Gut, 1991, 32, 1406–1411

Gall bladder emptying patterns in response to a normal meal in healthy subjects and patients with gall stones: ultrasound study

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Abstract

In this study gall bladder emptying patterns in response to a solid meal were studied using ultrasound. A similar triphasic pattern was seen in eight healthy control subjects and eight patients with gall stones, with 'early' and 'late' net emptying phases separated by a period of net refilling with peak postprandial gall bladder volumes occurring at (mean (SD)) 33·1 (17·9) minutes and 27·4 (18·8) minutes in control subjects and patients, respectively. A phase of slower net emptying followed, which was complete at 146 (33) minutes in control subjects and 125 (33) minutes in the gall stone patients (not significant). Superimposed upon this overall triphasic pattern, postprandial gall bladder emptying was punctuated by repeated short lived episodes of filling and emptying. The mean (SD) estimated postprandial bile outputs were 0·83 (0·34) ml/min in four control subjects and 1·2 (1·1) ml/min in seven patients with gall stones. We propose a 'washout' model to reconcile this large turnover of bile with the concentrating and storage functions of the gall bladder and predict that the extent rather than the rate of gall bladder emptying is important in determining stasis of bile in the gall bladder.

Impaired gall bladder motility may be important in the pathogenesis of gall stones.[1] Thus abnormal gall bladder emptying has been implicated in gall stone formation during pregnancy,[2,3] in patients taking female sex hormones,[3,4] and in those on longterm parenteral nutrition.[5,6] Furthermore, in the prairie dog in vitro gall bladder muscle contractility and in vivo gall bladder emptying, measured cholescintigraphically, are impaired before and during gall stone formation.[7–9] Previous studies, however, of gall bladder dynamics in patients with gall stones have yielded conflicting results. Enhanced,[10] normal,[11] or impaired gall bladder emptying[11–15] have all been described. In theory these conflicting conclusions may be due either to variations in gall bladder emptying after oral or intravenous stimuli or to differences in methodology, or both. Several parameters have been used to measure gall bladder emptying including the difference between initial and final volumes (the 'delta volume'), the ejection fraction, the emptying rate constant (k) and the t½ of emptying. They all assume that the gall bladder empties in a steady progressive fashion. This assumption has been challenged by Jazrawi *et al*,[16] who used a dual isotope technique, and by our own preliminary ultrasound studies of gall bladder motility.[17] The results of these two studies show that the gall bladder undergoes repeated episodes of filling and emptying over short periods of time, both in the fasting state and in response to meals. Jazrawi and coworkers have since confirmed their initial observations by quantifying absolute gall bladder filling and emptying in gall stone patients using the combination of duodenal perfusion and isotope techniques, with two separate markers to label gall bladder bile and hepatic bile.[18]

If meal stimulated gall bladder emptying under physiological conditions is indeed complex, and punctuated by repeated episodes of refilling, the actual volume of bile handled by the gall bladder will have been underestimated in previous studies. The aim of this study, therefore, was to examine gall bladder emptying in response to a normal meal in healthy control subjects and patients with gall stones, using ultrasound.

Methods

SUBJECTS

Gall bladder emptying was studied in eight patients with gall stones (one man and seven women) with a mean (SD) age of 54 (13·6) years and in eight healthy men with a mean (SD) age of 32 (6·7) years. None was taking regular medication, although one patient occasionally took antacids for heartburn. All premenopausal patients were studied during the first 10 days of the menstrual cycle.[3]

ESTIMATION OF GALL BLADDER VOLUME BY ULTRASOUND

Images of the gall bladder were obtained using a real time ultrasound system with a 128 mm 3·0 MHz 'linear array' transducer (System 285, Diagnostic Sonar, Livingston, Scotland). At the end of inspiration the gall bladder was scanned along its long axis, the resultant image 'frozen' on the oscilloscope screen and traced onto a cellophane sheet. Gall bladder volume was estimated from the silhouette using the sum of cylinders method described by Everson *et al*[19] and a commercial plotter (Datacorp Type 4 Datalizer, GTCO, Rockville, Maryland) connected to an Apple II computer.

VALIDATION OF METHOD USED TO ESTIMATE CHANGES IN GALL BLADDER VOLUME

To confirm that the observed changes in gall bladder volume were real and not artefactual, the

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Accepted for publication 14 December 1990

magnitude of the minute-by-minute fluctuations in gall bladder length and width were measured for 20 minutes before, and for 20 minutes after, a continuous 20 minute infusion of 100 Ivy dog units of cholecystokinin (Pancreozymin, Boots, Nottingham). Since cholecystokinin induces gall bladder contraction it would be expected to decrease the minute-by-minute fluctuations in the length and width of the gall bladder. Therefore, any increase in these minute-by-minute flunctuations would indicate that the changes were due to alterations in the dimensions of the gall bladder itself rather than to artefact.

Table I gives the results of minute-by-minute estimations of maximum gall bladder length and width before and after cholecystokinin in two control subjects. In the first, overall gall bladder length and width both decreased after cholecystokinin. But there were minute-by-minute fluctuations in the length and width of the gall bladder, and the amplitude of these fluctuations increased after the gall bladder had contracted in response to cholecystokinin. The mean (SEM) minute-by-minute fluctuations in width increased from 1·12 (0·16) mm to 2·11 (0·36) mm ($p<0·025$) and in length from 1·81 (0·34) to 2·91 (0·52) mm ($p<0·05$). In the second subject there were again significant falls in overall gall bladder length and width in response to cholecystokinin. But the minute-by-minute fluctuations in length increased from 1·66 (0·21) mm to 6·20 (0·91) mm ($p<0·01$). The fluctuations in measurements of width also increased, but the increase was not significant.

TABLE I *Mean (SEM) minute-by-minute fluctuations in gall bladder length and width in two healthy subjects before and after cholecystokinin infusions*

	Gall bladder			
	Mean length (mm)	*Mean width (mm)*	*Mean fluctuation in length (mm)*	*Mean fluctuation in width (mm)*
Subject 1:				
Before cholecystokinin	37·70 (0·37)	28·09 (0·27)	1·81 (0·34)	1·12 (0·16)
After cholecystokinin	36·08 (0·68)	17·55 (0·57)	2·91 (0·52)	2·11 (0·36)*
Subject 2:				
Before cholecystokinin	61·03 (0·36)	27·32 (0·30)	1·66 (0·21)	1·53 (0·27)
After cholecystokinin	43·18 (1·22)	16·68 (0·63)	6·20 (0·91)†	2·12 (0·29)

*$p<0·01$; †$p<0·001$.

TABLE II *Estimated minute-by-minute changes in volume in two large and apparently 'static' gall bladders in two patients with gall stones. The changes in diameter (delta diameters) of model cylinders of the same mean length and volume as the gall bladders that would be required to 'generate' the observed changes in gall bladder volume have been calculated*

	Patient 1	*Patient 2*
Mean gall bladder length (mm)	81·3	57·9
Mean gall bladder volume (ml)	47·6	46·5
Calculated diameter of model cylinder of equivalent length and volume (mm)	27·3	32·0

Delta volumes (ml) (actual gall bladder volume – mean gall bladder volume)		*Delta diameter (mm) (change in diameter of model cylinder required to generate delta volume)*	
Patient 1	*Patient 2*	*Patient 1*	*Patient 2*
+0·85	−1·97	0·19	0·54
−2·71	−5·17	0·62	1·42
−0·68	−4·05	0·15	1·11
+2·48	−4·00	0·55	1·09
+4·41	−2·64	0·98	0·72
−3·21	+1·31	0·74	0·35
−2·81	+0·62	0·64	0·17
−2·86	+5·79	0·65	1·53
+1·03	+4·24	0·23	1·12
+3·54	+5·87	0·79	1·55
Mean (SD)		0·55 (0·28)	0·96 (0·49)
Overall mean (SD) (mm)		0·76 (0·44)	

ESTIMATION OF OBSERVER ACCURACY IN DETERMINING GALL BLADDER SIZE

The fact that the volume of a cylinder is proportional to its length and to the square of its diameter was used to estimate the experimental error in estimating gall bladder volume in vivo. For 'large' gall bladders a given change in diameter would have proportionally greater effects on the calculated volume than the same change in length. Therefore, if the gall bladder is regarded as a cylinder of constant length, the magnitude of the changes in volume can be calculated. The error in measuring gall bladder volume was estimated by making repeated volume measurements of two large 'static' fasting gall bladders over 10 minutes. The changes in diameter of a cylinder of the same mean volume and length that would be required to 'generate' the observed gall bladder volume changes were than calculated.

Table II shows the results of minute-by-minute observations in two patients with gall stones with large static gall bladders, each of whom was studied over a 10 minute period. Assuming that these large gall bladders were of constant volume throughout the 10 minutes of observation and equating them to cylinders of the same mean length and volume, the changes in diameter ('delta diameters') of the model cylinders required to produce the observed changes in gall bladder volume were calculated. In the first patient the mean (SD) change was 0·55 (0·28) mm and in the second 0·96 (0·49) mm. Therefore, the overall mean (SD) of the individual delta diameters was 0·76 (0·44) mm. This figure was taken as a measure of the observer accuracy in estimating gall bladder size.

GALL BLADDER EMPTYING IN RESPONSE TO A NORMAL MEAL

The control subjects and patients were studied after an overnight fast of not less than 12 hours. Table III gives the composition of the test meal. Scans were taken before the meal was eaten to estimate the 'fasting' gall bladder volume. It soon became apparent, however, that there was preingestion gall bladder evacuation and therefore, in order to determine the extent of this 'cephalic phase' emptying, serial measurements of gall bladder volume were made before the meal. The immediate preingestion gall bladder volume was taken as the 'fasting volume.' Time 0 was arbitrarily defined as the mid-point of meal ingestion. The control subjects and patients then lay semirecumbent throughout the study and frequent scans were taken for not less than two hours, or until there was net refilling of the gall

TABLE III *Composition of test meal*

Constituent	Fat (g)	Protein (g)	Carbohydrate (g)	Fibre (g)	Energy (kJ)
60 g bread	1·0	4·7	29·8	1·6	579·0
10 g butter	8·2	0·04	–	–	310·0
5 g bran	0·3	0·7	1·3	2·2	43·3
120 g baked beans	0·6	6·1	12·4	8·8	322·6
250 ml milk	9·5	8·2	11·7	–	682·5
Total	19·6	19·8	55·2	12·6	1937·4

bladder. During the first two studies in control subjects scans were taken every 10 to 15 minutes. When it became clear that the gall bladder was undergoing rapid changes in volume, the sampling interval was reduced to five minutes or less. In four of the control subjects and in seven of the patients scans were performed at one minute intervals.

Results

GROSS GALL BLADDER EMPTYING PATTERNS

Cephalic phase emptying

In five of eight control subjects and in all eight patients with gall stones there was evidence of net gall bladder emptying before the start of the meal, but in the remaining three control subjects there was no such cephalic phase.

Postprandial gall bladder emptying patterns

The pattern of gall bladder emptying was triphasic, with early emptying, early refilling, and late emptying phases. This pattern was similar in control subjects and patients. Figure 1 shows an individual example, while Figure 2 shows the mean overall filling and emptying patterns in both groups.

Postprandial gall bladder emptying was complex and did not conform to a simple exponential pattern. In six out of eight control subjects and in all eight patients there was an early net emptying phase with a mean (SD) fall in gall bladder volume from 10·7 (4·8) ml immediately before the meal to 7·5 (4·4) ml at 14·1 (7·7) minutes in control subjects and from 22·0 (19·4) ml to 17·3 (17·2) ml at 18·5 (12·4) minutes in patients. This was followed by a period of early net refilling in all 16. As a result, the peak gall bladder volumes were 15·1 (9·9) ml at 33·1 (17·9) minutes in the control subjects and 24·9 (23·4) ml at 27·4 (18·8) minutes in the patients. After this net refilling there was a slower phase of net emptying during which gall bladder volume fell to reach a late nadir of 2·4 (1·9) ml at 146 (33) minutes in control subjects and 13·2 (18·8) ml at 125 (33) minutes in the patients.

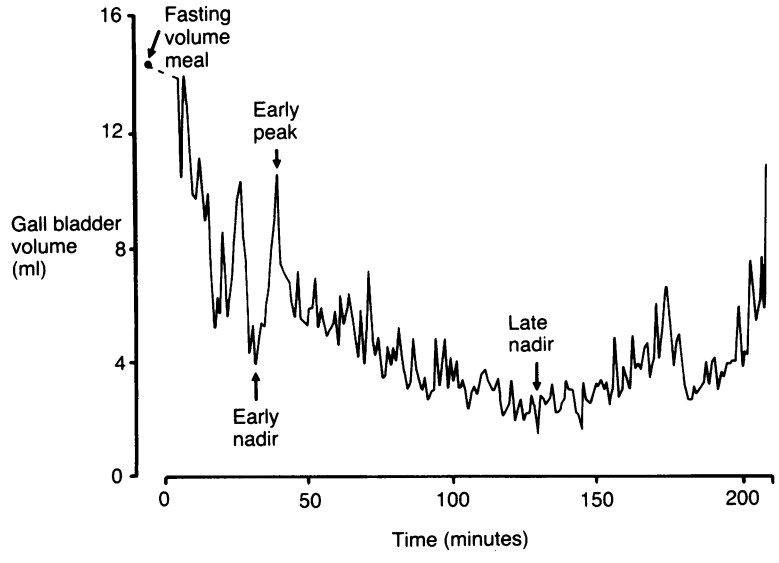

Figure 1: A representative example of a gall bladder emptying profile in a normal volunteer in which measurements of gall bladder volume were made at one minute intervals.

TABLE IV *Estimated postprandial delta volumes, ejection fractions, total outputs, and mean minute outputs of bile in four control subjects and seven patients with gall stones from meal ingestion to the time of the late nadir volumes. The 'delta volume' is defined as the difference between the immediate preingestion or fasting gall bladder volume (FV) and the 'late' nadir volume. The ejection fraction is the overall decrease in gall bladder volume expressed as a percentage of the fasting volume*

	Delta volume (ml)	Total output (ml)	Ejection fraction (%FV)	Mean output (ml/min)
Healthy control subjects:				
1	14·3	57·5	90·9	0·41
2	11·3	89·8	70·8	0·75
3	11·9	93·9	80·8	0·96
6	4·5	156·0	44·4	1·22
Mean (SD)	10·5 (4·2)	86·8 (60·9)	71·7 (20·0)	0·83 (0·34)
Patients with gall stones:				
9	12·2	70·6	78·4	0·67
10	15·4	74·3	89·9	0·75
12	10·2	230·3	19·9	2·07
13	6·8	382·7	13·0	1·22
14	6·5	64·3	87·9	0·38
15	14·0	145·0	63·0	1·10
16	2·6	44·8	65·5	0·40
Mean (SD)	9·7 (4·6)	144·6 (123·0)	59·6 (17·8)	1·2 (1·1)

Minute-by-minute gall bladder filling and emptying

Superimposed on the overall triphasic pattern, postprandial gall bladder emptying was punctuated by repeated, short lived episodes of filling and emptying throughout in all 16 patients and subjects studied. Table IV gives the estimated total output from the time of eating the meal to the time of the late nadir in the 11 who were studied at one minute intervals. In the healthy subjects the mean total output was eight times greater than the average delta volume and in the patients it was about 15 times greater. The mean (SD) estimated bile outputs per minute were 0·83 (0·34) ml/min and 1·2 (1·1) ml/min in control subjects and patients respectively. There were no correlations between (i) delta volume and mean minute output ($r=0·012$; $n=11$), (ii) fasting volume and delta volume ($r=0·10$; $n=11$), (iii) fasting volume and ejection fraction ($r=-0·19$; $n=11$), or (iv) mean minute output and ejection fraction ($r=-0·053$; $n=11$). There was, however, a significant positive correlation between fasting volume and mean minute output ($r=0·77$; $p<0·01$; $n=11$).

Contour changes in the gall bladder during emptying and refilling

Figure 3 shows two sets of superimposed gall bladder silhouettes, one obtained from a healthy

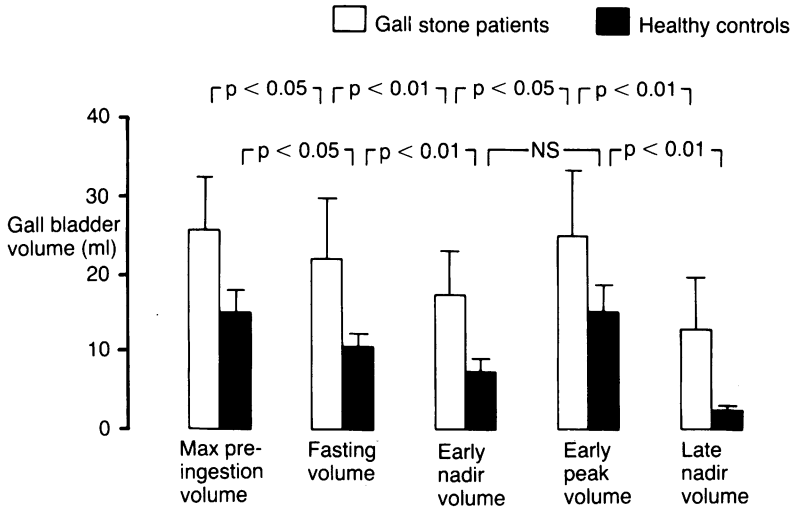

Figure 2: Overall filling and emptying patterns in patients with gall stones and control subjects. Means (SEM) are shown.

control subject and one from a patient with gall stones. Scans were obtained at one minute intervals before the meal and until the time of the late nadir. As the silhouettes show, the most obvious gall bladder wall movements occurred in the infundibulum and neck, with less movement seen in the fundus of the gall bladder.

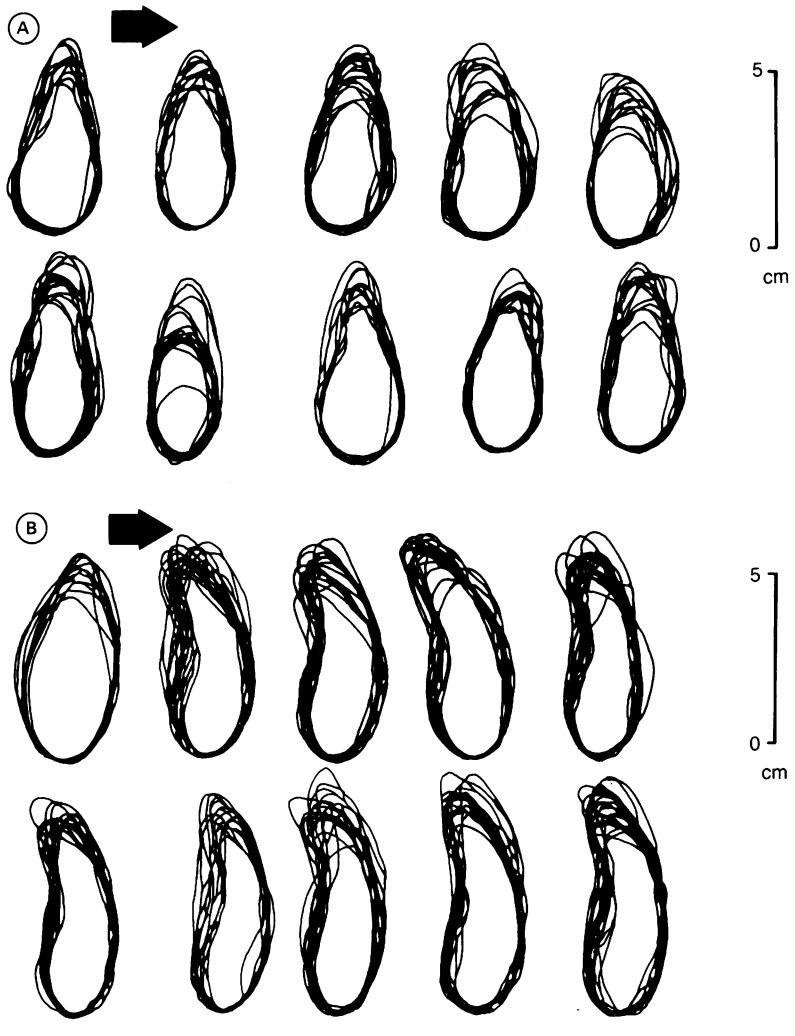

Figure 3: Superimposed gall bladder silhouettes obtained (A) from a healthy control subject and (B) from a patient with gall stones. Pre and postprandial scans are separated by the arrow. Scans were obtained at one minute intervals.

Discussion

The aim of this study was to examine the patterns of gall bladder emptying in healthy subjects and patients with gall stones in response to a solid meal. Ultrasound is safe, non-invasive, and allows repeated measurements of gall bladder volume at short time intervals. It also provides information about the gall bladder during filling and emptying. On the other hand, ultrasonography requires patient cooperation and observer skill to obtain satisfactory images which can only be recorded intermittently, rather than continuously. The calculation of gall bladder volume also depends on certain assumptions about gall bladder geometry.

For the present study it was therefore necessary to show that the observed changes in volume were due to changes in the gall bladder itself rather than to observer error or to measurement artefact. This was done in two ways. Firstly, we showed that the minute-by-minute fluctuations in the length and width of the gall bladder actually increased after gall bladder contraction had been induced by cholecystokinin. Secondly, we estimated the apparent 'error' in widthwise measurement from repeated measurements of large 'static' gall bladders. The estimated mean (SD) error in measuring the diameter of two large gall bladders was 0·76 (0·44) mm. For any particular study, however, observer error is likely to remain constant. Furthermore, individual variations in gall bladder configuration would cause a systematic error in the computation of gall bladder volume so that ultrasound enables valid conclusions to be drawn concerning gall bladder dynamics in any given subject.

In the present study the overall patterns of gall bladder emptying were qualitatively similar in both healthy controls and gall stone patients. Our finding of a 'cephalic phase' of gall bladder emptying has also been reported by others.[20-22] It is generally thought that the main stimulus to gall bladder contraction is an increase in circulating cholecystokinin in response to food (although Ivy and Oldberg considered that other factors may also be important).[23] The fact that gall bladder contraction occurs before the meal, and hence before a meal induced rise in plasma cholecystokinin concentration, suggests that neural rather than hormonal influences are important in the cephalic phase. Furthermore, there is still some controversy as to whether cholecystokinin acts directly on muscle cells[24] or indirectly by releasing acetylcholine from cholinergic neurones.[25]

The initial net emptying is probably due mainly to meal stimulated cholecystokinin release but it might also be due partly to a neurally mediated extension of the cephalic phase. At first sight the early net refilling of the gall bladder was surprising, particularly as it was followed by a slower phase of net emptying to a final nadir about two hours after meal ingestion. Bile flow does increase postprandially,[26] and a major factor influencing this flow is the osmotic effect of biliary bile acids.[27-29] Hence an explanation for the 'early' net refilling of the gall bladder is that it occurs as a result of the mobilisation of bile acids from the intestine, with their subsequent re-uptake and secretion by the liver. This

hypothesis is supported by the results of studies by Setchell *et al.*[30] They showed an increase in serum (unconjugated) bile acids which peaked between 30 and 60 minutes after a meal. In other words, the peak reabsorption of unconjugated bile acids occurred at about the same time as the early peak in gall bladder volume.

The observation that there are minute-by-minute fluctuations in gall bladder volume is not new. 'Spontaneous' contractions of the gall bladder were first noted by Doyon in 1883 in curarised animals.[31] Similar contractions were also noted in the dog by Bainbridge and Dale (1905), Ivy and Oldberg (1928), Crandall (1931), and Voegtlin *et al* (1933).[23 32-34] Spontaneous rhythmic contractions of gall bladder muscle were accentuated by (a) the nerve toxin chrysotoxin,[32] (b) volume distension of the gall bladder,[31 35] and (c) cholecystokinin.[23] Indeed, rhythmic contractions seem to be an intrinsic property of the muscle itself and can be seen in isolated gall bladder muscle strips.[36-38]. More recently, Takahashi *et al*[39] implanted strain gauges into the gall bladder wall of conscious dogs and observed periodic contractions of the gall bladder both in the interdigestive state (in relation to the migrating motor complex) and also postprandially. We believe that this paper is the first to describe periodic minute-by-minute gall bladder filling and emptying in humans.

Our data suggest that under physiological conditions the gall bladder handles most of the hepatic bile secreted by the liver before it is delivered into the duodenum. Previous estimates of the percentage of hepatic bile partitioned into the gall bladder vary from 35–50% in the baboon[40] and up to 75% in humans.[41] Lanzini *et al*, however, suggest that the percentage may be as high as 80% or more.[18] The human liver produces at least 1000 ml of bile per day but although common bile duct flow rates are low during fasting (0·5 to 1·0 ml/min), they increase after a meal to between 2 and 3 ml/min,[26] which is in agreement with our estimate of approximately 1 ml/min. This large flux through the gall bladder (which may be many times the fasting volume per hour) raises the question of how the gall bladder manages to concentrate bile. Although the absorptive capacity of the human gall bladder is not known, Svanvik *et al*[42] estimated that in conscious monkeys the daytime absorption rate of water is approximately one third of the fasting gall bladder volume per hour. Nevertheless, it is known that bile becomes stratified in the gall bladder, with the most concentrated viscous bile lying deep within the fundus.[43-46]

How might the gall bladder handle such a large volume and concentrate bile at the same time? One possible explanation is that fresh hepatic bile is not freely miscible with gall bladder bile and that the rate of turnover of bile in the gall bladder varies – the 'washout model' of gall bladder emptying. In this model the highest turnover of bile would be in the neck and infundibulum with much slower rates of turnover in the fundus, thus permitting concentration of bile in the deeper 'unstirred' layers of the fundus. In this way fresh hepatic bile would enter the infundibulum and become enriched through contact with more concentrated bile. As the gall bladder contracted the deeper, more concentrated layers of bile would be exposed to the washout effect of fresh hepatic bile entering the gall bladder. This would ensure a relatively constant postprandial output of bile in response to a meal.

This study was supported in part by grants from Gipharmex SpA, Milan and by the Special Trustees of Guy's Hospital. We thank Mr L Usiskin, Guy's Hospital Dental School, for use of the digitiser for calculating gall bladder volume.

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