Hepatic Bile Entry Into and Transit Pattern Within the Gallbladder Lumen: A New Quantitative Cholescintigraphic Technique for Measurement of Its Concentration Function

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The aim of the project was to study hepatic bile entry into and the transit pattern within the gallbladder lumen during fasting and to introduce a new quantitative scintigraphic test for measurement of its concentration function. **Methods:** Each of 10 control subjects and 10 chronic acalculous cholecystitis (CAC) patients received 111–185 MBq ^{99m}Tc-mebrofenin as a hepatic bile marker. Gamma-camera image data were collected in the anterior view on a $128 \times 128 \times 16$ computer matrix at 1 frame per minute for 60 min for the hepatic phase and 30 min for the gallbladder phase. The radiolabeled hepatic bile area within the gallbladder lumen was traced, and the net transit area and transit time were noted. The hepatic bile transit rate was calculated (as mm^2/min) and normalized to 1,000 mm^2 of the anterior gallbladder area. The cholecystokinin-8–induced ejection fraction was calculated nongeometrically using counts. **Results:** Hepatic bile entered the gallbladder continuously during fasting with a mean $\pm$ SD of 71% $\pm$ 20% in control subjects and 59% $\pm$ 27% in CAC patients, which were not significantly different ($P > 0.05$). The maximum frontal gallbladder area was 1,699 mm^2 in control subjects and 1,610 mm^2 in CAC patients ($P > 0.05$). Radiolabeled hepatic bile entered the gallbladder first along its central long axis in both groups, at a mean of 15 min and 16 min, respectively, and traveled toward the periphery in a lamellar fashion at a normalized mean rate of 38 mm^2/min and 40 mm^2/min in control subjects and CAC patients, respectively. The mean ejection fraction of 17% in CAC patients was significantly lower than the mean value of 56% in control patients ($P < 0.00001$). **Conclusion:** Hepatic bile enters the gallbladder continuously during fasting. In patients with CAC, the gallbladder maintains the normal concentration function but the contraction and emptying are reduced significantly. This new cholescintigraphic technique enables measurement of both functions sequentially with a single dose of ^{99m}Tc-mebrofenin.

Key Words: hepatic bile flow; gallbladder concentration; gallbladder contraction; cholescintigraphy; cholecystitis

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$\mathbf{T}$he liver is not only the largest organ but also carries out the most complex biologic function in the body. It secretes continuously approximately 600 mL of bile per day (0.4 mL/min), a major portion of which enters the gallbladder during fasting (*1*). This preferential bile flow enables sequestration of biologically important solutes (bile salts) within the gallbladder during fasting. Solutes are discharged into the duodenum soon after a meal because of the release of endogenous cholecystokinin (CCK). After a nearly complete emptying following a meal, the gallbladder refills to its normal capacity of 40–50 mL, usually within the first 4–6 h of fasting, but continues to receive hepatic bile during the entire period of fasting. This ability to accommodate a constant inflow of hepatic bile, in excess of its normal volume, is attributed to absorption of water and electrolytes through its epithelium and has been referred to as the concentration function of the gallbladder (*2*). The mechanisms by which the hepatic bile enters and then travels within the gallbladder lumen are not well defined. Does the hepatic bile enter the gallbladder continuously or intermittently, with alternating periods of emptying and refilling, much like a bellows? Does the hepatic bile mix with the gallbladder bile instantly, as soon as it enters, or does it follow a predefined path? Does the hepatic bile movement pattern change in diseases affecting the gallbladder? Answers to these questions are unclear because a technique that permits the study of such functions is not available.

We have previously introduced a noninvasive, nongeometric, and quantitative cholescintigraphic technique for measurement of the contraction and emptying (ejection fraction) functions of the human gallbladder (*3*). We now describe a new quantitative cholescintigraphic technique to study its concentration function.

MATERIALS AND METHODS

Ten healthy subjects (control subjects) and 10 patients with chronic acalculous cholecystitis (CAC) were chosen retrospectively from a group of 309 patients who were studied with ^{99m}Tc-mebrofenin for a variety of hepatobiliary diseases over an 18-mo

period, and data were stored on a laser disk. All patients were referred to the Nuclear Medicine Department by hospital medical staff for assessment of hepatobiliary diseases by cholescintigraphy.

Control Subjects

Ten control subjects (9 women, 1 man) ranged in age from 29 to 93 y, with a mean age of 52 y (Table 1). Normal hepatobiliary status was confirmed in all subjects by obtaining normal sonographic studies of the liver and gallbladder and normal hepatobiliary functional parameters, which included the hepatic extraction fraction, excretion half-time, and normal gallbladder ejection fraction (>35%) with a 3-min infusion of the octapeptide of CCK (CCK-8). None of the subjects was suspected clinically of having parenchymal liver disease and none was found to have any biliary disease that required treatment during the ensuing 3- to 12-mo period of clinical follow-up.

CAC Patients

Ten patients (8 women, 2 men) ranged in age from 22 to 65 y, with a mean age of 41 y. The main presenting symptom was postprandial right upper quadrant pain. Parenchymal liver disease was not suspected clinically, and all patients had normal sono-

graphic studies of the liver and gallbladder and normal hepatobiliary functional parameters, as referred to for the control subjects, with the sole exception of the gallbladder ejection, which was low (<35%). Solely on the basis of right upper quadrant abdominal pain and a low ejection fraction, all 10 patients underwent laparoscopic cholecystectomy. No gallstones were found at surgery, and gallbladder wall sections stained with hematoxylin–eosin showed changes typical for CAC, which include Rokitansky–Aschoff sinuses, wall infiltration with chronic inflammatory cells, and fibrosis of the submucosa. No microcrystals were found, and the epithelium was fairly intact.

All control subjects and CAC patients were contacted by telephone 3–12 mo after the study and asked to grade relief of symptoms as a percentage of pain before cholescintigraphy (control subjects) or surgery (CAC patients). Abdominal pain had cleared in all 10 control subjects without need for any specific therapy for hepatobiliary disease. Eight of 10 CAC patients had 100% pain relief, and the remaining 2 had >80% pain relief after laparoscopic cholecystectomy. The institutional interprofessional review board approved the request to contact control subjects and patients with CAC by telephone for follow-up information.

TABLE 1
Concentration and Contraction Functions of Gallbladder (GB) in Control Subjects and in Patients with CAC

No.	Fl no.	Age (y)	Sex	Wt. (kg)	Hepatic bile flow % GB	Hepatic bile flow % Inst.	GB appear. (min)	IHB area (mm²)	MHB area (mm²)	Trans area (mm²)	Trans time (min)	Trans rate (mm²/min)	mm²/min/1,000 mm² Obs 1	mm²/min/1,000 mm² Obs 2	GBEF (%)
Control															
1	202	42	F	104	92	8	15	164	2,606	2,442	23	106	43	39	40
2	209	29	F	115	96	4	10	55	2,551	2,496	23	108	43	42	37
3	216	57	F	71	94	6	15	54	1,357	1,303	28	47	34	26	51
4	224	44	F	83	55	45	12	99	1,445	1,346	20	67	47	39	41
5	226	93	F	60	77	23	16	139	1,654	1,515	20	76	46	47	65
6	230	53	F	80	60	40	14	131	1,894	1,763	32	55	29	34	75
7	241	50	F	91	44	56	13	164	1,390	1,226	20	61	44	34	43
8	262	37	F	100	57	43	12	197	1,905	1,708	33	52	27	26	79
9	273	65	M	77	48	52	15	77	1,062	985	31	32	30	33	89
10	311	47	F	72	83	17	27	164	1,128	964	21	46	41	44	38
Mean*					71	29	15	124	1,699	1,575	25	65	38	36	56
SD					20	20	5	50	542	540	5	25	8	7	20
CAC															
1	45	58	M	86	42	58	11	115	1,656	1,541	18	86	52	51	2
2	51	65	F	62	87	13	17	115	1,451	1,336	15	89	61	50	14
3	57	46	F	67	49	51	26	207	2,168	1,961	30	65	30	39	29
4	58	29	M	109	25	75	11	55	1,883	1,828	24	76	40	50	24
5	65	44	F	136	68	32	26	99	1,533	1,434	20	71	47	57	10
6	68	46	F	68	90	10	15	88	1,818	1,730	23	75	41	36	16
7	83	22	F	95	66	34	14	54	1,428	1,374	30	46	32	39	17
8	116	50	F	67	20	80	16	77	602	525	31	17	28	25	20
9	132	38	F	80	48	52	14	100	1,664	1,554	24	65	42	32	21
10	138	38	F	63	98	2	12	120	1,905	1,785	30	60	31	34	14
Mean*					59	41	16	103	1,611	1,507	25	65	40	41	17
SD					27	27	6	43	422	402	6	21	11	10	8
P					0.3	0.3	0.58	0.32	0.68	0.75	0.81	1	0.64	0.23	0.00001

*n = 10.

Fl no. = file number; Wt. = weight; IHB = initial hepatic bile; MHB = maximum hepatic bile; Trans = transit; GBEF = GB ejection fraction; Inst. = intestine; Obs = observer.

Cholescintigraphic Data Collection

After an overnight fast (minimum, 4 h), each subject received 111–185 MBq (3–5 mCi) [99mTc]-mebrofenin (Bracco Diagnostics, Princeton, NJ) intravenously while lying supine underneath a large-field-of-view gamma camera fitted with a low-energy, all-purpose, parallel-hole collimator (4). Anterior view planar hepatic phase images were acquired at 2 s per frame for 60 s (between 0 and 1 min) and at 1 frame per min for the next 59 min (between 2 and 60 min) after injection of the radiotracer, and the data were stored on a 128 × 128 × 16 computer matrix (Fig. 1).

Gallbladder phase data in the anterior view were acquired separately, between 61 and 90 min after radiotracer injection, at 1 frame per min for 30 min and stored on a 128 × 128 × 16 computer matrix. CCK-8 (Sincalide; Bracco Laboratories, Princeton, NJ) was infused at a dose of 10 ng/kg over 3 min (dose rate, 3.3 ng/kg/min for 3 min) through an infusion pump, beginning at 63 min. The gallbladder ejection fraction was calculated nongeometrically using counts to represent the bile volume as described (4). An ejection fraction value of <35% was considered abnormal and indicative of CAC. All 10 CAC patients had a cholecystectomy on the basis of biliary pain and a low ejection fraction.

Data Analysis

The liver and gallbladder functional parameters were obtained quantitatively by applying semiautomatic hepatobiliary software. The hepatic extraction fraction and excretion half-time were calculated from the hepatic phase data as described (5). The total hepatic bile secreted during the 60 min after radiotracer injection and its differential flow into the gallbladder versus the intestine were obtained by selecting 1 region of interest (ROI) over the gallbladder and another over the rest of the abdomen (excluding the liver, gallbladder, bile ducts, and urinary bladder) and dividing counts in each ROI by the sum of their total counts. The time–activity curves were generated over both regions. The gallbladder filling pattern during fasting (hepatic phase imaging) was scrutinized carefully by checking the direction of its time–activity curve (Fig. 2).

Calculation of Hepatic Bile Transit Rate Within Gallbladder

The computer data from the gamma camera were transferred to a MedView station (MedImage, Inc., Ann Arbor, MI) for analysis. The data from 0 to 1 min (30 frames) that show the blood supply to the
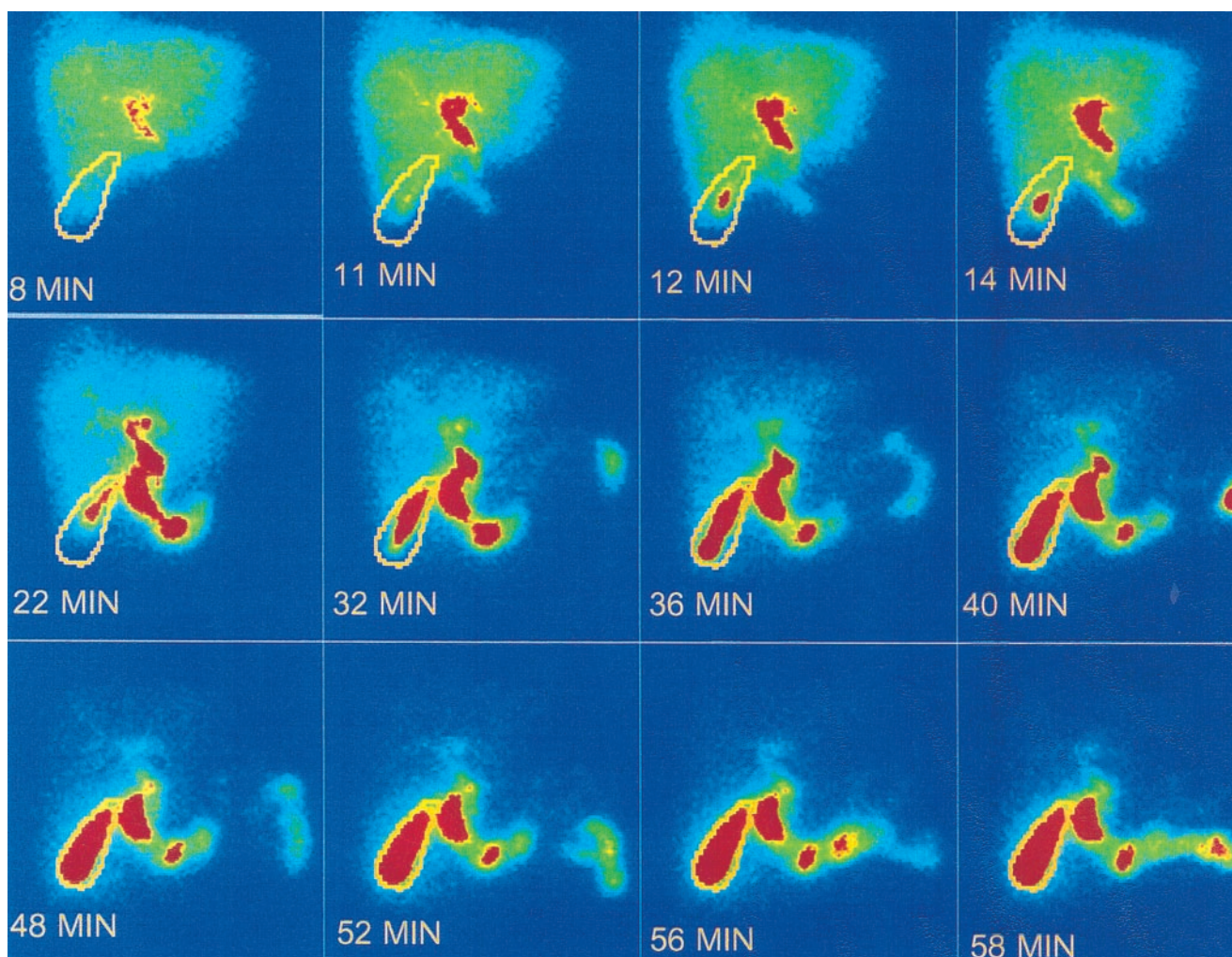

FIGURE 1. Hepatic bile transit pattern within gallbladder lumen. Outline of fully filled gallbladder is superimposed onto selected earlier frames along with their time of appearance from injection of [99mTc]-mebrofenin. Radiolabeled hepatic bile first enters gallbladder at 12 min (upper threshold set for red color) along central long axis of images at 14, 22, 32, 36, and 40 min. Once filled fully with radiolabeled bile, not much change in size of gallbladder is shown in frames 48 through 58 min.
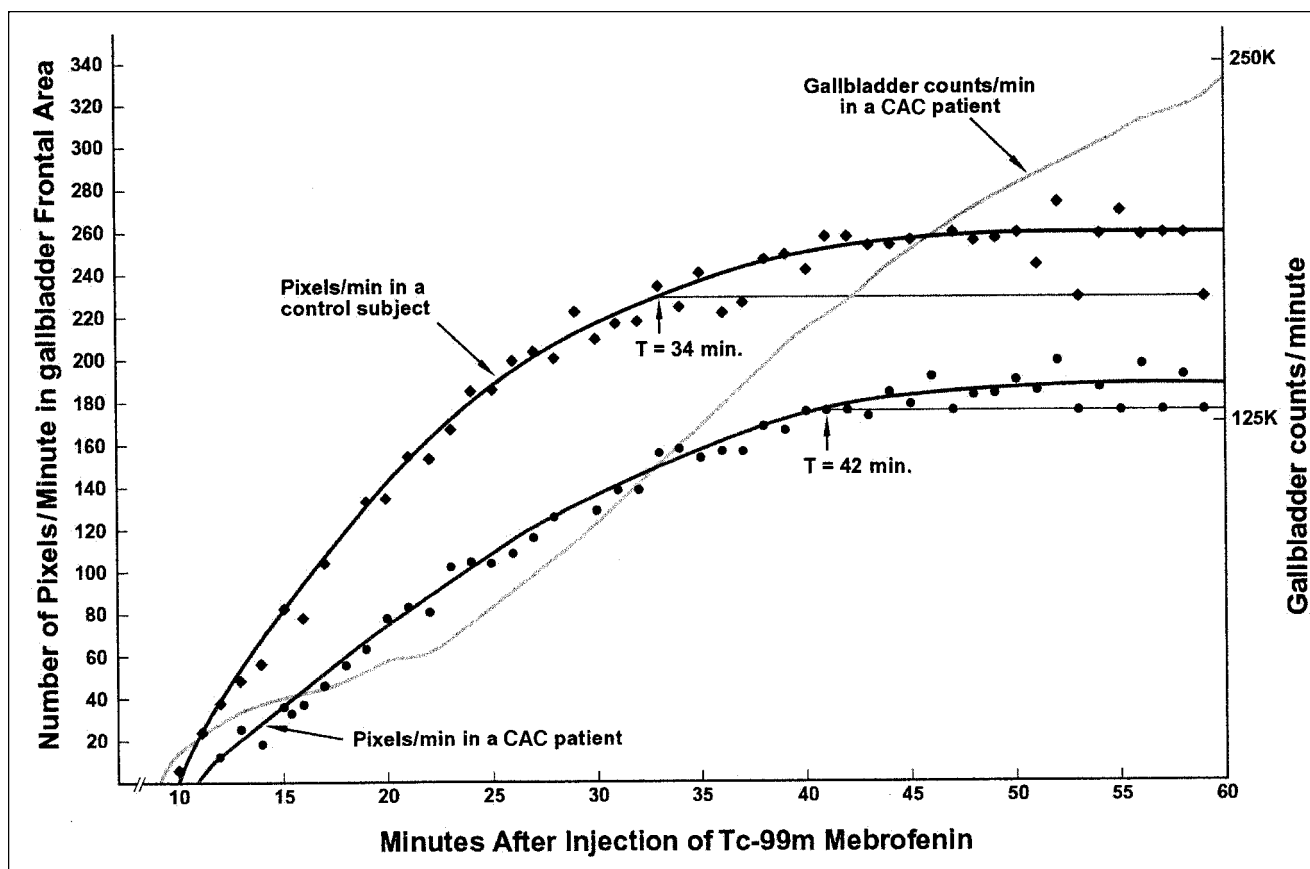
FIGURE 2. Hepatic bile entry pattern into gallbladder. *x*-Axis shows time in minutes after ^{99m}Tc-HIDA injection. *y*-Axis shows gallbladder area as number of pixels per minute on left and its volume as counts per minute on right. Gallbladder volume curve shows uninterrupted upslope (curve for control not shown to avoid crowding), indicating continuous bile entry during fasting. Transit time (T) is noted (arrow) at intersection of pixel-per-minute line and extrapolation line passing through lowest number of pixels between 50 and 60 min, by which time radiolabeled hepatic bile usually fills gallbladder to its full volume. Note that variation in gallbladder area (due to respiratory movement) beyond 45 min is not accompanied by any reduction in bile volume (counts per minute).

liver through the hepatic artery and portal vein were ignored for the purpose of this study, and the data from 2 to 60 min (frames 31–89) were used for the analysis. Each frame was magnified electronically 400% to facilitate reliable and easy tracing of the hepatic bile boundary within the gallbladder lumen (Fig. 1). The hepatic bile upper and lower threshold limits were set on 1 of the last 10 frames obtained between 51 and 60 min, by which time the radiolabeled hepatic bile has usually filled the entire gallbladder lumen (Fig. 1). This chosen frame was least affected by respiratory movement and no adjacent structure (duodenum or bile ducts) superimposed on this frame. All of the remaining frames were analyzed without changing preset hepatic bile upper and lower threshold limits (usually 0 for the lower limit and 15–20 for the upper limit).

Hepatic Bile Area Within Gallbladder Lumen

The time of arrival of the radiolabeled hepatic bile (RHB) into the gallbladder lumen first was considered the gallbladder appearance time (Fig. 1). By selecting a polygonal ROI curser, the RHB outer boundary was traced for all of the frames, and the total number of pixels within each frame was noted. The first frame showing the RHB within the gallbladder lumen was termed the initial hepatic bile area (IHB area), and the first frame showing the maximally filled gallbladder was termed the maximum hepatic bile area (MHB area). The total number of hepatic bile pixels in each

frame and the time of its appearance are given in Table 2. The hepatic bile area in the IHB frame and in the MHB frame was obtained by multiplying the total number of pixels by the area of each pixel (10.95 mm^2 for the Ecam camera [Siemens Medical Systems, Hoffman Estates, IL] and 20.25 mm^2 for the Genesis camera [ADAC Laboratories, Milpitas, CA]). The area of the IHB was subtracted from the area of the MHB to obtain the net hepatic bile transit area. The time difference between the MHB area and the IHB area represents the transit time. The net hepatic bile transit area was divided by the transit time to obtain the transit rate and is expressed as mm^2/min. The value was normalized to 1,000 mm^2 to account for the difference in gallbladder size (area) among patients, and the final hepatic bile movement value was expressed as mm^2/min/1,000 mm^2 of gallbladder frontal area (Table 2). Two observers independently obtained the normalized transit values by entering the data in a tabular form to test the reproducibility of the technique (Table 1).

By plotting time on the *x*-axis and the total number of pixels in each frame on the *y*-axis, a pixel–time graph was created (Fig. 2). A best-fitting line passing through most of the data points was drawn. The lowest number of pixels in any frame between 51 and 60 min was considered to represent the maximum anterior longitudinal area of the gallbladder accommodating the MHB area. This

TABLE 2
Hepatic Bile Transit Within Gallbladder Lumen in Control Subject and in Patient with CAC

Time after ^{99m}Tc-HIDA (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
No. of pixels																				
Control subject 209	—	0	0	0	0	0	0	0	0	0	5	24	38	49	57	81	79	106	120	133
CAC patient 138		0	0	0	0	0	0	0	0	0	0	11	24	18	35	36	44	54	61

Time after ^{99m}Tc-HIDA* (min)	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
No. of pixels*																				
Control subject 209	135	154	152	167	184	186	198	204	199	221	199	213	216	233	224	238	220	225	246	248
CAC patient 138	78	81	80	101	107	105	109	115	126	130	128	137	138	155	155	151	155	162	154	168

Time after ^{99m}Tc-HIDA* (min)	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
No. of pixels*																				
Control subject 209	240	255	255	251	251	253	259	252	252	258	242	273	233	256	269	255	257	258	237	267
CAC patient 138	166	172	174	171	182	179	190	174	180	182	189	182	199	174	184	179	194	179	190	180

*Data are continuations of subsequent set of data.
Calculations for control subject 209:
  Hepatic bile area = Number of pixels × pixel area.
  Initial hepatic bile area = $5 \times 10.95 = 55$ mm^2.
  Maximum hepatic bile area = $233 \times 10.95 = 2,551$ mm^2.
  Net hepatic bile transit area = $2,551 - 55 = 2,496$ mm^2.
  Hepatic bile transit time = $34 - 11 = 23$ min.
  Hepatic bile transit rate = $2,496/23 = 109$ mm^2/min.
  Adjusted transit rate = $108 \times 1,000/2,551 = 42$ mm^2/min/1,000 mm^2.
 Note that minimum number of hepatic bile pixels in frames between 51 and 60 min was 233 at 53 min. However, this low number was seen first at 34 min, indicating that gallbladder complete filling with radiolabeled hepatic bile occurred earlier at this time interval.

chosen frame is affected the least by gallbladder movement due to respiration. The earliest time taken to reach the maximum gallbladder longitudinal area was noted from either Table 1 or the graph in Figure 2.

Calculation of Gallbladder Ejection Fraction

A count-based nongeometric method was used as described (*3*). The ejection fraction was obtained by subtracting minimum counts (after CCK-8) from maximum counts (before hormone injection), dividing the difference by the maximum counts, and multiplying the quotient by 100. An ejection fraction value of <35% was considered indicative of CAC. Mean values between the control subjects and the CAC patients were tested by an independent Student *t* test, and $P < 0.05$ was considered statistically significant.

RESULTS

After intravenous injection of the radiotracer, radiolabeled hepatic bile entered the gallbladder at a mean time of 15 min in control subjects and 16 min in CAC patients. Gallbladder time–activity curves in all healthy subjects and CAC patients showed a continuous upslope (Fig. 2), indicating uninterrupted bile entry during fasting. None of the control subjects or CAC patients showed a downslope of the time–activity curve to suggest spontaneous gallbladder emptying during fasting. The quantity of hepatic bile entering the gallbladder varied from 44% to 96% in control subjects and from 20% to 98% in CAC patients. The mean ± SD hepatic bile flow into the gallbladder of 71% ± 20% in control subjects and 59% ± 27% in CAC patients did not differ significantly ($P = 0.3$). In control subject 2 and in CAC patient 10, all hepatic bile entered the gallblad-

der, resulting in nonvisualization of the small intestine at 60 min. The intestinal bile entry values of 4% and 2%, respectively, for these 2 individuals (Table 1) merely reflect the background count, which was not subtracted.

Bile Transit Rate Within Gallbladder

The hepatic bile first entered the gallbladder lumen along its long central axis and traveled slowly toward the periphery (Fig. 1). The initial mean ± SD hepatic bile area within the gallbladder lumen was 124 ± 50 mm^2 in control subjects and 103 ± 43 mm^2 in CAC patients ($P > 0.05$). The maximum mean hepatic bile area was 1,699 ± 542 mm^2 in control subjects and 1,611 ± 422 mm^2 in CAC patients. The mean net hepatic bile transit area and mean transit times were 1,575 ± 540 mm^2 and 25 ± 5 min for the control subjects and 1,507 ± 402 mm^2 and 25 ± 6 min for CAC patients, respectively (Table 1). The mean hepatic bile transit rate was 65 ± 25 mm^2/min for control subjects and 65 ± 21 mm^2/min for CAC patients. The adjusted mean rate was 38 ± 8 mm^2/min/1,000 mm^2 for control subjects and 40 ± 11 mm^2/min/1,000 mm^2 for CAC patients. None of these values was significantly different between the control subjects and the CAC patients (Table 1). A good correlation ($r = 0.71$) of the adjusted mean transit rate ($P < 0004$) was obtained independently by 2 observers (Fig. 3).

Gallbladder Emptying

The gallbladder mean ejection fraction of 17% ± 8% for CAC patients was significantly lower ($P < 0.00001$) than the mean value of 56% ± 19% for control subjects. Fluctuations in the total number of pixels in each frame beyond

40 min merely represented gallbladder size variation primarily due to respiratory movement and not due to bile emptying. This was confirmed in all 20 subjects by a continuous upslope of the time–activity curve, which represents bile volume within the gallbladder (Fig. 2).

DISCUSSION

The results of our study show that hepatic bile enters the gallbladder continuously during fasting and does not show any evidence to suggest that it empties and fills much like a bellows. However, the quantity of hepatic bile entering the gallbladder (vs. duodenum) during fasting is widely variable depending on the tonus of the sphincter of Oddi and the absorptive capacity of the gallbladder epithelium. The mean hepatic bile entry values of 71% (range, 44%–96%) in control subjects and 59% (range, 10%–98%) in CAC patients were not significantly different ($P > 0.05$). Assuming a normal bile production rate of 0.4 mL/min (600 mL/24 h), mean values of 59% for CAC patients and 71% for control subjects would represent hepatic bile entry into the gallbladder of approximately 0.23 and 0.28 mL/min, respectively. Because the gallbladder is already filled to its full volume before radiotracer injection, the gallbladder epithelium must therefore absorb 0.23–0.28 mL of water per minute to be able to accommodate this constant inflow of hepatic bile. Our results indicate that the mean hepatic bile transit rate of 65 mm^2/min for control subjects is approximately equivalent to an absorption rate of 0.28 mL/min of water through the gallbladder epithelium. In control subject 2 and in CAC patient 10, all hepatic bile entered the

gallbladder, resulting in nonvisualization of the intestine during the hepatic phase imaging. Therefore, in these 2 patients the entire 0.4 mL/min of hepatic bile entered the gallbladder during fasting. Intestinal bile flow values of 4% and 2% for these 2 patients (Table 1) merely represent uncorrected background counts.

Water and electrolytes are transported from the gallbladder lumen into the interstitium through lateral water channels situated between the columnar epithelial cells (6,7). Our results suggest that these water channels function normally in patients with CAC and that the fluid transfer rate is very similar to that of the control subjects. Under these circumstances, one would expect that the total volume of hepatic bile and the number of [99mTc]-labeled hepatic iminodiacetic acid (HIDA) counts entering the gallbladder in a cholescintigraphic study would be similar for control subjects and patients. This was found to be true in our study: The quantity of hepatic bile entering the gallbladder was not significantly different between control subjects and CAC patients. Furthermore, Fisher et al. (8) have shown a mean counting rate of 72,000 counts per minute in healthy subjects and 78,100 counts per minute in patients with gallstones at 60 min after intravenous injection of 185 MBq (5 mCi) [99mTc]-HIDA.

[99mTc]-Mebrofenin is a lidocaine analog that is taken up by hepatocytes by mechanisms that are very similar to those of other organic anions, such as bilirubin, bile acids, indocyanine green, and sulfobromophthalein sodium. Uptake by hepatocytes occurs along the basolateral domain primarily through receptor-mediated endocytosis. Bile acid and free
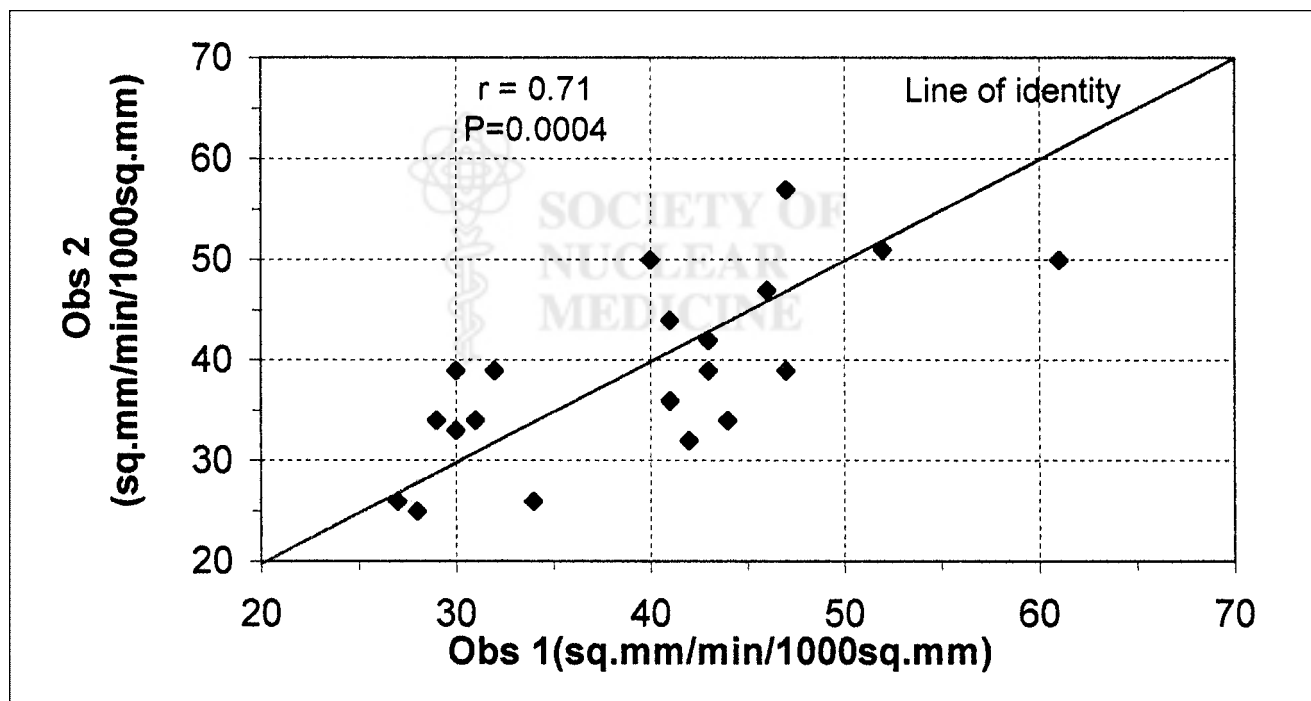

FIGURE 3. Reproducibility of bile transit rate measurement. Transit rate measured independently by 2 observers (Obs 1 and Obs 2) shows good correlation ($r = 0.71$; $P = 0.0004$).

fatty acid pathways are also used (*9*). After uptake, ^{99m}Tc-mebrofenin is secreted into bile canaliculi in its native form by an as-yet undefined mechanism and serves as an excellent in vivo radiolabeled hepatic bile marker. After traveling through intrahepatic and extrahepatic bile ducts, radiolabeled hepatic bile enters the gallbladder and duodenum. Approximately 98% of the injected dose of ^{99m}Tc-mebrofenin is normally taken up by the liver and secreted into bile, allowing an excellent delineation of the entire hepatobiliary system. ^{99m}Tc is an ideal radionuclide for gamma-camera imaging and quantification.

Hepatic bile entering the gallbladder is confined initially to its central long axis (Fig. 1) and does not appear to be perturbed during each respiration, despite the gallbladder's movement up and down with the liver. Hepatic bile gradually travels towards the periphery of the gallbladder in a lamellar fashion at a mean rate of 65 mm^2/min in control subjects and CAC patients. The adjusted rate of 38 mm^2/min/1,000 mm^2 for the control subjects and 40 mm^2/min/1,000 mm^2 for the CAC patients does not differ statistically between the 2 groups.

Selective absorption of water and electrolytes by the epithelium results in a relatively higher solute concentration of gallbladder bile compared with the newly entering hepatic bile during fasting. The concentration of some solutes (bile salts) can be 8–10 times higher in gallbladder bile than in hepatic bile (*10*). The mean transit time of 25 min (travel time from the central axis to the wall) for control subjects and CAC patients indicates that hepatic and gallbladder bile mixing, in vivo, does not occur instantly as soon as hepatic bile enters the gallbladder. Instead, mixing appears to occur in a lamellar pattern. The difference in solute concentration between the 2 bile solutions within the gallbladder appears to keep them apart, until a space is created at the periphery by the absorption of water and electrolytes by the epithelium. The time taken for creation of this space at the periphery appears to account for the lamellar pattern of hepatic bile movement within the gallbladder lumen (Fig. 1). This finding has an important implication while hepatic bile flow into or out of the gallbladder is measured using either chemical (indocyanine green) or radiolabeled (^{99m}Tc-HIDA) hepatic bile markers (*4,11*). Our findings suggest that one must allow at least 25–30 min after the first appearance of the tracer within the gallbladder lumen for complete mixing to occur before measuring gallbladder emptying with either a fatty meal or CCK-8 stimulation. In contrast to this delay in in vivo mixing, in vitro mixing occurs quite rapidly. In a simple experiment, a drop of India ink placed on the top of a saline column in a 50-mL syringe mixes with saline within 2 min. Complete mixing occurs almost instantly when the syringe is shaken gently to simulate gallbladder movement during respiration.

In control subjects and CAC patients, the gallbladder showed a continuously rising time–activity curve during the entire 60-min period of hepatic phase imaging. Fluctuations in the number of pixels (area) between 40 and 60 min, after the radiolabeled hepatic bile reaches the gallbladder wall, are merely an indication of gallbladder movement during respiration and should not be interpreted as evidence of its emptying and refilling during fasting. A continuous upslope of the gallbladder time–activity curve is confirmation of this physiology (Fig. 2). Gallbladder emptying during fasting, if it occurs, would be evident on the time–activity curve as a downslope. Primarily, on the basis of variation in gallbladder size found by sonography, some authors have proposed a theory that the gallbladder fills during fasting much like a bellows (*12*). Our findings do not support this hypothesis. Earlier, we have suggested alternative explanations for size variations found by sonography, including technical artifacts, inability to track the identical long axis for all frames during measurement, and possible gallbladder contraction without evacuation (*13*). Our results confirm that the apparent size variation during fasting of a fully filled gallbladder is mainly caused by respiratory movement and not by bile emptying and refilling. On the rare occasion when the gallbladder does empty spontaneously during fasting, it is usually accompanied by a reduction in size and total number of counts (G.T. Krishnamurthy and S. Krishnamurthy, unpublished data, 2000). Size variation alone, unaccompanied by a simultaneous change in counts, is a mere reflection of gallbladder movement due to respiration.

CAC is primarily a functional abnormality of the gallbladder without any easily demonstrable morphologic abnormalities and thus differs from that of cystic duct syndrome (CDS). CDS, described by Cozzolino et al. (*14*) in 1963, is characterized by impedance to bile flow out of the gallbladder due to mechanical obstruction within the cystic duct, which can be shown readily by histopathologic examination. More recent studies have provided new insights into our understanding of the pathophysiology of CAC. Amaral et al. (*15*) have shown a correlation between in vivo and in vitro gallbladder response to CCK-8. The decrease in emptying of the gallbladder in CAC patients is caused by a defect in the smooth muscle contractile apparatus, not by any reduction in the total number of CCK-A receptors in the wall smooth muscle. Our results now document that the concentration function remains normal, but contraction and emptying of the gallbladder are abnormal. CAC affects predominantly women in their middle age. Eight of 10 CAC patients in our study were women. The physical examination and liver function tests were normal, and the gallbladder did not show any morphologic abnormalities on either CT or sonographic examination. Physicians unfamiliar with quantitative cholescintigraphy may not obtain these functional studies and, therefore, the diagnosis of CAC may be delayed for many months or years. These patients usually undergo significantly more diagnostic procedures, often invasive, than patients with gallstones (*16, 17*). Cholecystectomy is the therapy of choice for pain relief. In our study, 8 of 10 patients had 100% pain relief and the 2 remaining had >80% pain relief from laparoscopic cholecystectomy. Similar results have been reported earlier (*18*).

Because of the high specific activity of radiolabeled hepatic bile, identification of its time of arrival into the gallbladder and the time to reach the gallbladder wall (transit time) are easy to recognize on cholescintigraphy. The determination of the maximum number of pixels and the net transit area in each frame and the calculation of the transit rate are quite simple by entering time and the number of pixels in the tabular format shown in Table 2. Manual calculation usually takes approximately 15–20 min for each patient. However, the entire technique for calculation of functional parameters, as outlined in Table 1, is amendable to simplification by developing semiautomatic computer software for tracing of the hepatic bile boundary for each frame (isocontour lines).

We have introduced a new, noninvasive, and quantitative test for measurement of the concentration function of the gallbladder, supplanting our previous work on contraction function (*3*). All of the ingredients (gamma camera, computers, radiopharmaceuticals, and the hormone cholecystokinin) necessary for measurement of concentration and contraction functions of the gallbladder are approved by the Food and Drug Administration and are readily available in most nuclear medicine departments throughout the world. All of the quantitative functional parameters that we described can be measured as part of a single ^{99m}Tc-HIDA study in the evaluation of hepatobiliary diseases. We anticipate that this new technique will promote a better understanding of gallbladder function in health and during disease. We have studied patients with only CAC and shown that the concentration function is normal, but contraction and emptying are abnormal. This test may provide new insights into our understanding of hepatic bile movement within the gallbladder in patients with cholesterol versus pigment stones or in those patients who are receiving different medications that either promote or prevent formation of microcrystals, bile sludge, or gallstones within the gallbladder. The potential ability to absorb the entire volume of hepatic bile by the gallbladder epithelium may explain why the common bile duct may not dilate for many days in some patients with acute obstruction due to a stone dislodged from the liver or gallbladder.

CONCLUSION

Hepatic bile continuously enters the gallbladder during fasting and moves from the long central axis toward the periphery in a lamellar fashion. Measurement of the rate of movement (mm^2/min) of ^{99m}Tc-HIDA–labeled hepatic bile within the gallbladder lumen from the central axis to the periphery may serve as an indicator of the rate of absorption of water by the gallbladder epithelium and, hence, its concentration function. The gallbladder in patients with CAC maintains a normal concentration function, but the contraction and bile emptying are abnormal. The new technique has the potential to provide information about the concentration and the contraction functions of the gallbladder after a single dose of ^{99m}Tc-HIDA.

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