Correlation between Release of Cholecystokinin and Contraction of the Gallbladder in Patients with Gallstones

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The role of endogenously released cholecystokinin (CCK) in mediating gallblader (GB) contraction was evaluated in 12 normal volunteers and 24 patients with gallstones (11 additional gallstone patients were excluded because of failure of adequate ultrasonographic visualization). CCK concentrations before and after oral administration of fat (Lipomul®) were measured by a specific radioimmunoassay. CCK release was correlated with changes in GB volume determined simultaneously by ultrasonography. On the basis of gallbladder contraction and operative findings, gallstone patients were divided into "contractors" (14), "noncontractors" (6), and "hydrops" (4). Lipomul caused prompt release of CCK in normal volunteers and all groups of gallstone patients. The changes (basal to peak) in plasma CCK (pg/ml) for the different groups were as follows: normal volunteers (108 ± 9 to 200 ± 16), contractors (77 ± 10 to 128 ± 13), noncontractors (59 ± 7 to 159 ± 38), and hydrops (43 ± 5 to 113 ± 47). The total integrated output of CCK (0–60 min) was greater in normal volunteers (3975 ± 762 pg–min/ml) than in contractors (1530 ± 567 pg–min/ml). Lipomul caused similar GB contraction in normal volunteers and contractors (from basal volumes to maximal contraction); these changes were from 19.5 ± 2.3 ml to 5.6 ± 1.0 ml in normal volunteers, and from 19.6 ± 3.2 to 5.2 ± 1.0 in contractors. Plasma concentrations of CCK and GB volume were highly correlated in the 12 normal volunteers ($r = -0.89$, $p < 0.01$) and in the 14 contractors ($r = -0.99$, $p < 0.01$)), but the GB was significantly ($p < 0.01$) more sensitive to changes in plasma CCK in the gallstone contractors than in the normal volunteers. The authors suggest that there may be two groups of gallstone patients, noncontractors and contractors. Stasis may be important in the pathogenesis of gallstones in the noncontractors, whereas in contractors, the authors speculate that an abnormality in the CCK–gallbladder relationship (characterized by diminished CCK release and increased GB sensitivity to CCK) may be involved in the evolution of the disease.

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THE PATHOGENESIS OF GALLSTONES is still incompletely understood, despite recent important advances, especially in studies on the lithogenicity of bile.[1] The role of the gallbladder itself in the development of gallstones is controversial.[2] Thureborn[3] suggested that hormonal and other metabolic disturbances might lead to temporary disorders of the extrahepatic biliary tree that produce stasis and then stone formation.

The chief humoral agent responsible for gallbladder contraction is cholecystokinin (CCK).[4] We have recently shown that release of CCK in man, measured by radioimmunoassay, is correlated closely with contraction of the gallbladder, as identified by ultrasonography.[5] Further studies revealed that plasma concentrations of CCK during infusion of pure cholecystokinin in man are similarly correlated with the gallbladder contraction that is demonstrated on cholecystosonography.[6]

The purpose of the present study was to use this method to explore the relationship between the release of CCK after a fatty meal and gallbladder contraction in patients with gallstones. The authors wish first to determine this relationship, then to compare the relationship in normal subjects and patients with those with gallstones. If it is abnormal in patients, we will attempt to relate this abnormality to the pathogenesis of gallstones.

Materials and Methods

Twelve healthy control subjects and 35 patients with gallstones volunteered for this study. The protocol was approved by the Institutional Review Board (the Human Research Committee, The University of Texas

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Medical Branch), and informed consent was obtained from each person. The presence of gallstones in the 35 patients was demonstrated by ultrasonography or by oral cholecystography, and was confirmed in each instance at the time of subsequent cholecystectomy. Eleven of the 35 gallstone patients were excluded from the study because adequate ultrasonographic images of the gallbladder could not be obtained. In these 11 patients, stone-filled or fibrotic, shrunken gallbladders contained insufficient bile to permit accurate measurement of gallbladder dimensions. The remaining 24 patients, consisting of 21 women and three men, with a mean age of 39 (range 19–70), were the study group. Of the 12 persons in the control group, ten were women, and the mean age was 31 years (range 19–48).

Each gallstone patient was given a fat-free diet while in the hospital. Control volunteers and gallstone patients fasted for at least 12 hours before the study. Blood samples were taken for determination of plasma CCK, and ultrasonographic images of the gallbladder were obtained. These studies were performed simultaneously in the basal state and at intervals for 60 minutes after oral Lipomul®, given in a dose of 1.5 ml/kg (Lipomul corn oil, Upjohn, Kalamazoo, MI, 71% fat/weight medium chain triglyceride).

Estimation of Gallbladder Volume

Gallbladder volume was estimated by repeated ultrasonographic measurements,[7–9] which were performed before and after ingestion of Lipomul. A real-time Varian ultrasonographic unit (Model D-3000), with a 2.25-MHz transducer, which permits a depth of view from 7 to 21 cm, was used to obtain anteroposterior and transverse echograms of the gallbladder. The gallbladder was visualized through an intercostal space over the right lobe of the liver to obviate blockage of sound transmission by colonic gas. The transducer was maintained at a constant position on the patient's skin surface and was angled to obtain images of the largest transverse diameter of the gallbladder. To estimate the approximate volume of the gallbladder, the authors used the method previously described by Everson and associates,[9] which uses width, length, and height, even though accurate measurement of the length of the gallbladder was not always possible in the contracted state. When changes in length could not be measured, it was assumed, for calculation, that the gallbladder length was constant. Images were recorded on Polaroid® film. Gallbladder volume was calculated from dimensions of length and cross-sectional areas by using the formula of an elliptic cylinder.[5] The volumes were then corrected to the more accurate "sum of cylinders" volumes by the formula $y = 0.6427x + 1.1$ (y = "sum of cylinders" volume; x = "single cylinder" volume)[9] (Fig. 1).

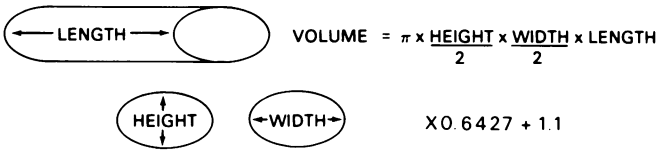

FIG. 1. Equation for calculating the approximate "single-cylinder" volume of the gallbladder, which may then be converted to the "sum of cylinders" volume by multiplying by 0.6427 + 1.1.[9]

Radioimmunoassay for CCK

Blood samples were collected in iced tubes containing 100 KIU of Trasylol® (Bayer, Leverkusen, Germany) and sodium heparin, 15 units/ml of blood. Plasma was separated by centifugation and stored at 4 C for later radioimmunoassay for CCK. All samples were measured in duplicate in the same radioimmunoassay.

The method used for radioimmunoassay of CCK was developed in the authors' laboratory and has been described in detail previously.[10] The method uses an antibody, UT 132, that recognizes most of the N-terminal part of the CCK molecule and has little affinity for gastrin. Validation of the CCK radioimmunoassay has been reported.[5,10–12]

Statistical Analysis

Results are expressed as the mean ± SEM. Correlations were evaluated by linear regression analysis. Differences between means and slopes of regression lines were evaluated by the Student's t-test, and those with a p value of <0.05 were considered to be significant.

Results

Correlation of the ultrasonographic studies with operative findings permitted division of the 24 gallstone patients into three groups, based on the contraction of their gallbladders (Fig. 2). Four patients were found to have hydrops of the gallbladder at operation; ultrasonographic studies on these patients showed large noncontracting gallbladders, with a mean volume of 99 ± 34 ml. Six other patients demonstrated slight, but not significant, contraction of the gallbladder and were analyzed separately ("noncontractors"). In the remaining 14 patients ("contractors"), the gallbladders, which had a mean basal volume of 19.6 ± 3.2 ml, were found to empty at the same rate after ingestion of Lipomul as those of the normal volunteers (basal volume 19.5 ± 2.3 ml). The residual volume after maximal contraction was

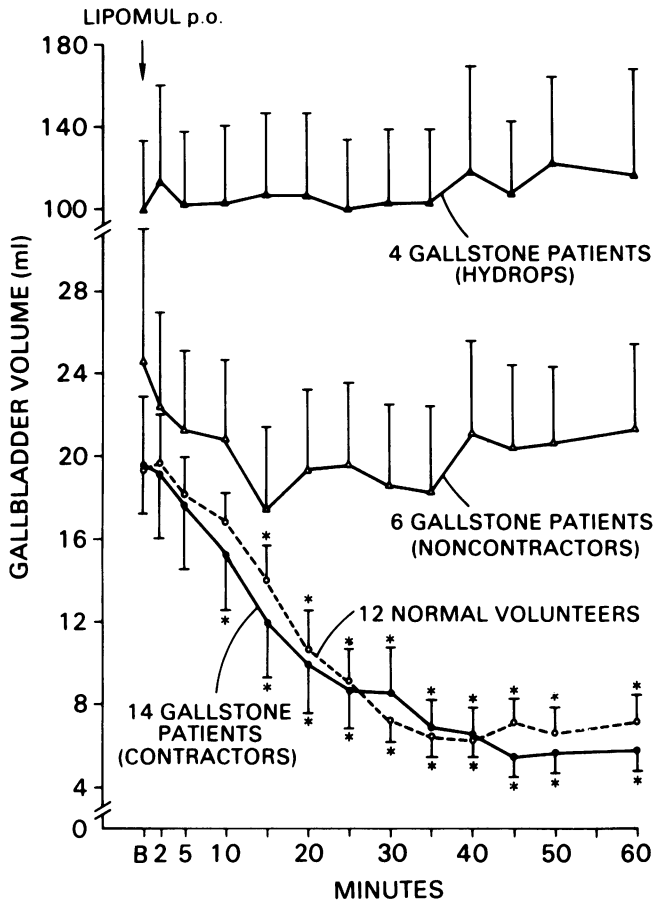

FIG. 2. Gallbladder volume in response to oral Lipomul in normal volunteers and in patients with gallstones (hydrops, noncontractors, and contractors). * = significant decrease from basal volume.

calculated in each person. This volume was found to be 5.2 ± 1.0 ml in the gallstone patients (contractors), which was not significantly different from the maximally contracted volume in normal volunteers (5.6 ± 1.0 ml). The normal volunteers showed a mean maximal emptying of their basal gallbladder volume of 70 ± 5%, and gallstone patients showed a maximal emptying of 70 ± 4%.

Plasma concentrations of CCK increased after administration of Lipomul in both normal subjects (Fig. 3) and gallstone patients (contractors) (Fig. 4), and this increase in CCK was closely associated in time with emptying of the gallbladder. At every sampling time, however, the gallstone patients (contractors) demonstrated significantly smaller release of CCK than did the normal subjects (Fig. 5). Not only are peak CCK concentrations much higher in the normal volunteers, but concentrations remain high throughout the 60-minute period, whereas CCK begins to fall at 40 minutes in the gallstone patients (contractors). (Note the difference in the scale of the right-hand (CCK) ordinate in Figures 3 and 4.) Furthermore, the integrated output of CCK during the 60 minutes after oral administration of Lipomul was much less (1530 ± 567 pg-min/ml) in the gallstone patients (contractors) than in the normal subjects (3975 ± 762 pg-min/ml). When the integrated CCK output for noncontractors is compared as well, there is no difference from that of the normal volunteers (Fig. 6).

Plasma CCK concentrations were highly correlated with gallbladder volume in normal subjects and in gall-

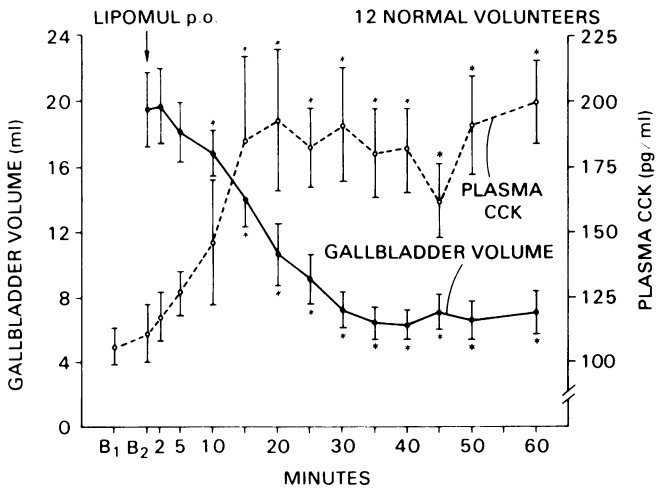

FIG. 3. Effect of oral Lipomul on simultaneously measured plasma concentrations of CCK and gallbladder volume in normal volunteers. * = significant change from basal.

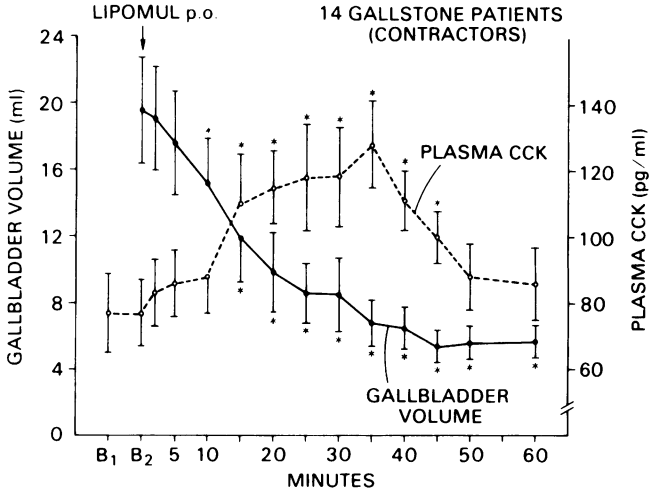

FIG. 4. Effect of oral Lipomul on simultaneously measured plasma concentrations of CCK and gallbladder volume in gallstone patients (contractors). * = significant change from basal.

stone patients (contractors), as shown in Figure 7. Sensitivity of the gallbladder to endogenously released CCK can be estimated from the slope of the linear regression lines that relate gallbladder volume to CCK. Gallstone patients (contractors) were significantly more sensitive to a change in plasma CCK than were the normal volunteers ($p < 0.01$).

Discussion

In previous studies we have shown a good correlation between plasma concentrations of CCK (released in response to oral and intraduodenal fat) and gallbladder kinetics.[5] Since multiple physiologic mechanisms are activated by fat in the gut, pure exogenous CCK was next infused into man, and again demonstrated a close correlation between rising plasma concentrations of CCK and gallbladder contraction, thus demonstrating a causal relationship.[6] The present study reveals that gallstone patients also show good correlation between concentrations of CCK and gallbladder size (providing that the gallbladder is capable of contracting). In patients whose gallbladder contracts, release of CCK is diminished, and gallbladder sensitivity to CCK is increased in comparison with normal subjects. The authors attempt here to relate these findings to the pathogenesis of gallstone disease.

Admirand and Small[1] have described the physicochemical requirements for the formation of cholesterol gallstones in man. Their study showed the importance

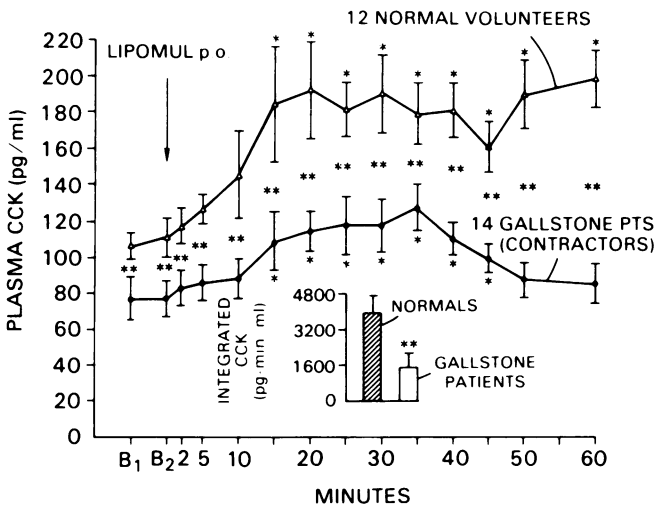

FIG. 5. Plasma concentrations of CCK in response to oral Lipomul in normal volunteers and in gallstone patients (contractors). The inset shows the integrated CCK output during the 60 minutes after Lipomul administration. * = significant increase from basal. ** = significant difference between normals and patients.

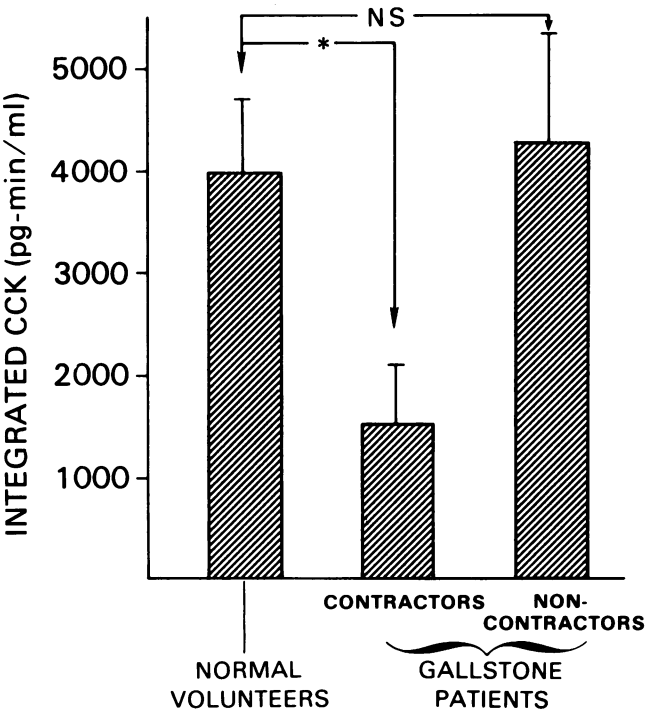

FIG. 6. Integrated output of CCK during one hour after oral Lipomul administration in 12 normal volunteers and 14 gallstone patients (contractors). * = significant difference between normal volunteers and gallstone patients (contractors).

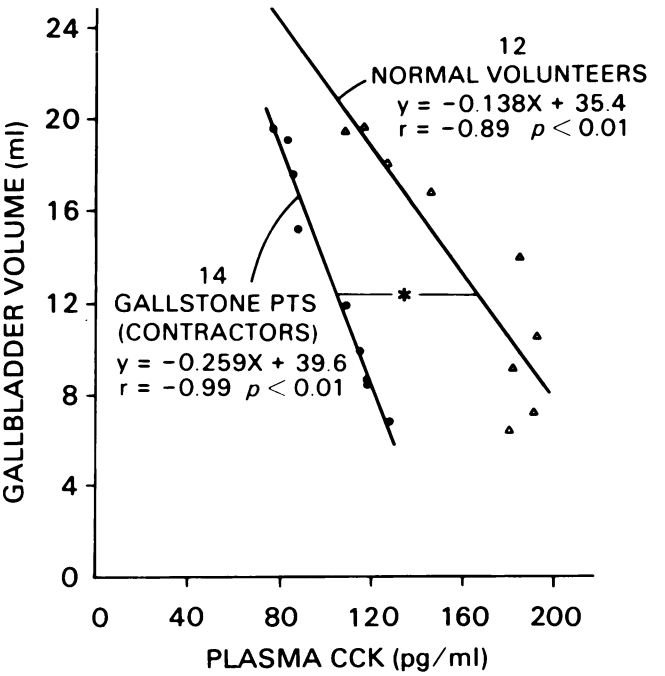

FIG. 7. Linear regression analysis of gallbladder volume *versus* plasma concentrations of CCK 0–30 minutes after Lipomul administration. * = significant difference between slopes of regression.

of alterations in the relative concentrations of bile acids, phospholipids, and cholesterol in determining the solubility of cholesterol in bile. The size of the pool of bile acid is an important determinant of cholesterol solubility. Vlahcevic and colleagues[13] have compared bile acid pool size in patients with and without gallstones, and found a diminished pool in patients with gallstones. They suggested that this diminished pool size may be critical in the pathogenesis of cholesterol cholelithiasis.

Gallbladder kinetics play an important role in the recycling of the bile acid pool. Since the bile acid pool size seems to be determined in part by the recycling frequency,[14] the gallbladder may play an important role in the pathogenesis of cholesterol gallstones by affecting bile acid pool size and bile acid output. LaMorte and colleagues[2] have reviewed the evidence supporting the interrelationship between gallbladder kinetics and bile composition. It has been suggested that gallblader hypotonicity may result in sequestration of bile in the gallbladder, thus diminishing the size of the circulating bile salt pool. This would result in hepatic secretion of supersaturated bile. These conditions were present in the group of patients with noncontracting gallbladders, although no judgment can be made as to whether stasis causes gallstones or results from diseased gallbladders.

On the other hand, the authors found no difference in the volume of bile emptied by the gallbladder in response to oral fat in gallstone patients (contractors) compared with normal volunteers in this study (Fig. 2) and in previous studies.[5,6] In gallstone patients (contractors), they cannot support the concept of an abnormality of motility or its contribution to the lithogenicity of bile. These findings are in contrast to those of Maudgal and colleagues,[15] who reported an increased rate of gallbladder emptying in response to a meal in patients with gallstones. Differences in experimental design may account, partially, for these differences. The authors obtained frequent images of the gallbladder by means of ultrasonography, whereas Maudgal and colleagues[15] measured gallbladder size at only three points in time, by means of oral cholecystography, and in response to a mixed meal, rather than to the liquid corn oil that was used in the present study.

Of the 35 gallstone patients, 14 had had gallbladders that contracted in a normal fashion after oral administration of fat. In 11 patients (31%), they were unable to obtain accurate cholecystosonographic images. This is slightly higher than previous reports of 8 to 20% nonvisualization by ultrasound in patients with gallstones.[16,17] Four of the remaining 24 patients had ultrasonic images of large, noncontracting gallbladders, which at operation proved to represent hydrops of the gallbladder. Of the remaining 20 patients, 14 showed brisk contraction after oral administration of fat, and six did not. We found no difference in the gross appearance of the 20 gallbladders; they were separable only by their contractile response to fat. We learned later that gallstone patients with contracting gallbladders released much less CCK in response to the Lipomul stimulus than did either the normal control subjects or the gallstone patients whose gallbladders failed to contract significantly (Fig. 6).

This finding is in opposition to the report of Maudgal and colleagues,[18] who found (by bioassay) that the maximal postprandial concentration of plasma CCK was higher in gallstone patients than in control subjects. It was found in the present study that the integrated postprandial output of CCK was equal to (noncontractors) or less than [contractors and hydrops patients (data not shown)] that of the control volunteers. The diminished CCK release of the contractors appears clear-cut (Fig. 5). The radioimmunoassay for CCK is sensitive and specific,[6,10] the standard errors are small, and the differences in both the individual curves (Fig. 5) and the integrated outputs (Fig. 6) are clearly significant.

Since the patients whose gallbladders contracted were able to empty their gallbladders to an extent equal to that of normal subjects, in spite of diminished release of CCK, the contracting, diseased gallbladder appeared to be more sensitive to changes in plasma concentrations of CCK than the normal gallbladder (as may be seen from the slopes of the linear regressions in Figure 7). This finding is supported by the experiment of Northfield and colleagues,[19] which showed that patients with gallstones required a lower threshold dose of exogenous CCK to initiate gallbladder emptying than did control subjects.

This study has, in summary, corroborated the finding that some patients with gallstones have gallbladders that contract and some do not. The pattern of contraction in response to fat in gallstone patients (contractors) is nearly identical with the pattern in normal subjects. The demonstration of diminished release of CCK and increased gallbladder sensitivity to CCK in gallstone patients who contract is evidence of a humoral-motility abnormality, but at present it cannot be determined whether the abnormality results from, or causes, gallstone disease.

As a final thought, we speculate on the possibility that there may be two distinct groups of gallstone patients, one in whom gallbladder motility is diminished, so that stasis is a major factor in the pathogenesis of stones. In the other, gallbladder motility is normal, but

release of CCK is diminished and gallbladder sensitivity is increased. The diminished release of CCK in these patients may contribute to gallstone formation in some other way, perhaps by altering the hepatic secretion of bile or by reducing gut motility, which may lead to diminished enterohepatic circulation of bile salts.

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Discussion

Dr. Paul H. Jordan, Jr. (Houston, Texas): The interesting observation revealed by this paper is that the gallbladders with stones, exhibiting no gross differences in appearance, fall into two categories: those that contract and those that do not. Equally interesting is the greater sensitivity to CCK of the contracting gallbladders with gallstones than is observed in the normal gallbladders.

Why should some gallbladders with stones be more sensitive to CCK, while some gallbladders will not contract at all? What is the feedback mechanism that controls the relationship between the CCK and the gallbladder emptying, such that the release of CCK is the same in normal patients and those with noncontracting gallbladders, but is less than that released in those with contracting gallbladders? Obviously, there is some type of derangement in the hormonal-motility relationship controlling gallbladder function.

Does Dr. Thompson wish to speculate as to the nature of this mechanism. Can he tell us if the two categories, that is, the contractors and the noncontractors, persist when challenged with exogenous CCK? And if so, does this have a clinical application with respect to the selection of patients for operation?

Since gallstone disease increases with age, I wonder, if the controls were more closely matched with patients with gallstone disease, would you find some noncontractors, and perhaps even some contractors with increased CCK sensitivity, among the so-called normal controls?

Dr. R. Scott Jones (Durham, North Carolina): I would like to ask Dr. Thompson whether the operative findings help to explain the mechanism of noncontractor or hydrops. Were stones impacted in the cystic duct, and were the cystic ducts patent in these patients?

One very important finding in this study was the significantly lower CCK—cholecystokinin—concentration and integrated cholecystokinin output in the contractors, compared with the controls. This raises the question of whether impaired CCK secretion participates in the development of gallstones, or whether the presence of gallstones in a contractible gallbladder in some way interferes with optimal CCK release from the intestine.

Some insight into that question might be gained by restudying CCK response to Lipomul in "contractors" following cholecystectomy. Because previous studies demonstrated that in gallstone patients with functioning gallbladders the bile salt pool size returns to normal following cholecystectomy, and because in the present study noncontractors had normal CCK responses, I would speculate that cholecystectomy might return CCK response to normal in the contractors.

Dr. James C. Thompson (Closing discussion): The questions that have been asked are the ones that have plagued us, namely, is there some defect in the motility of the gallbladder that leads to failure of CCK release, or is there some primary failure of the CCK mechanism?

We suspect that it's not the latter, because that would require two things being wrong, and would run counter to the dictate of Occam's razor. Occam, the medieval philosopher, said that if you have a simple