# Gastro-Intestinal Flora and Diarrhoea After Vagotomy*

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## SUMMARY

**Twenty patients, 7 of whom had diarrhoea after vagotomy and drainage, were investigated by culture of gastric and jejunal aspirates and measurements of faecal fat, free bile acids in jejunal fluid, urinary indican excretion, serum folate and serum vitamin $B_{12}$. The haematological findings were compared with those in 20 patients with duodenal ulcer who had not undergone surgery and in 25 healthy controls.**

**The pattern of small bowel flora was normal after vagotomy; this was consistent with the normal results of the urinary indican estimations. There was no relationship between diarrhoea and the bacteriological results. Steatorrhoea occurred in 6 of the 7 patients with diarrhoea and in only 1 of 13 without diarrhoea, suggesting a relationship between diarrhoea and faecal fat excretion after vagotomy and drainage.**

**The mean serum folate and vitamin $B_{12}$ levels of the patients after vagotomy were significantly lower than those in healthy controls. Some of the folate levels in the duodenal ulcer controls were subnormal.**

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Bowel frequency increases significantly after vagotomy and a drainage procedure for duodenal ulcer in the majority of patients. Most patients regard the change as welcome, if unexpected, relief of the state of 'constipation' which they believed they suffered before operation. However, a few patients after vagotomy develop a bowel habit which causes real distress and can be properly described as diarrhoea—in some this consists of many bowel movements daily, often associated with urgency, and in others there are frequent episodes of severe diarrhoea which are clinically similar to acute enteritis.

The cause of these changes remains unknown and we report the results of our investigation of the possibility that alterations in alimentary flora might be responsible. We made a series of measurements in selected patients after vagotomy, who were divided into two groups on the basis of their bowel habits. In some there had been only a minimal change after operation; others had shown a marked change and complained of diarrhoea. In comparing the results from these two groups, we are conscious of the imprecise nature of the term 'diarrhoea', which is a clinical complaint and cannot be quantified. Our hope was to find a factor which could be directly associated with the complaint and thereby put its treatment on a rational basis.

As well as the counts of gastric and jejunal bacteria, measurements of faecal fat excretion and bile acids were made to see if they could be related to bowel habit. In addition, serum vitamin $B_{12}$, serum folate and vitamin $B_{12}$ absorption were measured to see whether the flora had effects similar to those reported after partial gastrectomy.[1-3]

## PATIENTS AND INVESTIGATIONS

### Patients

Twenty volunteers from our Gastric Clinic participated in this investigation. There were 17 men and 3 women (mean age 48 years; range 33 - 70 years). All had undergone truncal vagotomy with a drainage procedure for duodenal ulcer at least 6 months to 4 years previously. The drainage procedure was a Heineke-Mikulicz pyloroplasty in 14 cases and a retrocolic gastrojejunostomy in the remainder. In all patients, the vagotomy was judged to be complete on the basis of the insulin test, interpreted according to the criteria of Hollander.[4]

The results of the pre- and postoperative gastric acid secretion tests were available in most patients. The stimulant was pentagastrin injected intramuscularly in a dose of 6 $\mu$g/kg body weight.[5]

In each patient a careful history was taken with special emphasis on bowel habit.

For the haematological comparisons blood samples were obtained from 25 healthy young adult volunteers and 20 unoperated patients with a radiologically proven duodenal ulcer.

### Investigations

The following investigations were carried out in each patient.

**Culture of gastric and jejunal aspirates:** A sterile Miller-Abbot tube was passed via the mouth into the stomach of the fasting patient. The resting gastric aspirate was discarded and a further sample was then taken for bacterial culture.

Under radiographic control, the Miller-Abbot tube was then guided through the outlet of the stomach so that the tip of the tube lay just distal to the duodeno-jejunal flexure in patients with pyloroplasty and approximately 30 cm

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distal to the anastomosis in those with gastrojejunostomy. The initial aspirate was discarded and a fasting specimen of jejunal juice taken for culture. A standard meal was then taken orally. One hour later, after discarding the initial aspirate, a further specimen of jejunal juice (post-cibal) was collected for culture. Aliquots of the fasting and post-cibal jejunal aspirates were deep-frozen immediately to −40°C for subsequent thin-layer chromatography of the bile acids.

Serial dilutions of the aspirates were plated within 30 minutes of collection and cultured aerobically and anaerobically according to the methods described by Gorbach *et al.*[6] Samples were kept at −4°C until they were plated.

The organisms cultured were identified by standard bacteriological methods and the numbers of organisms per ml of aspirate were recorded.

**Bile acids:** Twenty ml of freshly thawed jejunal aspirates were subjected to thin-layer chromatography and the bile acids were determined by fluorimetry.

**Faecal fat estimation:** A 4-day stool collection was analysed for fat content according to the method of Van de Kamer *et al.*[7] Each patient took a diet containing 75 - 100 g fat during the 4 days preceding and throughout the period of stool collection.

**Urinary indican estimation:** Urinary indican excretion was measured in a 24-hour urine collection by the method of Curzon and Walsh.[8]

## Serum Vitamin $B_{12}$ and Folate Estimations

In each patient (vagotomy and drainage cases, healthy volunteers and unoperated patients with proved duodenal ulcer), blood samples were obtained for haemoglobin, serum vitamin $B_{12}$ and folate estimations. Haemoglobin determinations were by standard methods.[9] Serum $B_{12}$ levels were measured by the microbiological assay described by Anderson[10] using *Euglena gracilis* as test organism. Serum folate levels were estimated by the microbiological assay using *Lactobacillus casei* as described by Waters and Mollin.[11]

In some patients, vitamin $B_{12}$ absorption studies were performed, using the urinary excretion method of Schilling.[12] An oral dose of 1 $\mu$g $^{57}$Co-labelled radioactive $B_{12}$ was given together with 40 $\mu$g of hog intrinsic factor (Radiochemical Centre, Amersham).

# RESULTS

## Bowel Habit

Some alteration in bowel habit occurred in all 20 patients (Table I). In 13 it was slight and consisted either of relief of pre-operative constipation or in a slightly increased frequency of defaecation. Two of these patients had urgency of very mild degree. Diarrhoea with marked urgency was a feature in 7 patients; it was continuous in 2 and episodic in 5.

## Acid Secretion

There was a significant reduction ($P<0.01$) in the basal and maximal acid output following vagotomy (Table II). However, the percentage reductions in mean basal and

**TABLE I. MAIN FEATURES OF BOWEL HABIT IN 20 PATIENTS AFTER TRUNCAL VAGOTOMY WITH DRAINAGE**

| Drainage procedure | No. of patients | Increased bowel frequency without diarrhoea | Increased bowel frequency with diarrhoea and urgency | | |
|---|---|---|---|---|---|
| | | No. | No. with urgency | Continuous (stools>5/day) | Episodic diarrhoea |
| Pyloroplasty ... ... ... ... | 14 | 9 | 2 | 1 | 4 |
| Gastrojejunostomy ... ... ... | 6 | 4 | 0 | 1 | 1 |
| Total ... ... ... ... ... | 20 | 13 | 2 | 2 | 5 |

**TABLE II. ACID SECRETION, FAECAL FAT AND URINARY INDICAN RESULTS (MEANS + SD)**

| | | All patients | Without diarrhoea | With diarrhoea |
|---|---|---|---|---|
| Basal acid (mEq/h) | before vagotomy | 5·9 ± 4·1 | 5·6 ± 3·9 | 6·4 ± 4·8 |
| | after vagotomy | 1·4 ± 1·4 | 1·0 ± 0·9 | 2·0 ± 1·9 |
| | % reduction | 76·3% | 81·7% | 68·3% |
| Peak acid (mEq/h) | before vagotomy | 43·0 ± 16·7 | 44·1 ± 16·5 | 41·0 ± 18·1 |
| | after vagotomy | 12·6 ± 8·7 | 13·9 ± 9·5 | 10·2 ± 4·8 |
| | % reduction | 70·7% | 68·5% | 75·1% |
| Faecal fat (g/24 hours) | | 6·2 ± 2·6 | 4·2 ± 2·7 | 8·5 ± 2·6* |
| Urinary indican (mg/24 hours) | | 60·7 ± 45·1 | 58·6 ± 58·5 | 64·7 ± 35·1 |

* Significantly higher ($P<0.01$) than the faecal fat excretion in patients without diarrhoea. There are no other statistically significant differences between patients with and without diarrhoea.

maximal acid outputs in patients with diarrhoea were not significantly different ($P<0.05$) from those in patients without diarrhoea.

## Faecal Fat

The mean faecal fat excretion (Table II) in patients without diarrhoea (4·2 g/day) was significantly lower ($P<0.01$) than the corresponding value in those with diarrhoea (8·5 g/day). One patient in the former group had a value of 8·5 g/day, but the remainder were all within the normal range (6 g/day). In only one patient with diarrhoea was faecal fat excretion within the normal range.

## Urinary Indican

The mean values (Table II) in the patients with and without diarrhoea did not differ significantly from each other ($P<0.05$) and were both within the normal range of 23 - 92 mg/day.

One patient without diarrhoea had a urinary indican value of 240 mg. This may have been due to the presence of *E. coli* in his jejunum ($9 \times 10^7$), and he also had a high dietary intake of protein which may have contributed to his indicanuria. He was clinically well. Two patients with diarrhoea had an indican excretion above the upper limit of normal.

## Microflora of Stomach and Small Intestine

Table III is a summary of the type and range of concentration of organisms cultured and the number of sterile cultures obtained from both stomach and jejunum of all 20 patients. The number of sterile aspirates from patients with diarrhoea appear to be less than those obtained from patients without diarrhoea. However, the numbers are too small to give statistical confirmation of this observation.

Table IV shows the counts of all organisms ($\log_{10}$) cultured from the gastric and jejunal aspirates in patients without and with diarrhoea. The mean counts in the two groups of patients were not significantly different.

The type of drainage procedure, pyloroplasty or gastrojejunostomy, did not influence the bacteriological findings. Post-cibal jejunal cultures did not reveal occult organisms, as judged by those found in the fasting jejunal cultures.

**TABLE III. ALIMENTARY FLORA CULTURED**

| Micro-organisms | | Vagotomy and pyloroplasty | | | | Vagotomy and gastrojejunostomy | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No diarrhoea (9) | | Diarrhoea (5) | | No diarrhoea (4) | | Diarrhoea (2) | |
| Probable origin | Species | No. neg. cultures | Highest count | No. neg. cultures | Highest count | No. neg. cultures | Highest count | No. neg. cultures | Highest count |
| | **Strep. viridans** | 2 | $2 \times 10^8$ | 0 | $1 \times 10^7$ | 0 | $8 \times 10^6$ | 1 | $2 \times 10^6$ |
| | **Staph. albus** | 5 | $3 \times 10^6$ | 2 | $8 \times 10^4$ | 1 | $5 \times 10^4$ | 1 | $1 \times 10^6$ |
| Oropharyngeal | **Staph. aureus** | 7 | $5 \times 10^2$ | 4 | $4 \times 10^4$ | 4 | 0 | 2 | 0 |
| | **Micrococcus** | 9 | 0 | 4 | $1 \times 10^5$ | 4 | 0 | 1 | $1 \times 10^6$ |
| | **Candida sp.** | 4 | $1 \times 10^5$ | 2 | $2 \times 10^4$ | 0 | $8 \times 10^3$ | 0 | $1 \times 10^5$ |
| | **Strep. faecalis** | 6 | $6 \times 10^4$ | 3 | $2 \times 10^5$ | 4 | 0 | 2 | 0 |
| Faecal | **E. coli** | 8 | $1 \times 10^5$ | 4 | $2 \times 10^5$ | 3 | $9 \times 10^7$ | 2 | 0 |
| | **Bacteroides sp.** | 9 | 0 | 4 | $1 \times 10^5$ | 4 | 0 | 2 | 0 |
| | **Lactobacillus sp.** | 0 | $6 \times 10^6$ | 1 | $3 \times 10^5$ | 3 | $2 \times 10^5$ | 0 | $1 \times 10^6$ |

**TABLE IV. COUNTS OF ALL ORGANISMS CULTURED**

| Total counts | Gastric (fasting) | | Jejunal (fasting) | | Jejunal (post-cibal) | |
|---|---|---|---|---|---|---|
| | Without diarrhoea | With diarrhoea | Without diarrhoea | With diarrhoea | Without diarrhoea | With diarrhoea |
| **108 or over** | 1 | 0 | 0 | 0 | 0 | 0 |
| **107** | 1 | 1 | 1 | 0 | 1 | 0 |
| **106** | 4 | 3 | 9 | 5 | 11 | 4 |
| **105** | 0 | 0 | 4 | 1 | 2 | 1 |
| **104** | 1 | 1 | 1 | 0 | 1 | 0 |
| **103** | 2 | 0 | 2 | 1 | 1 | 1 |
| **102** | 0 | 0 | 0 | 0 | 2 | 0 |
| **101** | 0 | 0 | 0 | 0 | 2 | 1 |
| **0** | 11 | 2 | 3 | 0 | 0 | 0 |

### Bile Acids

Quantitative thin-layer chromatography of the jejunal fluid revealed no free bile acids in any of the patients.

### Vitamin $B_{12}$ Absorption

This was tested in 5 patients, 3 with *E. coli* present in the jejunal fluid in counts ranging from $10^4$ - $10^7$ organisms per ml. In all 5, vitamin $B_{12}$ absorption was normal ranging from 9·9 to 17·6%.

### Serum Vitamin-$B_{12}$ Levels

Serum vitamin-$B_{12}$ levels in all patients after vagotomy were within the normal range, ranging from 240 - 624 $\mu\mu$g/ml. However, the mean level of the vagotomy patients (391 ± 124 $\mu\mu$g/ml), was significantly lower than the mean level (473 ± 39 $\mu\mu$g/ml) of the 20 control duodenal ulcer patients ($P<0·05$). The mean serum vitamin-$B_{12}$ level in the patients with diarrhoea after vagotomy was not significantly different from the mean level in those without diarrhoea ($P>0·05$).

### Serum Folate Levels

Serum folate levels in the vagotomy patients ranged from 2·0 to 9·4 m$\mu$g/ml (mean 4·9 ± 2·0 m$\mu$g/ml). Three of the patients had levels below the lower limit of the control range (Fig. 1) and the mean level was significantly less than that of the normal control group (mean 7·8 ± 3·3 ($P<0·01$). There was no relation between jejunal bacterial flora and serum folate level. None of the patients were anaemic and the only haematological abnormality among the 20 patients was the presence of occasional hypersegmented polymorphs in the peripheral blood films of 4 patients who had serum folate levels of 2·0, 2·0, 3·2 and 5·2 m$\mu$g/ml respectively.

The mean serum folate level of the unoperated duodenal ulcer patients was 5·9 ± 3·1 m$\mu$g/ml, which was not significantly different from that of the normal control group ($P>0·05$) or that of the vagotomy patients ($P>0·05$). However, 4 of the 20 unoperated patients had serum folate levels below the lower limit of those of the control group.

## DISCUSSION

This study suggested that bacterial colonization of the stomach and upper small intestine is not associated with diarrhoea after vagotomy and a drainage procedure. Vagotomy, although markedly decreasing acid secretion, was not associated with any gross alteration in the normal pattern of small bowel flora. The type and concentration of organisms found after vagotomy in this study did not differ from the findings of others in normal subjects.[13-16]

The action of bacterial enzymes on tryptophan in the intestinal lumen leads to the production of indoles which are absorbed and excreted in the urine. The normal levels of urinary indican excretion and normal $B_{12}$ absorption with intrinsic factor found in this investigation were consistent with the finding of normal bacterial cultures. In addition, none of the patients had subnormal serum-$B_{12}$ levels as may be found in post-gastrectomy patients with large numbers of faecal bacteria in the jejunum. The relatively low mean serum-$B_{12}$ level among the vagotomy patients compared with the matched unoperated duodenal ulcer patients does suggest that some degree of malabsorption of $B_{12}$ follows vagotomy but absorption tests in the same patient before and after operation are needed to confirm this.

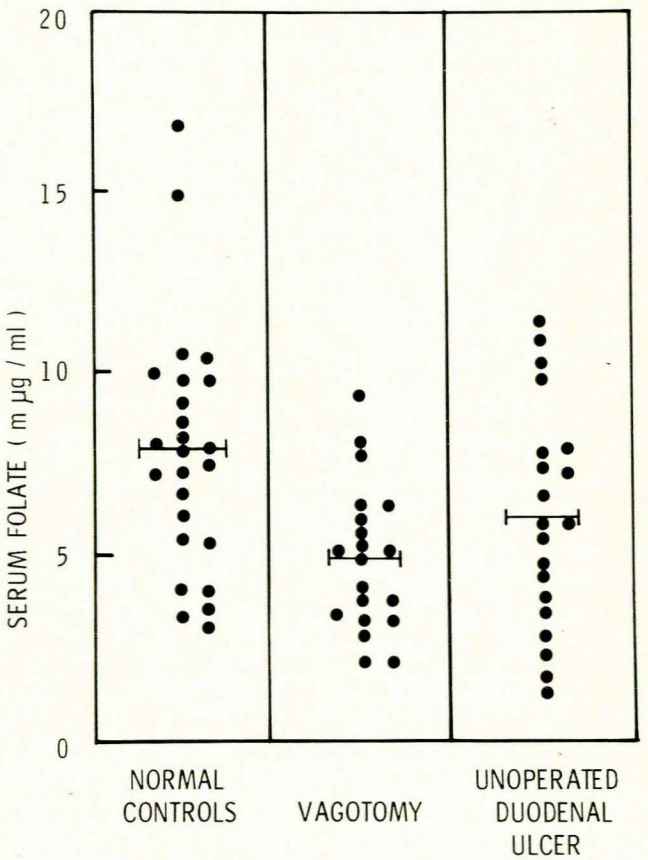


**Fig. 1. Serum folate (m$\mu$g/ml) levels in 25 normal controls, 20 patients after vagotomy and drainage and 20 unoperated duodenal ulcer patients. The horizontal bars represent the mean serum folate levels in these 3 groups.**

Steatorrhoea was a feature in those patients who had diarrhoea but was absent in all but one of those without diarrhoea. Therefore steatorrhoea after vagotomy appeared to be related to diarrhoea rather than to bacterial colonization, but the mechanism of the increase in faecal fat excretion remains obscure.

High serum folate levels have been reported in patients after gastrectomy with large numbers of faecal organisms

(*E. coli* $10^6$/ml or more) in the jejunal fluid.[2] These high levels are probably due to the absorption of folate synthesized by the bacteria. The absence of such high levels in the vagotomy patients studied here is consistent with their normal jejunal bacterial flora.

On the other hand, 3 vagotomy patients had low serum folate levels and the mean serum folate level of the 20 patients was also subnormal. Low serum folate levels in vagotomized patients have previously been reported.[17] In none of the patients here studied was folate deficiency sufficiently severe to cause anaemia or gross morphological changes in the peripheral blood film. Whether the mild deficiency is due to a poor dietary intake of folate or to malabsorption of dietary folate is uncertain. A similar incidence of low levels was found in patients with duodenal ulcers who had not undergone operation, so it may be that the deficiency is more related to the diet of an ulcer patient than to the operation of vagotomy.

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