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Bile reflux in columnar-lined Barrett's oesophagus

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Total and individual bile acid concentrations in the oesophageal aspirates from 30 patients with Barrett's oesophagus were compared with those from 15 patients with oesophagitis and 15 normal subjects. The highest total bile acid concentrations were found in the Barrett's patients and this was statistically significant when compared with controls but not oesophagitis patients. However, when the 95th percentile value of bile acid concentration in the normal subjects was taken as the 'cut-off' level, a significantly higher number of Barrett's patients (15/30) were bile refluxers than were the oesophagitis patients (3/15). Glycocholic and taurocholic acids were the predominant bile acids detected, but taurochenodeoxycholic acid was also present in significant amounts in the patients with oesophagitis. It is possible that bile reflux contributes to the development of Barrett's oesophagus.

Patients with Barrett's or columnar lined oesophagus (CLO) tend to have severe acid reflux, although there does not appear to be a difference between the degrees of acid exposure in patients with complicated and uncomplicated disease *(1)*. However, CLO can develop after total gastrectomy when no acid can be produced *(2)*, and significantly raised levels of intragastric bile have been found in patients with Barrett's oesophagus *(3)*. Despite claims that acid and pepsin are the most important factors responsible for oesophageal damage in gastro-oesophageal reflux, there is sound experimental evidence for the role of bile acids as well, either in an alkaline environment or in combination with acid *(4,5)*. *In vitro* studies on oesophageal biopsy tissue revealed that high concentration of bile salts damaged the oesophageal mucosal cells irrespective of pH, whereas low concentration only caused damage at an acid pH *(4,6)*. Bile salts are also known to increase ionic permeability of oesophageal mucosa and make it prone to damage from refluxate *(7)*. Similarly, in monkeys, it has been shown that bile concentration of gastric juice makes oesophagitis of all grades more likely *(8)*. It is therefore likely that factors other than acid are operating in the pathological process associated with gastro-oesophageal reflux (GOR), and in the study described here the possible role of bile acids is addressed.

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Subjects and methods

The subjects in this study consisted of 30 patients with Barrett's oesophagus, 15 patients with oesophagitis, two patients who had previously undergone gastrectomy, and 15 healthy volunteer controls. All patients with Barrett's oesophagus had circumferential columnar epithelial lining of the lower oesophagus extending at least 5 cm from the gastro-oesophageal junction and the median length of CLO was 7.5 cm (range 5–19 cm). The sex ratio and age range of each group is shown in Table I. After a full explanation of all the investigations involved, each subject gave written consent to participation in the study.

The method of collecting bile from the oesophagus was similar to that described by Gotley *et al. (9)*. In the week

Table I. Sex ratio and age range of Barrett's, oesophagitis and control subjects

	Male	*Female*	*Mean ± SD (years)*	*Median (years)*	*Range (years)*
CLO ($n=30$)	15	15	62.5 ± 10.2	65	35–78
Oesophagitis ($n=15$)	10	5	59.5 ± 7.3	61	30–79
Control ($n=15$)	10	5	20.7 ± 1.3	21	19–23

preceding the study, all medication that may have affected gastrointestinal motility or secretion was stopped, and the subjects fasted for 24 h before investigation. On the day of the study the position of the lower oesophageal sphincter (LOS), was determined in each subject by manometry using the station pull-through technique *(10)* and during the afternoon oesophageal intubation was performed per nares with a paediatric replogle tube so that the end of the tube was 5 cm above the LOS. Oesophageal secretion was collected by continuous aspiration at 40 mmHg and stored as 2-hourly aliquots for analysis.

After a rest period of 45–60 min to allow for the effects of the intubation to subside, the study began at 1600 and oesophageal aspirate collected up to this time was discarded. At 1800 a high fat test meal was eaten and at 2200 the subject retired to bed, being allowed to sleep with their normal arrangement of pillows. The subject was woken at approximately 0700 and from then on sat upright. A high fat breakfast was eaten at 0800 and the study was terminated at 1000.

Each oesophageal aspirate aliquot was separated from mucus by a fine wire mesh funnel, centrifuged at 2500 rpm for 20 min and stored at −20°C. The aliquots were then analysed individually by reversed phase ion paired/ion suppression high-performance liquid chromatography to determine the concentration of six conjugated bile acids, ie glycocholic acid, taurocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, glycochenodeoxycholic acid and taurochenodeoxycholic acid.

Each thawed sample was diluted in 100% HPLC grade methanol (May & Baker Ltd) in relative proportions 1:2 to precipitate out the protein. The sample was centrifuged in an Eppendorf spinner for 2–3 min, and the supernatant filtered through a 0.2 μm filter (Flowpore, Flow Laboratories) or occasionally a 0.45 μm filter (Acro LC3, Gelman Sciences). The HPLC columns contained Micropak SP C18-5 and Asahipak ODP-50 in series, and the mobile phase consisted of 55 ml HPLC grade water, 40 ml HPLC grade acetonitrile and 1 ml 0.4 M tetrabutylammonium phosphate. The mobile phase was degassed in an ultrasonic bath and the system was allowed to stabilise. The condition of the mobile phase (flow rate of 1.0 ml/min, recorder chart speed of 10 mm/min, absorption wavelength of 210 nm and absorption sensitivity between 0.05 and 1.0 absorption units) was maintained throughout the study. Quantification below 10 μM was considered to be trace value.

Of the 500 μM standard bile acid mixture, 50 μl was injected for HPLC analysis and this was repeated at least twice more during analysis of any one subject's samples; 50 μl of each filtered sample was injected to an appropriate absorption sensitivity to detect conjugated bile acids. Each sample run was repeated at least once with sensitivity being altered if peaks were non-quantifiable. Between each sample, or standard and sample 100% HPLC grade methanol was injected and left for the full run time (15 min) to wash the columns. Bile acid peaks were identified by comparison with the standard peaks. For each peak the height and width were measured and sensitivity of run recorded. The possibility that substances in saliva were dissociating at the same peaks as produced by bile acids was entertained but repeated HPLC analysis of saliva proved that such fears were unfounded.

For each subject, the total concentration of conjugated bile acids at each oesophageal aspirate collection was calculated. Total bile acid concentrations (BAC) in the CLO patients were compared with those in oesophagitis patients and in controls. In addition, the total BACs for each study period (fasting, postprandial, supine and upright) was recorded for each group of subjects and the groups compared. Concentrations of individual bile acids were similarly analysed. For comparative purposes, BAC in oesophageal aspirate samples from the two gastrectomy subjects were also analysed.

Statistical analysis

Owing to variable distribution of the data, a non-parametric test, ie Mann–Whitney U test was employed. All the analysis for P values were two-tailed and corrected for ties. To define bile refluxers, the 95th percentile of the control values was taken as a cut-off point, and to compare the number of subjects thus defined as bile refluxers the χ^2 test or Fisher's exact test was applied as appropriate. Correlation analysis was done using Pearson's rank correlation test.

Results

Total bile acids

The median concentration of total bile acid obtained by oesophageal aspiration from the control group was 465.95 μM/l (range 20.5–2300.2 μM/l). There was a significantly higher level in the CLO subjects (median 1351.7 μM/l, range 0–14011.5 μM/l) compared with the controls, but there was no demonstrable significant difference between the oesophagitis patients (median 817.3 μM/l, range 123.8–5721.14 μM/l) and the CLO patients or the controls (Fig. 1). However, when the 95th percentile of the control group (1105.8 μM/l) was taken as the upper limit of normal, only three out of the 15

(20%) oesophagitis patients had abnormally high bile acid concentrations, whereas 15 out of the 30 (50%) CLO patients fell into this category. This constitutes a significant difference (Fisher's exact test $P<0.05$) (Table II). The two patients who had previously undergone partial gastrectomy had total bile acids concentration of 14655 μM/l and 18620 μM/l, which were higher than any of the other subjects in the study.

Bile acids in preprandial oesophageal aspirate

The median total bile acid concentration in the preprandial oesophageal aspirates of the control subjects was 124.5 μM/l (range 0–440.4 μM/l) and the 95th percentile value was 365.4 μM/l. There were no significant differences between the CLO subjects (median 115.8 μM/l, range 0–1556.8 μM/l), the oesophagitis patients (median 124.9 μM/l, range 0–1531 μM/l) and the controls. Seven patients in the CLO group (23.3%) had abnormally high concentration of bile acid, whereas this was the case in only one of the oesophagitis group (6.7%). This did not, however, reach statistical significance by Fisher's exact test (Table II).

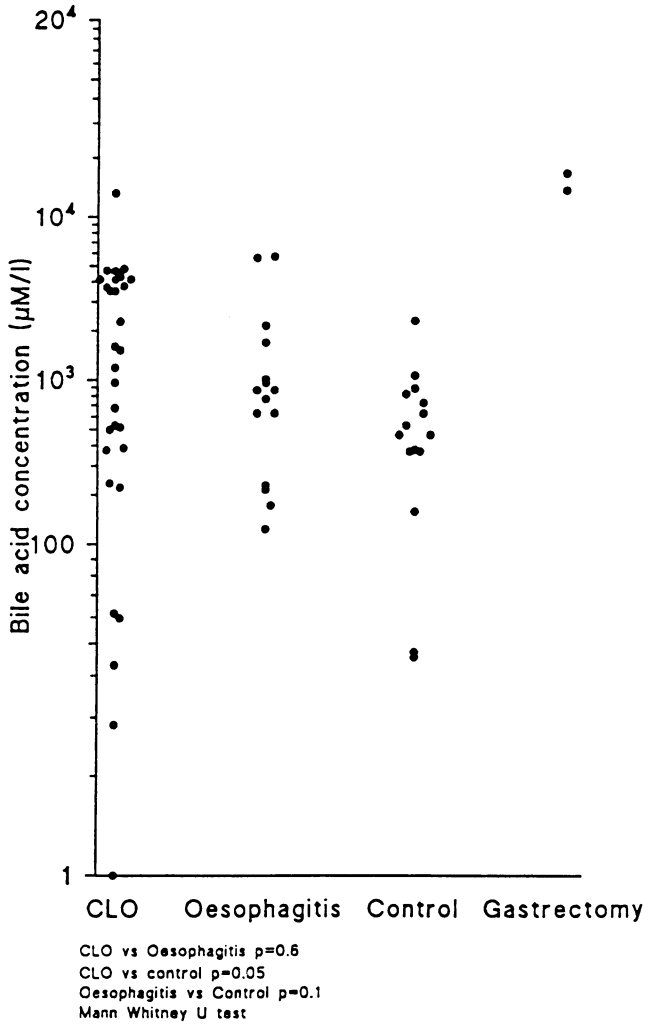

Figure 1. Comparison of total bile acid concentration in the oesophageal refluxate of CLO, oesophagitis and control subjects.

Bile acids in postprandial oesophageal aspirate

The median total bile acid concentration in the postprandial period in the control group was 57.1 μM/l (range 0–628.9 μM/l) and the 95th percentile was 348.5 μM/l. The median total bile acid concentration in the CLO patients during this period was 161.5 μM/l (range 0–7554 μM/l) which was significantly higher compared with the normal controls; the oesophagitis group (median 75.4 μM/l, range 0–961.96 μM/l) did not differ statistically from either CLO or control subjects. Ten patients with CLO (33.3%) had abnormally high levels of bile acid compared with 4 (27%) of the oesophagitis patients, but, again, this was not statistically significant.

Bile acids in oesophageal aspirate in upright posture

The median total bile acid concentration in the oesophageal aspirate of normal controls in the upright posture was 170.5 μM/l (range 9.1–753.3 μM/l) and the 95th percentile was 697.2 μM/l. The median total bile acid concentration in the CLO group in the upright posture was 508.5 μM/l (range 0–7654.2 μM/l) and statistically this was significantly higher than the control group. Again the oesophagitis group (median 262.3 μM/l, range 0–2493.1 μM/l) did not differ statistically from either the CLO or control groups. Ten patients in the CLO group (33.3%) had abnormally high levels of bile acid compared with one of the oesophagitis group (6.7%), and this was significant by Fisher's exact test (Table II).

Bile acids in oesophageal aspirate in supine posture

The median bile acid concentration in the control group was 309.3 μM/l (range 10.6–1612.9 μM/l) and the 95th percentile concentration was 1008.3 μM/l. The median concentration of bile acids in the CLO group was 804.5 μM/l (range 0–6357.4 μM/l) and was significantly higher than the controls, but the oesophagitis group (median 249.9 μM/l, range 0–5721.14 μM/l) was not statistically different to the CLO and control groups. Thirteen patients with CLO (43.3%) had abnormally high amount of bile acid compared with three patients in the oesophagitis group (20%) but this was not statistically significant.

Concentration of individual bile acids in the oesophageal aspirate

When individual bile acids were evaluated, glycocholic and taurocholic acids were found to be predominant. Glycocholic acid was present in a significantly higher concentration in the postprandial study period in the CLO patients (median 142.8 μM/l, range 0–1535.8 μM/l) compared with the control subjects (median 35.4 μM/l, range 0–628.8 μM/l). However, the CLO, oesophagitis and control groups had comparable concentrations of glycocholic acid in all other study periods.

Oesophagitis patients had a significantly higher concentration of taurocholic acid (median 3.8 μM/l, range

Table II. Bile acid concentration

Subject group	Study period	Median BAC (μM)	Range (μM)	Number of bile refluxers*(%)
CLO	18 h	1351.7	0–14011	15/30 (50)
	Fasting	115.8	0– 1556.8	7/30 (23.3)
	Postprandial	161.5	0– 7554	10/30 (33.3)
	Upright	508.5	0– 7654.2	10/30 (33.3)
	Supine	804.5	0– 6357.4	13/30 (43.3)
Oesophagitis	18 h	817.3	123.8– 5721.4	3/15 (20)
	Fasting	124.9	0– 1531.1	1/15 (6.7)
	Postprandial	75.4	0– 961.9	4/15 (27)
	Upright	262.3	0– 2493.0	1/15 (6.7)
	Supine	249.9	0– 5721.1	3/15 (20)
Control	18 h	465.95	20.5– 2300.2	
	Fasting	124.5	0– 440.4	
	Postprandial	57.1	0– 628.8	
	Upright	170.5	9.1– 753.3	
	Supine	309.3	10.6– 1612.9	
Gastrectomy	18 h	16638	14655.4–18620.7	2/2 (100)
	Fasting	241.4	241.1– 241.8	0/2 (0)
	Postprandial	7663.0	7662.9– 7663.2	2/2 (100)
	Upright	7904.5	7904.2– 7904.8	2/2 (100)
	Supine	8733.5	6750.6–10716.4	

* Those with BAC above the 95th percentile

0–92.3 μM/l) in the postprandial study period compared with the control subjects (median 0 μM/l, range 0–24.9 μM/l). Otherwise the concentration of taurocholic acid was comparable between the three groups in all other study periods.

Only traces of glycochenodeoxycholic, taurodeoxycholic and glycodeoxycholic acids were present in the CLO, oesophagitis and control groups and the concentrations were comparable between the three groups in all study periods. However, taurochenodeoxycholic acid was present in a significantly higher overall concentration in the oesophagitis group (median 0.12 μM/l, range 0–222.7 μM/l) compared with the CLO (median 0.0 μM/l, range 0–9.14 μM/l) and control groups (median 0.0 μM/l, 0–0.32 μM/l) (Fig. 2) and significantly more patients in the oesophagitis group (53%) had a higher concentration of taurochenodeoxycholic acid than the 95th percentile level of the controls compared with the CLO group (10%) ($P = 0.05$ Fisher's exact test). In the gastrectomised subjects, all six bile acids were present in higher concentrations compared with the other three groups.

Discussion

Duodenogastric reflux is a normal physiological phenomenon *(11,12)* but increased concentration of bile salts and bile acids have been found in the gastric contents of patients with reflux oesophagitis *(9,13,14)* and CLO patients *(3)*. However, quantitative assessment of individual bile acids in the gastro-oesophageal refluxate of the CLO patients has not been performed previously, and in this study HPLC analysis has been undertaken to more fully evaluate the nature of biliary gastro-oesophageal reflux.

BAC were found in 14 of the 15 normal control subjects but, of these, two had only traces of detectable bile acids. Thus, overall, 12 normal subjects refluxed bile into their oesophagus, which is in concordance with Mittal *et al.*'s *(15)* finding of detectable bile acids (range 300–4250 μM) in 10/13 healthy asymptomatic individuals. However, Gotley *et al. (9)* demonstrated bile acids in the oesophageal aspirates in only 2/10 normal controls and concluded that in Mittal's study the high levels of detectable bile acids were probably due to the use of a non-specific enzymatic method of bile acid assay which was picking up steroids from the test meal. This conclusion, however, is not supported by the present study, in which HPLC was utilised.

The 95th percentile level of BAC in our control group was 1105.8 μM, but although this is considerably higher than that described by Gotley *et al. (9)*, a significantly higher concentration of total BAC was nevertheless present in the oesophageal aspirate of the CLO group (median 1351.7 μM) compared with the control group (465.95 μM). In addition, using the 95th percentile, a significantly higher number of CLO patients (15/30) were classified as bile refluxers when compared with the oesophagitis patients. Although no difference in BAC concentration was found in the fasting period, in the

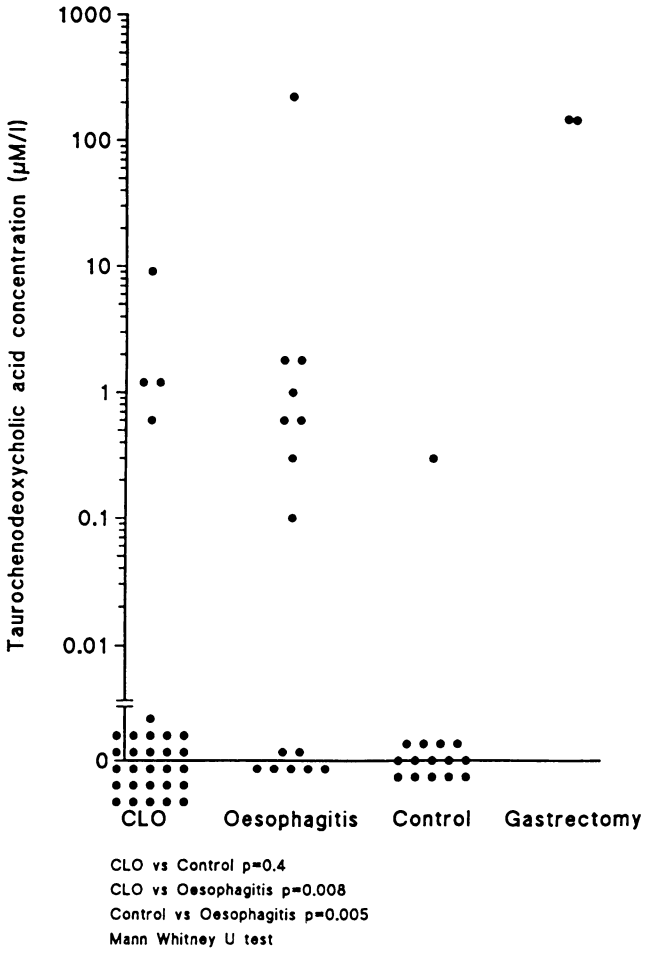

Figure 2. Comparison of taurochenodeoxycholic acid concentration in the oesophageal refluxate of CLO, oesophagitis and control subjects.

postprandial period CLO patients had higher levels of BAC detectable in their oesophageal aspirate than controls; a finding similar to Gillen *et al.* *(3)* who detected raised concentrations of BAC in the gastric aspirates from CLO patients after meals. One of the problems with clinical studies of gastro-oesophageal reflux is the age discrepancy between the controls and the patients, and the present study is no exception. However, there are ethical and practical difficulties associated with oesophageal intubation in asymptomatic elderly subjects, and previous studies have used controls in a lower age range than the study patients *(3,15)*. There is general agreement that having younger individuals in the control group, although not ideal, is acceptable, as gastro-oesophageal reflux is not increased in an ageing population.

CLO subjects had higher levels of BAC in the oesophageal aspirates in the upright and supine study periods compared with controls, but no such difference was observed when compared with oesophagitis patients. However, there was a significantly higher number of subjects classified as bile refluxers among the CLO patients when compared with the oesophagitis patients in the upright study period, suggesting that the increased BAC detected in the oesophageal aspirates of CLO subjects reflects the degree of reflux rather than accumulation of small amounts of bile due to poor oesophageal emptying.

In this study, predominantly glycocholic and taurocholic acids were detectable in high quantities and glycocholic acid was present in significantly higher amounts in the oesophageal aspirates of CLO patients compared with control subjects but only in the postprandial period. In contrast, oesophagitis patients had comparable levels of glycocholic acid but higher levels of taurocholic acid compared with controls, again postprandially. It is interesting that the majority of oesophageal aspirate samples from the CLO, oesophagitis and control subjects contained only one or two bile acids, whereas both gastrectomy patients had all six bile acids detectable in their oesophageal aspirate. It would seem likely that some bile acids are degraded in the stomach, and only those which are resistant to the gastric environment appear in the oesophagus.

In the final analysis of this study, it remains to ask if it is possible to identify factors which determine whether or not a reflux patient develops Barrett's epithelium. Although larger numbers of Barrett's patients had high concentrations of bile acids (as defined by the values in the normal controls) when compared with the oesophagitis patients, the considerable overlap between the two groups indicates that bile reflux cannot be the only cause. However, as it is concentration of bile acid rather than total amount which has been measured, this phenomenon cannot merely reflect the severity of reflux in volume terms.

It is possible that a high concentration of bile in the refluxate has a contributory role in the development of the metaplastic Barrett's epithelium, which itself is probably related to the severity of damage caused. This accords with *in vitro* studies which show that bile acids can damage oesophageal mucosa, and that the damage is more profound in an acid environment *(4)*. Thus, it is reasonable to suggest that patients who would otherwise have simple reflux oesophagitis are more likely to develop Barrett's epithelium if there is concomitant significant duodenogastric reflux. Other factors which determine the composition of gastro-oesophageal refluxate almost certainly play a part, however, and there is now evidence that CLO patients have greater pepsin concentrations than oesophagitis patients or normal controls *(16)*.

In this study, it has not been possible to show that CLO is associated with any particular pattern of bile acid in the refluxate when compared with oesophagitis patients. It is true that the oesophagitis patients did have significantly greater levels of taurochenodeoxycholic acid than the CLO patients, but as the levels were so small it is difficult to ascribe any 'preventative' effect to this compound. The proportion of the chenodeoxycholic acid in the duodenal bile has been shown to be high in patients with colorectal neoplasia *(17)*, and it is conceivable that the precise composition of bile in the oesophagus will prove to be important in the development of dysplasia or carcinoma in patients with Barrett's oesophagus.

References

1 Gillen P, Keeling P, Byrne PJ, Hennessy TPJ. Barrett's oesophagus: pH profile. *Br J Surg* 1987; **74**: 774–6.

2 Meyer W, Vollmar F, Bar W. Barrett oesophagus following total gastrectomy. *Endoscopy* 1979; **11**: 121–6.

3 Gillen P, Keeling P, Byrne PJ, Healy M, O'Moore RR, Hennessy TPJ. Implications of duodenogastric reflux in the pathogenesis of Barrett's oesophagus. *Br J Surg* 1988; **75**: 540–43.

4 Hopwood D, Bateson MC, Milne G, Bouchier IAD. Effects of bile acids and hydrogen ion the fine structure of the oesophageal epithelium. *Gut* 1981; **22**: 306–11.

5 Rees W, Rhodes J. Bile reflux in gastro-oesophageal disease. *Clin Gastroenterol* 1977; **6**: 179–200.

6 Lalyre Y, Subach S, Schmidt L, Barrett T, Layden T. Mechanisms of combined bile salt and acid induced esophageal injury. *Gastroenterology* 1984; **86**: 1149.

7 Safaie-Shirazi S, Den Besten L, Zike WL. Effect of bile salts on the ionic permeability of the oesophageal mucosa and their role in the production of oesophagitis. *Gastroenterology* 1975; **68**: 728–33.

8 Gillison EW, De Castro VAM, Nyhus LM, Kusakasi K, Bombeck CT. The significance of bile on reflux oesophagitis. *Surg Gynecol Obstet* 1972; **134**: 419–24.

9 Gotley DC, Morgan AP, Cooper MJ. Bile acid concentrations in the refluxate of patients with reflux oesophagitis. *Br J Surg* 1988; **75**: 587–90.

10 Dodds WJ. Instrumentation and methods for intraluminal esophageal manometry. *Arch Intern Med* 1976; **136**: 515–23.

11 Muller-Lissner SA, Fimmel CJ, Sonnenberg A *et al.* Novel approaches to quantify duodenogastric reflux in healthy volunteers and in patients with type I gastric ulcer. *Gut* 1983; **24**: 510–18.

12 Little AG, Martinez EI, De Meester TR, Blough RM, Skinner DB. Duodenogastric reflux and reflux oesophagitis. *Surgery* 1984; **96**: 447–54.

13 Crumplin MKH, Stol DW, Murphy GM, Collis JL. The pattern of bile salt reflux and acid secretion in sliding hiatal hernia. *Br J Surg* 1974; **61**: 611–16.

14 Kaye MD, Showalter JP. Manometric configuration of the lower esophageal sphincter in normal human subjects. *Gastroenterology* 1971; **61**: 213–23.

15 Mittal RK, Reuben A, Whitney JO, McCallum R. Do bile acid reflux into oesophagus? *Gastroenterology* 1987; **92**: 371–5.

16 Gotley DC, Ball D, Cooper MJ. Peptic activity in the refluxate of patients with uncomplicated gastro-oesophageal reflux. *Br Soc Gastroenterol Autumn Meeting* 1988; **FV 94**: 61–2.

17 Moorhead RJ, Cambell GR, Donaldson JD, McKelvey STD. Relationship between duodenal bile acids and colorectal neoplasia. *Gut* 1987; **28**: 1454–9.

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