (IE) 2, and an empty (E) granule 1. The product of the number of each granule type observed multiplied by its scoring factor was calculated separately for the four different granule types, summarized and divided by the total number of granules. (a) G-cell scoring 2.12. (b) G-cell scoring 1.22.

ELECTRON MICROSCOPY

For electron microscopy the tissues were immediately fixed by immersion in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate with 0.03% calcium chloride. After three hours the tissues were washed overnight in the same buffer and transferred to 2% osmium tetroxide in sodium cacodylate buffer. After rinsing the tissues were dehydrated in a graded series of acetone and embedded in Vestopal. Ultrathin sections were cut on an LKB-Ultramicrotome, picked up on formvar-coated copper grids and stained with uranyl acetate and lead citrate (Coggeshall and Venable, 1965). The sections were viewed in a Zeiss EM 9 S with a built-in condenser.

G-cell granule density index

In an attempt to obtain a quantitative assessment of the overall granule population in the different G-cell activity states a G-cell granule density index, based upon the electron density of granule content, was devised. A 'full' granule scored 4, an 'intermediate full' 3, an 'intermediate empty' 2, and an 'empty' granule 1. The product of the number of each granule type observed within one single cell and its scoring factor was calculated separately for the four different granule types, summarized, and

divided by the total number of granules. Thus, a granule index of 1-2 indicates G-cells containing mainly electron lucent or 'empty' granules, while an index of 3 indicates G-cells containing predominantly electron dense or 'full' granules. The calculation of the G-cell granule density index in two different G-cells is demonstrated in Fig. 3. Based upon the evaluation of 10 G-cells per case, the G-cell granule density indices were calculated in 11 healthy volunteers and nine DU patients.

STATISTICAL ANALYSIS

All values are presented as means $\pm$ SEM. Student's *t* test for unpaired values and least square correlation coefficient was used for calculation of *P*-values.

Results

IRG CONCENTRATION IN ANTRAL AND DUODENAL MUCOSA

Mucosal IRG concentration varied considerably both in controls and DU patients. As immediate freezing of the excised specimens was not easy to realize (especially in surgical biopsies) the stability of IRG in untreated tissue specimens was investigated. Biopsies of antral mucosa obtained from the same prepyloric region of man, dog and rat were kept at room temperature or +4°C up to 24 hours

Table 1 Antral mucosal IRG concentration ($\mu\text{g/g}$ wet weight) in different specimens obtained from the same prepyloric region and kept for different time intervals until freezing

Time	Man*						Dog*		Rat†		
	Case no: I	II	III	IV	V	VI	I	II	I	II	III
0	6.5	8.3	4.2	8.7	11.6	4.9	11.1	2.3	13.6	12.8	8.3
15 min	9.4	23.2	12.6	6.1	35.0	11.3	17.4	4.3	—	—	—
30 min	8.0	8.8	7.7	8.3	7.5	10.0	—	—	—	—	—
1 h	—	—	—	—	—	—	—	—	12.8	8.9	4.8
2 h	—	—	—	—	—	—	—	—	7.6	12.8	5.1
5 h	—	—	—	—	—	—	—	—	11.5	9.0	3.2
24 h	—	—	—	—	—	—	11.6	4.0	14.4	6.2	2.1

*Kept at room temperature.

†Kept at $+4^{\circ}\text{C}$.

Table 2 IRG concentration and number of G-cells in antral and duodenal mucosa of controls and patients with duodenal ulcer

	Antral IRG ($\mu\text{g/g} \pm \text{SEM}$)	Duodenal IRG ($\mu\text{g/g} \pm \text{SEM}$)	Antral G-cells per area $\pm \text{SEM}$	Duodenal G-cells per area $\pm \text{SEM}$
Controls	15.9 ± 2.6 (n = 21)	1.8 ± 0.5 (n = 10)	38.7 ± 3.4 (n = 18)	10.6 ± 1.5 (n = 9)
Duodenal ulcer	$35.93 \pm 5.2^*$ (n = 38)	3.2 ± 0.8 (n = 15)	41.2 ± 2.6 (n = 58)	9.9 ± 1.0 (n = 29)

*Significant difference from controls ($P < 0.005$).

(Table 1). In all species the concentration was not uniform for the same individual. However, the concentration did not decrease with the time elapsing until freezing. Also the method of freezing (liquid nitrogen, dry ice, or simple deposition in the deep freeze) did not influence the IRG concentration. From these results it is concluded that the wide range of IRG concentration is due to irregular distribution of the G-cells.

Accordingly, a correlation between the IRG concentration of sample I and II in 31 subjects of whom two biopsies were obtained was only low ($r = 0.5092$; $P < 0.01$). The mean IRG concentration in the antral and duodenal mucosa of controls and DU patients is listed in Table 2. The IRG concentration of 38 DU patients was significantly ($P < 0.005$) higher than in the antral mucosa of 21 controls. However, the overlap was considerable.

Fig. 4 IRG concentration in antral and duodenal mucosa of DU patients and controls.

Fig. 5 Number of G-cells in antral and duodenal mucosa of DU patients and controls. The area in which the duodenal G-cells are counted is 6.6 times larger than in the antrum.

Only one-third of the DU patients had an IRG concentration which was higher than the highest control value (Fig. 4). This tendency was apparent also in the duodenal IRG concentration (Table 2, Fig. 4). However, the difference was not statistically significant.

No difference could be found between surgical and endoscopic biopsies. The cases with high mucosal IRG concentration did not have especially high values for basal or stimulated gastric acid output or for fasting or meal stimulated serum IRG levels.

G-CELL NUMBER IN ANTRAL AND DUODENAL MUCOSA

The number of G-cells in antral and duodenal mucosa was identical in controls and DU patients (Fig. 5). The number of G-cells was significantly less in the duodenal than in the antral mucosa. Figure 5 does not reflect this relationship correctly because the area counted for G-cells in the duodenal mucosa was 6.63 times larger than in the antral mucosa. The number of G-cells in antral and duodenal mucosa obtained during endoscopy did not differ significantly from the findings in the much larger samples obtained during operation.

No relationship existed between IRG concentration and the number of G-cells in antral or duodenal mucosa both in controls and DU patients. Cases with a high number of antral or duodenal G-cells did not have raised levels for basal or stimulated

gastric acid output or for fasting or meal-stimulated serum IRG levels.

FRACTIONATION OF MUCOSAL IRG

The distribution of immunoreactivity in gastrin components was investigated in extracts of antral and duodenal mucosa of six control subjects and six DU patients. The Sephadex G-50 gel filtration patterns are listed in Table 3 (gastric mucosal extracts) and Table 4 (duodenal mucosal extracts). Two major immunoreactive components corres-

Table 3 Distribution of immunoreactivity in gastrin components (%) in antral mucosal extracts from controls and DU patients

	G-45	G-34	G-17	G-13
Controls				
Ha. (insulinoma)	—	4	96	—
Kl. (chron. pancreatitis)	—	9	82	9
Br. (insulinoma)	—	4	96	—
Ko. (insulinoma)	2	5	81	12
Bo. (volunteer)	—	—	100	—
Sch. (volunteer)	—	3	97	—
$\bar{x}$		5.0	92.0	
SEM		1.0	3.3	
DU patients				
Bro.	—	—	100	—
Man.	—	1	99	—
Hen.	—	3	97	—
Kan.	—	8	80	12
Cas.	2	2	90	6
Kes.	—	6	94	—
$\bar{x}$		4.0	93.3	
SEM		1.3	3.0	

Mucosal gastrin and G-cells in duodenal ulcer

Table 4 Distribution of immunoreactivity in gastrin components (%) in duodenal mucosal extracts from controls and DU patients

	G-45	G-34	G-17	G-13
Controls				
Ha. (insulinoma)	—	29	71	—
Kl. (chron. pancreatitis)	—	27	55	18
Br. (insulinoma)	—	41	59	—
Ko. (insulinoma)	—	35	65	—
Go. (chron. pancreatitis)	—	47	45	8
$\bar{x}$		35.8	59.0	
SEM		3.7	4.4	
DU patients				
Bro.	—	34	56	—
Man.	—	68	32	—
Hen.	—	44	56	—
Kan.	—	47	47	6
Cas.	—	30	70	—
Barc.	—	78	20	—
$\bar{x}$		50.1	47.2	
SEM		7.8	7.2	

ponding to the elution pattern of G-17 and G-34 were found in the mucosal extracts of all cases, both controls and DU patients. Component I (G-45) and G-13 were detected only in several cases (Fig. 6).

The predominant IRG component in the antral and duodenal mucosa of controls and DU patients was G-17. Ninety-two per cent (range 81-100%) of the total IRG was present as G-17 in the antral mucosa of controls and 93.3% (range 80-100%) in the antral mucosa of DU patients. Only 5% (range 3-9%) was present as G-34 in the antral mucosa of controls and 4.0% (range 1-8%) in the antral mucosa of DU patients. Thus, the patterns of IRG in antral mucosa of controls and DU patients were identical.

In the mucosa of the proximal duodenum G-34 amounted to 35.8% (range 27-47%) of the total IRG in controls and to 50.1% (range 30-78%) in DU patients. Correspondingly, G-17 was only 59.0% (range 45-71%) of the total IRG in controls and 47.2% (range 22-70%) in DU patients. However, the higher percentages of G-34 in the duodenal mucosa of DU patients did not reach statistical significance.

ULTRASTRUCTURE OF ANTRAL G-CELLS

The secretory granules of antral G-cells display a broad scale of varying electron density. In DU patients electron lucent 'empty' granules seemed to predominate in comparison with controls. By applying a morphometric method for assessment of the granule population (Fig. 3), a significant lower index of the granule density was calculated for the DU patients (Table 5). In the same DU patients the IRG concentration of the antral mucosa was significantly higher than in the controls, while the

Fig. 6 Sephadex G-50 gel filtration patterns of immunoreactive gastrin in mucosa extracts of antrum and proximal duodenum from a control subject (patient Kl. of Tables 3 and 4). The void volume of the column started at fraction 60. No significant IRG levels were detected before fraction 105.

Table 5 G-cell granule density, IRG concentration and number of G-cells in antral mucosa of controls and DU patients

	G-cell granule density index	IRG concentration (μ g/g)	G-cells per area
Controls (n = 11)	2.52 $\pm$ 0.14	17.5 $\pm$ 3.6	37.2 $\pm$ 3.5
DU patients (n = 9)	1.43 $\pm$ 0.03†	59.8 $\pm$ 14.7*	42.9 $\pm$ 5.2

*Significant difference from controls ($P < 0.025$), †($P < 0.005$).

number of G-cells was not different (Table 5).

This finding suggests that G-cells with electron dense secretory granules do not necessarily contain more gastrin than G-cells with electron lucent granules.

Discussion

A substantial variation of the mucosal IRG concentration has been observed by different investigators (Berson and Yalow, 1971; Creutzfeldt *et al.*, 1971; Jackson *et al.*, 1972; Malmström and Stadil, 1975). Already Berson and Yalow (1971) have discussed the reasons which may be responsible for this: initial rapid loss of IRG in excised unfrozen tissue or random variation among a small number of samples. In this investigation the IRG concentration was stable for many hours in three different species (Table 1). Even rapid freezing in liquid nitrogen did not change the result. This finding contradicts the conclusion of Malmström and Stadil (1975) who state that gastrin is rapidly degraded in untreated specimens. According to our investigation, the variation in the IRG concentration is mainly due to an uneven distribution of the G-cells in the mucosa. This conclusion agrees with the morphological findings: an identical variation has been found regarding the number of G-cells stained by immunohistology (Fig. 5). In this case IRG degradation could not have influenced the results because the tissue had been fixed immediately. Thus, single values of IRG concentration in antral or duodenal mucosa may be misleading and multiple biopsies are necessary for evaluating an individual case. However, this sampling error can be disregarded if groups of patients are compared.

In a group of 38 patients with duodenal ulcer a significantly higher IRG concentration has been found in the antral mucosa. This result disagrees with published investigations using a radioimmunoassay for gastrin. Malmström and Stadil (1975) compared 12 controls with 28 DU patients and Hughes and Hernandez (1975) 32 controls with 18 DU patients. The absolute values of Malmström and Stadil (1975) were higher, but both groups found no differences between controls and DU patients. The disagreement between these results and our findings is difficult to explain. The IRG concentration of the controls was in the same range in all investigations. The higher mean value of the 38 DU patients in our series resulted from 11 cases with values higher than the highest control values. These 11 DU patients were not different from the whole group regarding basal or stimulated gastric acid secretion, fasting or meal-stimulated serum IRG levels, and number of antral G-cells.

The IRG concentration of the duodenal mucosa amounted to roughly one-tenth of that of the antral mucosa both in controls and in DU patients. In the latter the mean value was higher but this did not reach statistical significance.

Contrary to the differences of the IRG content,

the number of G-cells identified by immunohistology in antral and duodenal mucosa was identical in controls and DU patients. This finding does not confirm suggestions of more abundant G-cells in the antral mucosa of DU patients (Pearse *et al.*, 1970; Pearse and Bussolati, 1970; Polak *et al.*, 1972). However, systematic investigations on this question have not yet been published. In only three of 58 DU patients of this series did the number of G-cells exceed the range of the 18 controls and only one of these three had an antral IRG concentration in the upper range. From this finding it can be concluded that the high IRG concentration found in one-third of the DU patients is not due to an increased G-cell mass.

A greater G-cell mass or antral G-cell hyperplasia has been described as cause for recurrent ulcer disease with fasting hypergastrinaemia (Polak *et al.*, 1972; Ganguli *et al.*, 1974). However, this entity seems to be extremely rare (Hansky, 1974) and has not yet been found in this laboratory. The number of G-cells in the mucosa of the duodenal bulb was much smaller than in the antral mucosa (less cells were counted in an area which was 6.6 times larger than in the antrum). This corresponds well with the much smaller IRG concentration in duodenal mucosa.

Berson and Yalow (1971) have described the distribution of the major gastrin components in the antral and duodenal mucosa. They found in the antrum predominantly G-17 (component III) and in the duodenum predominantly G-34 (component II). It has not been investigated systematically whether the molecular forms of gastrin differ between DU patients and controls. No significant differences were found in the antral (Table 3) and duodenal (Table 4) mucosa between six controls and six DU patients. The pattern in the antral mucosa was identical in the two groups. The higher percentage of G-34 in the mucosa of the duodenal bulb in the DU patients did not reach statistical significance. The percentages reported here for G-34 are underestimations of the real values because the antigastrin serum used in these studies has a 70% lower affinity, on a molar basis, for G-34 compared with that for G-17 (Mayer *et al.*, 1974). The findings do not support recent suggestions of Stadil *et al.* (1975). These authors found in pooled sera increased levels of G-17 in DU patients. Such a difference does not correspond with the mucosal patterns of gastrin components described here.

The discrepancy between normal G-cell number in antral and duodenal mucosa together with elevated mucosal IRG concentration suggests a higher gastrin content for the single G-cell. According to the secretory cycle of the G-cell formulated by Forss-

mann and Orci (1969), the number of electron dense secretory granules of the G-cells should be higher in DU patients than in controls. Forssmann and Orci (1969) had observed in cats and rats that, in the fasting state, the G-cells contained predominantly electron dense secretory granules and after feeding predominantly electron lucent membraneous sacs. This had been interpreted as discharge of the stored hormone from the granules into the cytoplasm and from there into the bloodstream. However, these morphological findings have not been supported by hormone estimation by Forssmann and Orci. In recent rat experiments a low granule density index of the antral G-cells was found together with an elevated antral IRG concentration and increased levels of serum IRG after stimulation by feeding (Creutzfeldt *et al.*, 1975b). From this finding it had been concluded that a low granule density index denotes only that gastrin has been released recently from granule stores; however, as gastrin biosynthesis occurs very rapidly, it gives no indication of the total amount of gastrin in the cell. As demonstrated in Table 5, the DU patients had a significantly lower granule density index than the controls and at the same time a significantly higher IRG content of the antral mucosa without an increase in number of G-cells. It cannot be decided yet whether the IRG in the G-cells is present in the electron lucent 'empty' granules or in a non-granular form in the cytoplasm.

In conclusion, the exaggerated IRG response of DU patients to different stimuli is not the consequence of an increased G-cell mass. The G-cells are not increased in the antral or duodenal mucosa (Table 1) and the antrum of DU patients is not larger than that of controls (Capper *et al.*, 1966). Also different molecular forms of gastrin do not explain the higher IRG serum levels after stimulation in DU patients. However, the low density index of the secretory granules and the elevated mucosal IRG concentration (Table 5) may be interpreted as signifying higher functional activity of the G-cells in DU patients. Such an increased activity could be due to a defect in autoregulation of gastrin release and gastric acid secretion at low pH (Walsh *et al.*, 1975) or to a higher vagal tone.

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