

Mutagenicity in gastric juice

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SUMMARY Mutagenicity has been measured in the gastric juice of 228 patients using the Ames bacteriological test system; while mutagenicity in control and duodenal ulcer patients did not differ from saline controls, mutagenicity was significantly increased compared with controls in patients suffering gastric ulcer ($p < 0.002$), carcinoma ($p < 0.002$), and in patients after gastric resection ($p < 0.01$). A transient rise in mutagenicity was seen following H_2 antagonist ingestion ($p < 0.002$). Increased levels of mutagenicity were found to correlate closely with gastric juice pH and bacterial count. Histidine concentrations in gastric juice did not explain the mutagenicity results.

The Ames' test¹ utilises a bacterium to detect mutagens. There is a close association between mutagenicity and carcinogenicity.² The application of this technique to gastric juice has previously been reported by Montes *et al.*³ *Salmonella typhimurium* strains TA 100 and TA 1538 were used and a mutagenic effect was detected in the gastric juice of volunteers in areas of the Colombian Andes where there is a high incidence of gastric carcinoma. This effect was only present in gastric juice samples with detectable amounts of nitrite, but was not produced by nitrite alone. Samples from volunteers resident in low risk areas failed to produce this mutagenic effect. It was suggested that intragastric n-nitrosation was responsible for the mutagenic effect. The same workers had previously reported a correlation between high gastric juice concentrations of nitrite, high pH, atrophic gastritis, and gastric carcinoma.

The potential risk of carcinogenesis by H_2 antagonists has also been investigated by this technique. DeFlora and Piciotto⁴ reported a mutagenic response by preincubating sodium nitrite and cimetidine in human gastric juice from untreated individuals or by adding nitrite in great excess to the gastric juice of patients receiving cimetidine. Ascorbic acid prevented this mutagenic effect. This finding would imply that patients taking cimetidine could have raised mutagen concentrations and thus possibly have an increased risk of

gastric carcinoma. There is an increased risk of malignancy in the gastric stump after gastrectomy⁵ and so this group may be worthy of further study.

The strain of bacteria used in our tests was *Salmonella typhimurium* TA 100 which requires histidine in its growth medium. This organism can undergo mutation which allows it to grow in a histidine free medium.

Methods

PATIENTS

Gastric juice was obtained at endoscopy or by nasogastric tube before operative procedure or gastric function test. (Endoscopes and their channels were cleared, immersed in cidex, and rinsed in water before use.) After transfer to a sterile container the sample was transported urgently to the laboratory. The pH was measured and a 1 in 10 dilution was prepared in 10% glycerol infusion broth before freezing at -70°C for subsequent bacterial study. The remaining specimen was frozen to -20°C , in some cases the specimen was also Ames tested immediately.

Gastric juice was sterilised by filtration (pore size $0.22\ \mu\text{m}$). In the first 26 cases pH was adjusted to 1, 4, and 7 with NaOH, but pH 7 was used thereafter. One half millilitre of juice was added to the same volume of test strain ($10^8/\text{ml}$) $0.5\ \text{mM}$ solution histidine and biotin and incubated at 37°C for 20 minutes. The samples were then mixed with 2 ml of molten overlay agar and layered on a histidine free agar base. Incubation was continued at 37°C for two

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days. Each test was performed in triplicate and two control solutions were tested in triplicate with every batch. Physiological saline was used as a negative control and a metronidazole solution containing 50 mg/l was used as a positive standard (this concentration was chosen after construction of a standard curve to produce approximately a three times rise in colony count). Strain tests were included in each batch to ensure that no alteration in the character of the salmonellae had occurred. Resistance to ampicillin is a feature of the TA 100 strain, while sensitivity to gentian violet remains. Counting was done manually. Mean colony counts were divided by the mean numbered colonies of the saline control to produce a mutagenicity ratio. Histidine was assayed using precolumn derivatisation with u-phthaldehyde/2-mercaptoethanol and reversed phase liquid chromatography.⁶ Histidine concentration and mutagenicity of 33 samples of gastric juice were measured. The effect of including increasing concentration of histidine in the test was studied in the range 25–500 $\mu\text{m/l}$. The histidine was added to a saline control to simulate the presence of histidine in the gastric juice.

In considering differences in mutagenicity ratios between different patient groups the mean of the three colony counts for each patient was taken and the Mann Whitney test used. Median numbers of gastric bacteria are plotted in Figure 1. While standard errors are given in Tables 1 and 2 these have not been used in the statistical method.

Two hundred and twenty eight patients were studied and of these 45 had duodenal ulcer, 22 a gastric ulcer, and gastric carcinoma was present in 21. In 16 patients no abnormality was detected at endoscopy and these are included as 'normals'. Forty nine patients taking H_2 antagonists were studied either within one to three hours of ingestion ($n=31$) or 12 hours after ingestion ($n=18$). Forty two patients were investigated who had previously undergone gastric surgery: partial gastrectomy 18, vagotomy 34.

In an additional 33 patients both gastric juice mutagenicity and histidine concentration was assayed.

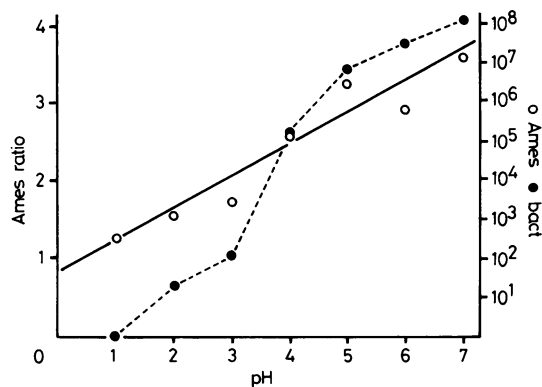


Fig. 1 Relationship of gastric juice pH to mutagenicity (○), bacteria (●).

Results

Of the twenty six samples of gastric juice tested at both pH 4 and pH 7 no significant difference in mutagenicity was found in the number of colonies (Table 3) (mean 146 and 142 colonies respectively). Testing was not possible at pH 1 because the organism was consistently killed.

The results of mutagenicity testing on both fresh and frozen gastric juice are provided in Table 4. In this case the ratios of the colony count produced by the gastric juice specimen to the saline control are shown. The ratio to the saline control was 1.48 fresh and 1.40 frozen. The remainder of the Ames tests in this article were performed on frozen gastric juice.

While the results of the gastric juice histidine were variable (Fig. 2) no particular pattern was seen. Increasing concentrations of histidine in the sample produced a rise in mutagenicity represented by the dotted line in Figure 2, but in no case were the concentrations of histidine detected high enough to account for the mutagenicity results. Indeed, while in comparison with the non-mutagenic samples (shown as open circles in Fig. 2) histidine levels in highly mutagenic juices tended to be a little higher eight of the nine juices with a mutagenicity ratio

Table 1 Mutagenicity results in diagnostic groups

	Control	Duodenal ulcer	Gastric ulcer	Carcinoma	H_2 antagonist	
					1–3 h	12 h
Patients	16	45	22	21	31	18
Mean mutagenicity ratio to saline	1.16	1.28	2.51	3.86	2.74	1.2
SE*	0.07	0.05	0.28	0.6	0.25	0.05

* For guidance only, not used in statistical analysis.

Table 2 Relationship of gastric juice mutagenicity and bacterial flora to pH

	pH						
	1	2	3	4	5	6	7
n	102	22	12	17	11	12	43
Mean mutagenicity ratio to saline	1.24	1.52	1.70	2.56	3.24	2.9	3.57
SE*	0.03	0.13	0.15	0.31	0.85	0.42	0.34
Total bacteria/ml (median)	0	600	1025	3×10^5	9×10^6	5.7×10^7	1.3×10^8

* For guidance only, not used in statistical analysis.

over 2 (shown as closed circles in Fig. 2) contained less than $150 \mu\text{m/l}$, which itself would only account for a mutagenicity ratio of around 1.1. The pH of the gastric juice samples tested for mutagenicity and histidine concentration varied between 1 and 7 (mean 2.8) and 12 samples had a pH of 4 or greater. Of the nine juices with a ratio of >2 , eight had a gastric juice pH exceeding 4.

Mutagenicity was then assessed in the different groups of patients (Table 1, Fig. 3). For each patient the mean of the three colony counts was compared with the mean saline control, to provide a mutagenicity ratio. Each result is plotted in Fig. 3. There was considerable variation in these ratios for individual patients within a group but some significant differences between groups are evident. There is no difference between the 45 duodenal ulcer patients (saline ratio 1.28) and 16 asymptomatic controls (ratio 1.16). The levels of mutagenicity seen in these groups was only slightly higher than for the saline controls.

Considering patients taking the H_2 antagonists (either cimetidine 400 mg or ranitidine 150 mg between which we have noticed no difference), we found that in patients within one to three hours of drug ingestion the mutagen ratio to saline control was raised at 2.74 (Table 1, Fig. 3). These results are significantly more mutagenic than the duodenal ulcer and control groups ($p < 0.002$). In contrast, 12 hours after drug ingestion the saline ratio was 1.2, which is not different from the controls.

The 22 patients with gastric ulcer were found to

have a mean mutagenicity ratio to saline control of 2.51 (Table 1, Fig. 3). These ratios are significantly higher than those of controls ($p < 0.0002$).

We tested 21 patients with carcinoma of the stomach, the mutagenicity ratios are the highest of any group, 3.86 to saline control (Table 1, Fig. 3). These ratios are significantly higher than for controls ($p < 0.002$).

We have studied gastric juice mutagenicity in 42 patients after peptic ulcer surgery. The results for those who have had a partial gastrectomy have been separated from the remainder who have had some form of vagotomy. For the 34 patients in the vagotomy group the mean pH was $2.69 (\pm 0.36 \text{ SE})$, and the saline control ratio $1.77 (\pm 0.14)$. These mutagenicity results are only a little higher than the duodenal ulcer or control groups. The difference in saline control ratio just achieving statistical significance ($p < 0.01$). The 12 patients who underwent gastric resection, however, have a mean saline control ratio of $2.63 (\pm 0.24)$. These results are significantly higher than the vagotomy group ($p < 0.002$) or than the controls ($p < 0.002$). The mean pH of the gastric resection group was $5.6 (\pm 0.46)$.

We next studied the relationship between all our mutagenicity results and pH of the sample (Table 2, Fig. 1). A gradual increase in saline ratio is seen with the increasing alkalinity (Fig. 1). A regression line has been constructed and this is different from a horizontal line (which would indicate no relationship between mutagenicity and pH) for saline ratio

Table 3 Comparison of Ames test results at pH 4 and 7

	pH	
	4	7
n	26	26
Mean colony count	146.4	142.5
SE	6.2	8.8

Table 4 Comparison of Ames test results in fresh and frozen gastric juice samples

	Ratio to control	
	Fresh gastric juice:saline	Frozen gastric juice:saline
n	23	23
Ratio	1.48	1.40
SE	0.12	0.1

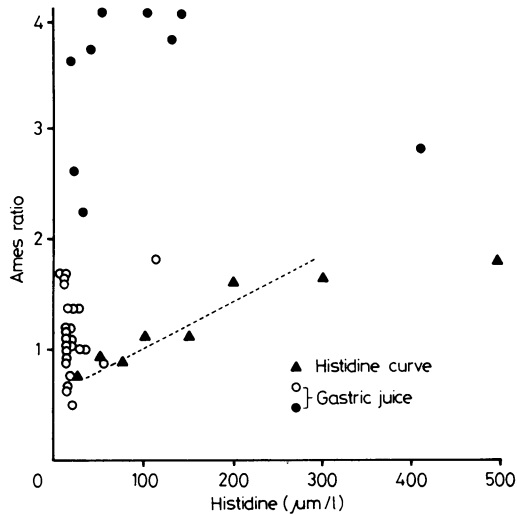


Fig. 2 Relationship of mutagenicity to histidine concentration.

($p < 0.01$). Median total bacterial count has also been included in Figure 1 at each pH to allow comparison with mutagenicity results.

We found no significant differences between different diagnostic groups at a given pH.

Discussion

We have shown significant increases in mutagenicity

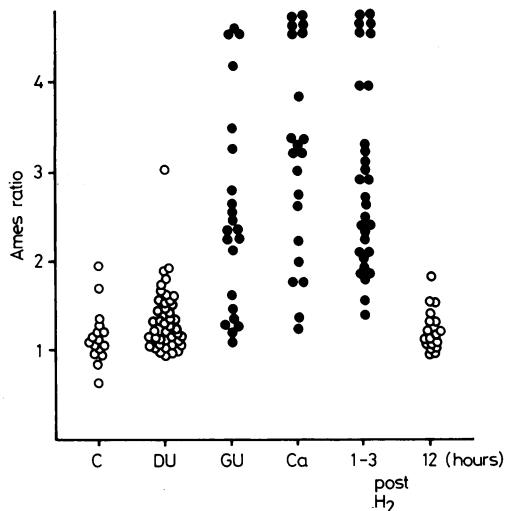


Fig. 3 Mutagenicity.

in the gastric juice of patients with gastric ulcer and after peptic ulcer surgery, patients with gastric ulcer, and those who have undergone gastric resection have an increased risk of developing gastric carcinoma and so this test may indeed be measuring carcinogens in gastric juice. The finding of extremely high levels of mutagenicity in carcinoma patients may not be important because the gastric juice at the time of presentation with malignancy may bear no relationship to that of the initiation of the tumour.

The interpretation of studies relying on single samples of gastric juice are fraught with some hazard, and indeed large variations within patient groups have been seen, but the difference between groups (Fig. 3) are relatively large. The measurement of mutagenicity in 24 hour studies would, however, be interesting. The histidine concentrations measured in gastric juice do not explain the rises in mutagenicity.

While it was shown that one to three hours after ingestion of H_2 antagonists a significant rise in mutagenicity is present, there was no evidence of this in the group tested 12 hours after ingestion. By comparison the permanent hypochlorhydria produced by gastric resections is more worrying and even vagotomy appears to produce a statistically significant though small rise. Exactly what is being measured in gastric juice by the Ames test is not clear, but the close correlation with pH and bacterial flora would be compatible with a bacterial metabolite.

The number of organisms present which are capable of reducing nitrate was found to follow the total bacterial count closely. It is interesting to note that at pH 4 and above where high mutagenic ratios were present, it is at this same pH range where the total bacterial count is high and significant numbers of nitrate reducing organisms are present.

The relationship with pH does explain the significant differences between diagnostic groups, and no differences were seen between different groups at a given pH. It is stressed, however, that all these gastric juice samples were buffered to the same pH of 7 and so this cannot merely be a 'killing' effect by acid on the test bacteria. Further evidence that this is not a 'killing' effect by low pH is that we have found gastric juice samples of pH 4 and above are considerably more mutagenic than the saline control.

While it is not established that carcinogenicity equates with mutagenicity in gastric juice, we can now perhaps regard mutagenicity as an index of various noxious gastric juice factors and this index may be of use in the study of the aetiology of gastric carcinoma.

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