Release of Cholecystokinin in Man

Correlation of Blood Levels with Gallbladder Contraction

ISIDORO WIENER, M.D., KAZUTOMO INOUE, M.D.,* CHARLES J. FAGAN, M.D.,†
PER LILJA, M.D.,‡ LARRY C. WATSON, M.D., JAMES C. THOMPSON, M.D.

From the Department of Surgery and the Department of Radiology The University of Texas Medical Branch Galveston, Texas

Although it is generally assumed that release of cholecystokinin (CCK) is the chief mechanism by which a fatty meal causes contraction of the gallbladder, measured release of CCK and gallbladder contraction have never been correlated. We have achieved this correlation in eight adult male volunteers, by means of a specific radioimmunoassay for CCK and by ultrasonographic imaging of the gallbladder. This study validates our CCK radioimmunoassay and correlates measured concentrations of CCK with changes in gallbladder size measured by ultrasonographic examination. Basal concentrations of CCK (82.6 ± 10.4 pg/ml) rose significantly to a maximum of 411.1 ± 79.9 pg/ml at 16 minutes after intraduodenal instillation of medium-chain triglyceride (Lipomul®). Mean basal volume of the gallbladder was 34.6 cm^3; maximum reduction of gallbladder volume (to one-third of original) was achieved at 18 minutes. Elevated CCK concentrations began to fall toward basal, and the gallbladder began to refill at 25 minutes. Results obtained after oral ingestion of Lipomul provided similar results. Linear regression analysis demonstrated excellent correlation between concentrations of CCK and gallbladder size during both contraction and relaxation phases. Future study of this correlation may be useful in patients with manifest dysfunction of the gallbladder, as well as in individuals known to be at risk of gallbladder disease.

SINCE 1928, WHEN FAT WAS demonstrated to cause contraction of the gallbladder in man[1], oral administration of fat has been used to stimulate gallbladder contraction during cholecystography. Exogenous cholecystokinin (CCK) may be used as an alternate stimulus in the radiographic study of gallbladder contraction[2]. Presumably, a fatty meal causes contraction of the gallbladder by release of CCK, although direct measurement of endogenous release of CCK by specific radioimmunoassay has never been correlated with gallbladder contraction.

In the present study, in normal subjects, we correlated radioimmunoassayable changes in endogenous concentrations of CCK in response to fat, with gallbladder contraction measured by ultrasonography.

Methods

Eight healthy male volunteers from our group of investigators participated in this study. Their ages ranged from 23 to 52 years, with a median age of 31 years. The protocol was approved by the Institutional Review Board (Human Research Committee, The University of Texas Medical Branch), and informed consent was obtained from each individual. After a fat-free evening meal, volunteers fasted for at least 12 hours before the study.

In the first study, a #12 French catheter was inserted through the mouth into the second portion of the duodenum, under fluoroscopic guidance. Blood samples were drawn before and at intervals after intraduodenal instillation of fat, and the plasma was stored at 4 C for future radioimmunoassay of CCK. Transverse and longitudinal echotomograms of the gallbladder were obtained byuse of a Varian®, real-time ultrasound unit (Model D-3000), with a 2.25 MHz phased-array transducer. To avoid blockage of sound transmission by gas in the colon, the gallbladder was visualized through an intercostal space and the right lobe of the liver. The transducer was aimed at a fairly constant position on the the patient's skin surface, but angled to obtain images depicting the largest transverse and longitudinal diameters of the gallbladder. Assuming that the gallbladder approximates an elliptical cylinder, we determined an approximate volume for each gallbladder, using its length, width, and height[3,4] (Fig. 1), even

Presented at the Annual Meeting of the American Surgical Association, Chicago, Illinois, April 22–24, 1981.

*Visiting Scientist from the First Department of Surgery, Kyoto University Medical School, Kyoto, Japan.

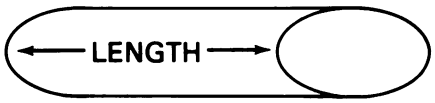
†Department of Radiology, The University of Texas Medical Branch, Galveston, Texas.

‡Visiting Scientist from the Department of Surgery, University of Lund, Lund, Sweden.

Supported by grants from the National Institutes of Health (AM 15241) and General Clinical Research Center Branch, DHEW Grant (RR 00073).

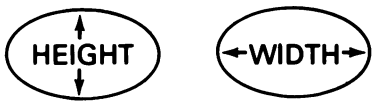
Reprint requests: James C. Thompson, M.D., Department of Surgery, The University of Texas Medical Branch, Galveston, Texas 77550.

0003-4932/81/0900/0321 $00.85



$$\text{VOLUME} = \pi \times \frac{\text{HEIGHT}}{2} \times \frac{\text{WIDTH}}{2} \times \text{LENGTH}$$

FIG. 1. Equation for calculating the approximate volume of the gallbladder.



though accurate measurement of the length of the gallbladder was not always possible. Results of calculations are expressed here and in previous papers[3,4] as gallbladder volume, but it should be recognized that the volume is only an estimate based on the assumption that length is constant. (Oral cholecystograms usually show a reduction in the length of the gallbladder after contraction in response to a fatty meal.) Calculations of volume, therefore, are approximate. The method provides results that are quite reproducible, and the small standard errors provide evidence that the changes in gallbladder size among the volunteers were relatively uniform.

After basal plasma samples were collected and baseline sonograms were obtained, Lipomul ® (71% fat/weight medium chain triglyceride (Lipomul-corn oil, Upjohn, Kalamazoo, MI) was instilled via the orodduodenal tube into the second portion of the duodenum in the dose of 1.5 ml/kg body weight. Echotomograms were then obtained at two, four, six, eight, ten, 12, 14, 16, 18, 20, 25, 35, 45 and 60 minutes after intraduodenal instillation of Lipomul (Figure 2 shows an example of gallbladder echotomograms obtained during contraction). Blood samples were drawn at the same times for radioimmunoassay of CCK.

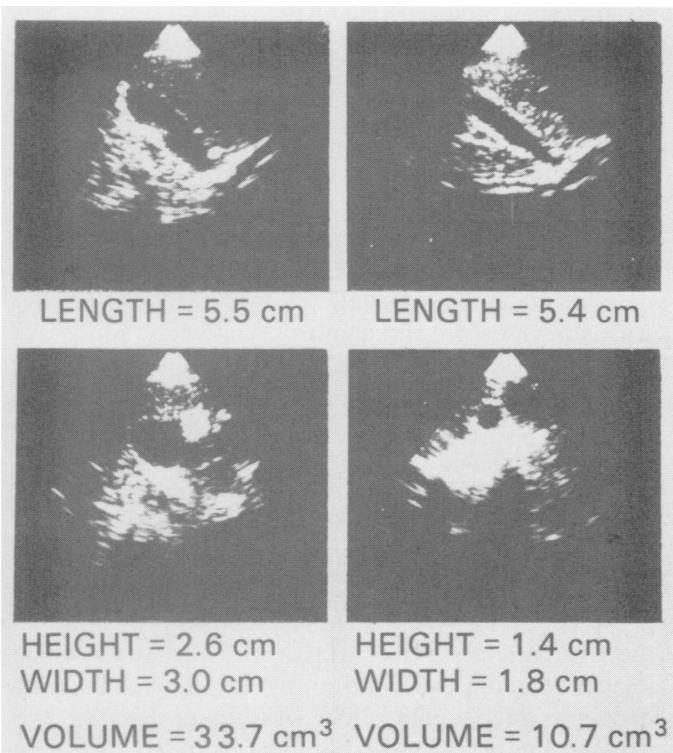


FIG. 2. Sequential echotomograms of the gallbladder showing the calculated volume at baseline and 18 minutes in two volunteers after intraduodenal Lipomul administration.

The protocol was repeated in the same volunteers on a different day without duodenal intubation. In this study, the subjects drank the same volume of Lipomul. Echotomograms were obtained and blood samples were collected at zero, two, five, ten, 15, 30, 45 and 60 minutes after ingestion of Lipomul.

The method employed for radioimmunoassay of cholecystokinin has been reported elsewhere[5]. Briefly, it employs CCK antibody UT 132, directed against the N-terminal portion of the CCK molecule and, therefore, has little or no affinity for gastrin or for the C-terminal octapeptide of CCK. Validation of the CCK radioimmunoassay has been reported[6,7].

The results are expressed as the mean ± one standard error of the mean. Student's paired t-test was used to analyze the data for statistical differences. Differences with a probability value of less than 0.05 were considered significant. Linear regression analysis was performed using the method of least squares.

Results

After intraduodenal instillation of Lipomul, mean basal concentrations of CCK (82.6 ± 10.4 pg/ml) rose significantly to 158.0 ± 26.3 pg/ml at six minutes (Fig. 3); maximal concentration (411.1 ± 79.9 pg/ml) was achieved at 16 minutes. Gallbladder volume decreased as CCK concentrations rose. The mean basal volume of the gallbladder was 34.6 ± 6.0 cm^3. At six minutes, gallbladder size was decreased significantly to 29.5 ± 5.5 cm^3 (88% of the original size); contraction continued for 25 minutes. The smallest gallbladder volume was observed at 18 minutes (11.5 ± 6.0 cm^3, one-third the mean original volume). Maximum gallbladder contraction occurred two minutes after maximum CCK concentrations were observed. During the contraction phase (0–16 minutes after instillation of Lipomul), rising concentrations of CCK were correlated

strongly with diminishing gallbladder volumes (r = −0.9818, Fig. 4).

Two subjects had a late release of CCK, with peak levels at 60 minutes, and with corresponding gallbladder contraction at 60 minutes in one subject and minimal contraction at 18 minutes in the other. (Results from all subjects are included in all calculations.) All subjects perceived borborygmi with intermittent hyperperistalsis and transitory feelings of oversatiety or mild nausea. All volunteers, except the two who had late release of CCK, had an average of three loose bowel movements from 40 minutes to 12 hours after the intraduodenal administration of Lipomul. These symptoms were similar to those noticed during previous infusion of exogenous pure CCK[5].

Refilling of the gallbladder began about 25 minutes after instillation of Lipomul (Fig. 3). As CCK concentrations fell toward basal levels, mean gallbladder volumes also began to return toward basal. As measured by linear regression analysis, CCK concentrations again correlated closely with gallbladder volumes in the 18–60 minute phase of relaxation (r = −0.9595, Fig. 4).

Oral ingestion of Lipomul caused significant release of CCK at two, 15, 30, 45, and 60 minutes, as compared with the basal concentration of 71.8±10.5 pg/ml (Fig. 5). The mean gallbladder volume was 20.4±2.2 cm³. The maximum release of CCK (196.4±39.0 pg/ml) occurred 15 minutes after ingestion of Lipomul. At the same time, the gallbladder was 7.5±1.2 cm³, 36% of the basal volume. One subject who had late release after intraduodenal instillation also had a late release of CCK after oral ingestion, with corresponding late gallbladder contraction. The other subject with late release on intraduodenal instillation, released only small amounts of CCK after oral ingestion of Lipomul. The volunteers were aware of increased bowel motility as the only symptom during the study; no diarrhea ensued. Linear regression analysis showed excellent correlation between CCK concentrations and gallbladder size (r = −0.9437) during the −15 minute contraction phase (Fig. 6), and during the 30–60 minute relaxation phase (r = −0.9010, Fig. 6).

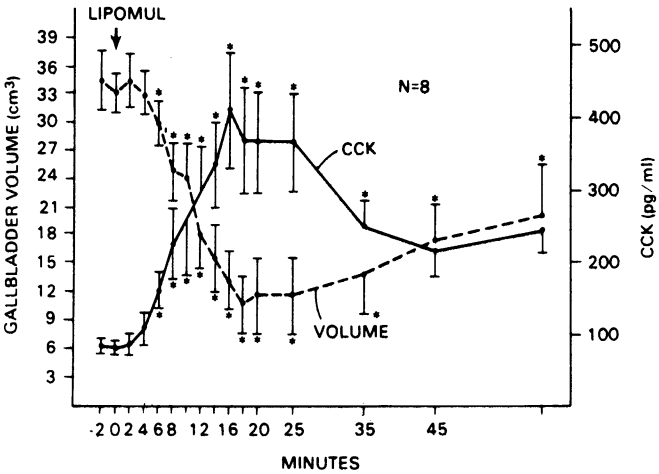


FIG. 3. Concentrations of plasma CCK (solid line) (mean ± SE) and gallbladder volume (broken line) in 8 normal men before and after intraduodenal instillation of Lipomul. Asterisks indicate $p < 0.05$.

Discussion

In 1919, Lyon[8] introduced direct study of bile in man by the technique of duodenal aspiration in gallbladder disease. Using a radiopaque iodinated compound that

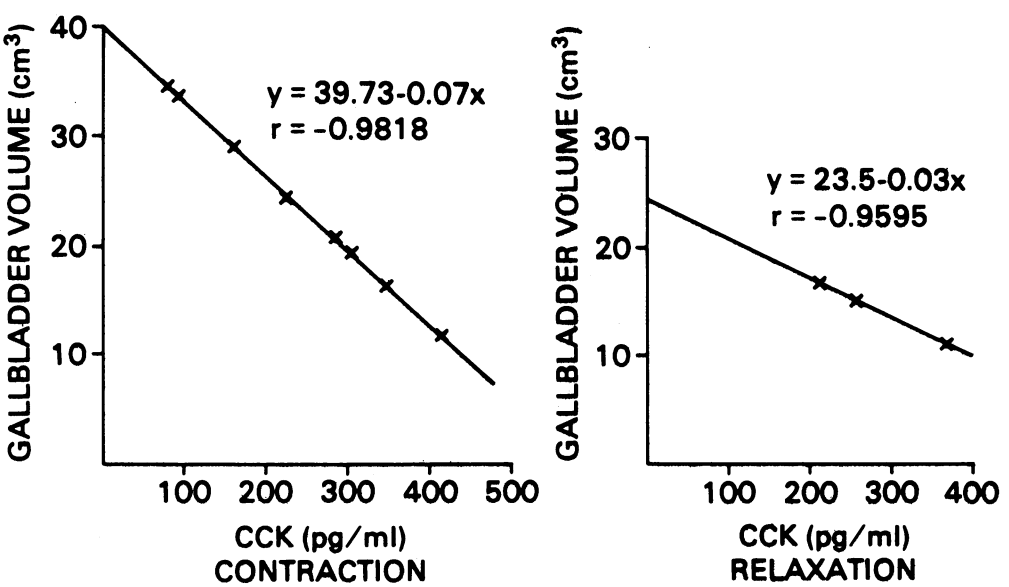


FIG. 4. Linear regression analysis of CCK levels and gallbladder volume during contraction phase (0–16 min) and relaxation phase (18–60 min) in 8 men after intraduodenal instillation of Lipomul.

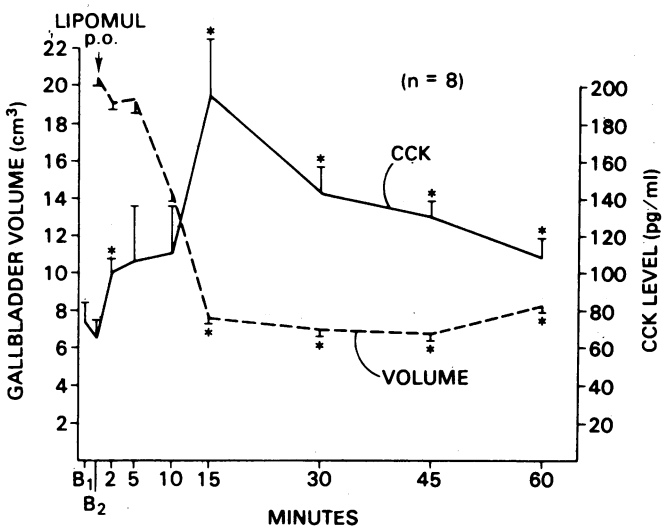


FIG. 5. Concentrations of plasma CCK (solid line) (mean ± SE) and gallbladder volume (broken line) in 8 normal men before and after oral administration of Lipomul. Asterisks indicate $p < 0.05$.

was excreted in the bile, Graham, Cole and colleagues, in 1925[9,10], developed oral cholecystography, which provided for the first time a simple, reliable clinical method for diagnosis of gallbladder disease.

Ivy and Oldberg[11] reported, in 1928, that extracts of duodenal mucosa caused contraction of the gallbladder. They named this activity cholecystokinin, but it was not until 1964 that Jorpes and Mutt isolated CCK in its pure form[12]. CCK is released from the duodenal mucosa in response to the presence of fat and certain amino acids in the upper small intestine. One of the main physiologic effects of CCK is to stimulate gallbladder contraction.

Studies of CCK in man have been restricted by lack of sensitive and precise methods for measuring blood levels and physiologic effects of the hormone. Some of the difficulties encountered in developing such methods reside in similarities of structure and actions shared by CCK and gastrin[13]. Furthermore, secretin is released simultaneously with CCK, and their actions may be difficult to separate.[14] In order to unravel the interrelationships among these hormones it is first necessary to achieve specific measurements of plasma concentrations; this is available by radioimmunoassay. Then, as a working standard, a biologic reference preparation is a requisite in order to permit correlation of the specific measurement of the hormone with its biologic action. Several attempts have been made to obtain a bioassay that would be reliable in measuring the effect of CCK on the gallbladder; one such method is the *in vitro* gallbladder strip[15].

We have developed and validated a specific radioimmunoassay technique for CCK[5]. The assay is further validated in the present study by the stable baseline concentrations of CCK, and the relatively narrow differences in the standard error of the mean concentrations.

The availability of a safe, reliable, clinical bioassay for CCK awaited development of gallbladder imaging by ultrasonography. Discrepancies in previous reports concerning timing of gallbladder contraction after a fatty meal result, most likely, from insensitive measuring techniques. After exogenous administration of CCK, maximum gallbladder contraction was seen at 15[2]–30[17] minutes, when measured by cholecystography, and at 20–25 minutes, when measured by intragallbladder balloon[18]. After a fatty meal, maximal contraction, as measured by oral cholecystography, occurred

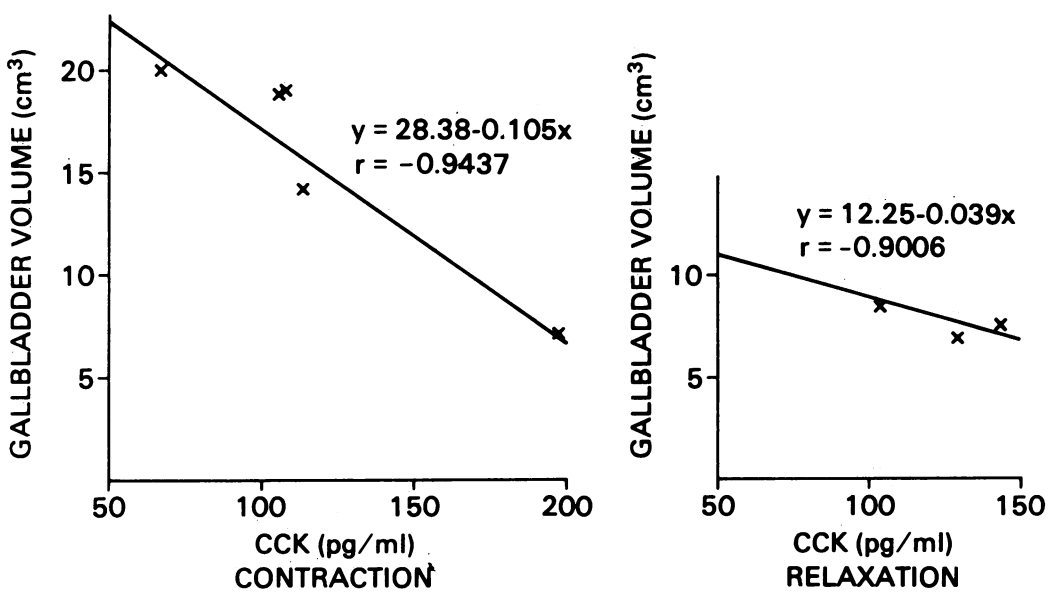


FIG. 6. Linear regression analysis of CCK levels and gallbladder volume during contraction phase (0–15 min) and relaxation phase (15–60 min) in 8 men after oral Lipomul.

40[16] to 60[17] minutes. Braverman, Johnson, and Kern[3] showed that ultrasonography is an acceptable way of assessing the approximate gallbladder volume[4]. The results of our present study provide strong corroboration. Maximal gallbladder contraction (one-third its original size) occurred at 18 minutes after intraduodenal instillation of Lipomul. The contraction response was rapid and nearly linear. Relaxation and refilling of the gallbladder have been reported to occur approximately 30 minutes after CCK administration[2,17]. In the present study, relaxation began approximately 25 minutes after endogenous CCK was released, at a time when plasma concentrations of CCK began to fall.

Concentrations of CCK were not as high after oral administration of Lipomul as those measured after intraduodenal instillation. The amount of Lipomul available to the duodenum at any one time in the oral study may be smaller, and it may be diluted with gastric juice. This could account for the higher concentrations of CCK observed during the intraduodenal stimulation. The time of maximal release of CCK was the same with both intraduodenal and oral administration of Lipomul.

The next step in our study was to correlate specific values of CCK obtained by radioimmunoassay with biologic activity of CCK (contraction of the gallbladder), measured by noninvasive ultrasonographic image as a bioassay in man. The sequential images provide a quantitative measurement of contraction of the gallbladder when it is exposed to CCK, and allow creation of a mathematic relationship between changing CCK concentrations and gallbladder contraction in normal man. This correlation provides a method for investigation into certain possibilities, not previously considered, in the mechanism of gallbladder disease. The method may make possible early detection of patients at risk of gallbladder disease, such as pregnant women, patients who have had vagotomy, or women taking oral contraceptives. The good correlation seen when Lipomul was given by mouth makes intraduodenal instillation unnecessary.

We conclude that CCK, as measured by a specific radioimmunoassay, is released by fat given by intraduodenal and oral routes. These studies in man demonstrate, for the first time, that both contraction and relaxation of the gallbladder are strongly correlated with measured plasma concentrations of CCK. This method may provide predictive information for gallbladder disease in subjects at risk.

References

1. Boyden EA. Analysis of reaction of human gallbladder to food. Anat Rec 1928; 40:147–191.
2. Edholm P. Gallbladder evacuation in the normal male induced by cholecystokinin. Acta Radiol Scand 1960; 53:257–265.
3. Braverman DZ, Johnson ML, Kern F, Jr. Effects of pregnancy contraceptive steroids on gallbladder function. N Engl J Med 1980; 302:362–364.
4. Ylostalo P, Kirkinen P, Heikkinen J, Maentausta O. Gallbladder volume in cholestasis of pregnancy. N Engl J Med 1981; 304:359.
5. Lonovics J, Guzman S, Devitt P, et al. Release of pancreatic polypeptide in humans by infusion of cholecystokinin. Gastroenterology 1980; 79:817–822.
6. Miyata M, Rayford PL, Thompson JC. Hormonal (gastrin, secretin, cholecystokinin) and secretory effects of bombesin and duodenal acidification in dogs. Surgery 1980; 87:209–215.
7. Rayford PL, Schafmayer A, Teichmann RK, Thompson JC. Cholecystokinin radioimmunoassay. *In* Bloom SR (ed) Gut Hormones. Edinburgh, Churchill Livingstone. 1978. pp.208–212.
8. Lyon BBV. Diagnosis and treatment of diseases of the gallbladder and biliary ducts, preliminary report on a new method. JAMA 1919; 73:980.
9. Graham EA, Cole WH. Roentgenologic examination of the gallbladder. Preliminary report of a new method utilizing the intravenous injection of tetraiodophenolphthalein. JAMA 1924; 82:613–614.
10. Graham EA, Cole WH, Copher GH. Cholecystography. The use of sodium tetraiodophenolphthalein. JAMA 1925; 84:1175–1177.
11. Ivy AC, Oldberg E. A hormone mechanism for gallbladder contraction and evacuation. Am J Physiol 1928; 86:599–613.
12. Jorpes JE, Mutt V, Toczko K. Further purification of cholecystokinin and pancreozymin. Acta Chem Scand 1964; 18:2408.
13. Thompson JC. Chemical structure and biological actions of gastrin, cholecystokinin and related compounds. *In* Holton P (ed) The International Encyclopedia of Pharmacology and Therapeutics. Oxford, Pergamon Press Limited. 1973. pp 261–286.
14. Meyer JH. Release of secretin and cholecystokinin. *In* Thompson JC (ed) Gastrointestinal Hormones, Austin, University of Texas Press. 1975. pp.475–489.
15. Lonovics J, Devitt P, Rayford PL, Thompson JC. Actions of VIP, somatostatin, and pancreatic polypeptide on gallbladder tension and CCK-stimulated gallbladder contraction in vitro. Surg Forum 1979; 30:407–409.
16. Sacchetti G, Mandelli V, Roncoroni L, Montanari C. Influence of age and sex on gallbladder emptying induced by a fatty meal in normal subjects. Am J Roentgenol Radium Ther Nucl Med 1973; 119:40–45.
17. Park CY, Pae YS, Hong SS. Radiological studies on emptying of human gallbladder. Ann Surg 1970; 171:294–299.
18. Jansson R. Effects of gastrointestinal hormones on concentrating function and motility in the gallbladder. Acta Physiol Scand Suppl 1978; 456:1–38.

Discussion

Dr. Philip Sandblom (Lausanne, Switzerland): I hope you will not find it preposterous of me to tell you about my first scientific work, done exactly 50 years ago, because it shows much of the same result that we just heard, but done, naturally, much less elegantly and with much cruder methods.

In those days, cholecystography had just started, led by Graham and Cole. I also had just read about A. C. Ivy's discovery of the hormone, cholecystokinin, which he found effective in cats and dogs.