

Intragastric N-nitrosation is unlikely to be responsible for gastric carcinoma developing after operations for duodenal ulcer

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SUMMARY Three groups of patients studied after operations which had cured their duodenal ulcer were compared with a control group (no operation, n=8). The surgical procedures included: proximal gastric vagotomy (n=7), truncal vagotomy and pyloroplasty (n=7), truncal vagotomy and antrectomy (n=8). Samples of gastric juice were aspirated half hourly or hourly over 24 hours for measurement of pH, counts of all identified bacteria, nitrite and total N-nitrosocompounds. Although the pH over 24 hours was significantly higher after proximal gastric vagotomy ($p<0.05$) and truncal vagotomy and antrectomy ($p<0.001$) than controls, there was no difference between truncal vagotomy and pyloroplasty and controls. Counts of nitrate reducing bacteria over 24 hours were also significantly higher after truncal vagotomy and antrectomy than controls ($p<0.1$) but no differences were observed between the other groups. Only after truncal vagotomy and antrectomy was nitrite over 24 hours significantly increased compared with controls ($p<0.01$). Despite these higher values after truncal vagotomy and antrectomy, there was no significant difference in total N-nitrosocompounds between any of the four groups. Whereas bacterial counts and nitrite increased with pH, no correlation was found between total N-nitrosocompounds and pH. These results provide no evidence that exposure to total N-nitrosocompounds is increased after operations for duodenal ulcer.

There is some evidence that patients who have had a gastric resection for peptic ulcer have a greater risk of developing gastric cancer than the population at large.^{1,2} These 'stump cancers' which arise in the gastric remnant usually occur 15-20 years after a previous resection for benign disease. The prognosis of such lesions is usually very poor and many patients present with advanced disease.³ One report has also suggested that there is a small but significant increased risk of developing gastric cancer after a previous vagotomy.⁴ Many patients develop intestinal metaplasia or dysplasia after gastric operations⁵ which may represent premalignant changes. The concept of an intragastric cocarcinogen being responsible for development of

malignancy after a latent period of 10-20 years is thus attractive.⁶

Bacterial proliferation in gastric juice is common after previous gastric resection as a consequence of persistent hypochlorhydria.⁷ Some of these organisms are capable of reducing nitrate to nitrite.⁸ This reaction may encourage the production of N-nitrosocompounds from available substrates: amines, amides, and ureas.⁹ Certain N-nitrosocompounds, particularly nitrosamides, are locally carcinogenic in experimental animals. Studies based upon single fasting gastric samples have indicated that stable N-nitrosamides^{10,11} are found in significantly higher concentrations after partial gastrectomy than controls. There is some doubt about extrapolating data based upon single gastric samples in fasted subjects and previous studies when conducted over 24 hours have indicated considerable variation in pH, nitrite, bacterial

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counts, and N-nitrosocompounds.¹² We have therefore performed a series of studies on half hourly or hourly gastric aspirates collected over 24 hours in three groups of subjects who have had a previous successful operation for duodenal ulcer and controls. All subjects received a controlled diet and intra-gastric pH, counts of individual bacteria, nitrite concentrations and total N-nitrosocompounds were measured.

Methods

PATIENTS

We have selected 22 asymptomatic male patients who have undergone one of three operations more than two years before the study to cure their previous duodenal ulcer. Hence all patients had a proven duodenal ulcer before operation and no evidence of recurrent ulcer was shown. The operations included proximal gastric vagotomy (n=7), truncal vagotomy and Heineke Mickulicz pyloroplasty (n=7), and truncal vagotomy with Bilroth I antrectomy (n=8). Results were compared with eight male controls who had no clinical evidence of peptic ulceration. Controls were selected to provide a suitable range of ages which matched the postoperative groups. All individuals signed a consent form indicating their willingness to participate in the study and the enquiry had the approval of our local ethical committee.

Gastric aspirates were obtained by an indwelling nasogastric tube which was passed at 0900 hours. Half hourly aspirates were collected and analysed for pH, nitrite, and total N-nitrosocompounds. Because of insufficient half hourly gastric volumes, total and individual gastric bacterial viable counts were measured hourly. The study was discontinued at 0900 on the following morning. In order to measure the unstable N-nitrosocompounds we converted a side ward adjacent to the area where patients were being studied so that the gastric samples could be analysed within five minutes of collection. We therefore used a cohort of chemists who worked in shifts throughout the night to analyse these samples. The annexe also provided a suitable area for preparing food. All food administered during the study was bought in bulk and stored deep frozen so as to reduce any variation in content. All subjects ate identical meals, which consisted of commercially available processed or frozen food, conforming to a typical hospital meal. The food was heated in a microwave oven after prior weighing of non-itemised materials. Those who smoked regularly were allowed to smoke as usual during the study. A standard volume of beer was included in the specified beverages. The following meals and

snacks were provided: breakfast (0930h), coffee and biscuit (1130h), lunch (1330h), tea and biscuit (1530h), dinner (1800h), and late night beverage (2230h).

pH

The pH was measured with a glass electrode (Radiometer, Copenhagen).

MICROBIOLOGY

Gastric juice samples were collected into sterile containers and homogenised with a vortex mixer to break up mucus and food and to disperse organisms. The samples were then frozen at -80°C and analysed later. Frozen samples were used as previous validation studies in this laboratory have indicated that the difference in total viable counts between fresh and frozen gastric aspirates is less than 10-fold.¹³ Serial 10-fold dilutions of gastric juice were prepared after thawing the samples in an anaerobic cabinet. Dilutions were inoculated on eight separate selective media and incubated for 48 hours.⁷ Each species was identified, the number of colonies was counted and then tested for their ability to reduce nitrate.

NITRITE

The gastric juice sample was immediately buffered with borax to raise the pH to more than 7.0 so as to prevent destruction of nitrite. Samples were then stored at -10°C and were analysed within one week by polarography.¹²

N-NITROSOCOMPOUNDS

Immediate measurement (within five minutes of gastric aspiration of the total N-nitrosocompounds) was performed by the method of Bavin *et al.*^{12 14} Samples were prepared by adding hydrazine sulphate to destroy any nitrite present and titrating to pH 4.0. An aliquot was added to HBr/acetic acid and the nitric oxide evolved was measured by a chemiluminescent method to provide a figure for the total N-nitrosocompounds. Only using this immediate analysis is it likely that the unstable but more important contact carcinogens such as the nitrosamides and nitrosureas would be detected.

STATISTICAL ANALYSIS

Because of the highly fluctuating nature of the responses in this study for each parameter in each subject for the whole 24 hour period the area under the response vs time curve was calculated using the trapezoidal method (straight line point to point). The periods of time over which observations were recorded varied slightly, therefore the area under the curves were divided by the time spanned by the

curves to give area under curves/hour. These modified areas were used in all analyses. Analysis of variance was carried out on the area under curves/hour for each parameter to determine any differences between the four subject groups. To satisfy the assumption underlying the use of analysis of variance pH was analysed as $\log_e (\text{pH}-1)$ and nitrite as $\log_e (\text{NO}_2+1)$.

Results

pH

There was a small but significant difference in the area under the curve for pH values after proximal gastric vagotomy, compared with controls ($p < 0.05$). The reduction in pH compared with controls was 41%. There was no significant difference in the area under the curve for pH values between truncal vagotomy and pyloroplasty and controls nor in the reduction in pH compared with controls (31%). By contrast the median pH values after truncal vagotomy and antrectomy were constantly raised usually between 4.0 and 6.0 throughout the 24 hours

and almost all samples were bile stained. The difference between truncal vagotomy and antrectomy and controls was highly significant ($p < 0.001$) and the reduction in pH compared with controls was 90%. In each of these three groups there was a marked buffering effect by food, at 0900, 1400 and 1800 (Fig. 1).

BACTERIAL COUNTS

Changes in the total counts of bacteria closely followed intragastric pH. For pH values less than 3.5 the only species commonly isolated included lactobacilli, *Streptococcus viridans* and occasionally yeasts. The organisms in addition to those which were commonly identified in hypochlorhydric samples included: *Streptococcus faecalis*, beta haemolytic streptococci, *Micrococcus sp*, *Staphylococcus albus*, *Staphylococcus aureus*, *Neisseria sp*, *Haemophilus sp*, aerobic non-sporing bacilli, *Bacteroides oralis* or *B melaninogenicus* and veillonellae. *Escherichia coli*, Klebsiella and proteus were extremely rare and only occurred after truncal vagotomy and antrectomy. The principal nitrate

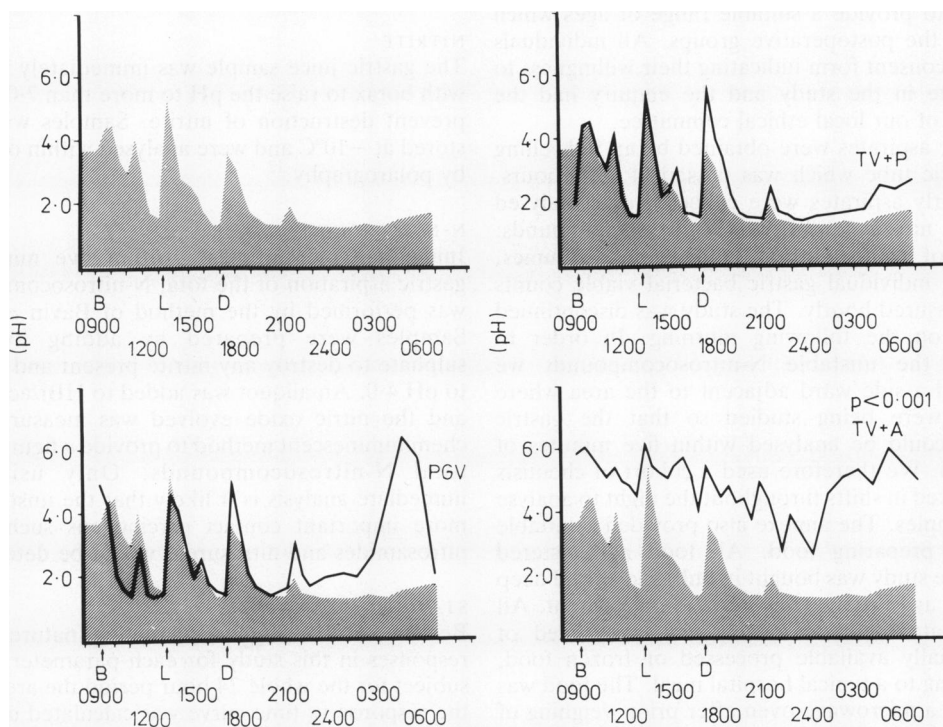


Fig. 1 Hourly median pH values in controls (top left), after proximal gastric vagotomy (bottom left), truncal vagotomy and pyloroplasty (top right), and truncal vagotomy and antrectomy (bottom right) over 24 hours. B, L, and D refer to breakfast, lunch and dinner.

reducing bacteria were micrococci, veillonellae, *Staphylococcus albus* and *Staphylococcus aureus*. There was no significant difference between the median nitrate reducing bacterial counts in each group and our controls except after truncal vagotomy and antrectomy ($p < 0.01$) (Fig. 2). Although there was a persistent total bacterial population throughout the 24 hours after truncal vagotomy and antrectomy of between 10^2 and 10^6 organisms per millilitre, the nitrate reducing bacteria usually disappeared from samples between 2300 and 0200.

NITRITE

Nitrite concentrations varied considerably throughout each 24 hour period and between subjects. Nitrite values increased significantly with intragastric pH. The median values for each group compared with controls is illustrated (Fig. 3). Analysis of area under the curve for nitrite indicated that only after truncal vagotomy and antrectomy were gastric nitrite concentrations significantly greater than controls ($p < 0.01$).

N-NITROSOCOMPOUNDS

Total N-nitrosocompounds also varied considerably throughout each 24 hour period and between subjects. Mean values varied between 0.1 and 6.0 $\mu\text{m/l}$. There was no correlation between total N-nitrosocompound concentrations and pH (Fig. 4). Although mean values for total N-nitrosocompounds appeared higher in the postoperative groups than in controls during the early hours of the morning and at their peak at 1900 and 2000 (Fig. 5), there was wide variation in individual values and statistical analysis based on individual area under the curves for total N-nitrosocompounds during the 24 hours of study indicated that no statistically significant differences were present between the groups. Hence despite higher values for pH, nitrate reducing bacterial counts and nitrite after truncal vagotomy and antrectomy the end products of N-nitrosation were not increased.

Discussion

This detailed study involving immediate analysis of

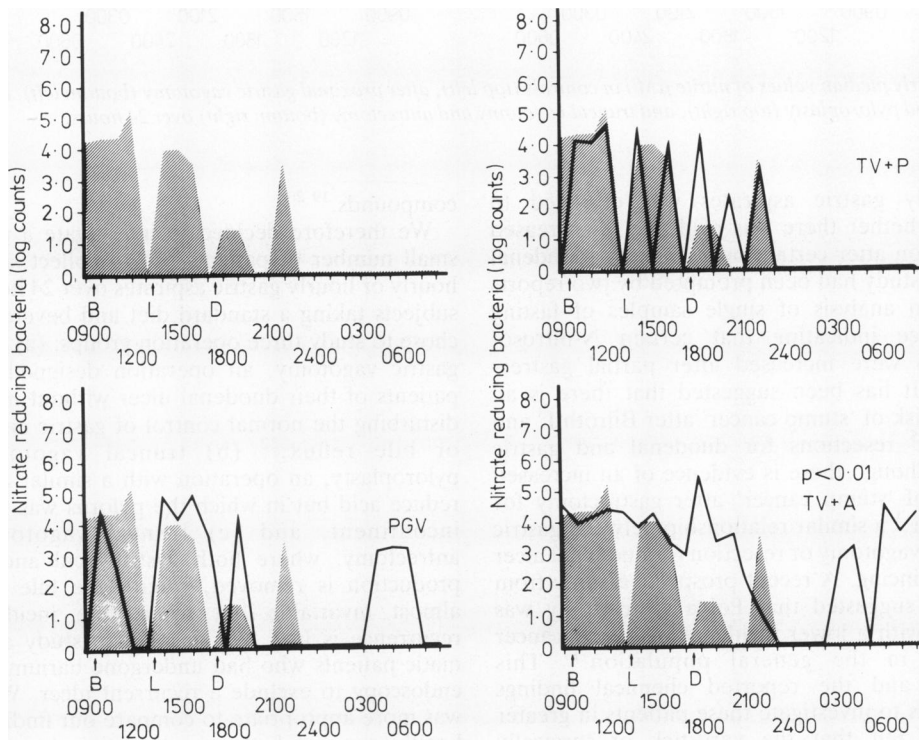


Fig. 2 Hourly median counts of nitrate reducing bacteria (\log_{10}) in controls (top left), after proximal gastric vagotomy (bottom left), truncal vagotomy and pyloroplasty (top right), and truncal vagotomy and antrectomy (bottom right) over 24 hours.

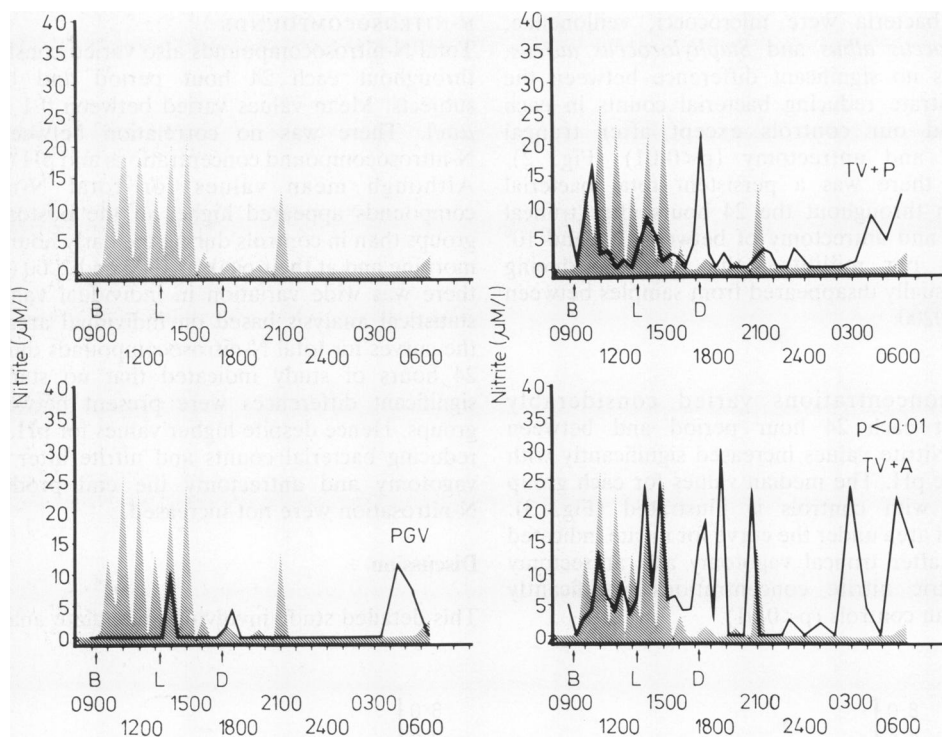


Fig. 3 Hourly median values of nitrite $\mu\text{M/l}$ in controls (top left), after proximal gastric vagotomy (bottom left), truncal vagotomy and pyloroplasty (top right), and truncal vagotomy and antrectomy (bottom right) over 24 hours.

half hourly gastric aspirates was designed to establish whether there was evidence of increased N-nitrosation after certain operations for duodenal ulcer. The study had been prompted by two reports based upon analysis of single samples of fasting gastric juice indicating that certain N-nitroso-compounds were increased after partial gastrectomy.^{10 11} It has been suggested that there is an increased risk of 'stump cancer' after Bilroth I³ and Bilroth II¹⁵ resections for duodenal and gastric ulcer.¹⁶ Although there is evidence of an increased incidence of 'stump cancer' after gastrectomy for gastric ulcer,¹ a similar relationship between gastric cancer and vagotomy or resection for duodenal ulcer is less convincing. A recent prospective study from Edinburgh suggested that Polya gastrectomy was associated with a lower incidence of gastric cancer than that in the general population.¹⁷ This suggestion and the reported chemical findings prompted us to investigate these patients in greater detail. We felt that the yardstick of surgically induced hypochlorhydria¹⁸ should be compared with the effect of administering H_2 -receptor antagonist drugs on concentrations of N-nitroso-

compounds.^{19 20}

We therefore decided to investigate a relatively small number of patients but to collect serial half hourly or hourly gastric aspirates over 24 hours with subjects taking a standard diet and beverages. We chose to study three operation groups: (a) proximal gastric vagotomy, an operation designed to cure patients of their duodenal ulcer without materially disturbing the normal control of gastric emptying²¹ or bile reflux;²² (b) truncal vagotomy and pyloroplasty, an operation with a similar ability to reduce acid but in which the pylorus was rendered incontinent; and (c) truncal vagotomy and antrectomy, where both gastric acid and gastrin production is removed, where free bile reflux is almost invariable but where the incidence of recurrence is low. We decided to study asymptomatic patients who had undergone barium meal or endoscopy to exclude a recurrent ulcer. We felt it was more appropriate to compare our findings with healthy age matched asymptomatic volunteers than with patients who had an active duodenal ulcer, as the aim of operation is to render duodenal ulcer patients normal. Furthermore, the cancer risks after

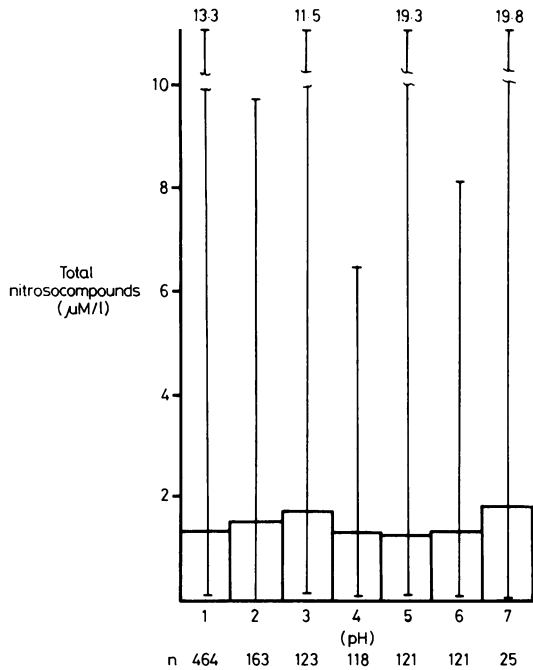


Fig. 4 Median values for total N-nitrosocompounds according to gastric juice pH.

gastric operations have been compared with the normal population. Patients were studied relatively early after operation, too early for development of carcinoma. The study was designed, however, to identify factors which might be responsible for the development of gastric malignancy 10–20 years later, not established epithelial malignancy.

There was neither sufficient space in our anaerobic cabinets nor adequate numbers of staff to undertake immediate viable counts on all bacteria. In view of the low frequency of strict anaerobes in gastric juice, even when cultured immediately, and our own studies regarding the adequacy of immediate freezing and rapid thawing of gastric samples for delayed quantitative bacteriology, we felt satisfied that metabolically important bacteria would not be lost using these techniques.

The fluctuations in gastric pH, particularly the buffering effect of food, were observed in all groups except those who had had vagotomy and antrectomy. After truncal vagotomy and antrectomy gastric juice samples were invariably bile stained with a pH which was usually between 4.0 and 6.0. The correlation between pH and bacterial counts was closer for total counts than for the numbers of nitrate reducing bacteria. The nitrate reducing organisms were all oral in origin (staphylococci or

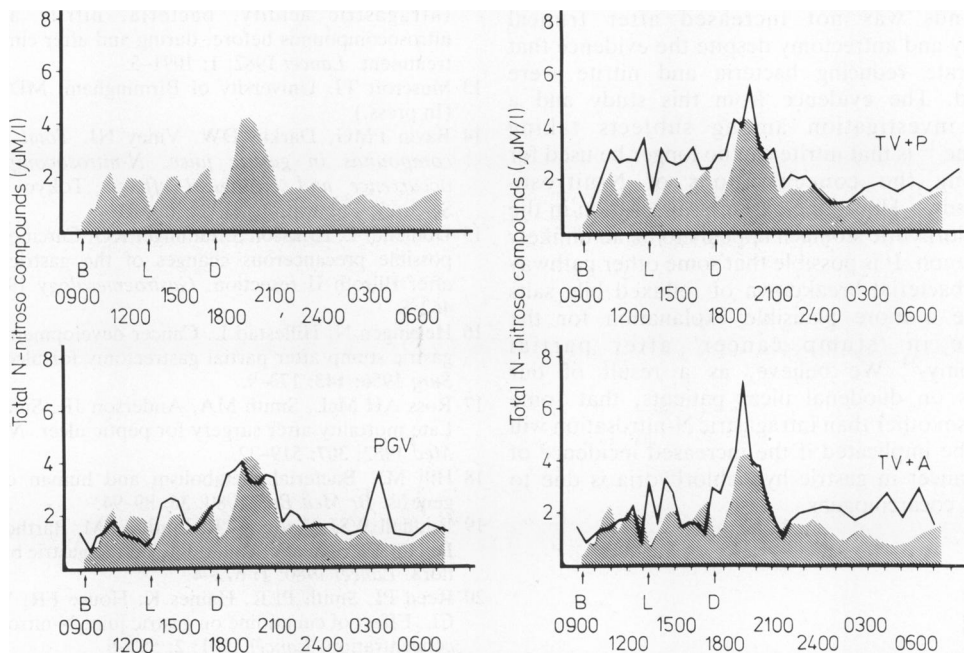


Fig. 5 Hourly mean values of total N-nitrosocompounds µM/l in controls (top left), after proximal gastric vagotomy (bottom left), truncal vagotomy and pyloroplasty (top right) and truncal vagotomy and antrectomy (bottom right) over 24 hours.

veillonellae) and the coliforms rarely contributed to the nitrate reducing count. It is possible that bile could have had an inhibitory effect on certain nitrate reducing bacteria after truncal vagotomy and antrectomy.

The importance of these observations on total N-nitrosocompounds when compared with those reported by Reed¹⁰ is that the unstable and thus more locally active cocarcinogens such as nitrosamides will have been detected by our method of collection. This difference is highlighted by the concentrations of N-nitrosocompounds reported in the two studies, as the values obtained in this investigation were approximately five times greater than those reported by Reed. The difference in handling the gastric juice samples might also explain the discrepancies between the studies with regard to changes in N-nitrosocompound values with the pH of gastric juice.

The method of statistical analysis, based on area under the curve, was selected in an attempt to assess total exposure to N-nitrosocompounds over 24 hour period. This does not allow for identification of very high values in any individual but, as values were so variable between individuals and between groups, any analysis of peak values would have been based on an arbitrary definition of 'high value'.

The most important finding of this study was, therefore, that total exposure to N-nitrosocompounds was not increased after truncal vagotomy and antrectomy despite the evidence that pH, nitrate reducing bacteria and nitrite were increased. The evidence from this study and a similar investigation among subjects taking cimetidine¹² is that nitrite can no longer be used for predicting the concentrations of N-nitrosocompounds.²³ Hence increased N-nitrosation in the hypochlorhydric stomach appears to be an unlikely phenomenon. It is possible that some other pathway such as bacterial breakdown of refluxed bile salts might be a more plausible explanation for the increase in 'stump cancer' after partial gastrectomy.²⁴ We believe, as a result of our enquiries on duodenal ulcer patients, that some mechanism other than intragastric N-nitrosation will have to be implicated if the increased incidence of gastric cancer in gastric hypochlorhydria is due to chemical cocarcinogens.

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