

# Relation between gastric histology and gastric juice pH and nitrite and N-nitroso compound concentrations in the stomach after surgery for duodenal ulcer

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**SUMMARY** Formation of N-nitroso compounds in gastric juice has been implicated in the pathogenesis of cancer in the stomach after operation. Gastric juice was aspirated from 85 subjects: 23 were controls, 51 had previously undergone vagotomy and gastrojejunostomy, and 11 had previously undergone vagotomy and pyloroplasty. The gastric juice samples were analysed for pH, nitrite, and total N-nitroso compounds.

A significant correlation was found between pH and nitrite concentration ( $p < 0.01$ ). No significant correlation was found between pH and total N-nitroso compound concentration or between nitrite and N-nitroso compound concentration. The vagotomy and gastrojejunostomy patients had higher pH values and higher concentrations of nitrites and N-nitroso compounds than controls ( $p = 0.01$  in all cases).

The 51 vagotomy and gastrojejunostomy patients also underwent endoscopy and biopsy. They were divided into three groups: group 1 (21 patients) had no intestinal metaplasia and no more than mild dysplasia; group 2 (20 patients) had intestinal metaplasia; and group 3 (10 patients) had moderate or severe dysplasia. Groups 2 and 3 both had higher pH values and higher nitrite concentrations than group 1 ( $p = 0.01$  in all cases). There was no significant difference, however, between either group 2 or 3 and group 1 for total N-nitroso compound concentration.

Since there was no simple linear relation between pH and N-nitroso compound concentration, it was concluded that formation of N-nitroso compounds at high pH was unlikely to be involved in the pathogenesis of gastric cancer in the hypochlorhydric stomach after operation. The relation between nitrite and histological abnormality was not associated with a similar relation between N-nitroso compounds and histological abnormality. It therefore appears that there is no simple relation between N-nitroso compounds and the pathogenesis of premalignant gastric mucosal changes.

There is substantial evidence that gastric surgery for benign peptic ulcer leads to an increased incidence of gastric cancer in the long term.<sup>1-4</sup> There have also been several reports of possible premalignant conditions of the gastric mucosa in the stomach after operation.<sup>2,5</sup> Farrands *et al*<sup>6</sup> found 11 cases of epithelial dysplasia in 71 patients following gastric surgery. Work from our own centre has shown that patients with duodenal ulcers who have been treated surgically have a higher incidence of moderate and severe dysplasia and intestinal metaplasia than

medically treated patients.<sup>7</sup>

The cause of this high incidence of premalignant and malignant change in the stomach after operation is controversial. Domellof<sup>8</sup> has suggested bile reflux as a possible cause. Others have suggested that hypochlorhydria allows bacterial proliferation with subsequent reduction of dietary nitrate to nitrite and then to N-nitroso compounds,<sup>9</sup> which are known to be carcinogenic, at least in experimental animals.<sup>10</sup> Contradictory data, however, have appeared in the published work on the relation between pH and N-nitroso compound concentration in gastric juice.<sup>9,11</sup>

The purpose of this study was to investigate the relation between pH and concentrations of nitrites and N-nitroso compounds in gastric juice and to relate N-nitroso compound concentration to histological changes in a group of patients who had undergone vagotomy and gastrojejunostomy.

### Patients and methods

#### PATIENTS

Eighty five subjects entered the study. Fifty one patients (42 men, nine women; mean age 60.43 years) had previously had a vagotomy and gastrojejunostomy and 11 had had vagotomy and pyloroplasty (five men, six women; mean age 57.0 years). All the operations had been carried out electively for duodenal ulcer in the Royal Victoria Hospital, Belfast, between 1957 and 1967. The 23 control subjects (14 men, nine women; mean age 56.8 years) were healthy volunteers who had no history of previous surgery to the gastrointestinal tract or of previous or current gastrointestinal symptoms. Analysis of variance showed no difference between the groups for age distribution.

Gastric juice was taken from all subjects for analysis. After an overnight fast a size 14 Salem sump nasogastric tube was passed and the position checked radiologically. Where possible three samples of gastric juice were then aspirated at 15 min intervals. The pH of each sample was measured using a Corning pH meter model 113. Samples were then divided into two aliquots for nitrite and total N-nitroso compound analysis. Nitrite analysis was carried out as described elsewhere (p 596).

A total of 82 vagotomy and gastrojejunostomy patients underwent endoscopy, and the 51 patients who entered the current study are those who agreed to return for gastric juice sampling. At the time of endoscopy seven biopsies were taken. The methods used for endoscopy and biopsy, assessment of biopsy specimens, and details of endoscopic and histological findings in all 82 vagotomy and gastrojejunostomy patients have been reported previously.<sup>7</sup> For the purposes of this study the 51 patients were divided into three groups. Group 1 had no intestinal metaplasia and no more than mild dysplasia in any biopsy specimen. All these patients had various degrees of gastritis and atrophy and the histological changes were considered less serious than those in groups 2 and 3. There were 21 patients in this group (14 men, seven women) and their mean age was 59.6 years. Group 2 had intestinal metaplasia in one or more biopsy specimen but no more than mild dysplasia. There were 20 patients in this group (19 men, one woman) and their mean age was 62.25 years. The patients in group 3 all had moderate

(nine patients) or severe (one patient) dysplasia in one or more biopsy specimen. There were 10 patients in this group (nine men, one woman) and their mean age was 58.5 years. Analysis of variance showed no difference between the groups for age distribution.

#### TOTAL N-NITROSO COMPOUND ANALYSIS

Total N-nitroso compounds were assayed using the technique described by Bavin.<sup>14</sup> Aliquots of gastric juice (5 ml) were placed in a tube containing hydrazine sulphate (Analar) in excess (100 mg made up in 1.5 ml water) in order to remove nitrite. The pH was adjusted to 4 using 1.0 N aqueous potassium hydroxide. Potassium hydrogen phthalate was added as a buffer to stabilise the pH. One millilitre of this mixture was then analysed for total N-nitroso compounds using a chemiluminescence analyser (Scott Model 125). The apparatus was in a laboratory adjacent to the room occupied by the patient so that analysis could start as soon as samples became available.

The assay was linear for solutions of N-nitrosopyrrolidine made up in gastric juice between concentrations of 0.05 and 1  $\mu\text{mol/l}$  (coefficient of correlation 0.99) and between 0.5 and 25  $\mu\text{mol/l}$  (coefficient of correlation 0.99). Gastric juice, known to give no response with the chemiluminescence analyser, had N-nitrosopyrrolidine added to give a concentration of 0.25  $\mu\text{mol/l}$ . Eight aliquots were analysed, giving a coefficient of variation of 7.4%. When 10.5 ml aliquots of gastric juice were each spiked with 0.05  $\mu\text{mol}$  of N-nitrosopyrrolidine, the mean recovery was 104.25% (coefficient of variation 7.58%).

### Results

#### pH, NITRITE, AND TOTAL N-NITROSO COMPOUNDS

Sufficient gastric juice was obtained for analysis of nitrite and total N-nitroso compounds in 236 samples (66 from controls, 30 from vagotomy and pyloroplasty patients, and 140 from vagotomy and gastrojejunostomy patients). The relation between pH and nitrite concentration for all of these samples is shown in Fig. 1. The highest concentrations of nitrite were found in the higher pH ranges. Fig. 2 shows the relation between pH and total N-nitroso compound concentrations. The highest concentrations of N-nitroso compounds were found in the regions of pH 3–3.99 and 7–7.99. The mean values of all the samples from each patient were calculated for pH, nitrite concentration, and total N-nitroso compound concentration. With these figures the Spearman rank correlation coefficient between pH and

nitrite concentration was 0.76 ( $p < 0.01$ ). No correlation was found between pH and total N-nitroso compound concentration or between nitrite and total N-nitroso compound concentration.

The control group and the vagotomy and gastrojejunostomy group were compared using the mean values for pH and nitrite and total N-nitroso compound concentrations, which were calculated for each patient or subject in both groups (Table 1). With the Mann-Whitney U test, the vagotomy and gastrojejunostomy patients had significantly higher pH values than the control group ( $p = 0.01$ ). Using the same statistical test, we found significantly more nitrite and total N-nitroso compound in the vagotomy and gastrojejunostomy group ( $p = 0.01$  in both cases).

RELATION BETWEEN HISTOLOGY AND pH AND NITRITE AND TOTAL N-NITROSO COMPOUND CONCENTRATIONS IN GASTRIC JUICE

Values for pH and nitrite and total N-nitroso compound concentrations were compared between the histological groups (within the vagotomy and gastrojejunostomy patients) (Table 2). Groups 2 and 3 both had higher pH values and nitrite concentrations than group 1. There was no difference, how-

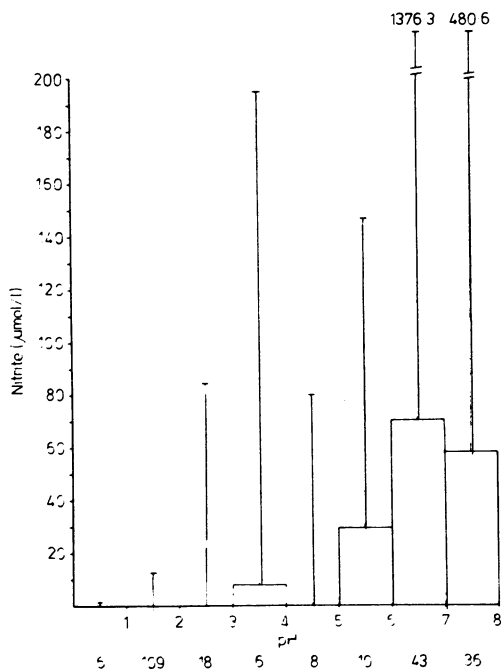


Fig. 1 Relation between gastric juice pH and nitrite concentration using medians and ranges. The lower range was zero in all pH groups. The figures below the pH scale refer to the numbers of samples.

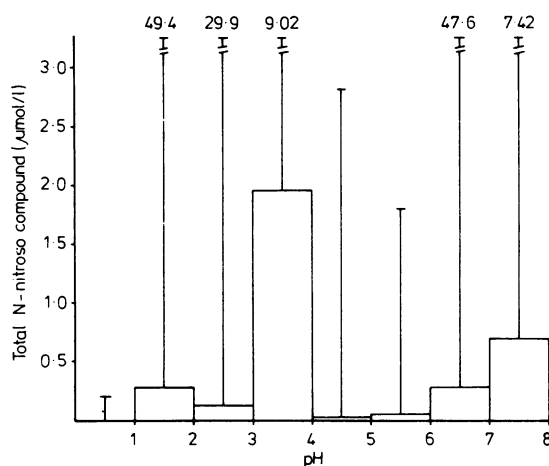


Fig. 2 Relation between gastric juice pH and N-nitroso compound concentration using medians and ranges. The lower range was zero in all pH groups. The figures below the pH scale refer to the numbers of samples.

ever, between group 1 and either group 2 or 3 for total N-nitroso compound concentrations.

Discussion

It has been suggested that N-nitroso compounds can be formed at low pH by ordinary chemical means and at high pH by bacteria. Mirvish<sup>15</sup> stated that the chemical formation of N-nitroso compounds is greatest in the region of pH 3-4 and Hawksworth and Hill<sup>16</sup> showed that bacteria could produce N-nitroso compounds at neutral pH. In the current study N-nitroso compounds were found in both high and low pH samples with peaks in the regions of pH 3-3.99 and pH 7-7.99. It is possible that the peak occurring at the lower pH (which unfortunately contained only six samples) was due to ordinary chemical nitrosation whereas the peak at higher pH was due to bacterial formation of N-nitroso compounds. Data from several laboratories,<sup>11,17</sup> including our own, have shown that bacteria proliferate in gastric juice to an appreciable extent only if the pH is in the region of 4 or above. It would seem unlikely from these data that bacterial reduction of nitrate to nitrite and then to N-nitroso compounds is the major factor responsible for carcinogenesis in the stomach after operation. This, however, does not rule out the possibility that chemical formation in the region of pH 3-4 could be important.

The reason for vagotomy and gastrojejunostomy patients having higher concentrations of N-nitroso compounds than controls might be that a smaller proportion of them (17 of 53 compared with 16 of

Table 1 Comparison of pH and nitrite and total N-nitroso compound concentrations in control subjects and vagotomy and gastrojejunostomy patients\*

	Control subjects	Vagotomy and gastrojejunostomy patients
pH	1.57 (1.19; 3.64)	5.18 (1.77; 6.86) p = 0.01
Nitrite ( $\mu\text{mol/l}$ )	0 (0; 10.57)	13.1 (1.13; 74.06) p = 0.01
Total N-nitroso compounds ( $\mu\text{mol/l}$ )	0.2 (0.1; 0.39)	0.97 (0.14; 2.73) p = 0.01

\*Values given as median (upper and lower quartiles).

Table 2 Comparison of pH and nitrite and total N-nitroso compound concentrations in the histological groups of the vagotomy and gastrojejunostomy patients\*

	Group 1	Group 2	Group 3	Significance	
				Group 1 v group 2	Group 1 v group 3
pH	1.77 (1.52; 3.35)	6.32 (2.56; 6.93)	6.98 (6.47; 7.27)	p = 0.01	p = 0.01
Nitrite ( $\mu\text{mol/l}$ )	2.77 (0; 11.46)	50.24 (1.65; 80.00)	61.9 (34.5; 168.4)	p = 0.01	p = 0.01
Total N-nitroso compound ( $\mu\text{mol/l}$ )	1.05 (0.07; 2.99)	0.52 (0.18; 2.68)	1.22 (0.92; 3.86)	NS	NS

\*Values given as median (upper and lower quartiles).

23 controls) had a mean gastric juice pH below 2, which is below both peaks of N-nitroso compound formation. The histological groups were chosen because of a strong suspicion that both intestinal metaplasia<sup>18,19</sup> and appreciable degrees of dysplasia<sup>20,21</sup> are premalignant. We have discussed the importance of these histological diagnoses elsewhere.<sup>7</sup> Mild dysplasia was included in group 1 as its importance is questionable<sup>21</sup> and since in a previous study we found no significant difference in the amount found in stomachs that had and those that had not undergone surgery.<sup>7</sup> The high pH values and nitrite concentrations found in patients with appreciable dysplasia and intestinal metaplasia plus the positive correlation between pH and nitrite are in agreement with previous work reported from this laboratory based on endoscopic samples of gastric juice.<sup>22</sup> Despite this, no relation was found between N-nitroso compound concentration and histology and this would tend to suggest that N-nitroso compounds, whether formed chemically or by bacteria, are not of primary importance in the aetiology of these possible premalignant conditions, even though the vagotomy and gastrojejunostomy group as a whole had higher concentrations of N-nitroso compounds than the healthy volunteers. It is possible that the primary abnormality is the histological change, which produces less acid secretion, with a consequent rise in gastric juice pH and nitrite concentration (but not N-nitroso compound concentration) occurring as secondary phenomena.

The relation between pH and nitrite found by us is in agreement with many other studies. It is generally accepted that the nitrite found at high pH is formed by nitrate reducing bacteria.<sup>17</sup> The lack of relation between pH and total N-nitroso compound concen-

tration found by us is in agreement with Milton-Thompson *et al.*,<sup>11</sup> but not with Reed *et al.*<sup>9</sup> or Stockbrugger *et al.*<sup>13</sup> The explanation for this important difference probably lies with the methodology for the measurement of total N-nitroso compounds. Reed *et al.*<sup>9</sup> and Stockbrugger *et al.*<sup>13</sup> did not give details of the time lapse between collection of samples and analysis, although the importance of early analysis has previously been emphasised by one of these groups.<sup>23</sup>

Milton-Thompson *et al.*<sup>11</sup> and ourselves analysed samples immediately without storage. Reed *et al.*<sup>9</sup> and Stockbrugger *et al.*<sup>13</sup> stored their samples with sulphamic acid, which reduced the pH of samples to below 2. Reed *et al.*<sup>9</sup> state that their method is unlikely to have measured total original concentration of N-nitroso compounds since N-nitrosamides are not stable at this pH. It is therefore possible that an artefactual positive correlation could have emerged, since the addition of sulphamic acid to low pH samples would bring the pH down to a lower value than in high pH samples. Less breakdown of N-nitrosamide would therefore occur in the higher pH samples, giving an apparently higher concentration at final analysis. In the method used by Milton-Thompson *et al.*<sup>11</sup> and ourselves, hydrazine sulphate was used instead of sulphamic acid and the pH was brought to 4 in all samples, thus allowing less possibility of artefact, since N-nitrosamides are more stable at this pH.

Keighley *et al.*,<sup>24</sup> who used the same method as Milton-Thompson *et al.*<sup>11</sup> for the analysis of gastric juice total N-nitroso compounds, found no difference between controls and Billroth II gastrectomy patients when measuring N-nitroso compounds over 24 h periods. There were, however, only eight sub-

jects in each group. In addition, the fact that Keighley *et al*<sup>24</sup> included postprandial samples and therefore exogenous N-nitroso compounds from food, as well as endogenous N-nitroso compounds, may explain the difference between their results and ours.

In conclusion, although a positive correlation exists between gastric juice pH and nitrite concentration, this is not accompanied by a simple linear relation between pH and N-nitroso compounds. We therefore consider that formation of N-nitroso compounds at high pH is not of primary importance in the pathogenesis of mucosal abnormality despite the association between nitrite and histological abnormality. Vagotomy and gastrojejunostomy patients overall had higher concentrations of N-nitroso compounds than controls. No relation was found, however, between N-nitroso compounds and histological groups within the vagotomy and gastrojejunostomy patients. This casts doubt on the importance of N-nitroso compounds, formed at any pH, in the production of gastric mucosal changes.

Sister E Crawford assisted in intubating patients. Miss S Campbell typed the script.

PCH Watt was in receipt of a Royal Victoria Hospital Research Fellowship during the study.

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