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Relationship between histology and gastric juice pH and nitrite in the stomach after operation for duodenal ulcer

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SUMMARY One hundred patients who had undergone operation for duodenal ulcer (68 vagotomy and gastroenterostomy; seven vagotomy and pyloroplasty; 22 gastrectomy and three gastroenterostomy) 10 or more years previously each underwent endoscopy. Biopsies were taken and gastric juice aspirated for measurement of pH and nitrite concentration. Patients were divided into five histological grades; chronic superficial gastritis (± minimal atrophic gastritis) (35), atrophic gastritis/intestinal metaplasia (30), mild dysplasia (21), moderate/severe dysplasia (13) and carcinoma (one). A wide spectrum of pH values was found with 35 patients having a fasting intragastric pH below 4·0 and 65 above 4·0. A strong relationship was found between histological grade and pH. Patients with chronic superficial gastritis had a fasting intragastric pH below 4·0 more frequently than those with moderate/severe dysplasia ($p<0·001$). Gastric juice nitrite concentrations were higher in the moderate/severe dysplasia group than in the chronic superficial gastritis group ($p=0·02$). The strong correlation between pH and nitrite concentration, previously documented, was confirmed. The implications of these findings in the pathogenesis of carcinogenesis in the postoperative stomach are discussed.

Patients who have had gastric surgery for benign ulcer disease have given rise for concern because of the possibility of late malignant change in the postoperative stomach.[1] Several endoscopic studies from Scandinavian centres have shown a high incidence of malignant and probable premalignant changes occurring in the long term after gastric surgery.[2–4] A number of endoscopic studies have now been reported from British centres. Savage *et al*[5] examined 63 patients but did not confirm the Scandinavian experience regarding stump cancer, although they did have a 21% incidence of significant dysplasia and one carcinoma *in situ*. More recently, however, Farrands *et al*[6] examined 71 patients and found one infiltrating carcinoma and three cases of carcinoma *in situ*. They also found other histological abnormalities such as gastritis and intestinal metaplasia to be common. This group recommended endoscopic screening of all patients more than five years after gastric surgery. One further British study,[7] examined the long term survival and cause of death (from death certificates) of a group of postgastric surgery patients and found no increase in death from gastric cancer.

Therefore, although there is general agreement that mucosal changes are common after gastric surgery the significance of actual gastric cancer as a cause of death, in postgastric surgery patients, remains in doubt at least in the British context. It has been suggested that hypochlorhydria[8] may be an important factor in the pathogenesis of gastric mucosal changes, as the bacterial flora which proliferate in an alkaline environment may produce compounds which could be carcinogenic. Both N-nitroso compounds[9] (metabolised from nitrate and nitrite) and secondary bile acids[10] formed by bacteria have been suspected.

As it is apparent that many patients after ulcer surgery are still capable of producing a low pH,[11] it seemed appropriate to relate the histological changes which occur in the postoperative stomach to intragastric pH. In addition, the relationship between intragastric nitrite and both pH and histological change appeared to merit further investigation.[12]

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Methods

PATIENTS

Only patients who had operation for duodenal ulcer 10 or more years previously were included. These patients formed part of a programme aimed at screening postgastric surgery patients for gastric carcinoma. Some were new referrals to surgical clinics for either new upper gastrointestinal complaints or unrelated complaints. Other were asymptomatic patients either still under regular review or whose reviews had lapsed, in which case they were recontacted. In all cases a symptom questionnaire was filled out and a drug history taken. Those who were on H_2 receptor blockers or on nitrite or nitrate containing drugs were excluded. Patients were then offered endoscopy and in those who accepted, informed consent obtained. Approval from the local hospital ethical committee was given for all aspects of this study.

Endoscopy was carried out using an Olympus GIF, Q or P gastroscope. Intravenous diazepam was used for sedation and the pharynx was sprayed with xylocaine. Between each endoscopy the suction channel of the endoscope was washed with Savlon (containing 4% isopropyl alcohol) and rinsed with water. Intermittent samples of water were taken for nitrite measurement and bacteriological assessment. Before and after each endoscopy session the suction channel was washed with activated glutaraldehyde solution and rinsed with water.

On entering the stomach, approximately 10 ml of juice was aspirated using either a sterile cannula passed through the biopsy channel or a trap introduced into the suction system. At the end of the endoscopy, seven gastric biopsies were taken, five in a circle around any stoma present and two from the proximal stomach. Three patients had less than seven biopsies (minimum five). Gastric juice was transported as rapidly as possible to the laboratory. pH was measured with a pH meter (Corning model 113) before nitrite analysis.

Protein was precipitated using zinc sulphate and potassium ferrocyanide solutions[13] and each sample centrifuged twice at 3000 rpm. Nitrite estimation was performed colourimetrically using the Griess reagent[8] and extinction was measured with a Pye Unicam model SP8-100 spectrophotometer at 540 nm. The method had good reproducibility (coefficient of variation at the 10 μM/l was 2·2% on 11 samples). The sensitivity of the method was taken to be 3 nmol which was the smallest amount which could confidently be determined from zero. Spiking of gastric juice with a known amount of nitrite gave a mean recovery of 98·5% at pH 6·94. (Coefficient of variation 5·2% on 11 samples).

The biopsy specimens were collected in formalin, fixed in mercuric formal, embedded in wax, and stained with haematoxylin and eosin. Biopsies were all examined by one consultant pathologist (JMS) who had no knowledge of pH and nitrite results. Biopsies were assessed for the presence of chronic superficial gastritis, atrophic gastritis and intestinal metaplasia. Dysplasia was assessed using the criteria of Morson *et al*,[14] who pointed out the difficulty of differentiating between mild dysplasia and epithelial regenerative change. Therefore, patients with mild dysplasia were separated from those with moderate or severe dysplasia where the diagnosis was more clear cut.

Biopsies from the region of the stoma were assessed separately from those from the body of the stomach. Each set of biopsies (either stomal or body) in each patient was given an overall histological grade depending on the most significant mucosal lesion present. Five grades were used which were, in ascending order of significance: chronic superficial gastritis (± minimal atrophic grastritis); atrophic gastritis/intestinal metaplasia; mild dysplasia; moderate/severe dysplasia; carcinoma.

Forty seven patients returned for further gastric juice sampling. This was carried out with a size 14 Salem Sump nasogastric tube which was carefully positioned in the proximal stomach under radiographic control and with reference to the position of the stoma found at endoscopy. The stomach was initially emptied and all juice discarded. After a half hour the stomach was again emptied and the juice discarded. Three samples for analysis were then taken at 10 minute intervals.

Statistical analysis was carried out using the χ^2 test (with Yates' correction), and the Mann-Whitney U test. Where other statistical tests were used they are mentioned in the text.

Results

One hundred patients were included in the study. Eight were new referrals with significant upper gastrointestinal symptoms; nine were new referrals with conditions unrelated to the upper gastrointestinal tract; three were still undergoing review; 80 were no longer being reviewed and were recontacted – of these 80, seven had significant symptoms.

Of the 15 patients with significant symptoms two were found to have recurrent ulcer on endoscopy; five had oesophagitis; one had a gastric carcinoma. Also, four had a clinical diagnosis of dumping syndrome and three a clinical diagnosis of bile reflux.

Biopsies from the stomal region showed a greater

degree of histological change (normal 0 patients; chronic superficial gastritis 39; atrophic gastritis/intestinal metaplasia 30; mild dysplasia 18; moderate/severe dysplasia 12; carcinoma one) compared with biopsies from the body of stomach (normal seven patients; chronic superficial gastritis 46; atrophic gastritis/intestinal metaplasia 30; mild dysplasia 11; moderate-severe dysplasia five; carcinoma one). For the purposes of this study each patient was given an overall histological grade, this being the higher grade found in either stomal or body biopsies in that patient (Table 1). No correlation was found between symptoms and histological change except in the carcinoma patient.

These histological groups were compared with respect to current age and years since surgery, also in Table 1. Analysis of variance showed no significant overall difference between the groups for these variables; group V was not included in this or other analysis as there was only one patient.

Operation types are detailed in Table 2. Marked dysplastic changes were more common in the vagotomy and drainage patients than in the gastrectomy patients. This trend was more definite when only patients with pH>4·0 were considered.

Values for pH ranged from 1·1 to 8·3. The patients fell into two groups with respect to pH, there being 30 patients in the pH<3·0 range and 62 in the pH>5·0 range. Only eight patients had pH values falling between these two groups. A pH of 4·0 was therefore used to divide patients into those capable of secreting acid in the fasting state and those not. This division has been used by others.[15] Patients who maintained a fasting pH below 4·0 were much more frequent in the vagotomy and drainage patients (31 out of 75) compared with the gastrectomy patients (one out of 22). The three patients who had drainage only all had a fasting pH below 4·0. Gastric juice nitrite values showed a wide range from zero to 276 μM/l.

Table 1 *Histological grades with age and years since surgery*

Histological grade	*No*	*Current age* Mean (range)	*Years since surgery* Mean (range)
I Chronic superficial gastritis (±minimal atrophic gastritis)	35	57 (39–71)	20 (13–34)
II Atrophic gastritis/intestinal metaplasia	30	60 (39–76)	22 (13–41)
III Mild dysplasia	21	60 (47–74)	19 (10–30)
IV Moderate/severe dysplasia	13	63 (42–79)	19 (13–30)
V Carcinoma	1	78	30

Samples of water taken through the suction channel of the endoscope on 17 occasions contained zero nitrite in 14 instances, and a maximum of 1 μM/l. Bacterial culture of these samples gave no significant growth.

PH AND HISTOLOGY

Within each histological group, the patients were classified according to whether their endoscopic fasting gastric juice pH was above 4·0 or below 4·0 (Table 3). The pH values within each group were compared with the chronic superficial gastritis as some degree of chronic superficial gastritis was found in all patients and appeared to represent the norm in the postoperative stomach. A greater degree of histological abnormality was found in the presence of high pH.

PH AND NITRITE

A strong correlation (Spearman's rank correlation coefficient = 0·53 $p<0·001$) was found between pH and nitrite in gastric juice taken at endoscopy (Fig. 1). Only when the pH of the gastric juice is above 4·0 is there potential to have very high concentrations of nitrite.

NITRITE AND HISTOLOGY

A significant difference was noted between the endoscopic gastric juice nitrite values occurring in the moderate/severe dysplasia group and the chronic superficial gastritis group (Table 3). This was expected as all patients in the former group were above pH 4·0, whereas more than half of the latter group were below pH 4·0. The nitrite values for patients within the other histological groups did not differ significantly from the chronic superficial gastritis group.

REPRODUCIBILITY OF FASTING PH AND NITRITE IN INDIVIDUAL PATIENTS

The pH of the gastric juice taken by nasogastric tube from 47 patients who returned to have gastric sampling was compared with the pH found at endoscopy in the same patients. In Fig. 2 the endoscopic pH is plotted against the mean of three pH values in samples taken by nasogastric tube. Although identical pH values were not obtained, only one patient had a change of pH range (from pH>4 to pH<4) between endoscopic and nasogastric tube samples, Also one patient had a change in pH values from pH<4 to pH>4 over the period of the three samples taken by nasogastric tube although the mean value was below pH<4. Endoscopic gastric juice samples, therefore, if taken with great care give a good representation of a patient's usual fasting intragastric pH.

Table 2 *Operation types in each histological grade*

	Vagotomy and drainage (75)		*Gastrectomy (22)*		*Drainage only (3)*
	Vagotomy and gastro-enterostomy (68) (3 later had roux loop)	*Vagotomy and pyloroplasty (7)*	*Billroth I (3)*	*Billroth II (19) (5 later had roux loop)*	*Gastro-enterostomy (3)*
Chronic superficial gastritis	22	3	1	8	1
Atrophic gastritis/intestinal metaplasia	22	1	1	6	-
Mild dysplasia	14	2	1	2	2
Moderate/severe dysplasia	10	1	-	2	-
Carcinoma	-	-	-	1	-

Nitrite values are similarly plotted in Fig. 3. In patients whose gastric juice was pH<4, the nitrite always tended to be low. In those patients whose gastric juice was pH>4, the nitrite values were much more variable, but tended to be higher than in the low pH patients.

Discussion

A surprisingly large proportion of the patients studied had a low fasting gastric pH. Obviously inadequate surgery may account for this but patients who had no acid secretion immediately after surgery may eventually regain the ability to secrete acid. Possible explanations include recovery of vagal function and perhaps hyperplasia of antral G cells was caused by continuous exposure to alkaline gastric juice.[11]

The fasting pH was surprisingly relatively constant above or below 4·0, whether taken at endoscopy or nasogastric aspiration. This may have been because endoscopy juice was taken under direct vision, usually from a pool lying in the fundus, and was therefore known to be from the stomach, rather than distal to the stoma. We avoided sampling obvious small bowel contents which had refluxed into the stomach. At the time of nasogastric tube sampling, the discarding of resting juice probably dealt with any enterogastric reflux occurring at the time of intubation. The continuing presence of a fine nasogastric tube in the stomach has been shown to have little effect on enterogastric reflux.[16] Thus in both cases we felt we achieved a reasonably true representation of fasting gastric secretion, largely unaffected by enterogastric reflux. The benefit, from the point of view of this study, is that it allows division of patients into two distinct groups with respect to pH. This division is also pertinent to nitrite as, although a low pH always means a low nitrite concentration, patients with a high pH have the potential to generate large amounts of nitrite. Therefore, although low nitrite values may occur in patients in the latter group (pH>4·0), the high pH indicates a tendency for higher average exposure to nitrite when compared with patients in the former group (pH<4·0). Paradoxically a single pH value may in fact give a better indication of an individual's exposure to nitrite than a single nitrite value.

The natural history of the histological changes found in the postoperative stomach is unknown.[17 18] Siurala *et al*[19] showed an increased incidence of gastric carcinoma in patients with atrophic gastritis. Morson[20] has suggested that gastric carcinomas

Table 3 *Gastric juice pH and nitrite values in each histological grade (endoscopic samples)*

	Patients (no) pH<4	*Patients (no) pH>4*	*Comparison with chronic superficial gastritis* (pH values)*	*Nitrite (μM/l) Median (range)*	*Comparison with chronic superficial gastritis† (nitrite values)*
Chronic superficial gastritis	22	13	—	3·9 (0–212)	—
Atrophic gastritis/intestinal metaplasia	7	23	p=0·003	13·1 (0–216)	NS
Mild dysplasia	6	15	p=0·03	13·0 (0–227)	NS
Moderate/severe dysplasia	0	13	p=0·001	90·1 (0–275)	p=0·02
Carcinoma	0	1	—	173·0	—

* χ^2 test with Yates' correction. † Mann-Whitney U test.

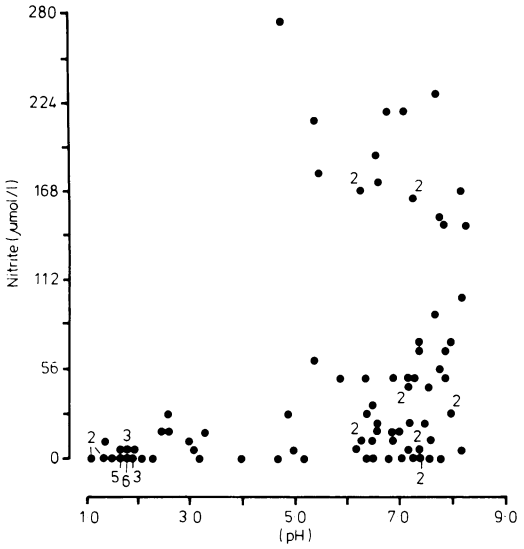

Fig. 1 *Scattergram to show the relationship between gastric juice pH and nitrite concentration in endoscopic samples of 100 postgastric surgery patients.**

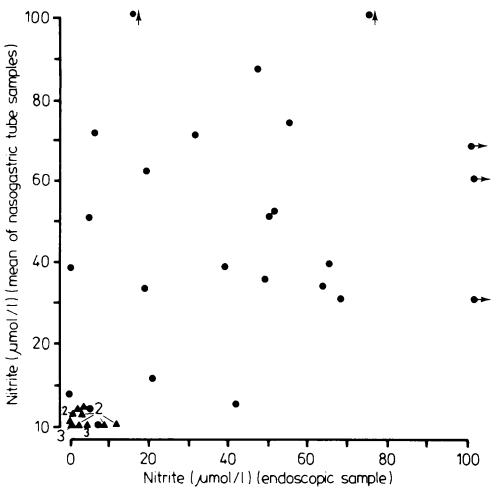

Fig. 3 *Gastric juice nitrite concentration at endoscopy plotted against mean nitrite concentration of three samples taken from the same patients by naso-gastric tube. Triangles represent patients whose endoscopic gastric juice was pH<4. Dots represent patients whose endoscopic gastric juice was pH>4.**

often occur in areas of intestinal metaplasia and he also stresses the importance of follow up on patients with dysplasia in view of the experience with the colon and cervix.[14]

As a number of studies[21 22] have shown that the commonest site of origin of postgastric surgery cancer is at the stoma, the majority of the biopsies in our study were from this site. Although dysplastic changes were more common around the stoma than in the gastric body, in our patients, a statistical test was not applied as the greater number of biopsies taken from the stoma would have made this analysis invalid. This is particularly true in view of the well recognised patchy distribution of the histological changes.

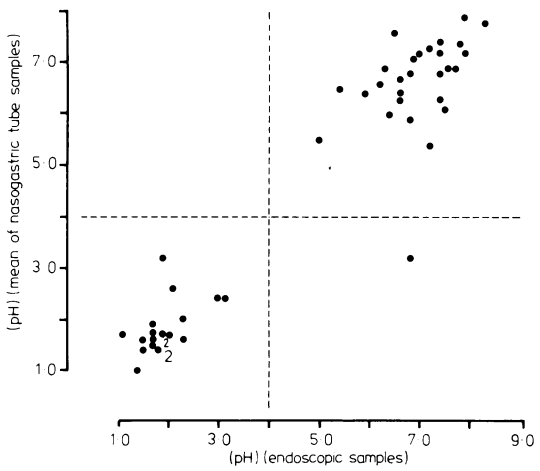

Fig. 2 *Gastric juice pH at endoscopy plotted against the mean pH of three samples taken from same patients by nasogastric tube.**

In our study we found these histological changes occurring after all types of operation. The gastrectomy patients tended to have lower grades of histological change than the vagotomy and drainage patients. This is surprising as the former group tended to have on average a higher pH. The majority of stomas, however, in the vagotomy and gastroenterostomy patients, and all the stomas in the vagotomy and pyloroplasty patients occur in antral mucosa. The antrum in duodenal ulcer patients before surgery has a higher incidence of gastritis than dyspeptic patients.[23] Therefore, the already abnormal antral mucosa may be more prone to further histological change. Also, it is well known that primary carcinoma of the stomach occurs most commonly in the antrum and pyloric region of the stomach, suggesting that antral mucosa may be more predisposed to malignant change than body mucosa. The increased incidence of gastric carcinoma after surgery has usually been reported in

* The small numbers on the scatter plots indicate where two or more patients fell on or very near the same point.

gastrectomy patients.[11] This increase has recently been reported after vagotomy and drainage[24] and our findings of histological abnormalities in these patients after a comparatively short follow up may be relevant to this.

A schematic diagram of the theories of the pathogenesis of gastric carcinoma in the post-operative stomach in shown in Fig. 4. There is general agreement that high concentrations of nitrite occur in the hypochlorhydric stomach and that this results from bacterial proliferation. Muscroft[15] has shown a correlation between nitrate reducing bacteria and nitrite concentrations in human gastric juice. Furthermore, in our laboratory we have initial data which show that if a bacterial culture is added to sterile gastric juice of high pH, but low nitrite, and incubated for 24 hours, nitrite is greatly raised, only if the bacteria survive. Whether these high concentrations of nitrite proceed to *in vivo* formation of N-nitroso compounds is less clear. In a sterile solution, nitrosation occurs at a low pH and the rate of formation is proportional to the square of the nitrite concentration.[25] Bacteria have been shown to produce N-nitroso compounds in conditions of high pH[26] and one study has shown increasing concentrations of N-nitroso compounds occurring with increasing pH in human gastric juice.[9] A later study,[27] using a different analytical technique, did not confirm this.

Unconjugated and secondary bile acids have been shown in the gastric juice of postgastrectomy patients.[28] These have been shown to have a carcinogenic effect on the colon in the experimental animal[29] and to have a damaging effect on canine gastric mucosa,[30] although there is no direct evidence for their long term effect on human gastric mucosa.

There is a great deal of uncertainty in this field and all data must therefore be interpreted with caution. We have shown a relationship between the histological changes occurring in the postoperative stomach and fasting pH. We have also confirmed the strong relationship between gastric pH and nitrite concentration found by others. This does not, of course, exclude the possibility that other pH related factors, such as bile metabolites are important in the pathogenesis of mucosal abnormality.

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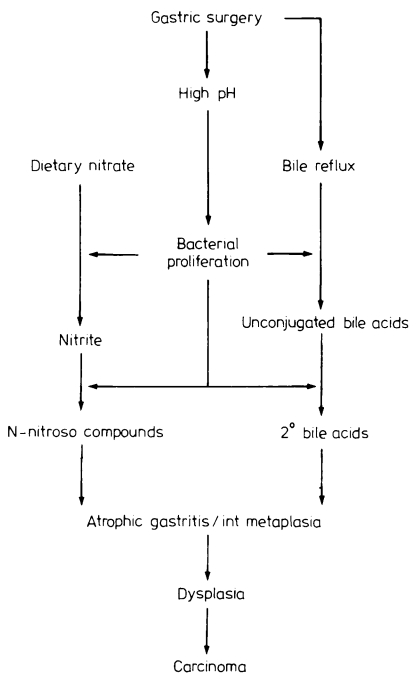

Fig. 4 *Flow diagram of the proposed theories of carcinogenesis in the postoperative stomach.*

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