Filling, Postcholecystokinin Emptying, and Refilling of Normal Gallbladder: Effects of Two Different Doses of CCK on Refilling: Concise Communication

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A study of 18 normal subjects was undertaken to evaluate the rapidity of gallbladder filling with Tc-99m IDA during fast, and the degree of emptying and incidence of refilling after intravenous infusion of either 20 or 40 ng/kg of octapeptide of cholecystokinin (CCK-8). During fast, the frequency of onset of gallbladder filling with Tc-99m IDA at 20, 30, 40, and 42 min was 68%, 84%, 95%, and 100%, respectively. Following CCK-8 infusion, the gallbladder mean ejection period of 11 ± 4 (s.d.) min and ejection fraction of 40% ± 23 were similar in subjects given either a 20 ng/kg or a 40 ng/kg dose. The frequency of onset of refilling at 20, 30, 40, and 50 min after CCK-8 was 33%, 63%, 71%, and 79%, respectively for the combined groups. At 50 min after CCK-8, all subjects given 20 ng/kg refilled, whereas only 63% of the 40-ng/kg group did so. There was no correlation between the degree of emptying (ejection fraction) and onset of refilling. We conclude that the frequency of post CCK-8 onset of gallbladder refilling, which reflects sphincter tone recovery, is widely variable, depending upon both the dose and the duration from the time of infusion of CCK-8 and should be taken into consideration if a preemptying procedure is adopted during hepatobiliary imaging with Tc-99m IDA agents.

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The gallbladder normally fills with hepatic bile during a fast, and empties its contents into the duodenum upon stimulation by cholecystokinin (CCK) given either exogenously or released endogenously following a meal. At present it is not clear how soon a normal gallbladder begins to empty and how long it continues to empty following infusion of CCK. Once the CCK infusion is stopped, it is not known how soon the sphincter of Oddi regains its tone, thus facilitating refilling of the gallbladder. The fraction of hepatic bile that enters the gallbladder is controlled primarily by the tone of the sphincter of Oddi. The temporal relationship between cholecystokinin stimulation and gallbladder emptying and refilling is poorly understood primarily because of unavailability of an appropriate test.

In recent years, the introduction of technetium-99m-labeled iminodiacetic acid analogs (Tc-99m IDA) as bile markers has provided investigators with a new method to study the biliary physiology thoroughly without the use of sedation, intubation tubes, or large quantities of intravenous contrast agents (*1*). The wider use of IDA analogs has resulted in the establishment of many new functional parameters (*2,3*). It has been suggested that the gallbladder be emptied routinely or when the patient has fasted longer than 24 hr, to facilitate refilling (*4,5*). In either case it is important to know how soon after an infusion of CCK, the gallbladder begins to fill again. If it does not begin to fill early enough, a normal gallbladder might suggest cystic duct obstruction.

The present study was undertaken to explore further the variables of normal biliary physiology with respect

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TABLE 1. GALLBLADDER FILLING DURING FAST, EMPTYING, AND REFILLING FOLLOWING 20 AND 40 NG/KG OP-CCK IN NORMAL SUBJECTS

Serial #	Age	Sex	Ht (cm)	Wt (kg)	Filling: Time of appearance (min)	Filling: Filling fraction (%)	Emptying: Ejection period (min)	Emptying: Ejection fraction (%)	Refilling index (RF/FF) after CCK-8: 20 min	30 min	40 min	50 min
Group A: 20 ng/kg												
1	46	F	172	63	18	10	16	24	0	1.99	—	—
2	38	F	167	63	22	8.5	12	46	0	1.25	1.25	1.74
3	30	F	162	76	24	26	10	20	0.96	0.96	1.0	2.11
4	47	M	182	70	18	4.8	5	17	0	0	0	1.33
5	39	M	172	77	20	22.8	9	59	1.04	1.44	2.31	2.31
6	38	M	188	79	20	14.3	10	45	0.82	1.23	1.42	1.66
mean ± s.d.				71 ± 7	20 ± 2	11 ± 8	10 ± 4	35 ± 17				
Group B: 40 ng/kg CCK-8												
1	53	M	188	90	20	5.1	11	23	0	0	0	0.25
2	22	F	168	57	14	7	5	87	0.63	0.83	0.91	0.91
3	27	M	171	58	14	5.6	5	19	1.79	1.79	2.57	2.79
4	28	F	166	68	14	3.1	10	45	0	0	0	0
5	28	M	182	79	34	0.8	7	15	0	3.5	3.5	—
6	27	M	170	54	14	1.8	10	50	0	0	0	0
7	50	F	175	98	22	8.1	13	15	0.8	—	—	—
8	40	F	170	63	16	1.8	10	45	0	—	—	—
9	48	F	165	104	14	1.6	15	27	0	—	—	—
10	28	M	170	68	16	5.4	16	68	0.11	0.61	—	—
11	48	F	165	102	42	2	21	88	0	0	—	—
12	40	F	170	64	32	1.5	13	34	0	0	—	—
Mean ± s.d. 37 ± 11			172 ± 7	75 ± 18	21 ± 10	3.7 ± 2.5	11 ± 5	43 ± 26				

to (a) how soon the normal gallbladder begins to fill with Tc-99m IDA in the fasting state; (b) how soon and how long it empties following infusion of two different doses of octapeptide of cholecystokinin (CCK-8); (c) how much it does empty; (d) how soon the sphincter of Oddi regains its tone to promote refilling of the gallbladder following cessation of CCK-8 infusion; (e) whether there is any correlation between the degree of emptying and the rapidity of refilling; and (f) whether pre-emptying hastens or delays gallbladder refilling.

Since the day-to-day tone of the sphincter of Oddi may be variable depending upon the duration of fast, among other factors, the present study was designed to study all three functions (filling during fast, post CCK-8 emptying, and refilling with Tc-99m IDA) after an overnight fast.

MATERIAL AND METHODS

Eighteen normal adult volunteers (8 male, 10 female) ranging in age from 22 to 53 yr (mean 33 yr) were chosen. Their good health was confirmed by medical history, physical examination, and liver function tests (total bilirubin, alkaline phosphatase, albumin, total protein, glutamic transaminase, and lactic dehydrogenase). Ultrasound study indicated normal liver and gallbladder. The subject's informed consent was obtained for the institutional subcommittee for human studies. Subjects were divided into A and B groups according to the dose of CCK-8 used to stimulate gallbladder emptying: six subjects in Group A received 20 ng/kg and 12 subjects in Group B received 40 ng/kg (Table 1).

Data collection. This was conducted in two phases. The first phase (for gallbladder filling) lasted for 60 min. The second phase (gallbladder emptying and refilling) lasted for another 60 min (Tables 1,2).

First phase. After an overnight fast (minimum 8 hr) each subject received 1.5 mCi i.v. of commercial Tc-99m parabutyl-IDA (PBIDA) or 5 mCi of Tc-99m dimethyl-IDA (HIDA). The difference in dosage was planned because the liver's excretion half-time is longer for PBIDA than for HIDA (107 min compared with 42). The intent was to have at least 500 μCi in the liver when the second phase started, at 60 min after Tc-99m IDA injection. Using a parallel-hole collimator, analog images were made every 2 min for an hour, while a computer stored images at one frame/min in a 64 × 64 matrix.

Second phase. After the first-phase collection, a 5-mm pinhole collimator was substituted and focussed over the gallbladder in a way that best separated the gallbladder from the bile ducts and intestine. Two-minute images and one-minute digital data were obtained as before, for another hour. A saline placebo was infused i.v. at 5 min after the second phase began. At 10 min, CCK-8 was infused over 3 min with a Harvard infusion pump, either 20 ng/kg (Group A) or 40 ng/kg (Group B).

TABLE 2. GALLBLADDER FILLING, EMPTYING, AND REFILLING IN NORMAL SUBJECTS GIVEN TWO DIFFERENT DOSES OF OP-CCK (MEAN ± S.D.)

CCK-8 Dose	Filling		Emptying		Frequency of refill at			
	Time of appearance (min)	Filling fraction (%)	Ejection period (min)	Ejection fraction (%)	20	30 min	40	50
Group A 20 ng/kg (n = 6)	20 ± 2	11 ± 8	10 ± 4	35 ± 17	50%	83%	83%	100%
Group B 40 ng/kg (n = 12)	21 ± 10	3.7 ± 2.5	11 ± 5	43 ± 26	25%	50%	63%	63%
Group A & B Combined (n = 18)	21 ± 0.8	7.1 ± 7	11 ± 4	40 ± 23	33%	63%	71%	79%

DATA ANALYSIS

First phase. Two equal regions of interest were chosen—one over the gallbladder and the other over the liver for background—and a net time-activity curve, (decay-corrected) was generated for the gallbladder (Fig. 1). Analog images were inspected carefully and the earliest time of the appearance of the gallbladder was recorded. The gallbladder filling fraction (FF), at the time of its appearance, was calculated by the following equation:

$$\text{GB Filling Fraction (\%)} = \frac{\text{1-min GB cts at its appearance} - \text{1-min baseline ct at 10 min}}{\text{1-min GB cts at 59-60 min}} \times 100$$

The filling fraction at appearance time essentially represents the fraction of the total 60-min gallbladder radioactivity required to visualize it on analog images. The early rise in the gallbladder curve is probably due to unsubtracted hepatic uptake anterior to the gallbladder. The 10-min count is considered to represent this foreground peak activity due to liver uptake (*6*).

Second phase. Two equal regions of interest were chosen: one over the entire gallbladder and the other superior and lateral to the gallbladder, representing liver background. The net gallbladder time-activity curve was obtained after subtraction of the normalized background, and the gallbladder's net count was decay-corrected. The gallbladder ejection fraction was calculated as (*2*).

$$\text{GB Ejection Fraction (\%)} = \frac{\text{net GB cts before CCK-net GB cts after CCK}}{\text{net GB cts before CCK}} \times 100$$

The period from beginning to end of gallbladder emptying (ejection period) was noted from the curve. The gallbladder refilling fraction (RF, Fig. 2) was calculated for every 10 min, beginning 20 min after CCK-8 infusion up to the termination of the study, using the equation:

$$\text{Refilling fraction (\%) after CCK-8 at time (t)} = \frac{\text{GB cts after CCK-8 at time (t)} - \text{Cts at the end of GB ejection period}}{\text{GB cts just before OP-CCK infusion}} \times 100.$$

We have chosen to calculate the refilling fraction for every 10 min. A refilling index (RI) was obtained by dividing refilling fraction by filling fraction. This normalizes the results and compensates for the differences in Tc-99m IDA dose between the two groups. Since the liver continuously excretes HIDA and PBIDA, thus decreasing specific activity of the hepatic bile, a refilling value equal to or greater than half of the filling fraction was considered to indicate gallbladder refilling. Table 1 shows the filling fraction and the refilling index. Refilling fraction = FF × RI.

RESULTS

The time of gallbladder appearance during fast was 20 ± 2 min (mean ± s.d.) in Group A, 21 ± 10 min in Group B ($p < 0.05$) and 21 ± 8 min for the combined groups. At 20, 30, and 40 min after injection of Tc-99m IDA, 68%, 84%, and 95% respectively of the gallbladders were visualized. By 42 min, all gallbladders were visualized (Table 1). Mean filling fraction was 11% ± 8% in Group A and 3.7% ± 2.5% in Group B, the differences reflecting mainly the effects of dose: 5 mCi for the former and 1.5 mCi for the latter.

The mean ejection period was 10 ± 4 min in Group A and 11 ± 5 min in Group B ($p > 0.05$) and 11 ± 4 min for the combined group. The mean ejection fractions were 35% ± 17% in Group A, 43% ± 26% in Group B ($p > 0.05$) and 40% ± 23% in the combined group. There was no significant difference between the two groups in the gallbladder appearance time, ejection period, and ejection fraction ($p > 0.05$). In Group A subjects (dose 20 ng/kg), the frequency of refill at 20, 30, 40, and 50 min post CCK-8 was 50, 83, 83, and 100% respectively. The corresponding values in Group B subjects (40 ng/kg) were 25, 50, 62, and 63%, respectively. When the two groups are combined, these frequencies become 33%, 63%, 71%, and 79%. There was no correlation between the degree of emptying (ejection fraction) and the 30-min refilling fraction (Fig. 3).

DISCUSSION

The fraction of hepatic bile entering the gallbladder is controlled primarily by the tone of the sphincter of Oddi and the resulting differences in hydrostatic pressure at various levels of the biliary tract. The resting mean pressures in the Oddi sphincter, common bile duct, and gallbladder are known to be about 15, 12, and 10 cm H_2O respectively (*7,8*). The sphincter tone fluctuates, thus releasing bile into the duodenum entry or diverting it into the gallbladder.

Of all of the known cholecystogogues, cholecystokinin is considered to be the principal hormone that controls gallbladder contraction and emptying. In man the serum half-time of exogenously administered cholecystokinin is 2.5 min (*9*). The mean ejection period of 11 min for our combined group suggests that gallbladder emptying lasts for about $4\frac{1}{2}$ serum CCK half-time. The mean ejection periods did not differ significantly between Groups A & B (10 compared with 11). Both groups also had similar ejection fractions. There was a clear difference, however,

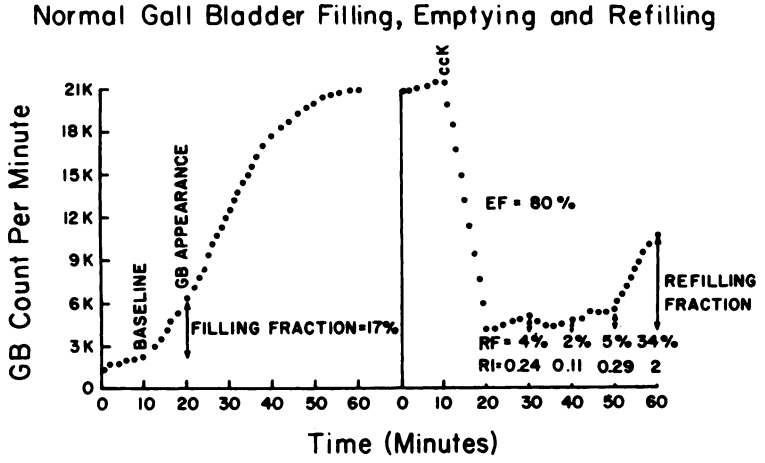

FIG. 1. Calculation of filling, emptying, and refilling: gallbladder filling fraction at 20 min when seen on analog images is 17% (left). Gallbladder empties 80% of its contents after CCK-8 infusion, with ejection period 10 min (right). Small fluctuating peaks at 30, 40, and 50 min are considered as noise and true gallbladder refilling starts at 60 min, with refilling index of 2.0.

with regard to the frequency of post-CCK refill. Group A (20 ng/kg) had 50% refilling at 20 min and 100% at 50 min. Group B (40 ng/kg) had 25% and 63%. This suggests that the larger CCK-8 dose may not promote greater emptying but it does delay refilling. This might result from prolonging the increased tone of the gallbladder, or prolonging the relaxation time of the sphincter of Oddi, or both.

Since all fasting subjects showed gallbladder filling within 42 min in but only 83% (A) and 63% (B) after CCK it seems logical to use fasting subjects to facilitate rapid gallbladder filling. If a CCK-8 dose of 40 ng/kg is used, a period longer than 50 min should be allowed before injecting Tc-99m IDA. Baker et al. and Klingensmith et al. showed that in normal subjects fasting hastened gallbladder filling and oral feeding delayed it, probably secondary to the prolonged effects of endogenous cholecystokinin (*10,11*). It appears logical, then, to increase sphincter tone as much as possible to facilitate rapid gallbladder filling. The factors that decrease sphincter tonus will delay filling. Note, however, that fasting longer than 24 hr may have an opposite effect, namely, the gallbladder with a patent cystic duct may not fill with Tc-99m IDA (*5*). In such cases, pre-emptying appears logical.

We do not know yet whether a diseased gallbladder responds to cholecystokinin like a normal one. Only if they react alike may one claim that fasting is the best method to facilitate rapid filling. Since the durations of action of cholecystokinin on the diseased gallbladder and the sphincter are not known, we cannot say whether pre-emptying hinders or helps GB refilling. Until this question has been settled, it may be best to use fasting to fill the gallbladder for routine Tc-99m IDA imaging but to consider pre-emptying when the duration of the fast is longer than 24 hr. In the latter case, the Tc-99m IDA injection should be given 30–60 min after the cholecystokinin, depending on the CCK dose.

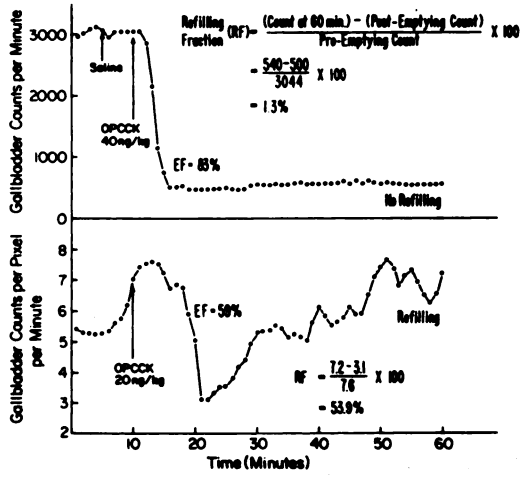

FIG. 2. Gallbladder in Subject A (upper) was seen at 20 min, with filling fraction 5.1%. Subject received CCK-8, 40 ng/kg. Refilling fraction at 50 min is 1.3%, with refilling index of 0.25 (1.3/5.1), considered "no refill." Gallbladder in Subject B (lower) was also seen at 20 min, with filling fraction of 22.8%. Subject received OP-CCK, 20 ng/kg, and ejection fraction was 53%. Refilling index in this subject is 2.31 (53.9/22.8), indicating definite refill.

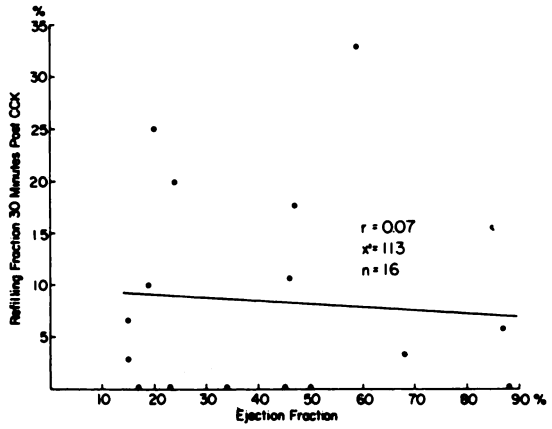

FIG. 3. Correlation between gallbladder ejection fraction (X) and 30-min refilling fraction (Y) is negligible (r = 0.07) suggesting that magnitude of emptying has no effect on degree of refill at 30 min after CCK-8.

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