Cholecystokinin Metabolism in Man and Dogs

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We have developed a sensitive, specific and reproducible radioimmunoassay for cholecystokinin (CCK) with which basal levels of CCK of between 400-800 pg/ml have been measured in normal man, in patients with diabetes and with duodenal ulcer disease, and in normal dogs. After a meal, circulating levels of CCK rose to 1000-1200 pg/ml in human subjects. Release of CCK was more rapid in diabetic and duodenal ulcer patients than in normal subjects, but elevated postprandial levels persisted much longer in normal subjects. Patients with the Zollinger-Ellison syndrome had elevated values of cholecystokinin which rose after a meal. Lack of correlation between elevated basal levels of gastrin and CCK in patients with the Zollinger-Ellison syndrome suggest that the hypercholecystokininemia may be absolute. The disappearance half-time of exogenous CCK was about 2½ minutes in normal subjects as well as in diabetic and duodenal ulcer patients. Studies in dogs demonstrated no uptake of basal levels of cholecystokinin by the kidney; on infusion of exogenous CCK-33, the kidney extracted 43% of the total CCK presented and 56% of the integrated CCK. We conclude that: 1) circulating basal and postprandial levels of CCK may be measured in a reproducible fashion; 2) postprandial release of CCK is more rapid in diabetic and duodenal ulcer patients than in normal man; 3) the disappearance half-time of exogenous CCK in man and dogs is about 2½ minutes; 4) the kidney is a major site for uptake of CCK.

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IN 1856 Claude Bernard reported that introduction of hydrochloric acid into the duodenum resulted in stimulation of bile flow.[1] Ivy and Oldberg[19] demonstrated in 1928 that extracts of small intestinal mucosa caused contraction of the gallbladder; they called the active material cholecystokinin. In 1943 Harper and Raper[15] extracted a potent stimulant of secretion of pancreatic enzymes from small bowel mucosa and they named the agent pancreozymin. The meticulous studies of Mutt and Jorpes[20,21] have culminated in the isolation and chemical characterization of a single polypeptide which possesses the biologic activities ascribed to both hormones. Because cholecystokinin was discovered first, it is now conventional to refer to the polypeptide hormone as cholecystokinin with the knowledge that it has multiple actions besides stimulating gallbladder contraction (stimulation of pancreatic enzyme secretion and stimulation of gastrointestinal smooth muscle,[3] *inter alia*[33]).

Precise studies on the metabolism of cholecystokinin (CCK) have been hindered by lack of precise methods for measuring physiologic concentrations of the hormone. Cholecystokinin is structurally similar to gastrin (the C-terminal 5-amino acid residues are identical), and the two hormones share several actions.[33] In addition, secretin may be released from small bowel mucosa at the same time as CCK and their actions overlap.[22]

Two years ago we reported development of a radioimmunoassay which allowed measurement of the endogenous release of cholecystokinin.[29] Difficulties with early radioimmunoassay methods for CCK have been recently reviewed;[9] our current method is reliable, sensitive and reproducible.[25] In the present study we report precise measurements of release of CCK in normal persons, in diabetic and duodenal ulcer patients and in patients with the Zollinger-Ellison syndrome. The disappearance half-

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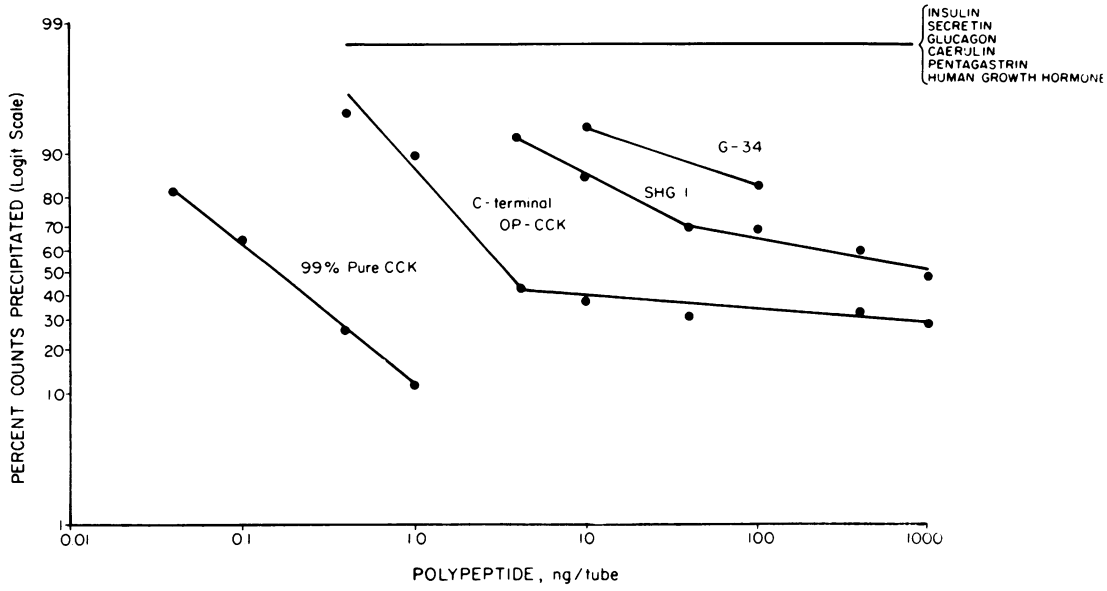

FIG. 1. Dose-response curves of graded amounts of 99% pure CCK and other polypeptide hormones in the CCK radioimmunoassay, with 99% pure CCK used as a labeled antigen.

time for CCK in man is calculated, and studies in dogs are reported which show that the kidney plays an important role in the catabolism of CCK.

Materials and Methods

Radioimmunoassay

The current radioimmunoassay technique[25] differs from our previously reported assay,[26,28,29] in that 99% pure CCK (3000 IDU/mg) (obtained from the Gastrointestinal Hormone Laboratory of the Karolinska Institute, Stockholm) is now used both as the labeled antigen and as the reference standard. The assay technique has been described in detail;[25] a summary of the method will be provided for clarity.

Antiserum was generated in New Zealand white rabbits by the method of Vaitukaitis and colleagues[38] in which 16% pure CCK (500 IDU/mg) (also obtained from the GIH Laboratory, Karolinska Institute) was used as an immunogen. Booster injections of the immunogen were given biweekly and the rabbits were bled on alternate weeks. The antiserum selected (UT122) bound 25%-35% of labeled antigen at a final dilution of 1:2500. Labeled 99% pure cholecystokinin was iodinated with carrier-free ^{125}I to specific activities of 30-50 μci/μg by the method of Greenwood, Hunter and Glover.[13]

For each CCK radioimmunoassay, 99% pure CCK was used as a reference standard and dose-response curves were generated by the method of Rodbard.[31] CCK concentrations were calculated as picograms per milliliter (pg/ml).

To test the specificity of the assay, we measured the ability of other polypeptide hormones (some whose molecular structure resembles that of CCK) to displace labeled CCK from combination with antiserum. A standard dose-response curve was generated with varying doses of 99% pure CCK, and was compared with that of other hormones (Fig. 1). Insulin, secretin, glucagon, caerulein, pentagastrin, and human growth hormone did not compete with labeled cholecystokinin for antibody sites in quantities ranging from 4 to 1000 ng per tube. The C-terminal octapeptide of CCK (Op-CCK, gift from Dr. Miguel Ondetti, Squibb Laboratory), synthetic 17-amino acid human gastrin I (SHG I) and natural 34-amino acid human big gastrin (G-34, gift from Professor R. A. Gregory) did effect displacement of labeled CCK from CCK antibody, but the lines of inhibition generated were not parallel to the dose-response curve of 99% pure CCK. Compared to the mass of 99% pure CCK required for an equivalent displacement of labeled cholecystokinin from combination with antibody, the mass of Op-CCK was 25 times greater, the mass of SHG I was 400-500 times greater, and the mass of G-34 was 1000-1250 times greater. Since there is only slight cross-reaction with Op-CCK, which contains the biologically active segment of the molecule, it would appear that this antibody (as well as all other CCK antibodies yet reported[9]) recognizes chiefly the nonbiologically active segment of the molecule.

The reproducibility of the assay was tested in a study in which 5 replicate measurements of CCK in human serum were made in 4 separate radioimmunoassays. The intra-assay coefficient of variation was 4.5% and the between-assay coefficient of variation was 12.5%.

In order to test the ability of the assay system to quantify CCK in serum, graded volumes (50-500 μl) of serum taken from a patient with the Zollinger-Ellison syndrome, were analyzed in the radioimmunoassay. This patient previously had been shown to have high levels of

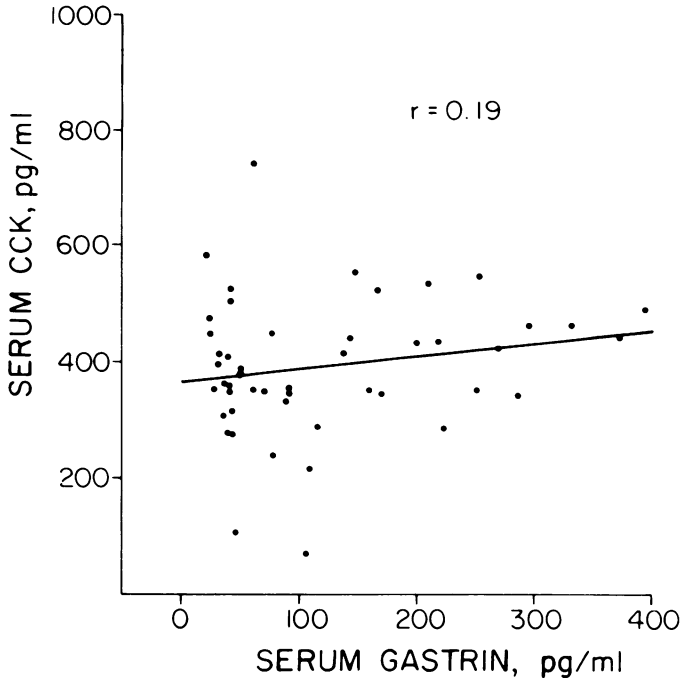

FIG. 2. Plot of simultaneously measured concentrations of CCK and gastrin from four individual patients in the fasting and in the postprandial state. Concentrations of gastrin were used as the independent variable and concentrations of CCK as the dependent variable.

CCK in serum. The dose-response curve of the endogenous CCK in serum and the dose-response curve generated by 99% pure CCK, were exactly parallel, which indicated that the assay system can accurately quantify CCK in serum.[25]

To test the ability of the assay to distinguish between endogenous gastrin and CCK in serum, we obtained 48 serum samples (from four individuals) collected before and at intervals after a meal to provide comparison of simultaneous gastrin and CCK concentrations. Serum concentrations (expressed as pg/ml) were measured with a specific radioimmunoassay for gastrin[24] and for CCK.[25]

The mean basal concentration of serum gastrin was 95±18 pg/ml; this increased to a peak postprandial level of 167±77 pg/ml. Mean basal CCK concentration was 413±66 pg/ml. This level rose to a postprandial peak of 526±42 pg/ml. Comparison of the gastrin and CCK concentrations by unweighted linear regression analysis, resulted in the calculation of a correlation coefficient (r) of 0.19 (Fig. 2). This weak correlation indicates that serum CCK levels measured in the radioimmunoassay system were not influenced by changes in serum gastrin values.

Concentrations of CCK in Man

Studies were performed on 69 subjects; informed consent was obtained from each person.

Release of CCK by food. The effect of the administration of a liquid test meal (250 g Sustagen) containing 166 g carbohydrate, 59 g protein, and 9 g fat on the circulating level of CCK, was studied in 16 normal subjects, 13 insulin-dependent diabetic patients, 12 patients with proven duodenal ulcer (preoperative), 8 proven duodenal ulcer patients studied 2-16 weeks after selective proximal vagotomy, and 11 patients with histologically proven Zollinger-Ellison syndrome, studied at various periods after total gastrectomy. They were fasted for at least 14 hours prior to the study and blood samples were collected twice before the meal and at regular intervals after eating.

Disappearance half-time (T½) of exogenous CCK. The rate of disappearance of exogenously administered CCK was studied in 4 normal subjects, 4 insulin-dependent diabetics, and 4 patients with proven duodenal ulcer before operation. Blood samples were collected from fasting patients immediately before the intravenous 5-minute infusion of either 0.5 or 1.0 IDU/kg/min of 16% pure CCK. Further samples were obtained at 3 minutes after the initiation of the infusion, at the end of the 5-minute infusion, and after the infusion at 1-minute intervals for 10 minutes and then at 2-minute intervals for an additional 10 minutes. Serum samples were collected and stored frozen until assayed for CCK. The disappearance half-time of exogenous cholecystokinin was calculated by linear regression analysis.[39] The mean basal concentration of cholecystokinin was subtracted from all subsequent values for each person in the study. The peak concentration of CCK at the end of the infusion was assigned a value of 100% and the value for each subsequent time period was calculated by dividing each CCK concentration by the peak value. Per cent values were then converted to natural logarithms and the data were analyzed by linear regression analysis with time used as the independent variable and the natural logarithm of the per cent of the peak CCK concentration as the dependent variable. This analysis resulted in an equation of the regression line:

$$y = a + bx$$

where: $y = \log_n$ per cent peak CCK concentration;
a = value of y when $x = 0$;
b = slope of the regression line;
and x = time in minutes.

The disappearance half-time was then calculated by dividing 0.693, the natural logarithm of 2, by the slope of the regression line.

Renal Uptake of CCK in Dogs

Four mongrel dogs weighing between 18-25 kg were anesthetized with pentobarbital sodium after an overnight fast. The right brachial vein was cannulated and a catheter was passed into the aorta via the left femoral artery. A midline laparotomy incision was performed and the left renal vein was cannulated by way of the left femoral vein. Basal blood samples were obtained from the aorta and the renal vein after which an infusion of

16% pure CCK (1 IDU/kg/min) was administered for 5 minutes to each dog. Samples were obtained from the aorta and renal vein at the end of the 5-minute infusion and at 1-minute intervals for the next 5 minutes.

Statistical Note

Results are expressed as the mean ± one standard error. The Student 't' test was used to analyze the data for statistical significance of differences between means. Differences with a P value of less than 0.05 were considered significant.

Results

Concentrations of CCK in Man

Release of CCK by food. The mean basal concentration of CCK in 16 normal subjects was 728±84 pg/ml. This level rose to a peak at 60 minutes after food to 1079±101 pg/ml and 4 hours later was still elevated (1146±143 pg/ml). Samples obtained at 30, 60, 90, 180, and 240 minutes after food were elevated significantly above basal (Fig. 3).

In 13 insulin-dependent diabetic patients, the mean basal concentration of CCK was 542±94 pg/ml, which rose briskly after a meal to a peak of 797±112 pg/ml at 15 minutes. Only the 5-minute and the 15-minute samples were elevated significantly above basal. At 90 minutes, the value was 604±78 pg/ml and at 180 minutes the concentration was 636±118 pg/ml (Fig. 4).

The mean basal concentration of CCK in 12 preoperative patients with duodenal ulcer was 630±71 pg/ml, which rose rapidly to a peak of 1003±194 pg/ml at 15 minutes. Postprandial concentrations of CCK in preoperative patients were elevated significantly above basal levels at 5, 15 and 30 minutes. At 180 minutes after food, serum CCK was 741±77 pg/ml (Fig. 5). In 8 duodenal ulcer patients after selective proximal vagotomy, the mean basal concentration of CCK was 709±95 pg/ml, which rose to 1305±299 pg/ml at 30 minutes. Only the 15-minute and 30-minute postprandial samples were elevated significantly above normal, and the concentration of CCK at 180 minutes after eating was 614±59 pg/ml (Fig. 5). The observed differences between values in preoperative and in postoperative duodenal ulcer patients were not significant.

The mean basal concentration of CCK in serum of 11 patients with the Zollinger-Ellison syndrome (after total gastrectomy) was 1628±320 pg/ml. Because of wide variations among patients, this concentration was assigned a value of 100% and postprandial levels were compared to it. The peak postprandial concentration of 140±18% was achieved at 15 minutes; samples at 15, 60 and 90 minutes after food were elevated significantly above basal. By 150 minutes, CCK concentrations had returned to basal (Fig. 6).

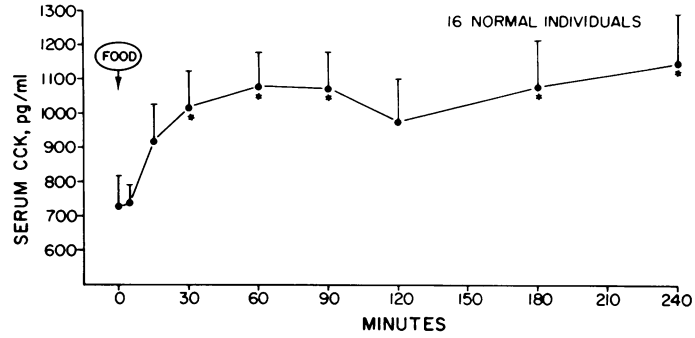

FIG. 3. Concentrations of serum CCK (pg/ml ± SE) in 16 normal subjects before and at different time periods after the ingestion of a high-protein, high-carbohydrate liquid meal. Asterisks identify values which are elevated significantly above basal.

Disappearance half-time of exogenous CCK. Exogenous CCK was removed rapidly from circulation and was virtually undetectable at 14-15 minutes after the infusion was stopped. The calculated disappearance half-time was 2.4 minutes in normal subjects, 2.7 minutes in diabetic patients and 2.4 minutes in duodenal ulcer patients (Fig. 7). All correlation coefficients (r) were significant.

Renal Uptake of CCK in Dogs

The mean basal concentration of cholecystokinin in serum obtained from the aorta in 4 dogs was 441±63 pg/ml; basal serum from the renal vein had a mean concentration of CCK of 463±76 pg/ml. At the end of the 5-minute infusion, the concentration in the aorta was 3053±417 pg/ml and the simultaneous concentration in the renal vein was 1729±368 pg/ml (Fig. 8). All postinfusion concentrations in the renal vein were lower than in the aorta. Differences at 5 and 7 minutes were significant.

In order to demonstrate the cumulative differences between concentrations of CCK in the aorta and in the renal vein during the 10-minute period after the infusion was

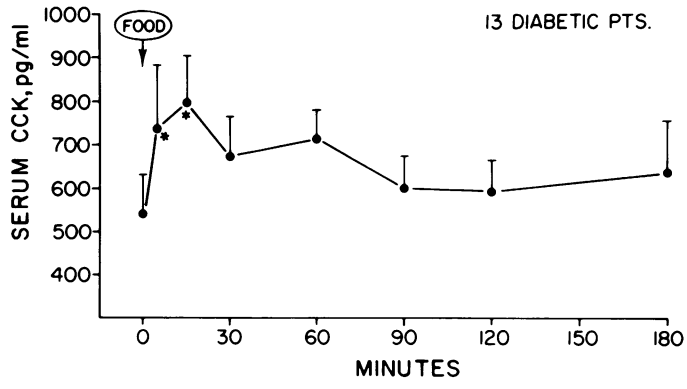

FIG. 4. Concentrations of CCK (pg/ml ± SE) in the serum of 13 insulin-dependent diabetic patients before and at different time periods after the ingestion of a high-protein, high-carbohydrate liquid meal. Asterisks identify values which are elevated significantly above basal.

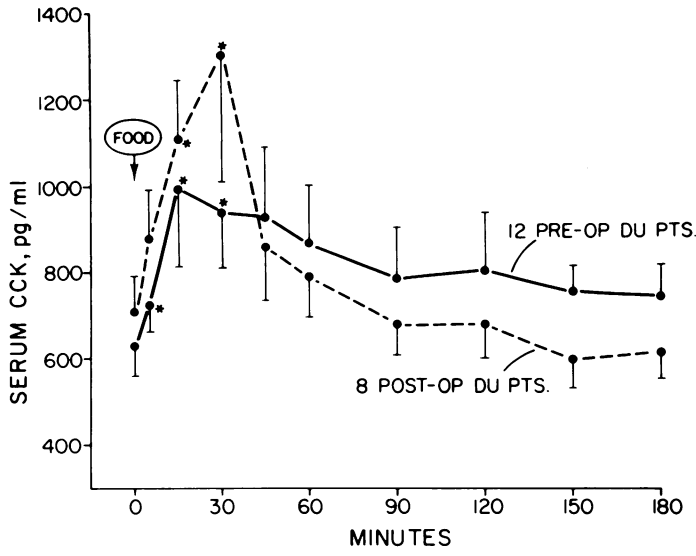

FIG. 5. Concentrations of serum CCK (pg/ml ± SE) in 12 preoperative patients with duodenal ulcer and in eight patients studied at varying time periods after selective proximal vagotomy. Samples were obtained before and at different time periods after the ingestion of a high-protein, high-carbohydrate meal. Asterisks identify values that were significantly elevated above basal. The differences in concentrations obtained from preoperative patients were not significant as compared with postoperative patients.

begun, the integrated cholecystokinin responses in the aorta and renal vein were calculated (Fig. 9).[35] The integrated response during a given period is the difference between the mean *stimulated* CCK concentration and the mean *basal* CCK concentration during the period of study. The units for expression of integrated CCK response are pg-min/ml. The integrated CCK response 0-10 minutes in the aorta was 14,626±1037 pg-min/ml and in the renal vein was 6394±1874 pg-min/ml; this difference was significant.

A linear relationship existed between the arterial serum CCK concentrations and the arteriovenous difference in serum CCK concentrations (Fig. 10). The mean regression equation was $y = -376.1 + 0.58x$ and the coefficient of correlation was 0.98 ($P<0.01$).

Discussion

Several radioimmunoassays for cholecystokinin have been described.[8,10,11,16-18,26,28,29,40] Our present assay[25] is an improvement over the method used previously.[26,28,29] The current method uses a different antibody and uses 99% pure CCK as both a labeled antigen and as a reference standard. The 99% pure CCK was found to be about 25 times more immunopotent than the 16% pure CCK used in previous assays.[25] Use of the less pure CCK preparation for dose interpolation resulted in higher estimates of concentration of CCK in test samples. Basal concentrations of CCK in normal subjects in this study were measured at about 700 pg/ml, a value that is between 1/7 and 1/25 that reported previously.[29] Harvey and colleagues have reported fasting levels in man of between 25 pg/ml[16] and 60 pg/ml.[17] This difference may be due to antibody selectivity. CCK has been reported to exist in at least two molecular forms (a 39-amino acid variation [CCK-39] and the currently available 33-amino acid molecule [CCK-33]).[6,23] The antibody used[16,17] might recognize only one molecular form (as Hansky and colleagues[14] found with a specific gastrin antibody). One CCK antibody, developed with porcine CCK as immunogen, was found not to cross-react with human CCK.[11]

Although our present antibody for CCK reacts only slightly with 17-amino acid synthetic gastrin and with natural 34-amino acid gastrin (Fig. 1), and although there seems to be little correlation between levels of serum gastrin and levels of CCK in the same patients (Fig. 2), it is still important to note that results of this assay when used for determination of serum CCK levels in patients with the Zollinger-Ellison syndrome (whose serum gastrin levels are often extremely high) should be interpreted with caution. In practice, we have found no correlation between high levels of gastrin and high levels of CCK in patients with the Zollinger-Ellison syndrome. (For example, one patient had a serum gastrin level of 26,000 pg/ml and a CCK level of 1200 pg/ml; another patient had a serum gastrin value of 4000 pg/ml and a serum CCK concentration of 1800 pg/ml). The mean basal concentration of 1628 pg/ml in the Zollinger-Ellison patients in Fig. 6, therefore, may represent absolute hypercholecystokininemia.

The administration of a high-protein, high-carbohydrate liquid meal evoked a release of CCK that was more rapid in diabetic patients and in patients with duodenal ulcer than in normal subjects, and there was a greater

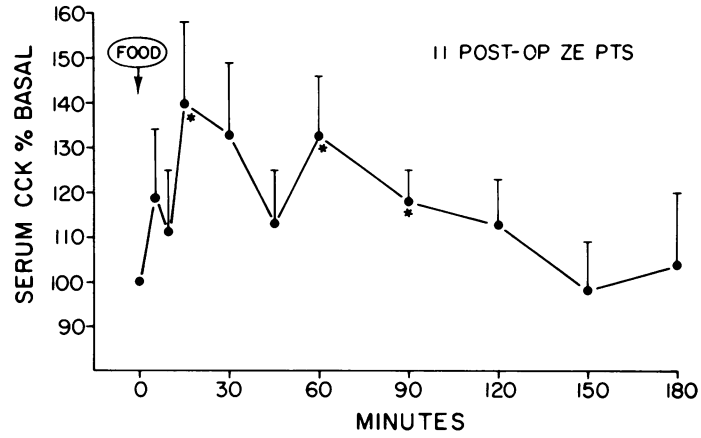

FIG. 6. Concentrations of serum CCK (pg/ml ± SE) in 11 patients with the Zollinger-Ellison syndrome studied at varying periods after total gastrectomy. Samples were obtained before and at different time periods after the ingestion of a high-protein, high-carbohydrate liquid meal. Asterisks identify values which were elevated significantly above basal.

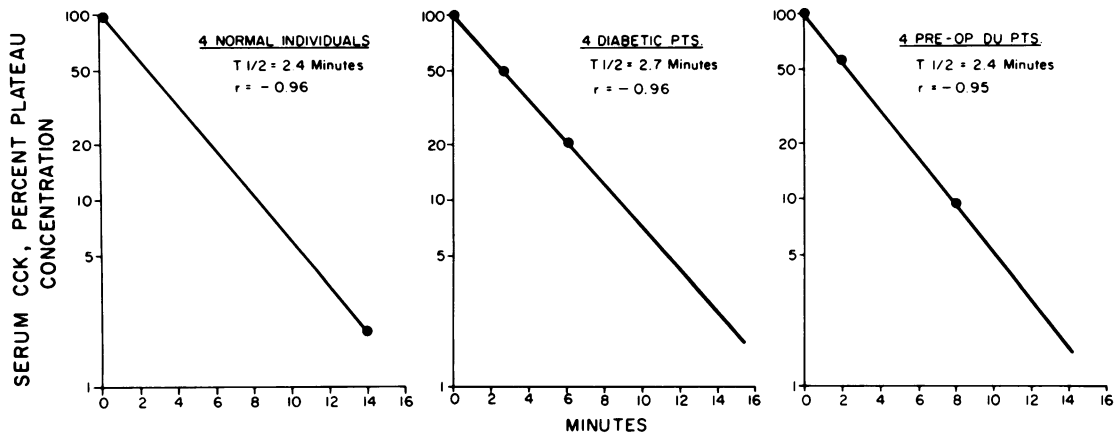

FIG. 7. Semilogarithmic plot of the disappearance half-time of exogenous CCK from circulation of four normal subjects, four insulin-dependent diabetic patients, and four preoperative patients with duodenal ulcer. Samples were obtained before, during, and at varying time periods after a 5-minute infusion of 16% pure CCK (0.5 or 1.0 IDU/kg/min). The slopes of the correlation coefficient (r) were calculated by unweighted linear regression analysis.

prolongation of elevated CCK levels in normal subjects than in diabetic or duodenal ulcer patients. Diabetic patients and ulcer patients showed significant elevation of CCK levels at 5 minutes after the liquid meal; significant elevations were not achieved in normal subjects until 30 minutes. These differences appear to be real, but their significance is not apparent. Studies on the rates of gastric emptying were not performed. An increase in the rate of emptying might be expected in ulcer patients, but not in diabetic patients.

We had previously reported[29] significant elevations of CCK at 30 minutes after a fatty meal of fried eggs, bacon, sausage, buttered toast and cream. In the present study, CCK levels in normal subjects increased steadily and significantly at 30, 60, 90, 180 and 240 minutes after food and the level was relatively constant from 30 minutes to the end of the 4-hour test period (Fig. 3). Harvey and colleagues[16] measured CCK levels in 5 healthy subjects after the ingestion of a pint of milk. They reported basal CCK levels of 25.8 pg/ml which rose to between 8 and 16 ng/ml within 35 minutes and thereafter declined rapidly, approaching basal within 45 minutes. The ratio of peak-to-basal CCK levels varied from 320:1 to 640:1,[16] as compared with the ratio of 1.5:1 in the current study. Since our subjects received a high-protein and high-carbohydrate liquid meal with higher osmolarity than the milk given by Harvey and associates,[16] the prolonged elevation of CCK in our patients may be due to retention of the hyperosmolar material in the stomach.

We have previously shown that Zollinger-Ellison patients after gastrectomy have, uniformly, a brisk serum gastrin response to a meal, a response that apparently results from release of tumor gastrin by the meal.[34,37] If the high CCK values measured after food in the Zollinger-Ellison patients represent true elevations of

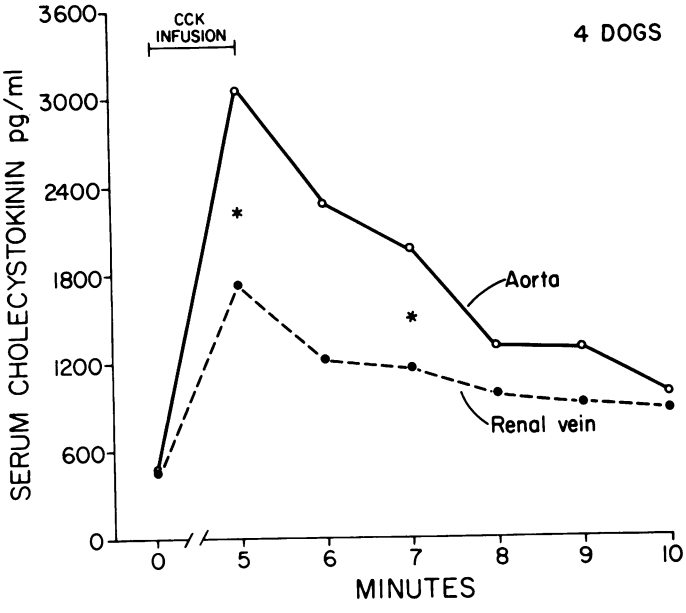

FIG. 8. Relationship between serum gastrin levels in the renal artery (aorta) and renal vein in the basal state (time 0) and after a 5-minute infusion of 16% pure CCK (1 IDU/kg/min) in 4 dogs. Asterisks denote points of significant differences.

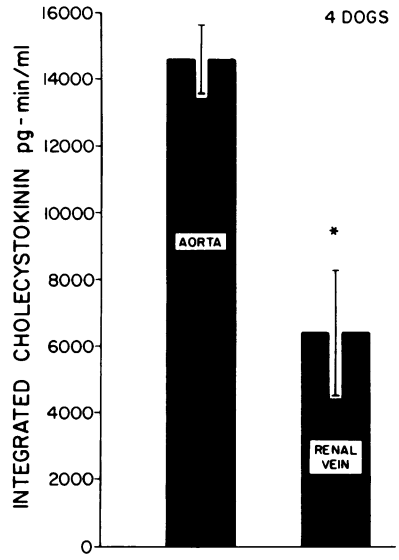

FIG. 9. Integrated cholecystokinin values, 0-10 minutes, for the study illustrated in Fig. 8. Integrated cholecystokinin value for the renal vein was significantly different than that of the renal artery (aorta).

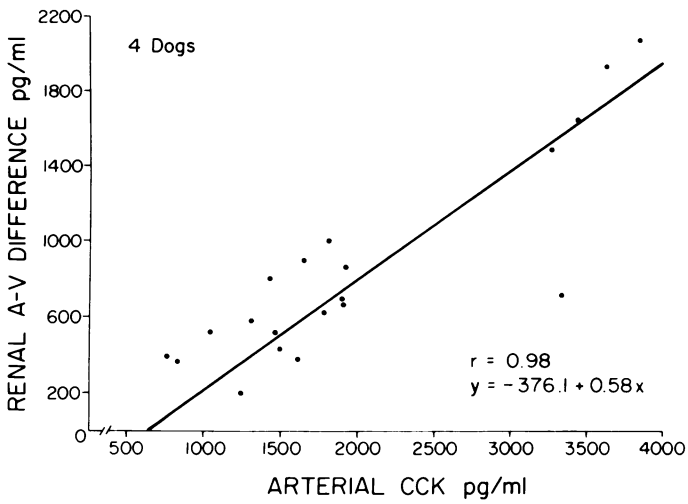

FIG. 10. Relationship of renal arteriovenous CCK difference, *y*, and arterial serum CCK concentration, *x*, in 4 dogs. r = coefficient of correlation.

CCK, the source of the large amounts of CCK might be the tumor. A CCK concentration of 0.85 μg/g of tumor has, however, been measured by us in a Zollinger-Ellison tumor that had a gastrin concentration of 587 μg/g (unpublished data). This gastrin/CCK ratio of 690:1 is within the range of cross-reactivity of the CCK assay (Fig. 1). This does not preclude the presence of significant concentrations of CCK in the tumor, nor does it support that possibility.

The disappearance half-time of exogenous CCK was remarkably similar in normal subjects and in diabetic and duodenal ulcer patients. The values agree well with the T½ we have previously reported[30] for exogenous CCK in dogs. Among the different molecular forms of gastrin, there is a direct relationship between molecular size and disappearance half-times (Table 1). Surprisingly, the T½ for exogenous 33-amino acid CCK corresponds more closely with the T½ for 17-amino acid gastrin (2.7 minutes) than it does for 34-amino acid gastrin (9 minutes[32] or 15 minutes[39]). Harvey and associates[16] suggested that the half-life of endogenous cholecystokinin in man was 5-7 minutes. We found the half-life of endogenous, that is mixed-molecular gastrin, to be 8.6 minutes in dogs.[36] Cholecystokinin has been reported to exist in at least two separate molecular forms (CCK-33 and CCK-39),[6,23] and physiologically-released CCK is likely to be a mixture of the different molecular forms.

Although levels of *endogenous* cholecystokinin, in response to food, rose more rapidly in diabetic and duodenal ulcer patients than in normal subjects and postprandial elevations persisted longer in normal subjects, the disappearance half-times of injected exogenous cholecystokinin were nearly identical in the three groups. This would suggest that if diabetic and duodenal ulcer

TABLE 1. *Disappearance half-times (T½) of Gastrin and CCK*

Gastrins*	CCK†
Big-big gastrin—90 min.[32]	Dogs—2.6 min.[30]
Big Gastrin (G-34)—15 min.[39]	Normal man—2.4 min. (this study)
Endogenous (mixed) gastrin—8.6 min.[36]	Patients with diabetes—2.7 min. (this study)
Little gastrin (G-17)—2.1 min.[27]	Patients with DU—2.4 min. (this study)
Minigastrin (G-13)—1.8 min.[7]	

*All gastrin T½ studies were performed in dogs.
†In all CCK T½ studies, the material injected was the natural porcine 33-amino acid form of CCK.

patients do have different metabolic rates for cholecystokinin, the difference must involve mechanisms other than the catabolism of the 33-amino acid form of cholecystokinin.

The brief half-life of cholecystokinin in circulation suggests catabolic mechanisms that are rapid and efficient. We have previously shown that although the kidney does not take up *basal* levels of gastrin, it does extract between 30%-40% of exogenously administered G-17[4] or endogenously stimulated mixed-molecular gastrin.[2] Nephrectomy was found to cause an increase in the disappearance half-time of G-17 from 2.85 minutes to 7.88 minutes.[5] The present study indicates that the dog kidney does not extract basal levels of CCK since the concentration of endogenous CCK was the same in the renal vein as it was in the aorta. At the end of a 5-minute CCK infusion, however, 43% of the peak total CCK concentration presented was extracted by the kidney. If only stimulated levels are considered, the differences in integrated CCK (Fig. 9) show that 56% of the exogenously elevated levels of CCK were extracted by the kidney over the 10-minute period. Urinary excretion of CCK was not measured in this study; only negligible quantities of gastrin are excreted via the urine.[4,36] The fate of CCK picked up by the kidneys is not known. It may be simply altered by the renal parenchymal cells and returned to circulation in an immunochemically unrecognizable form. It is likely that, as with gastrin, urinary excretion is not important. Grace and associates[12] have suggested that gastrin may be removed at least partially by direct uptake from peritubular blood, and CCK, because of its similar molecular conformation, may be handled in a similar fashion.

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Discussion

Dr. William Silen (Boston): I think Dr. Thompson has indeed validated an excellent assay for CCK. We certainly look forward to further studies from his very productive laboratory showing us the interactions between CCK, gastrin and soon perhaps even secretin.

I did note on some of his slides that the CCK concentrations were elevated for as long as six hours after a meal. I wonder whether Dr. Thompson has any data on the concentration of CCK at which we get physiologic effects in man. In other words, can we expect at those high levels to show how stimulation of contraction of the gallbladder or increase in pancreatic enzyme output perhaps that hasn't been studied yet.

It is of some interest that these levels are very high even at six hours.

Of particular interest, I think, are the elevations noted in the Zollinger-Ellison patients. Perhaps here we have another example of a tumor which might indeed elaborate in addition to gastrin, another hormone.

In the diabetic patients, I would ask whether there's any information