The relation between functioning parietal cell and gastrin cell masses in two groups of duodenal ulcer patients

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Summary

1. Serum gastrin concentrations before and after a standardized meal were determined in twenty-eight patients with duodenal ulcer and in ten normal control subjects.

2. In response to pentagastrin, thirteen of the duodenal ulcer subjects secreted acid within the limits of normal and fifteen secreted in excess.

3. The differences in the basal serum gastrin concentrations between the three groups, normal subjects, acid 'normosecretors' and hypersecretors were not statistically significant but that of the hypersecretors was suggestively low.

4. The integrated gastrin response and peak gastrin responses to meals were higher in duodenal ulcer patients with normal acid secretion than in the hypersecretors but the values for the latter were not different from normal subjects.

5. Stabilization of intragastric pH by infusion into the antrum of sodium bicarbonate during the test meal response period did not alter these differences between the two ulcer patient groups.

6. A significant inverse correlation exists between the maximal acid output and the integrated gastrin response in both normal subjects and hypersecreting duodenal ulcer patients.

7. The evidence (a) supports the existence of an inverse relationship between the functioning parietal cell and gastrin cell masses, (b) shows the gastrin response in normosecreting ulcer subjects to be inappropriately high, and (c) suggests that excessive vagotonia exerts trophic effects upon both parietal cell mass and gastrin cell mass.

Key words: duodenal ulcer, integrated gastrin responses, maximal acid output, pentagastrin.

Introduction

Patients with duodenal ulceration have an elevated gastric acid secretory capacity compared with normal subjects. This is due, in the main, to an increase in the parietal cell mass (Card & Marks, 1960). That subjects with duodenal ulceration may have a higher functioning gastrin cell mass is suggested by significantly higher than normal serum gastrin levels after meals (Byres, Young, Chisholm & Lazarus, 1970; Korman, Soveny & Hansky, 1971b; McGuigan & Trudeau, 1973; Fordtran & Walsh, 1973). Evidence from animal experiments indicates the trophic dependence of the parietal cell mass upon gastrin (Martin, Macleod & Sircus, 1970) and the ability of exogenously administered gastrin and the synthetic pentapeptide to increase its size (Crean, Marshall & Rumsey, 1969; Willems, Vansteenkiste & Limbosh, 1972). It is thus possible that the increased parietal cell mass in patients with duodenal ulcer may be due to a primary abnormality in the endocrine system of the gut.

A significant degree of parietal cell hyperplasia is not, however, universally present in patients with duodenal ulcer, as indicated by the wide range of acid secretory capacity that is encountered and which, at the bottom of the spectrum, overlaps that of

When the antrum acid-initiated mechanism inhibitory to the release of gastrin is blocked with sodium bicarbonate, vagal stimulation causes a rise in serum gastrin levels double that in normal subjects in the same conditions (Hansky, Korman, Cowley & Baron, 1971). These workers postulated that there must be an increase in both the gastrin cell and the parietal cell masses.

Excessive vagal tone has been postulated as the major pathogenesis of duodenal ulcer disease (Dragstedt, 1956) but Fordtran & Walsh (1973) suggested an alternative explanation of a defect in the mechanisms of the duodenum which exert control over the rates of gastric secretion and emptying. They based this on their observations that in duodenal ulcer subjects acid continues to be secreted at a high rate after more than 90% of a meal's buffer has been emptied from the stomach, a circumstance which is physiologically inappropriate.

Observations on a number of different contributory factors to the appearance of acid hypersecretion in individual patients with duodenal ulcer led one of us to a hypothesis of variable pathogenesis associated with an increase in gastrin cell mass in one group and of the parietal cell mass in another (Sircus & Small, 1964).

It may be that it is the inter-relationship between the parietal cell mass and the gastrin cell mass which is a crucial factor in the pathophysiology of duodenal ulceration, but this has not been defined. We have attempted this in the present study by correlating the gastrin response to stimulation by a standardized meal with the maximal acid secretory capacity in normal subjects and in patients with duodenal ulceration.

Method

The twenty-eight patients had duodenal ulceration proven by radiology, endoscopy and/or subsequent surgery; there were twenty-one males and seven females, with ages ranging from 14 to 62 years (mean 39.8). The controls consisted of ten healthy subjects (eight males and two females) with ages ranging from 18 to 52 years (mean 32.7).

Informed consent was obtained from all patients. Fasting commenced at 20.00 hours and, on the following morning at 09.00 hours, a small (19 G) butterfly needle was inserted under local anaesthesia into an antecubital vein and kept patent with a slow infusion of sodium chloride (150 mmol/l). After the patient had rested for 15 min the first blood sample was taken (zero – 10 min) and, 10 min later (zero time), a further sample was taken, subsequent to which the patient ate a standardized meal consisting of 50 g of protein, 45 g of carbohydrate and 40 g of fat. The meal consists of 20 g of Casilan (protein hydrolysate) mixed with an instant breakfast powder with 6 g of protein, 0.15 g of fat and 10 g of carbohydrate and homogenized in 300 ml of milk, together with 45 g of Cheddar cheese and 10 g of butter. (The calcium content is 1.3 g.) Further blood samples were taken at 15, 30, 45, 60 and 90 min after the commencement of the meal.

Subsequently the tests were repeated in subjects of both acid-secreting types but with simultaneous infusion of sodium bicarbonate into the antrum to eliminate the influence of a low pH of antral contents upon gastrin secretion. The technique for maintaining the gastric contents at a pH of 5.5 was that described by Fordtran & Walsh (1973) and included sampling and adjusting every few minutes, a Holter pump being used for delivery of the necessary amount of bicarbonate. After blood was taken at −75 min, sodium bicarbonate was infused at a constant rate through the naso-gastric tube into the stomach: 50 mmol was run in initially, and subsequently the bicarbonate was introduced at a constant rate of (20 + x) mmol/h, where x is the value of the maximum acid output in mmol/h of the individual patient. At zero time, while the bicarbonate was being infused, the test meal was served. The bicarbonate infusion was continued for 165 min, to the end of the measurement of the meal response.

On a separate day, after an overnight fast, the duodenal ulcer subjects and eight of the normal control subjects had their maximum acid output measured. An intramuscular injection of pentagastrin (6 μg/kg total body weight) was given and the total acid secretion (or maximum acid output) in the hour that followed was collected in 20 min periods by continuous aspiration with a motor pump and the acid content measured with an automatic titrator. The methodology of this part of the investigation was conventional (Baron, 1973).

In our laboratories the mean of the acid secretory response over a period of 1 h after pentagastrin, the maximal acid output, when expressed in mmol/kg total body weight in normal subjects is 0.30 ± 0.10 SD. We define the top limit of normality as 2 SD above
the mean value. 'Normosecreting' duodenal ulcer subjects therefore secrete less than 0.51 mmol of acid/kg body weight and 'hypersecretors' more than that amount, per post-pentagastrin hour. Expressing the value of maximum acid output in terms of body weight substantially reduces the differences in this value found between the sexes (Sircus, 1968). 'Normosecretors' and 'hypersecretors' nevertheless form a continuous range of values for maximum acid output in duodenal ulcer subjects with an unimodal curve of distribution.

**Serum gastrin estimation**

Gastrin estimations were performed in duplicate with a modification of the method of Yalow & Berson (1970). Antiserum was prepared in rabbits against small (50 μg) amounts of porcine gastrin I and II conjugated with egg albumin. The association constant of the antiserum used in the studies was \(5.2 \times 10^{-11}\) l/mol. Fractionation of serum samples indicated that this antiserum detected 'big big gastrin' (Yalow & Berson, 1972), component I and component II ('big gastrin') (Gregory & Tracy, 1972), heptadecapeptide gastrin I and II as well as component IV (Rehfeld, Stadil & Vikelsoe, 1974) (Fig. 1). Sensitivity was below 0.5 pg/ml of incubates (less than 5 pg/ml of serum). Cross-reaction by cholecystokinin was 2 \(\times 10^{-4}\) on a molar basis. Within-assay and between-assay coefficients of variation were 5.5% and 11% respectively. The sera separated from the blood samples were stored at \(-20°C\) until assayed. Samples being compared were usually determined within the one assay.

Where comparison was made amongst three groups of data, these were analysed by analysis of variance using either a one-way or two-way classification where appropriate. Subsequent SEM values were then computed and Student's t-test was performed where appropriate, with the residual (or pooled) variance obtained. Chi-square test was used to compare the ages of the normal and ulcer subjects. Results have been expressed as mean value ± SEM except where otherwise stated.

**Results**

Based on the criterion of normality for maximum acid output (see above), thirteen patients with duodenal ulceration were 'normosecretors' (mean maximum acid output 0.37 ± 0.01 mmol h\(^{-1}\) kg\(^{-1}\)) and fifteen patients were hypersecretors (mean 0.69 ± 0.06 mmol h\(^{-1}\) kg\(^{-1}\)). The mean value for the eight normal subjects who had their acid secretory capacity measured was 0.34 ± 0.04 mmol h\(^{-1}\) kg\(^{-1}\).

![Fig. 1. Fractionation of three samples of concentrated serum from a normal subject through a Superfine Sephadex G-50 column (1 cm x 150 cm) calibrated with human serum albumin ([125]IA), growth hormone ([125]GH), insulin ([131]Ins), pure porcine gastrin I ([125]PGI) and sodium iodide ([131]I). Eluate fractions (1 ml) were collected and the gastrin content was expressed as a percentage of the total amount of gastrin in the sample. □—□, Fasting; ○—○, 60 min after test meal; ————, 120 min after test meal.](image-url)
TABLE 1. Measurements of serum gastrin after a standardized meal
Mean values and SEM are shown for each point in Fig. 3, giving the differences between the duodenal ulcer patients and normal subjects.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Normal subjects (10)</th>
<th>Duodenal ulcer patients (13) Normosecretors</th>
<th>Hypersecretors (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>75</td>
<td>71</td>
<td>54</td>
</tr>
<tr>
<td>SD</td>
<td>18.5</td>
<td>10.1</td>
<td>10.1</td>
</tr>
<tr>
<td>SEM</td>
<td>5.9</td>
<td>2.8</td>
<td>5.9</td>
</tr>
<tr>
<td>15 min</td>
<td>90</td>
<td>143</td>
<td>78</td>
</tr>
<tr>
<td>30 min</td>
<td>102</td>
<td>134</td>
<td>50.5</td>
</tr>
<tr>
<td>45 min</td>
<td>109</td>
<td>154</td>
<td>49.0</td>
</tr>
<tr>
<td>60 min</td>
<td>119</td>
<td>181</td>
<td>48.5</td>
</tr>
<tr>
<td>90 min</td>
<td>115</td>
<td>214</td>
<td>46.5</td>
</tr>
</tbody>
</table>

**Basal serum gastrin.** The mean basal (zero time) serum gastrin of the whole series of twenty-eight duodenal ulcer patients was 61.9 ± 5.9 pg/ml, which is not significantly different ($t = 1.2025, P > 0.1$) from that of the normal control subjects (75.0 ± 7.8 pg/ml). There was no statistically significant difference ($F = 1.9494$) amongst the mean values of the basal serum gastrin levels of the normal subjects, the normosecreting duodenal ulcer patients and the hypersecreting duodenal ulcer patients, but the low value of the hypersecretors is highly suggestive of a real difference (mean values = 75.0, 71.0 and 54.0 pg/ml respectively, SEM 4.71) (Table 1).

**Gastrin response to protein meal.** The gastrin response to meal stimulation of the duodenal ulcer patients as a whole (Fig. 2) tended to be higher than that of the normal subjects but at no time reached statistical significance. Likewise, the difference in the integrated gastrin response, i.e. the total post-prandial release of gastrin represented by the whole area under the curve 0–90 min after the meal between the normal subjects and the mixed group of duodenal ulcer patients just failed to reach statistical significance ($0.1 > P > 0.05$).

Since the time of the peak gastrin response of the subjects studied varies and may be at 30, 45, 60 or 90 min after the meal, the mean peak gastrin response was determined in the ulcer subjects (199.6 ± 23.7 pg/ml) and the normal subjects (135 ± 14.9 pg/ml). Significant difference, however, was still not statistically demonstrable between the two groups ($0.1 > P > 0.05$). The data were further subjected to analysis of variance with a two-way classification, which takes into account the variation in time as well as the variation in response of all the subjects studied. A significant difference emerged ($F = 9.3159, P < 0.05$) (Table 2).

When the ulcer patients were typed and subdivided according to the acid secretory capacity as described above, significant differences appeared in
the peak gastrin responses of the normal subjects compared with the ulcer patients grouped together ($F = 18.3803, P < 0.01$). However, though the mean values of the peak gastrin response is significantly higher in the ulcer normosecretors than in the normal subjects ($t = 4.6285, P < 0.01$), and in normosecretors than in hypersecretors ($t = 5.6639, P < 0.01$), it is not significantly different between the normal subjects and the hypersecretor patients ($t = 0.4899, P > 0.05$) (Table 2). A significant difference in the integrated gastrin response was also observed amongst the normal subjects, normosecretors and hypersecretors ($F = 14.1727, P < 0.01$). The mean integrated gastrin response is significantly higher in normosecretors than in the normal subjects ($t = 3.0439, P < 0.01$) and the hypersecretors ($t = 3.7152, P < 0.01$), but is not significantly different between the normal subjects and the hypersecretor patients ($t = 0.3123, P < 0.05$) (Table 2). A significant difference exists in the gastrin response from zero to 90 min amongst the normal subjects, and both ulcer groups, is further supported by the results of the analysis of variance by a two-way classification which shows $F = 49.5469 (P < 0.01)$. The mean gastrin response at 15, 30, 45, 60 and 90 min is significantly different between the normal subjects and normosecreting ulcer subjects ($P < 0.01$ at all times), between the two groups of patients ($P < 0.01$ at all times), but is not significantly different neither between the two groups of patients ($P < 0.01$ at all times) nor between the normal sub-

### Table 2. Tests of significance of difference in serum gastrin concentrations between pairs of groups at given times after ingestion of a test meal, by means of Welch's test

Values given in this Table are those of the test statistic $t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$ where $\bar{x}_1, \bar{x}_2$ represent the mean values in the groups being compared, $s_1, s_2$ the SD (standard deviation) and $n_1, n_2$ the number of individuals in the two groups. The tabulated values do not show statistical significance at the 5% level, except where indicated by asterisks: *0.01 < $P$ < 0.05; **0.001 < $P$ < 0.01; ***$P$ < 0.001.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Time after ingestion of test meal (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Normal v. normosecretors</td>
<td>0.3</td>
</tr>
<tr>
<td>Normal v. hypersecretors</td>
<td>2.5*</td>
</tr>
<tr>
<td>Normosecretors v. hypersecretors</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Table 3. Results for duodenal ulcer patients and normal control subjects showing peak gastrin response and integrated gastrin response after ingestion of a test meal

Mean values ± SEM are shown.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Subjects</th>
<th>Maximum acid output (mmol/kg total body wt.)</th>
<th>Basal serum gastrin (pg/ml)</th>
<th>Peak gastrin response (pg/ml)</th>
<th>Integrated gastrin response (ng min⁻¹ ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects (10)</td>
<td>8</td>
<td>2</td>
<td>32.7(1)</td>
<td>0.34 ± 0.04 (8 subjects)</td>
<td>75.0 ± 4.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6 ± 0.46</td>
</tr>
<tr>
<td>Duodenal ulcer patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normosecreting (13)</td>
<td>9</td>
<td>4</td>
<td>39.0(1)</td>
<td>0.38 ± 0.02</td>
<td>71.0 ± 4.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.4 ± 0.46</td>
</tr>
<tr>
<td>Hypersecreting (15)</td>
<td>12</td>
<td>3</td>
<td>40.4(1)</td>
<td>0.69 ± 0.06</td>
<td>54.0 ± 4.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.1 ± 0.46</td>
</tr>
<tr>
<td>Grouped (28)</td>
<td>21</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) No significant difference (see text).
(2) Computed from residual variance.
Maximum acid output

In each of the duodenal ulcer patients and the eight normal subjects this correlated with the corresponding integrated gastrin response. A significant inverse correlation was observed in the normal subjects (\(P < 0.05, r = 0.75\)). The patients with duodenal ulcer also showed a trend towards an inverse correlation but because of the width of scatter this just failed to reach an acceptable level of significance (\(r = 0.35, P < 0.06\)). However, twenty-five of the twenty-eight patients with ulcers had values above and to the right of the line of regression for normal subjects and this is highly significant (\(\chi^2 = 18.8, P < 0.001\)) (Fig. 5).

The normal subjects and both groups of the patients were divided into four age groups (\(< 25, 26-35, 36-45, > 46\) years) and the \(\chi^2\) was calculated for each group. No significant difference was observed amongst the subjects studied in all the age groups (\(\chi^2 = 2.2608, 0, 0.6160, 1.000\) respectively).

Discussion

It has been conclusively demonstrated that the maximal acid output is an accurate index of the gastric parietal cell mass. The serum gastrin response to a large ‘protein’ meal in duodenal ulcer subjects and normal subjects has been claimed to be an index of the antral gastrin cell mass (Hansky & Korman, 1973; Byrnes et al., 1970; Feurle, Ketterer, Becker & Creutzfeldt, 1972), but there have not yet been any definitive studies on the relationship between gastrin cell mass and serum gastrin response to stimuli. If
Parietal and gastrin cell masses

These indexes are correct, our demonstration of an inverse relationship between the maximum acid output and serum gastrin response to protein suggests that, in both normal subjects and in patients with duodenal ulceration, the size of the parietal cell mass is a primary event and this, in turn, influences the size of the gastrin cell mass. Another and anatomical demonstration of this dependence of the gastrin cell mass on the parietal cell mass is the inverse relationship between the area of the stomach covered by cells secreting acid and pepsin and that bearing mucosa of antral structure (Capper, Butler, Buckler & Hallett, 1966). Although, in duodenal ulcer subjects, the density of antral gastrin cells varies considerably (Ganguli, Polak, Pearse, Elder & Hegarty, 1973), a recent study shows a high degree of correlation between cell density, total gastrin cell mass and area occupied (Polak, Bloom, Pearse & Welbourn, 1975). Alternatively, the relationship may be a physiological one. High rates of acid secretion are more likely to result in a lower mean intragastric pH, which in turn may reduce gastrin cell function either directly by the action of low pH on the antral mucosa or through the effect of acid in releasing duodenal hormones with an inhibitor effect upon the gastrin cells (Hansky, Soveny & Korman, 1971; Konturek, Biernat & Oleksy, 1974). Because of these mechanisms it is possible that hyperplasia of gastrin-secreting cells in response to neural or humoral trophic stimuli will be limited.

Thus it appears that, with the exception of the rare gastrinoma, the parietal cell mass is determined by factors other than gastrin. Recent clinical observations (Lam & Sircus, 1975a) have suggested that there may be a genetic predisposition to parietal cell mass hypertrophy marked by differences in the distribution of ABO blood groups in acid normosecreting and hypersecreting subjects with duodenal ulcer, but these data have not been confirmed in further studies (R. Prescott, C. L. Lai, S. K. Lam & W. Sircus, unpublished work).

The patients with duodenal ulceration also demonstrated a similar inverse relationship of maximum acid output on integrated gastrin response, although the correlation coefficient (-0.35) just failed to reach the accepted limits of statistical significance (P < 0.06). However, the validity of this relationship is supported by the significantly higher integrated gastrin response in the normosecretors compared with the hypersecretors. This overall difference was not due to more rapid acidification of the test meal by the hypersecretors because the gastrin response remained unchanged when the meals were accompanied by continuous intragastric bicarbonate (Fig. 5).

The most striking abnormality of gastrin release in the duodenal ulcer patients, especially those with a normal acid output, was that it was 'inappropriate' for any given level of gastric acid secretion, the response being significantly greater than in the normal subjects. This inappropriate gastrin response may reflect either an abnormally forceful trophic drive to the gastrin-secreting cells or impairment of the mechanism by which acid inhibits gastrin release. There is no histological abnormality to suggest that the gastrin-secreting cells in ulcer patients should have diminished responsiveness to hydrogen ions and, in fact, recent studies have shown that decreasing the antral pH in duodenal ulcer subjects does diminish gastrin release (Konturek et al., 1974). Therefore it seems likely that the inappropriate gastrin response
is due to an excessive stimulus to the cells. The most likely source of such a stimulus is the vagus nerves. It is tempting to speculate that an increased vagal tone also leads to parietal cell hyperplasia in some patients. Another study we have recently completed confirms the belief that all duodenal ulcer subjects have an increased vagal drive (Lam & Sircus, 1975b). We suggest that it is possible that this drive affects both gastrin and parietal cell masses, but that either, for the genetic reasons mentioned above, an individual subject may respond with more ready hyperplasia of one cell mass or the other, or there is a duration effect associated with this drive with a tendency for the parietal cell mass to undergo progressive hypertrophy with the passage of time (Sircus, 1960). Taken as a single group, all duodenal ulcer subjects secrete more acid than normal subjects (Lam & Sircus, 1975a) but many retain maximum acid output within 2 so of the mean value in normal subjects. The antral gastrin cell hyperplasia, described by Ganguli et al. (1973), may represent our acid normosecreting patients in whom the transition to a greater degree of parietal cell hyperplasia has begun but before the gastrin cell inhibiting effect of increased acid secretion has been morphologically effective.

Our findings may explain some of the anomalies in the reports of previous workers who have not taken into account the differing acid secretory capacities, such as in the variations reported by Novis, Marks, Bank & Sloan (1973) and between the results of Stern & Walsh (1972) and McGuigan & Trudeau (1973). It is of interest therefore that analysis of the data in the study by Fordtran & Walsh (1973) making a comparison of serum gastrin levels after meals and peak acid secretion after histamine shows that of their seven subjects with duodenal ulcer five were secreting acid significantly above the range of their six normal control subjects and two were not. Three of their duodenal ulcer subjects had post-prandial peak serum levels of gastrin above 150 pg/ml, and of these two were those with an acid response to histamine in their normal range. The other four had peak gastrin levels below 75 pg/ml, and of these three were hypersecreting acid in response to histamine. This analysis supports our findings in the present study.

References


Parietal and gastrin cell masses