Histamine and insulin dose-response studies of gastric secretion in Indian control subjects and patients with duodenal ulcer in the Ganges delta

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SUMMARY The gastric secretory responses to various doses of histamine and insulin have been studied in 11 control and 12 duodenal ulcer subjects belonging to the Ganges delta of India where the incidence of duodenal ulcer disease is known to be high. A dose of 0.04 mg/kg body weight of histamine acid phosphate was sufficient to produce peak gastric acid output both in the control and duodenal ulcer subjects. However, a dose as low as 0.025 U insulin/kg body weight was enough to produce peak rates of gastric acid output in duodenal ulcer subjects, whereas in the controls a minimum dose of 0.05 U insulin/kg body weight was sufficient. A greater proportion of the duodenal ulcer patients also showed a peak acid secretory response in the first hour after administration of insulin. Furthermore, increasing doses of insulin in this population did not produce lower levels of blood glucose but did produce increasingly high acid output as subjects did in the West. K values derived from the intravenous glucose tolerance test showed that 75% of duodenal ulcer patients and 54% of the controls had variable degrees of intolerance to glucose. Gastric acid secretion in response to a bolus of 50 ml 50% intravenous glucose was also studied in a separate group of 16 duodenal ulcer and 13 control subjects. A sharp rise in the volume, titratable acidity, and total acid output was observed in the early part of the fourth hour in the control and duodenal ulcer subjects. In a separate group of controls a bolus of intravenous hypertonic saline produced no such increase in gastric acid secretion.

Several studies published in recent years have now established that 0.2 U insulin/kg body weight provides an effective dose for stimulation of gastric acid secretion in subjects of the Western countries (Isenberg et al., 1969; Baron, 1970; Isenberg et al., 1970; Baron and Cowley, 1971; Baron et al., 1972; Cowley and Baron, 1973). However, in Indian subjects an effective dose of insulin to stimulate gastric secretion has not been established, although various studies of histamine stimulated gastric acid secretion have been reported (Vakil and Mulekar, 1965; Goyal et al., 1966; Desai et al., 1967; Desai et al., 1969). Of particular interest is the study of Desai et al. (1969) who showed that, in subjects weighing less than 60 kg, 0.04 mg/kg histamine acid phosphate provides a submaximal stimulus for gastric acid secretion.

The present report concerns gastric secretory studies in response to various doses of insulin and histamine in duodenal ulcer patients and control subjects living in the Ganges delta of West Bengal in India. In order to define the relationship of fluctuating blood glucose levels to gastric acid secretion, the effect of a bolus of intravenous glucose on the latter was also studied over a period of four hours in a separate group of control and duodenal ulcer patients. In five control subjects the effect of a bolus of intravenous hypertonic saline (2.5% NaCl (0.427 mol/l)) on gastric acid secretion was also examined.

The population living in the Ganges delta is known to have a high incidence of duodenal ulcer (Malhotra, 1964) and subsists mainly on a low calorie, predominantly carbohydrate, low fat, and a low protein diet, the main staple being rice (A Study of Food Habits in Calcutta, 1972).

Methods

Part 1

HISTAMINE AND INSULIN DOSE RESPONSE
STUDIES OF GASTRIC ACID SECRETION:
DUODENAL ULCER SUBJECTS AND CONTROLS
This part of the study was carried out on 23 male subjects. Of these, 12 (average age 29-9 years, range 17 to 40 years; average weight 49-32 kg, range 40 to 61 kg) gave a typical history of duodenal ulcer and had a niche or deformed duodenal cap on the barium meal study. The rest (average age 33 years, range 25 to 46 years; average weight 54.5 kg, range 38 to 65 kg) acted as controls. The control subjects were adult male volunteers working in the hospital and gave their informed consent to the investigation. They had no gastrointestinal symptoms, but, in view of the high incidence of duodenal ulcer disease in the community and the prevalence of asymptomatic duodenal ulcer, a limited barium meal study was carried out and revealed no evidence of duodenal ulcer. A fast of at least 12 hours preceded each test and studies were not conducted more frequently than every other day.

A Levin's tube (16-18 fr.) was passed and its position in the mid-antrum of the stomach was ascertained by fluoroscopy and water recovery test (Findlay et al., 1972). Gastric juice was collected by continuous hand suction and the aspirates were pooled into 15 minute samples. During the tests the patients mostly lay either on the left side or supine.

Subcutaneous histamine test
After collecting the basal secretion for 30 minutes, 100 mg mepyramine maleate was injected intramuscularly. At the end of one hour histamine acid phosphate was injected; 0-04 mg/kg, 0-05 mg/kg, and 0-06 mg/kg doses of histamine acid phosphate were used on separate occasions in each subject. After the administration of the drug, gastric juice was collected for two hours.

Intravenous insulin test
Soluble insulin (Boots, England) was diluted in 0-15 mol sodium chloride solution so that the concentration was 1 to 4 units per millilitre. The doses tested were similar to those of Isenberg et al. (1969) —that is, 0-025, 0-05, 0-1, 0-2, and 0-4 U/kg body weight. The insulin was rapidly administered by the intravenous route. Gastric secretion was collected for four hours after administration of the drug. The highest dose—that is, 0-4 U/kg body weight—of insulin could be used only on subjects (nine duodenal ulcer and eight control) who did not get severe hypoglycaemic symptoms at a dose of 0-2 U/kg body weight.

Gastric acid concentration was determined by titrating 1 ml gastric juice with 0-1 mol NaOH, using 0-1% phenol red as indicator. Gastric acid output was calculated from the volume and the concentration of acid.

Part IIa
GASTRIC ACID SECRETORY RESPONSE TO BOLUS OF INTRAVENOUS GLUCOSE AND HYERTONIC SALINE: CONTROL SUBJECTS
Five control subjects (mean age 30-8 years, range 29 to 33 years; mean weight 49-24 kg, range 44-2 to 53-2 kg) were given a gastric secretion test as described below with 50 ml, 50% bolus of intravenous glucose and 50 ml hypertonic saline (2-5% NaCl—that is, 0-427 mol). Gastric secretory response to a dose of 0-05 U/kg insulin, as described above, was also studied in these subjects.

Part IIb
GASTRIC ACID SECRETORY RESPONSE TO BOLUS OF INTRAVENOUS GLUCOSE: DUODENAL ULCER SUBJECTS AND CONTROL SUBJECTS
In a separate group of 29 subjects included in this part of the study 16 (mean age 33-5 years, range 18 to 50 years; mean weight 54-3 kg, range 31 to 74 kg) had duodenal ulcer and 13 acted as controls (mean age 28-3 years, range 22 to 45 years; mean weight 51-3 kg, range 41 to 68 kg).

Intravenous glucose tolerance test
Patients were given 50 ml 50% glucose intravenously over a period of five minutes. Blood samples were drawn at 0, 5, 15, 30, 60, 120, 180, and 240 minutes after administration of glucose. K values were calculated from semilogarithmic plots (Marks and Marrack, 1962).

Gastric acid secretion in response to intravenous glucose and hypertonic saline
The tests were performed in a similar way to those with intravenous insulin except that, instead of insulin, a bolus of 50 ml 50% glucose or 50 ml hypertonic saline (2-5% NaCl—that is, 0-427 mol/l) was given parenterally (intravenously).

These subjects also had a gastric acid secretion test in response to histamine (0-4 mg/kg body weight) and insulin (0-05 U/kg body weight).

Venous blood was sampled before the insulin was injected and 30 minutes afterwards. Blood sugar was determined by the modified Somogyi and Nelson method (Varley, 1967). Standard reference sera were run along with each batch as a control.

The peak acid output (PAO) was calculated by taking the sum of the two consecutive highest 15 minute outputs and multiplying them by two (Baron, 1963).
basal secretion of duodenal ulcer subjects was significantly higher than that of the controls. No relationship existed between the basal secretion and the weight of the patient or the fasting blood sugar. This was true for both the control and duodenal ulcer subjects. The mean coefficient of variation for repeated basal acid output in subjects with duodenal ulcer as well as in the controls was 65%.

**HISTAMINE DOSE RESPONSE**

A dose of 0.04 mg/kg body weight was found to be an effective dose for eliciting maximal gastric acid response both in the control and duodenal ulcer subjects (Figs. 1a and 1b, Table 2). The duodenal ulcer subjects, however, had a significantly higher PAO than did the controls (Figs. 1a and 1b, Table 2). In both the control and duodenal ulcer subjects no relationship could be shown between the weight of the patient and the PAO (results not shown).

**INSULIN DOSE RESPONSE**

The mean acid output after increasing doses of insulin for each 15 minute period both in the controls and the duodenal ulcer subjects is shown in Figs. 2a and 2b. In duodenal ulcer subjects the lowest dose (0.025 U/kg) itself elicited average maximal response, whereas in the controls 0.05 U/kg was required to elicit this.

The peak acid output in controls increased with an increase in the dose of insulin from 0.025 to 0.05 U/kg body weight, but there was no further significant rise to higher doses of insulin up to 0.4 U/kg. In duodenal ulcer subjects, however, the lowest dose—that is, 0.025 U/kg—elicited maximal response and there was no significant difference between a dose of 0.025 U/kg and any of the higher doses up to 0.4 U/kg. The acid output in the controls was significantly lower than in the duodenal ulcer subjects at a dose of 0.025 U/kg but was not different at 0.05 U/kg or higher doses (Table 3). The PAO for insulin was 80 and 76% of the average PAO after histamine in control and duodenal ulcer patients respectively.

The time of occurrence of the peak acid output varied from the third to the eighth 15 minute period after insulin, except in one instance where the peak came in the first period. When the time of the peak 15 minute acid response was analysed, it was seen that it occurred in the first hour in 43 out of 57 studies (75%) in the duodenal ulcer patients, compared to 27 out of 52 (52%) in the control subjects. This difference in the occurrence of the peak among the duodenal ulcer patients was significant ($\chi^2 = 6.32, p < 0.02$).

No relationship could be demonstrated between the weight of the patient and the peak acid output.

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**Fig. 1** Mean 15 minute acid output in (a) control and (b) duodenal ulcer subjects at three doses of histamine.

- - - : 0.04; O ... O : 0.05; △ ... △ : 0.06.

**Results**

**Part I**

**BASAL SECRETION**

Basal secretion was collected on each day of the study and data on at least seven collections were available on each patient. The values for basal secretion have been normalised by log conversion and analysed (Table 1). In any individual, the basal secretion varied from day to day. The mean log
Dose-response studies of gastric secretion in control subjects and patients

Table 1  Mean of log hourly basal gastric acid secretion in control and duodenal ulcer subjects (in serial order of estimation)

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Serial order of studies</th>
<th>Pooled mean SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4  5  6  7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11 0.50 0.42 0.42 0.36 0.17 0.32</td>
<td>0.3663 ± 0.04</td>
</tr>
<tr>
<td>±SE</td>
<td>0.09 0.07 0.14 0.10 0.14 0.12 0.12</td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>12 0.83 0.76 0.60 0.63 0.65 0.56</td>
<td>0.6234 ± 0.04</td>
</tr>
<tr>
<td>±SE</td>
<td>0.03 0.02 0.03 0.01 0.04 0.02 0.05</td>
<td></td>
</tr>
</tbody>
</table>

= No significant difference.
Note: 1. Log basal secretion in duodenal ulcer patients is significantly higher than in controls, \( p < 0.05 \).
2. For both control and duodenal ulcer, there is no difference between days of studies.
3. Log to the base 10.

Table 2  Estimation of peak acid output (mmol/h) in control and duodenal ulcer subjects at different doses of histamine

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Dose of histamine (mg/kg body weight)</th>
<th>Residual error of means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.04 0.05 0.06</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11 19.73 19.66 17.84 1 ± 1.826</td>
<td></td>
</tr>
<tr>
<td>±SE</td>
<td>1.96 2.70 2.28 1.5 * * *</td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>12 24.92 26.85 24.34 2 ± 2.120</td>
<td></td>
</tr>
<tr>
<td>±SE</td>
<td>2.20 2.03 2.56 2.3 * * *</td>
<td></td>
</tr>
</tbody>
</table>

= No significant difference  * Significant, \( p < 0.05 \).
Note: 1. No significant difference in PAO between different doses of histamine for either control or duodenal ulcer patients.
2. Average PAO is significantly higher in duodenal ulcer subjects at each dose.

after insulin (results not shown). The blood sugar was equally low at 30 minutes after injection of 0.025, 0.05, 0.1, and 0.2 units of insulin (Table 4). Both the control and duodenal ulcer patients exhibited similar responses.

**RELATIONSHIP OF BASAL SECRETION TO PEAK ACID OUTPUT AFTER HISTAMINE AND INSULIN**
To study the relationship of basal secretion to peak acid output, we analysed the mean basal secretion of each individual with his peak acid output in response to histamine and insulin. In the controls there was a significant rise in the peak acid output after insulin with an increasing basal output (\( p = < 0.05 \)). No such relationship could be shown for histamine stimulated output. The duodenal ulcer subjects showed this trend of increasing maximal output with increasing basal output both for histamine and insulin (\( p = < 0.05 \)).

**Part IIa**
Unlike intravenous glucose, which produced a sharp increase of gastric acid secretion in the middle of the fourth hour, hypertonic saline produced no such response (Fig. 3).

**Part IIb**
**INTRAVENOUS GLUCOSE TOLERANCE TEST**
The results of the intravenous glucose tolerance test in duodenal ulcer and control subjects have been shown in terms of their K values (Marks and Marrack, 1962) in Table 5. There is a trend towards borderline tolerance to intravenous glucose in the majority of both the control and duodenal ulcer subjects. Two patients with duodenal ulcer showed frank intolerance. However, no statistically significant difference could be shown in K values between the control and duodenal ulcer subjects.

**SECRETION OF GaSTRIC ACID AFTER INJECTION OF GLUCOSE 50 ML 50 % INTRAVENOUSLY AND ITS RELATIONSHIP TO BLOOD SUGAR LEVELS, HISTAMINE, AND INSULIN MEDIATED GaSTRIC ACID SECRETION**
Figure 4 shows that an insignificant increase in acid output occurred in the first half hour after parenteral administration of glucose and was maintained beyond the second hour up to the middle of the fourth hour when a sharp increase in acid output occurred in the majority of subjects. The increase in acid output was accounted for by an increase in volume as well as titratable acidity. In duodenal ulcer patients all the outputs were higher at every period and the peak was more pronounced (Fig. 4a).

Although a particular individual's secretory pattern could not be related to the blood sugar level, it is seen that at the second hour average blood sugar level was similar to the fasting level and beyond this
the average was below the line, at which stage also the peak gastric acid secretion occurred. Means of the peak acid output (± SE) in control subjects after histamine, insulin, and intravenous glucose were (in mmol), 21.2 (± 2.53), 12.51 (± 154), and 9.7 (± 1.2) and in duodenal ulcer patients were 23.8 (± 1.85), 14.63 (± 2.51), and 13.97 (± 1.87) respectively.

**Discussion**

We have been unable to confirm the finding of

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**Table 3**  Estimation of peak acid output (mmol/h) in control and duodenal ulcer subjects at different doses of insulin

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Dose of insulin (U/kg body weight)</th>
<th>Residual error of means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>11.52</td>
</tr>
<tr>
<td>±SE</td>
<td>1.21</td>
<td>2.26</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>17.70</td>
<td>19.63</td>
</tr>
<tr>
<td>±SE</td>
<td>2.12</td>
<td>1.32</td>
</tr>
</tbody>
</table>

= No significant difference.  + Significant, p <0.05.
* Mean of eight control and nine duodenal ulcer subjects.

**Fig. 2**  Mean 15 minute acid output in (a) control and (b) duodenal ulcer subjects at five doses of insulin.  
× × ×:0.025; △ △ △:0.05;  
○ ○ ○:0.1; ○ ○ ○:0.2; ■ ■ ■:0.4.
Desai et al. (1969) that to produce maximal stimulation of gastric acid, Indian subjects require a higher dose of histamine than do subjects of Western countries. Not only did our control subjects produce maximal secretion at a dose of 0.04 mg/kg, but there were no discernible differences between these and the ulcer patients where the dose of histamine required to produce peak acid output was concerned. However, PAO in response to histamine was higher in the duodenal ulcer patients. We could not demonstrate a relationship of maximal gastric acid secretion to the body weight of the patient. In this respect our results are similar to Goyal et al. (1966) and different from those reported by Desai et al. (1967). The differences between our results and those of Desai and his colleagues probably reflect the regional as well as the dietary differences that exist in the different parts of India.

Since Isenberg et al.'s (1969) systematic study of insulin mediated gastric secretion, a dose of 0.2 U/kg insulin to stimulate maximal gastric acid output is widely used in Western countries. As the smallest dose that we used—that is, 0.025 U/kg—produced peak rates of gastric acid secretion in duodenal ulcer subjects, we cannot define the minimum dose required to produce half maximal secretory response. However, this study suggests that the dose requirement of the Indian population is much lower than that of Western subjects. In this respect this population appears to be sensitive to insulin with respect to gastric acid secretion and the duodenal ulcer subjects appear to be more sensitive than the controls, as they produce peak acid output at a dose of 0.025 U insulin, whereas the dose required by the controls is 0.05 U/kg. For the present, we would suggest that, for this population, a dose of 0.05 U/kg is sufficient to produce peak acid output both in the control and in the duodenal ulcer subjects.

This difference in gastric acid secretory response to insulin between duodenal ulcer and controls is similar to the findings of Isenberg et al. (1972, 1975), who have shown that the dose of pentagastrin required to elicit half maximal secretory rates is significantly less in patients with duodenal ulcer than in normal subjects. The mechanism of this hypersensitivity of vagus nerve or hypothalamus to insulin in our population needs further study and at present remains a topic for speculation. One of this study's interesting findings is that the fall in blood sugar level after insulin was similar irrespective of the dose. Furthermore, the blood glucose levels that were obtained in this study 30 minutes after insulin were higher (1.94 mmol/l (35-40 mg%) than those reported from Western subjects (0.6-1.1 mmol/l (10-30 mg%)). Fasting blood sugar levels in these subjects were, however, in the normal range.

Table 4  Mean blood sugar levels half an hour after administration of graded doses of insulin

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Dose of insulin (U/kg body weight)</th>
<th>Residual error of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>42.52</td>
</tr>
<tr>
<td>± SE</td>
<td>4.99</td>
<td>5.02</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>12</td>
<td>44.38</td>
</tr>
<tr>
<td>± SE</td>
<td>4.90</td>
<td>4.49</td>
</tr>
</tbody>
</table>

= No significant difference.
* Not included in the analysis as was not done in all the subjects (mean of eight control and nine duodenal ulcer subjects).

Fig. 3  Mean acid output (mmol) in five control subjects after intravenous administration of insulin, glucose and hypertonic saline. — insulin, ... glucose.
Table 5 Classification of controls and duodenal ulcer subjects with respect to their carbohydrate tolerance after intravenous GTT (according to K values)

<table>
<thead>
<tr>
<th>Groups according to K values*</th>
<th>Control† (%)</th>
<th>DU‡ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>0</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>1 to 1.5</td>
<td>7 (53.8)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>&gt;1.5</td>
<td>6 (46.2)</td>
<td>4 (25)</td>
</tr>
</tbody>
</table>

*According to criteria of Marble (1971) subjects with K values < 1 are carbohydrate intolerant, 1 to 1.5 are borderline tolerant, and those with >1.5 are tolerant.

†No statistically significant difference between values in duodenal ulcer and control subjects were observed (Wilcoxon—rank sum test).

The mean percentage falls of blood sugar with a dose of insulin ranging from 0.025 to 0.4 u/kg, were 48%, 53%, 54%, 57%, and 60% respectively. This raises the possibility that this population has a relative resistance to the hypoglycaemic effect of insulin. Although the Boots insulin used in this study was not assayed for its insulin content, we believe that the findings obtained were not due to any methodological artefact, because even the lowest dose produced the maximal acid secretion by the stomach. Also, the saline dilution that we used was the same as that suggested by Isenberg et al. (1972) and the highest dilution used was for the lowest dose which also produced maximal acid response. In order to prevent any deterioration of activity, the dilutions were always freshly made before use. We are unable to compare our data with those from other parts of India, as no systematic study of this phenomenon has so far been reported. However the workers from Southern India (V. I. Mathan, personal communication) have made similar observations on subjects having dietary patterns similar to ours. These findings raise the possibility that this study population, which was on a predominantly carbohydrate diet, had become chemically diabetic and, as such, had developed a relative resistance to insulin. The
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data on intravenous glucose tolerance in these subjects showed that the blood sugar level at one hour was higher than the fasting level, both in the control as well as in the duodenal ulcer patients. Furthermore, the carbohydrate tolerance expressed as K values after intravenous glucose clearly showed that the propensity to carbohydrate intolerance was higher among our subjects, irrespective of whether they were controls or had a duodenal ulcer. Furthermore, our own unpublished data on oral glucose tolerance with 100 g glucose and a tolerance test after a rice meal showed a similar trend. Obviously a larger sample population is needed to critically evaluate whether this population on a relatively high carbohydrate diet has a tendency to chemical diabetes or not. Our results also suggest a trend towards a higher frequency of borderline carbohydrate intolerance to intravenous glucose in duodenal ulcer subjects. Many workers in the past (Evensen, 1942; Platt et al., 1949; Buchanan et al., 1967; Humphrey et al., 1972) have demonstrated a variable degree of intolerance to oral glucose in patients with duodenal ulcer. Recently, Creutzfeldt et al. (1977) identified two distinct populations among duodenal ulcer patients—one with normal and the other with impaired oral glucose tolerance. The latter were shown to have a marked increase in serum levels of immuno-reactive gastric inhibitory polypeptide (IR-GIP).

The mechanism of glucose mediated gastric acid secretion remains speculative and we are unable to offer any suitable explanation for this hitherto unreported phenomenon. Gastric acid response observed after administration of glucose was absent following a bolus of intravenous hypertonic saline in a group of control subjects; this suggests that this secretory response to parenteral glucose is real in our population. It is unlikely that these responses are non-specific, as they were regular, nearly at the same time, and had sharp peaks.

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References


