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# The effect of healing on bile reflux in gastric ulcer

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SUMMARY Bile reflux has been studied by serial estimations of bile acid concentrations in the stomach before and after a liquid meal. Thirteen normal subjects and 38 patients with gastric ulcer were examined. Thirty-one in the ulcer group had the test repeated once or twice within two months of the initial test to observe any change in reflux associated with healing. Patients with gastric ulcer had significantly higher concentrations of bile acids than normal subjects, both before and after the meal, although there was an overlap between the groups. In patients with an ulcer which healed or showed some healing over two months, no consistent change in reflux was found.

Bile is often present in the stomach of patients with gastric ulcer but this is an uncommon finding in normal subjects (Borg, 1959; Buckler, 1965; du Plessis, 1965; Rhodes, Barnardo, Phillips, Rovelstad, and Hofmann, 1969). Its presence in the stomach may be of no significance, although it has been suggested (du Plessis, 1965; Capper, 1967) that bile acids may be responsible, wholly or in part, for the gastritis which is almost invariably present in gastric ulcer (Konjetzny, 1925; Magnus, 1946; du Plessis, 1960). Reflux of bile into the stomach may precede or develop consequent upon ulceration. If the former were the case one might expect reflux to continue after healing but if reflux occurred because of the presence of the ulcer it should diminish with healing.

To examine these possibilities we have measured bile acid concentrations in the gastric juice of patients with active gastric ulcer and later when the ulcer was healing or healed. These results are compared with a group of normal subjects.

## Materials and Methods

### NORMAL SUBJECTS (GROUP A)

Thirteen normal subjects, seven female and six male, were examined. Each was interviewed and had no symptoms referable to the gastrointestinal tract. A full physical examination was carried out, with a chest radiograph and full blood count. None had associated serious disease. Their ages (26-68 years) were similar to those of the patients with gastric ulcer.

### PATIENTS WITH GASTRIC ULCER (GROUP B)

Thirty-two patients, 15 male and 17 female, aged 19-76 years, were examined. The ulcer, demonstrated radiologically, was on the lesser curve or posterior wall proximal to the angulus. There was no associated duodenal abnormality and the patients were not receiving analgesic therapy. Twenty-five outpatients were described as 'active' because they had had symptoms within the previous two weeks (Table I, nos. 14-38); one patient (no. 39) had had no pain for two weeks and a further patient (no. 40) was shown to have a healing ulcer. Five other patients with active ulcer (nos. 41-45) had been treated in hospital for up to nine days.

### PATIENTS WITH GASTRIC AND ASSOCIATED DUODENAL ULCER OR DEFORMITY (GROUP C)

Six patients (nos. 46-51) were examined. Four were outpatients, one of whom had no symptoms, and two had been in hospital for 10 days.

The bile reflux tests were carried out after the subject had fasted for at least 10 hours. A radio-opaque polyethylene nasogastric tube (OD 2 mm, W. Watson & Co., Barnet, Hertfordshire) with a steel olive on the end was passed when the nasal cavity had been anaesthetized with Xylocaine (Astra Chemicals Ltd., Watford, Hertfordshire). The tip of the tube was positioned radiologically to the left of the vertebral border.

A fasting sample was taken and a standard liquid meal instilled into the stomach. The meal, which was well tolerated, consisted of 22 g of corn oil and 56 g of dextrose made up to 500 ml with warm water. Ten-ml samples were taken immediately after the meal and every 30 minutes for two hours. The position of the tube was then checked radiologically, using Gastrografin (Schering, A.G., Berlin) if the tip had moved to the right of the midline. This

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| *Initial Test* | *Bile Acid Concentration (mM)* | | | | |
|---|---|---|---|---|---|
| | *Subject* | *Fasting* | *1 Hour* | *2 Hours* | *Maximum Postprandial* |
| *Group A: Normal Subjects* | | | | | |
| | 1 | 0 | 0·17 | 0 | 0·17 |
| | 2 | 0 | 0·1 | 0·47 | 0·47 |
| | 3 | 0 | 0 | 0 | 0 |
| | 4 | 0 | 0·24 | 0·12 | 0·27 |
| | 5 | 0·12 | <0·1 | 0 | <0·1 |
| | 6 | 0 | <0·1 | <0·1 | <0·1 |
| | 7 | 0 | 0·37 | 0·43 | 0·48 |
| | 8 | 0 | 0 | <0·1 | <0·1 |
| | 9 | 0 | <0·1 | 0·13 | 0·15 |
| | 10 | 0 | <0·1 | 0·23 | 0·23 |
| | 11 | <0·1 | 0·16 | 0·3 | 0·32 |
| | 12 | 0 | 0·15 | <0·1 | 0·15 |
| | 13 | 0 | 0 | <0·1 | <0·1 |
| *Group B: Gastric Ulcer* | | | | | |
| Outpatients | 14 | 0·74 | 0·1 | — | 0·1 |
| | 15 | 2·1 | 2·0 | — | 2·0 |
| | 16 | 0·9 | 0·98 | 0·52 | 0·98 |
| | 17 | 2·5 | 2·0 | 3·1 | 3·1 |
| | 18 | 0 | <0·1 | <0·1 | 0·1 |
| | 19 | 3·2 | 7·4 | 0·9 | 7·4 |
| | 20 | 1·6 | 0·77 | 0·29 | 0·77 |
| | 21 | 0·28 | 0·2 | 1·5 | 1·5 |
| | 22 | 0 | <0·1 | 0·14 | 0·14 |
| | 23 | 0·2 | 0·55 | — | 0·55 |
| | 24 | 1·0 | 0·26 | 0·28 | 0·4 |
| | 25 | 0·16 | 0·31 | 0·49 | 0·49 |
| | 26 | 1·1 | 0·23 | 0·32 | 0·32 |
| | 27 | 0·4 | 0·32 | 1·4 | 1·4 |
| | 28 | 2·8 | 0·25 | 0 | 0·25 |
| | 29 | 1·6 | 0·18 | 1·14 | 0·18 |
| | 30 | 0·28 | 0·48 | 0·11 | 0·55 |
| | 31 | 0·48 | 0·59 | 1·7 | 1·7 |
| | 32 | 0·71 | 0·51 | 0·46 | 0·64 |
| | 33 | <0·1 | <0·1 | 0·3 | 0·49 |
| | 34 | 0 | <0·1 | 0·14 | 0·14 |
| | 35 | 0·2 | <0·1 | 0·23 | 0·23 |
| | 36 | 0·11 | 0·15 | 0·2 | 0·2 |
| | 37 | 0·12 | 0·11 | 0·12 | 0·21 |
| | 38 | 0 | 0·13 | 0 | 0·13 |
| | 39[1] | 0 | 0·97 | 5·0 | 5·0 |
| | 40[2] | <0·1 | <0·1 | 0·4 | 0·4 |
| Inpatients | 41 | 0 | 0 | 0·21 | 0·21 |
| | 42 | 0·39 | 0·12 | 0·21 | 0·21 |
| | 43 | 0 | 0 | <0·1 | <0·1 |
| | 44 | 2·0 | 2·2 | 1·7 | 2·2 |
| | 45 | 0 | 0 | 0·46 | 0·6 |
| *Group C: Gastric Ulcer with Duodenal Abnormality* | | | | | |
| Outpatients | 46 | 0·43 | 0·55 | 0·40 | 0·55 |
| | 47 | 1·0 | 0·8 | 0·5 | 0·8 |
| | 48 | <0·1 | 1·8 | 4·9 | 4·9 |
| | 49[1] | <0·1 | <0·1 | <0·1 | <0·1 |
| Inpatients | 50 | <0·1 | <0·1 | 0·24 | 0·24 |
| | 51 | 0·92 | 0·34 | 0·34 | 0·34 |

Table I *Bile acid concentrations in the stomach, fasting and after a test meal, in normal subjects and patients with gastric ulcer*

[1]Ulcer not active (no symptoms). [2]Ulcer healing at initial test

occurred on two occasions and on one of these the tube was in the duodenum; the last sample of this test was rejected. During the test subjects either walked about the room or remained seated.

Tests were repeated after one and/or two months in 28 patients from group B and three from group C (Table I) to examine any change associated with healing. Patients who had follow-up tests had the barium meal repeated and were described as 'healed', 'healing', or 'not healing'. The area of a 'healing ulcer' was less than 50% of the original; any smaller reduction or enlargement of the ulcer was described as 'not healing'.

### PREPARATION AND STORAGE OF SAMPLES

The samples were allowed to stand for 30 minutes and corn oil was then aspirated from the surface. Samples were stored at −20°C. Periodically, a batch

of 12 samples was thawed, a 2 ml aliquot removed and freeze-dried in an Edwards SpeediVac centrifugal freeze drier (model 5PS, Edwards High Vacuum Ltd., Crawley, Sussex). This took six hours, after which the sample tubes were sealed with parafilm (Lindsay and Williams Ltd., London) and stored at −20°C for subsequent analysis.

MEASUREMENT OF BILE ACID CONCENTRATION

Bile acid concentrations were measured by the steroid dehydrogenase method of Iwata and Yamasaki (1964) with modifications similar to those of Turnberg and Anthony-Mote (1969). Our method differed from the latter technique in two respects: (1) a larger volume (2 ml) of pyrophosphate buffer was used, and (2) control tests (without enzyme) were used to compensate for the colour and turbidity of samples. From each gastric test, bile acids were measured in at least three samples (fasting, one hour, and two hours). In addition, heavily bile-stained samples which might contain the maximal concentration were examined. The colour of each sample was therefore noted by comparison with a colour chart, graded 0 to 5, which represented increasing concentrations of bile. All bile acid analyses were duplicated.

The freeze-dried samples were re-dissolved in methanol by shaking with a mechanical agitator; they were then centrifuged for five minutes. Twenty to 100 $\mu$l of the supernatant was used for analysis. This volume and also the volume of methanol were varied to achieve a satisfactory concentration for the enzyme reaction.

RELIABILITY OF THE METHOD

The accuracy, precision and sensitivity (Borth, 1952; Loraine and Bell, 1966) of the bile acid analysis were examined. Accuracy was assessed by recovery of cholic acid from two gastric samples. The precision of the whole method was measured at the beginning of the work and when two-thirds of the samples had been analysed. Twenty to 30 replicates of gastric samples were used on each occasion. The precision of the enzyme method was also calculated from the variation between duplicates (Henry, 1964). The sensitivity, which is the smallest result distinguishable from zero (Borth, 1957), was estimated twice when precision was measured.

ANALYSIS OF RESULTS

Comparison between the groups, gastric ulcer and normal, was assessed by the Wilcoxon rank sum test (Wilcoxon, 1945). Results from the same subject at different stages of healing were compared using Student's t test for paired observation.

## Results

METHODS

*Accuracy*

Recovery was between 103% and 106%.

*Precision*

The coefficient of variation for the whole method was 9% and 2·5% on the two occasions. The coefficient of variation between duplicates in the enzyme reaction alone was 4·8%, 3·7%, 3·2%, and 2·2% when assessed on four occasions.

*Sensitivity*

On the two occasions when total precision was measured sensitivity was 0·2 mM and 0·05 mM. We have chosen 0·1 mM as the sensitivity; this is approximately half the concentration in our lowest standard. In statistical analyses values less than 0·1 mM have been taken as 0·05 mM.

COLOUR 'GRADING' OF SAMPLES

The colour grades of samples and the corresponding bile acid concentrations are in Table II. In spite of considerable overlap between the grades, the colour enabled us to identify samples with negligible bile; of 124 grades 0 and 1 samples, 42 contained no bile acid and 45 had a concentration of $<0·1$ mM. However, of 89 grade 3 samples, only 27 had concentra-

| *Grade* | *Appearance of Specimen* | *Bile Acid Concentration (mM)* | | | | |
|---|---|---|---|---|---|---|
| | | *Mean* | *SD* | *Lowest* | *Highest* | *No. of Estimations* |
| 0 | Clear | 0·05 | 0·09 | 0 | 0·4 | 52 |
| 1 | Doubtfully bile stained | 0·11 | 0·12 | 0 | 0·55 | 72 |
| 2 | Minimally bile stained | 0·35 | 0·38 | 0 | 2·2 | 82 |
| 3 | Mildly bile stained | 1·1 | 1·2 | 0·14 | 3·9 | 89 |
| 4 | Moderately bile stained | 4·8 | 5·5 | 0·32 | 26 | 27 |
| 5 | Heavily bile stained | — | — | — | — | 0 |

Table II *Bile acid concentrations of specimens graded according to colour*

tions less than 0·5 mM and only one of grade 4 had a concentration of less than 1 mM.

BILE REFLUX TESTS

The bile acid concentrations from the first study in each subject are set out in Table I.

In Figs. 1 and 2 are the fasting and maximal postprandial concentrations for groups A and B. Group B is divided into active, healing, and healed ulcer. Active ulcer refers to the initial test for all except two patients; one had no symptoms and one a healing ulcer.

*Normal subjects (group A)*

Fasting levels were low (0-0·12 mM) and in 11 of 13 the concentration was zero. After the meal maximal levels were in most cases higher (0-0·48 mM).

*Gastric ulcer (group B)*

In this group both fasting (0-3·2 mM) and maximal postprandial concentrations (<0·1-7·4 mM) were higher than the normal group ($P < 0.001$ fasting; $P < 0.01$ postprandial) but in each category there was some overlap.

The pattern of bile reflux during the test is shown in Fig. 3, where normal subjects are compared with 24 outpatients with active ulcer. There is a marked difference between the groups. A rise in concentration after the meal was seen commonly in both groups; this occurred in 11 of the 13 normal subjects and in 16 of 24 with ulcer.

*Patients with duodenal abnormality and gastric ulcer (group C)*

The concentration of bile acids, pattern of reflux, and influence of healing did not differ from the results in group B.

THE EFFECT OF HEALING ON BILE REFLUX

The range of concentrations in patients with healed or healing ulcer was similar to the active group (Figs. 1 and 2). In the healing group, concentrations of 0-1·6 mM (fasting) and <0·1-3·8 mM (postprandial) were found. The corresponding values for the healed group were 0·3·9 mM (fasting) and <0·1-3·9 mM (postprandial).

The influence of healing on the maximal postprandial concentration of bile acids is in Fig. 4; patients nos. 39 and 40 are excluded. The ulcer healed in 11, was healing in 10, but in five was not healing. Healing did not affect bile reflux in any consistent way. With a healed ulcer, five showed increased reflux, two decreased, while four had little reflux at any stage. In patients with an ulcer which was healing, three had decreased reflux, two increased reflux, one remained the same and five had little reflux on any occasion. Of those who did not heal, reflux decreased in one, increased in one, and remained low in three. Similar results were found with fasting concentrations.

A comparison of results from patients when their ulcer was active and healing showed a slight fall in the mean bile acid concentration with healing. However, this was not significant for the maximal post-

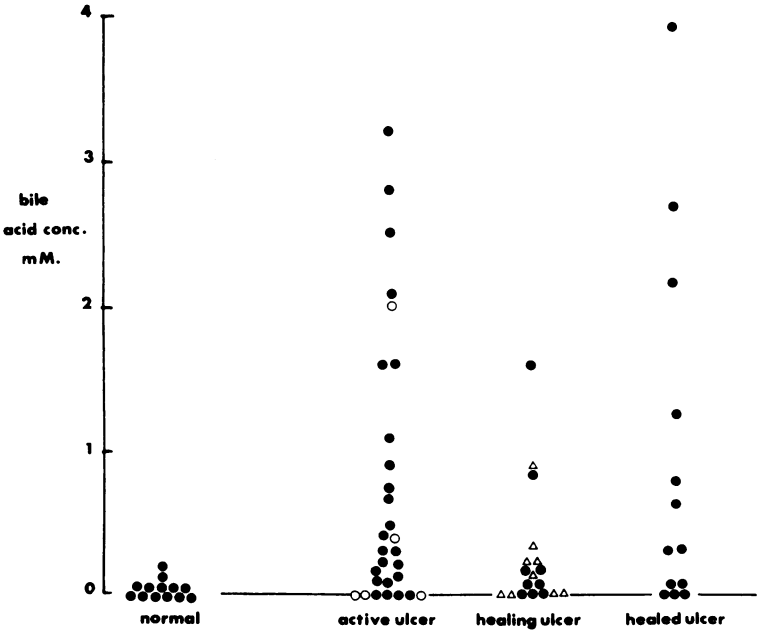


Fig. 1 *Fasting concentrations of bile acids in normal subjects and patients with gastric ulcer when the ulcer was active, healing, and healed.*
*Active ulcer outpatients* ●
*inpatients* ○
*Healing ulcer at 1 month* ●
*2 months* △
*Healed ulcer at 1 or 2 months* ●

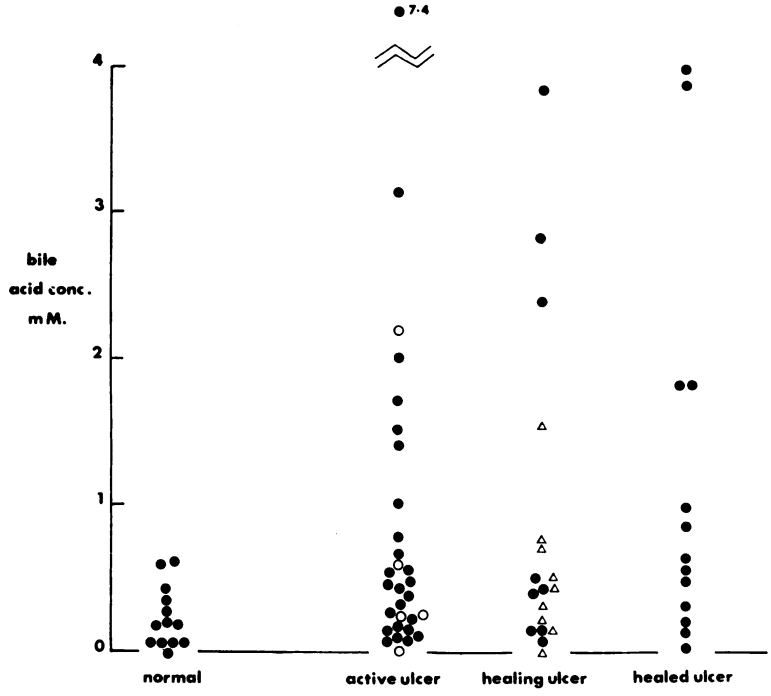


Fig. 2 *Maximal postprandial concentration of bile acids in normal subjects and patients with gastric ulcer. Symbols as in Figure 1.*

Fig. 3 *Pattern of bile reflux. Bile acid concentration in fasting samples and for two hours after a liquid meal. Thirteen normal subjects are compared with 24 outpatients with active gastric ulcer. In two subjects samples were obtained for only one and a half hours.*

Fig. 2

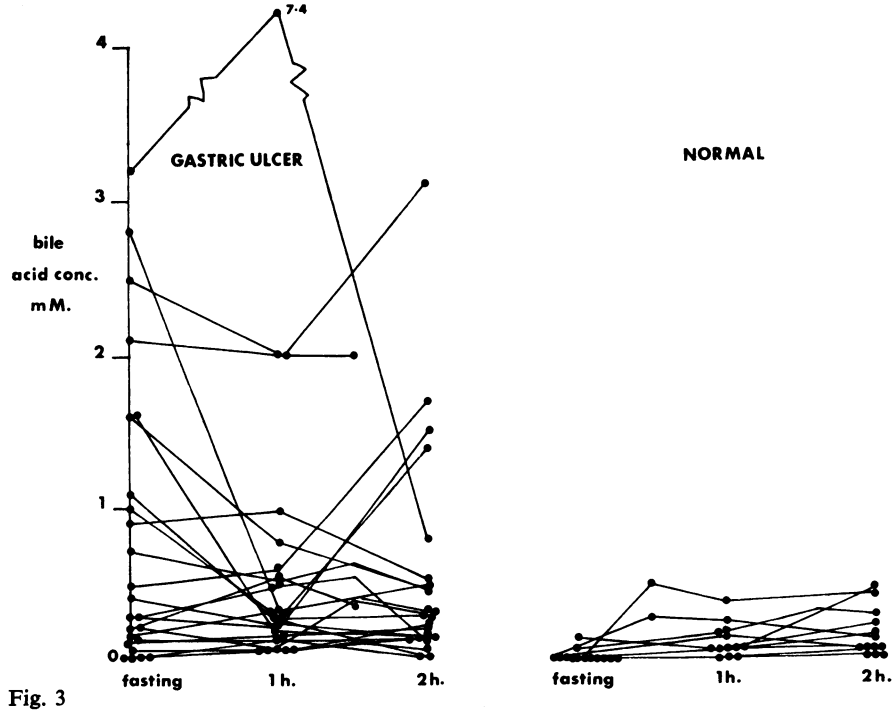


Fig. 3

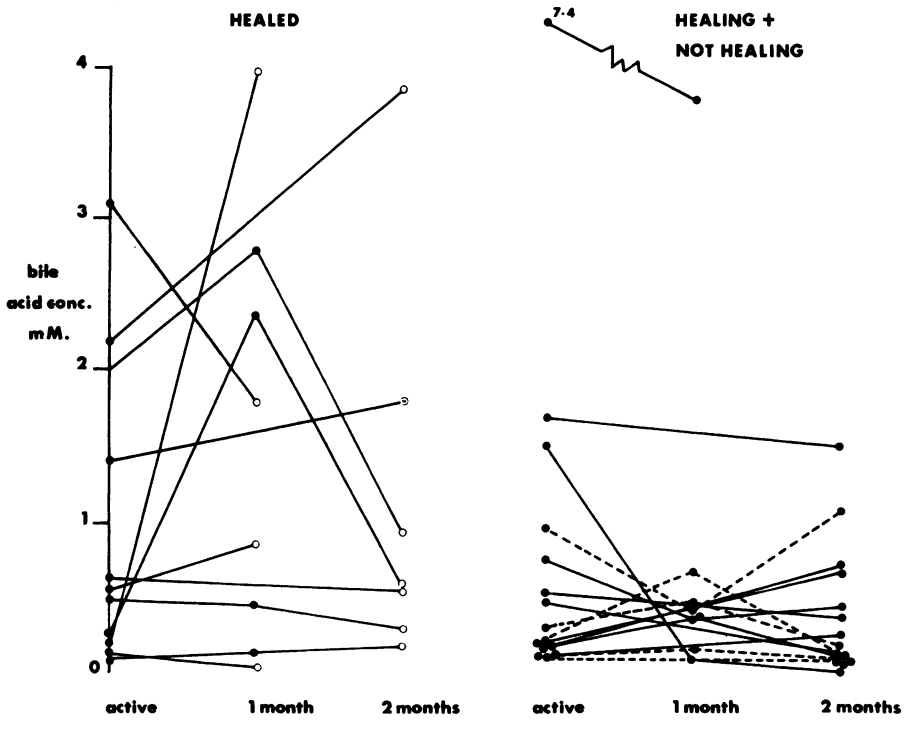


Fig. 4 *Maximal postprandial concentrations of bile acids in 26 patients who had tests repeated at one and/or two months.* *Ulcer present* ● *Ulcer healed* ○ *Ulcer healing* ●——● *Ulcer not healing* ● . . . ●

prandial levels and only marginally significant ($P < 0.05$) for fasting levels. A comparison of results in patients when the ulcer was active and healed showed a slight fall in fasting levels when healed, but a slightly greater rise in maximal postprandial levels. Neither of these differences was significant.

## Discussion

In gastric ulcer the stomach contains bile more often and at higher concentrations than normal. This is true cf fasting, nocturnal, and postprandial samples, as well as after histamine or insulin stimulation (Watkinson, 1951; Borg, 1959; Buckler, 1965; du Plessis, 1965; Rhodes *et al*, 1969).

The highest concentrations recorded are often, though not always, in the postprandial period (Rhodes *et al*, 1969). Although the range of concentration in gastric ulcer is considerable, the overlap with normal subjects is small (du Plessis, 1965; Rhodes *et al*, 1969). Repeated measurements in the same patient show considerable variation (Rhodes *et al*, 1969).

Measurement of bile concentrations in gastric juice has been rather unsatisfactory. The colour has been graded by earlier workers (Borg, 1959; Buckler, 1965), and, although this may be misleading, it is moderately reliable as an approximate measurement (Table II). We have tried unsuccessfully to measure bilirubin concentrations by a modification of the method of Michaëlsson, Nosslin, and Sjölin (1965). Bile acid concentrations have been measured by paper chromatography (du Plessis, 1965) and with radioactive labelling of the bile acid pool (Rhodes *et al*, 1969). We measured bile acid concentrations in a few samples only from each patient and it was therefore unnecessary to label the pool. The problem of extracting bile acids from samples, caused by their variable solubility at different *p*H levels (Dowling and Small, 1968; Hofmann and Small, 1967), was overcome by freeze drying and subsequently redissolving in methanol. Mucus and other protein in the samples, which might cause problems of turbidity in the enzyme reaction, were precipitated with methanol. The method has proved reliable in terms of accuracy and precision.

Our results are similar to those of previous work. Fasting and postprandial bile acid concentrations

were higher in patients with gastric ulcer than in normal subjects. However, in the present study, the overlap between the two groups was less with fasting, rather than postprandial samples, the reverse of previous findings (Rhodes *et al*, 1969). The study was particularly designed to examine whether healing of the ulcer was associated with a change in the reflux of bile. The results show no consistent change associated with healing, which would suggest that the ulcer does not cause the reflux. The slight fall in bile acid concentration associated with partial healing is probably of little significance since there was no corresponding fall in patients whose ulcer healed.

The hypothesis relating bile reflux and gastric ulcer is as follows. Gastric ulcer is associated with a gastritis (Konjetzny, 1925; Magnus, 1946; du Plessis, 1960) which extends proximally from the pylorus. Reflux of duodenal contents into the stomach, associated with a disturbance of antral motility (Garrett, Summerskill, and Code, 1966), is common in gastric ulcer and may persist after healing. Bile acids and some other components of bile break the gastric mucosal barrier (Davenport, 1968; Chapman, Rudick, Dyck, Werther, and Janowitz, 1969; Ivey, Clifton, Hubel, and Debensten, 1969; Davenport, 1970) and allow hydrogen ion to penetrate and damage the mucosa, giving rise to a gastritis (Lawson, 1964). With this background of recurrent bile reflux and diffuse gastritis, a discrete gastric ulcer may occur adjacent to acid-secreting mucosa. The importance of bile reflux in predisposing to, or perpetuating, gastric ulcer in man must be established by further work. However, bile has been shown to cause gastric ulcer in animals (Kirk, 1970). Elucidation of factors which cause the reflux or discovery of drugs which will reverse the motility disturbance may be important steps in the future of this story.

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**References**

Borg, I. (1959). Bile admixture in gastric juice in health and in peptic ulcer before and after operation according to Billroth II and Billroth I. *Acta chir. scand.*, Suppl. 251, 97-112.

Borth, R. (1952). The chromatographic method for the determination of urinary pregnanediol. *Ciba Fdn Colloq. Endocr.*, **2**, pp. 45-57. Churchill, London.

Borth, R. (1957). Steroids in human blood. *Vitam. and Horm.*, **15**, 259-290.

Buckler, K. (1965). Unpublished observations, cited by Capper (1967).

Capper, W. M. (1967). Factors in the pathogenesis of gastric ulcer. *Ann. roy. Coll. Surg. Engl.*, **40**, 21-35.

Chapman, M. L., Rudick, J., Dyck, W. D., Werther, J. L., and Janowitz, H. D. (1969). Electrolyte movements across the gastric mucosa: the effects of bile on the permeability of antrum and fundus. (Abstr.) *J. clin. Invest.*, **48**, 15a.

Davenport, H. W. (1968). Destruction of the gastric mucosal barrier by detergents and urea. *Gastroenterology*, **54**, 175-181.

Davenport, H. W. (1970). The proper study. *Gastroenterology*, **58**, 590-591.

Dowling, R. H., and Small, D. M. (1968). The effect of *p*H on the solubility of varying mixtures of free and conjugated bile salts in solution. *Gastroenterology*, **54**, 1291.

Du Plessis, D. J. (1960). Some aspects of the pathogenesis and surgical management of peptic ulcers. *S. Afr. med. J.*, **34**, 101-108.

Du Plessis, D. J. (1965). Pathogenesis of gastric ulceration. *Lancet*, **1**, 974-978.

Garrett, J. M., Summerskill, W. H. J., and Code, C. F. (1966). Antral motility in patients with gastric ulcer. *Amer. J. dig. Dis.*, **11**, 780-789.

Henry, R. J. (1964). *Clinical Chemistry. Principles and Technics*, p. 124. Harper and Row, New York.

Hofmann, A. F., and Small, D. M. (1967). Detergent properties of bile salts: correlation with physiological function. *Ann. Rev. Med.*, **18**, 333-376.

Ivey, K. J., Clifton, J. A., Hubel, K. A., and Debensten, L. (1969). Effect of bile salts on back diffusion of hydrogen ions in the human gastric mucosa. *J. Lab. clin. Med.*, **74**, 886-887.

Iwata, T., and Yamasaki, K. (1964). Enzymatic determination and thin-layer chromatography of bile acids in blood. *J. Biochem. (Tokyo)*, **56**, 424-431.

Kirk, R. M. (1970). Experimental gastric ulcers in the rat: the separate and combined effects of vagotomy and bile-duct implantation into the stomach. *Brit. J. Surg.*, **57**, 521-524.

Konjetzny, G. E. (1925). Entzündliche Genese des Magen Duodenalgeschwürs. *Arch. Verdau.-Kr.*, **36**, 189-226.

Lawson, H. H. (1964). Effect of duodenal contents on the gastric mucosa under experimental conditions. *Lancet*, **1**, 469-472.

Loraine, J. A., and Bell, E. T. (1966). General principles in hormone assay. In *Hormone Assays and Their Clinical Application*, edited by J. A. Loraine and E. T. Bell, pp. 2-16. Livingstone, Edinburgh.

Magnus, H. A. (1946). The pathology of simple gastritis. *J. Path. Bact.*, **58**, 431-439.

Michaëlsson, M., Nosslin, B., and Sjölin, S. (1965). Plasma bilirubin determination in the newborn infant. A methodological study with special reference to the influence of hemolysis. *Pediatrics*, **35**, 925-931.

Rhodes, J., Barnardo, D. E., Phillips, S. F., Rovelstad, R. A., and Hofmann, A. F. (1969). Increased reflux of bile into the stomach in patients with gastric ulcer. *Gastroenterology*, **57**, 241-252.

Turnberg, L. A., and Anthony-Mote, A. (1969). The quantitative determination of bile salts in bile using thin-layer chromatography and 3-α-hydroxysteroid dehydrogenase. *Clin. chim. Acta*, **24**, 253-259.

Watkinson, G. (1951). A study of the changes in *p*H of gastric contents in peptic ulcer using the 24 hour test meal. *Gastroenterology*, **18**, 377-390.

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biomet. Bull.*, **1**, 80-83.