

Relation between intragastric bile acid concentration and mucosal abnormality in the stomach after vagotomy and gastroenterostomy for duodenal ulcer

PCH WATT,* JM SLOAN,* TL KENNEDY†

*From the Departments of *Pathology and †Surgery, Royal Victoria Hospital, Belfast*

SUMMARY A high incidence of possible premalignant conditions of the gastric mucosa has been reported in the postoperative stomach and it has been suggested that bile plays a role in their pathogenesis.

Fifty eight patients who had had a vagotomy and gastrojejunostomy between 1957 and 1967 underwent endoscopy and biopsy. Subsequently, samples of fasting and postprandial gastric juice were taken from all patients for analysis of total bile acid. The patients were divided into three histological groups: group 1 (24 patients) had gastritis or mucosal atrophy but no more than mild dysplasia; group 2 (23 patients) had intestinal metaplasia in one or more biopsies; group 3 (11 patients) had moderate or severe dysplasia in one or more biopsies. Group 2 and group 3 patients had higher fasting intragastric bile acid concentrations than group 1 ($p < 0.01$ in both cases). There was no difference between fasting bile acid concentrations in groups 2 and 3. In the postprandial phase groups 2 and 3 also had higher peak intragastric concentrations than group 1 ($p < 0.01$ in both cases). Again, there was no significant difference between groups 2 and 3.

It is concluded that there is a relation between mucosal abnormality in the postoperative stomach and intragastric bile acid concentration. The possible aetiological link between bile acid and these mucosal abnormalities is discussed.

There is now considerable evidence that surgery for benign peptic ulceration is associated in the long term with a high incidence of gastric carcinoma.¹⁻³ This has prompted a number of endoscopic and biopsy studies of groups of patients who have had gastric surgery.⁴⁻⁸ These studies have shown a high incidence of mucosal abnormality and a number of unsuspected gastric cancers have been found.^{4,5}

The pathogenesis of premalignant and malignant conditions in the stomach after operation is controversial. Several studies have suggested that the bacteria which proliferate in the hypochlorhydric stomach are capable of reducing dietary nitrate to nitrite and of further reducing nitrite to N-nitroso compounds, which are known to be carcinogenic, at least in experimental animals.^{9,10} Other studies have suggested that bacteria break down the bile acids, which reflux into the stomach leading to metabolites which could be carcinogenic in the long term.¹¹

The object of this study was to investigate the relation between intragastric bile acid concentration

and histological abnormality in a group of patients who had undergone gastric surgery.

Patients and methods

In a previous study⁶ 82 patients who had undergone vagotomy and gastrojejunostomy were subject to endoscopy and biopsy. The methods used for endoscopy, biopsy, and histological assessment have previously been reported.⁶ Briefly, at the time of endoscopy seven biopsies were taken from each patient, and each biopsy was individually assessed for gastritis, atrophy, intestinal metaplasia, and dysplasia. Of these 82 patients, 58 returned for gastric juice sampling and they form the basis of this report. All had undergone vagotomy and gastrojejunostomy in the Royal Victoria Hospital, Belfast, between 1957 and 1967. There were 47 men and 11 women, with a mean age of 60.3 years (range 43-77 years). Most of the patients were asymptomatic, but five complained of diarrhoea, three of oesophagitis, three of dumping symptoms, and one had symptoms suggestive of bile reflux.

For the purposes of this study the 58 patients were divided into three histological groups. Group 1 had no intestinal metaplasia and no more than mild dysplasia in any biopsy. All these patients had various degrees of gastritis and mucosal atrophy. There were 24 patients in this group (17 men, seven women) and their mean age was 60.1 years. Group 2 patients had intestinal metaplasia in one or more biopsies, in addition to gastritis and mucosal atrophy. There were 23 patients in this group (21 men, two women) and their mean age was 61.3 years. Group 3 patients had moderate (10 patients) or severe (one patient) dysplasia in at least one biopsy. These patients also had various degrees of gastritis and mucosal atrophy and, in addition, seven also had intestinal metaplasia in one or more biopsies. There were 11 patients in group 3 (nine men, two women) and their mean age was 58.8 years. Analysis of variance showed no significant difference in the age distribution between the three groups.

Before gastric juice sampling all the patients fasted overnight. A size 14 Salem sump nasogastric tube was passed and the position checked radiologically. After 30–45 min the stomach was completely aspirated and the aspirate used only for analysis of nitrite and N-nitroso compounds, the results of which are to be reported. Gastric juice was then collected over a 30 min period. A meal containing 18 g of corn oil and 12 g of glucose made up to 100 ml with warm water was then given via the nasogastric tube. Aliquots (10 ml) of gastric juice were then aspirated 20, 40, and 60 min after the meal. The pHs of the preprandial samples were measured using a Corning model 113 pH meter.

BILE ACID ANALYSIS

Analysis of total bile acid was carried out on all fasting and postprandial samples using reagent mixtures as described for the commercial kit produced by Nyegaard (Sterognost 3 α Pho), which is based on the technique used by Fausa and Skalhogg.^{11a} This technique involves the oxidation of bile acids by the enzyme 3 α -hydroxysteroid dehydrogenase with concomitant reduction of NAD to NADH₂. The NADH₂ is produced mole for mole from bile acids and can readily be measured using a spectrophotometer (Pye Unicam SP8–100) set to measure extinction at 340 nm. All assays were carried out on 100 μ l samples of gastric juice at room temperature and pH 9.5. Readings were taken after a reaction time of 20–40 min as recommended by Fausa and Skalhogg.

The assay was linear (coefficient of correlation = 0.999) for cholic acid standard solutions up to a concentration of 1000 μ mol/l. The assay was also linear

for bile acids in gastric juice over a similar range (coefficient of correlation = 0.992).

To assess reproducibility 12 aliquots of a minimally bile stained sample of gastric juice were assayed. The mean concentration was 108.03 μ mol/l and the coefficient of variation was 3.7%. The same gastric juice was then spiked with cholic acid in order to raise the bile acid concentration by 200 μ mol/l. Twelve aliquots were assayed, giving a mean value of 284.8 μ mol/l (coefficient of variation = 2.02%). The recovery of cholic acid was 90.4%.

The effect of pH on recovery of bile acids was minimal. The addition of the corn oil to gastric juice, however, reduced recovery by about 13%.

Table Bile acid concentrations in preprandial and postprandial samples

	Bile acid concentration (μ mol/l)*	
	Preprandial	Postprandial
Group 1 (n = 24)	197.7 (0–11 985)	891 (0–20 987)
Group 2 (n = 23)	1448 (0–26 835)	18 364 (275–34 256)
Group 3 (n = 11)	8040 (1690–25 911)	11 066 (2640–30 217)
All patients (n = 58)	(0–26 835)	(0–34 256)

*Values given as median (range)

Results

PREPRANDIAL SAMPLES

The ranges of bile acid concentration for preprandial samples are shown in the Table. Values for all groups are plotted in Fig. 1. Significantly higher preprandial bile acid concentrations were found in group 2 compared with group 1 ($p = 0.01$) and in group 3 compared with group 1 (Mann-Whitney U test; $p = 0.01$). No significant difference was found between groups 2 and 3.

The pH values of the preprandial samples in all 58 patients showed a positive correlation with bile acid concentration (Spearman's correlation coefficient 0.79; $p < 0.01$).

POSTPRANDIAL SAMPLES

For the purposes of this study, the peak postprandial concentration was used for comparison between groups (Table). The bile acid concentrations for all groups are plotted in Fig. 2. The bile acid concentrations in group 2 and group 3 were both higher than in group 1 (Mann-Whitney U test; $p = 0.01$ in both cases). No significant difference was found between groups 2 and 3.

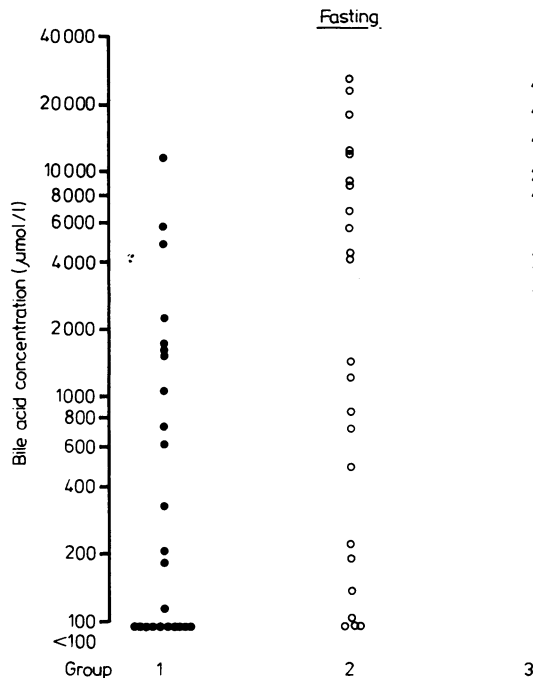


Fig. 1 Fasting gastric juice bile acid concentrations in histological groups of patients who have undergone vagotomy and gastrojejunostomy.

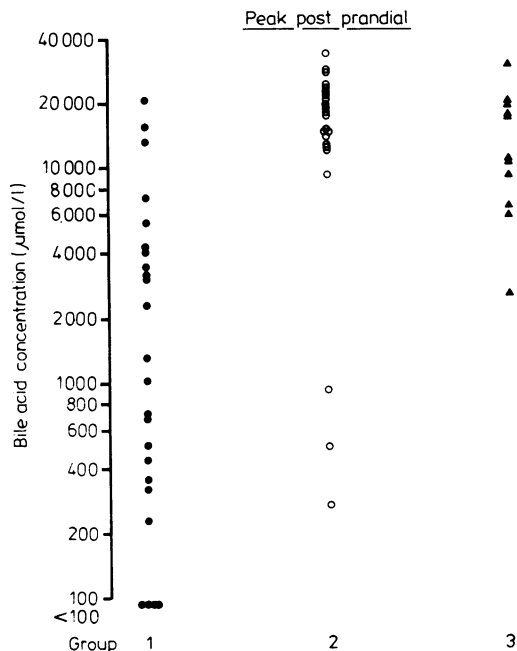


Fig. 2 Peak postprandial gastric juice bile acid concentrations in histological groups of patients who have undergone vagotomy and gastrojejunostomy.

Discussion

▲ Studies which have followed up over prolonged periods groups of patients who have had gastric surgery have given contradictory results as to whether these patients have a higher than normal incidence of gastric cancer. McClean Ross *et al*¹² found no increased incidence of gastric cancer whereas Krause¹ found an observed to expected ratio of 2:2 for gastric cancer. Schrupf *et al*⁴ found four cases of infiltrating carcinoma in 108 gastrectomy patients at endoscopy, which is more than they would have expected in the normal population. Stalsberg and Taksdal² have shown an increased frequency of previous gastric surgery at necropsy in patients with gastric cancer. These studies concerned either exclusively or mainly gastrectomy patients. Since gastrectomy patients lack the gastric antrum which is that part most likely to undergo malignant change, it might be expected that they would have a lower incidence of gastric cancer than the normal population. The fact that the incidence is at least equal to, if not more than, normal suggests that a carcinogenic process is in progress in the gastric stumps.

The nature of this carcinogenic process is controversial. Reed *et al*¹⁰ found a positive correlation between pH and N-nitroso compounds in gastric juice. Since these have been shown to be carcinogenic at least in experimental animals,¹³ this group has suggested that they might be responsible for the high incidence of gastric cancer in hypochlorhydric states such as pernicious anaemia and after gastric surgery. Other studies have found no correlation between gastric juice pH and total N-nitroso compound concentration.¹⁴ Work from our own laboratories on N-nitroso compounds has found no convincing role for them in the pathogenesis of premalignant mucosal change in patients who have undergone vagotomy and gastrojejunostomy included in the current study.

The uncertainty regarding N-nitroso compounds and the fact that bile acids have been suspected of having a major role in the pathogenesis of colonic cancer¹⁵ and in cancer following gastric surgery¹¹ stimulated this study on the relation between gastric juice bile acid concentration and gastric mucosal histology. The histological groups were chosen because of the strong suspicion that both intestinal metaplasia¹⁵ and appreciable degrees of dysplasia are premalignant conditions.¹⁶ Mild dysplasia was included in group 1 as its importance has been questioned¹⁷ and since in a previous study no significant difference was found in its incidence in vagotomy and gastrojejunostomy and control patients.⁶

In this study, considerable effort was made to dis-

tinguish dysplasia from epithelial regenerative change¹⁶ using the criteria of Whitehead.¹⁸ When epithelial regenerative change was diagnosed it was recorded and graded into two grades. Since identification of the lesion is subjective, the reproducibility of this grading was poor and therefore no correlation was attempted between intra-gastric bile acid and epithelial regenerative change. In a previous report we showed that our grading of dysplasia and its distinction from epithelial regenerative change was reproducible.⁶ Since epithelial regenerative change was found to some extent in virtually all these postoperative patients, it was considered inappropriate to analyse the differences in intra-gastric bile acid concentration between those who did and those who did not have the lesion. Mosimann¹⁹ has used the term foveolar hyperplasia, defined as "elongation and tortuosity of the gastric pits making them appear more frequently than normal in transverse sections." These authors found this change in all their patients who had had gastric surgery.²⁰ We found, however, that foveolar hyperplasia was difficult to assess in a reproducible way.

Owing to the difficulties in quantifying duodenogastric reflux,²¹ gastric bile acid concentration was used in the current study. We considered that concentration of bile acids was a more important factor in determining mucosal damage than the total quantity refluxing into the stomach as the latter does not take into account the dilutional effect of gastric juice. Hoare *et al*²² have shown that this type of technique for the collection of gastric bile acid is reproducible. The relation between bile acid concentration in gastric juice and histological abnormality is in agreement with the work of Ritchie,²³ who studied early postoperative patients. Thomas *et al*²⁴ found a relation between gastric mucosal dysplasia and bile reflux as measured by H I D A scan and our studies support their findings. Our findings also support the view of Domellof,¹¹ who suggested that bile metabolites may be important in the pathogenesis of carcinoma following gastric surgery. Domellof *et al*²⁵ subsequently showed the presence of unconjugated and secondary bile acids in the gastric juice of post-gastrectomy patients. Deoxycholic acid has been shown to promote carcinogenesis in the mouse rectum.²⁶ Hill *et al*²⁷ have shown that bacteria can metabolise bile acids into possible carcinogenic substances, and several studies have shown that bacteria proliferate in the hypochlorhydric stomach.^{10,28} There would therefore appear to be a theoretical basis for suggesting an aetiological role for bile acting as a carcinogen in hypochlorhydric stomachs and our data would tend to support this theoretical view.

In the fasting state patients in groups 1 and 2 appeared to have a wide range of bile acid concen-

trations, whereas group 3 patients all tended to have high intra-gastric bile acid concentrations, although there were only 11 patients in this group. Group 2 patients tended to have a greater response to the test meal. We are unable to explain this adequately, although a speculative suggestion is that the anatomical arrangement of the stoma in these patients might predispose them to a sudden increase of gastric bile acid concentration after contraction of the gallbladder.

We have found an association between intra-gastric bile acid concentration and histological abnormality. This does not rule out the possibility that some other substance is responsible for the mucosal change and that bile is acting merely as a marker for that substance. In particular, lysolecithin²⁹ has been suggested as a cause of gastric mucosal damage. Our data apply only to total bile acids, although we are currently investigating the association between mucosal damage and the various bile acid fractions. We have previously shown a relation between gastric mucosal abnormality and high pH.³⁰ The reason for this may be two fold. Firstly, the increased bile acid concentration in gastric juice found in these patients will raise the pH. Secondly, the mucosal abnormality, which is possibly caused by the bile reflux, leads to less secretion of acids.

In conclusion, we have found an association between probable premalignant gastric mucosal changes and intra-gastric bile acid concentration in a group of patients who have undergone vagotomy and gastrojejunostomy. This suggests the possibility of an aetiological role for bile in the pathogenesis of premalignant changes in the stomach after operation.

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Requests for reprints to: Dr PCH Watt, Department of Pathology, Royal Victoria Hospital, Belfast, BT12 6BA, N Ireland.