Effect of Cholecystokinin, Gastrin, Secretin, and Glucagon on Human Gallbladder Muscle *in Vitro** (33826)

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Cholecystokinin-pancreozymin (CCK-PZ) causes contraction of the human gallbladder *in vivo* (1, 2) and *in vitro* (3) but the influence, if any, of other gastrointestinal hormones on the organ is unknown. The C-terminal tetrapeptide of CCK-PZ may be identical with that part of the gastrin molecule (4, 5) which increases antral motility. However, gastrin has little effect on the gallbladder of the anesthetized cat or dog (6), although a synthetic gastrin-like pentapeptide has been reported to increase the bilirubin concentration of duodenal aspirates in man (7), a response consistent with either contraction of the gallbladder or choleresis. Gastrointestinal hormones have not been found to inhibit motility of the human gallbladder. Both secretin (8, 9) and glucagon (10, 11), which have structural similarities, inhibit the motility of human gastric and small intestinal smooth muscle but their effect on the gallbladder has not been established.

The present study was designed to investigate further the effects of cholecystokinin on motility of smooth muscle from the human gallbladder has not been established.

The present study was designed to investigate further the effects of cholecystokinin on motility of smooth muscle from the human gallbladder by using an *in vitro* system and to determine whether gastrin, secretin, or glucagon had any effects under these circumstances. The method was adapted from that used by Fishlock and Parks (12) for testing the action of pharmacologic agents on human gastrointestinal smooth muscle, and an experimental design suitable for statistical analysis of results was devised.

Material and Methods. Gallbladders without obvious abnormalities of the wall on gross inspection were obtained after cholecystectomy, usually for calculi, and were stored in Krebs solution at 4°. Strips stored thus for several hours behaved in a similar manner to fresh strips. The tests were performed on the same day the specimens were obtained. Serosa and underlying fat were dissected off, and up to eight matched, adjacent, parallel, full-thickness strips, 4 by 15 mm, were cut from each specimen. Bundles of muscle fibers in the gallbladder wall run in many different directions and are not separated into well-defined layers (Fig. 1). Fiber orientation could not be determined by naked eye inspection, so all strips were arbitrarily cut with their long axes at right angles to the long axis of the gallbladder. Figure 1 shows the range of variation found in 17 consecutive gallbladders examined histologically. The muscle was always well preserved, although varying in thickness, and the inflammatory change in the mucosa was variable.

The apparatus used to study motility (Fig. 2) was modified from that of Gaddum (13). The strips were suspended in an atmosphere of prewarmed, moistened 95% oxygen–5% carbon dioxide in an inner chamber which was placed in a water bath at 37°. The bathing fluid (Krebs solution with or without added hormone) dripped down the surface of the strip at a rate of 1.25 ml/min, so that the composition of the fluid in contact with the strips was relatively constant. The strip was connected to an isotonic writing lever with wettable thread at a tension of 2 g. Recordings were made by a kymograph using electrosensitive paper (Teledeltos).

Changes in strip length were compared by planimetry of 5-min periods of the kymogra-

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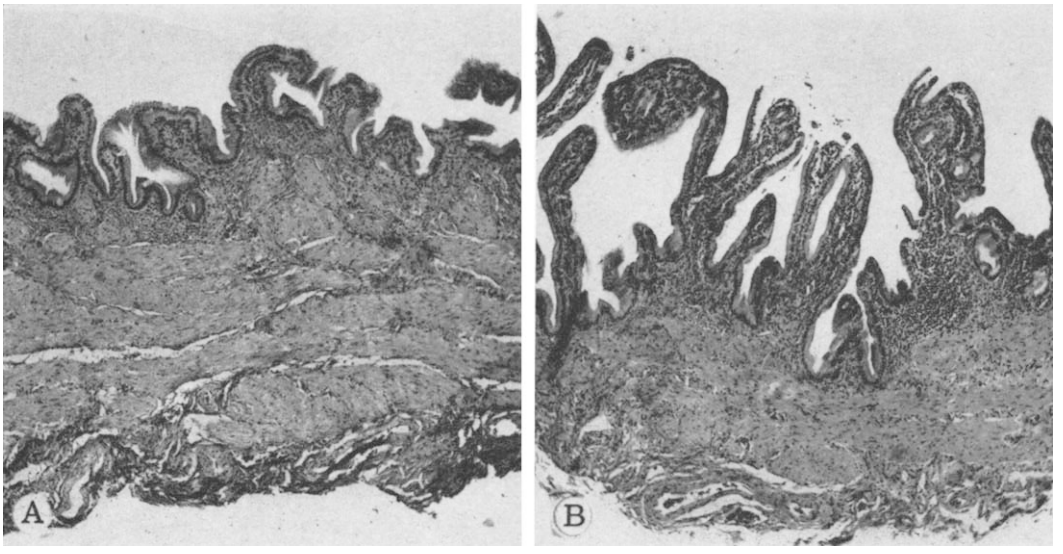

FIG. 1. Strips from two gallbladders used in study, to show range of variation found: muscle layer is thick (A) or thin (B); mucosal inflammatory infiltrate is slight (A) or marked (B). Sections were cut parallel to long axis of strips. Hematoxylin and eosin; ×100.

ph tracing, the area being divided by the time interval and correction being made for writing lever magnification (× 15). Spontaneous changes in strip length were usual, most strips lengthening initially in response to tension and some shortening later. Strips from the same gallbladder showed similar spontaneous changes. The analysis was therefore based on a comparison between test strips and control strips cut from the same specimen. The sequence of tests was randomized and the significance of results was calculated by the rank sum test or the signed rank sum test of Wilcoxon and Wilcox (14).

Before measurements were begun, all strips were bathed under tension at 37° for 30 min

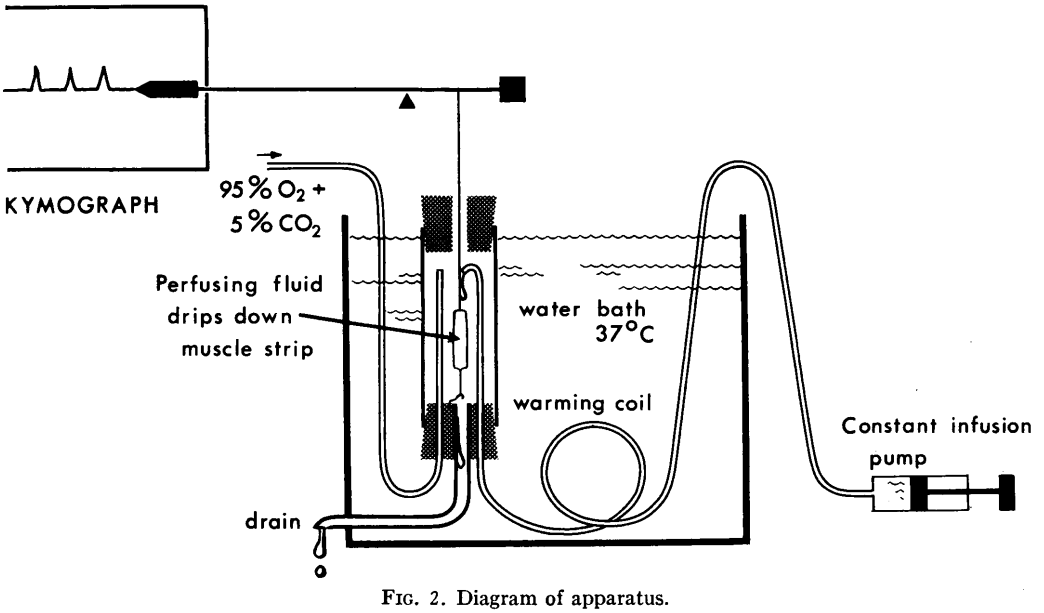

FIG. 2. Diagram of apparatus.

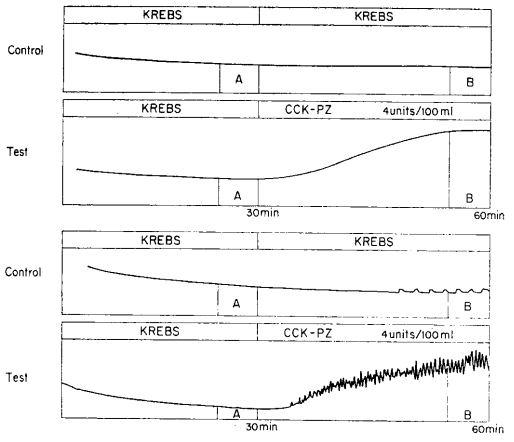

FIG. 3. Recordings of paired strips from two gallbladders: CCK-PZ caused shortening of test strips compared to controls. Strips from second gallbladder, both treated and untreated, show faster activity. Changes in length of each strip were compared by planimentry of areas A and B (see text).

in modified Krebs solution containing (mmoles): Na, 143; K, 5.9; Ca, 3.22; Mg, 2.0; Cl, 129; HCO_3, 25; SO_4, 2.42; H_2PO_4, 1.2; and dextrose, 5.55. The hormones were dissolved in 0.9% NaCl, stored frozen, and added to the Krebs solution immediately before each test. The hormone preparations used were natural CCK-PZ [Cecekin, batch no. 214007 (Vitrum, Stockholm)]; synthetic secretin with an activity of 2000 units/mg (15); pure synthetic human gastrin I (16); and crystalline natural glucagon (Eli Lilly and Company). The glucagon contained lactose which was shown to have no effect in the concentration present. The sensitivity of the method to CCK-PZ was first assessed and compared with the known effect of CCK-PZ on the gallbladder *in vivo*. Concentrations of other hormones were selected as being comparable to CCK-PZ in terms of the amounts known to produce their main physiologic effects *in vivo*.

Results. CCK-PZ. The effects of CCK-PZ on gallbladder motility were studied by comparing changes in length and wave patterns between control and test strips during 30-min intervals (Fig. 3). Strips were taken from 16 gallbladders. Only one test was performed with each strip, which was then discarded. Two 5-minute periods, A and B, were compared, allowance being made for spontaneous changes in strip length. Thus, the value (B—A) for the control strip subtracted from the value (B—A) for the test strip reflected the CCK-PZ effect (Fig. 3). Strips treated with CCK-PZ showed significant and progressive shortening after a brief latent period. The lowest concentration causing significant shortening compared to control strips was 4 units/100 ml ($p = 0.05$) (Fig. 4). Increasing the concentration above 10 units/100 ml did not increase strip contraction ($p > 0.05$), although the latent period was significantly shorter with higher concentrations (Table I).

Rapid regular or irregular waves were noted with approximately two of every three strips (Fig. 3). Their amplitude was usually 0.1–0.3 mm but occasionally reached 3 mm, and the rate was predominantly between 3 and 6/min. Larger waves (2–3 mm) were usually slower and more complex. The increase in the number of waves (1.4/min) after CCK-PZ at 10 units/100 ml was greater ($p = 0.05$, $n = 16$) than that occurring spontaneously for the control strips (0.7/min), but the hormone had no significant effect on wave amplitude. Strip shortening in response to CCK-PZ was comparable whether or not such additional activity was present (Fig. 3).

The duration of action of CCK-PZ was assessed in a further series of tests using paired strips from the same gallbladder, one being treated with the hormone (4 units/100 ml) for a 15-min period. In seven tests, the

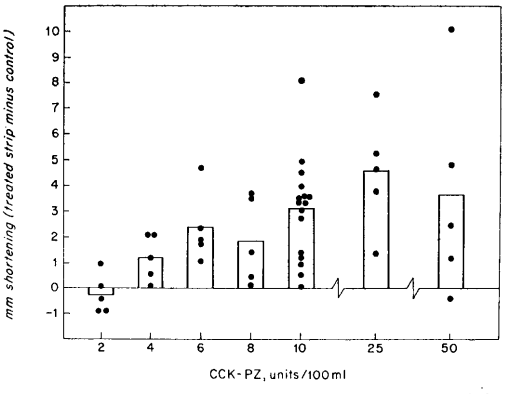

FIG. 4. Shortening of gallbladder strips treated for 30 min with different concentrations of CCK-PZ.

TABLE I. Effect of CCK-PZ Concentration on Latent Period.

CCK-PZ (units/100 ml)	No. of strips responding[a]	Mean latent period (min)	p
4–6	8	6.25	<0.025
8–10	16	2.92	<0.01
25–50	9	0.83	

[a] Strips not contracting in response to hormone are not included.

mean time taken for the treated strip subsequently to return to the same length relative to the control strip was 18 – 4.15 (SE) min.

Secretin, Gastrin, and Glucagon. After an initial 30-min equilibration in Krebs solution, matched strips from the same gallbladder were compared for three consecutive 20-min periods (Fig. 5). Strips from 18 gallbladders were used in this part of the study. Control strips were bathed first in Krebs solution, then in CCK-PZ at 10 units/100 ml, and then again in Krebs solution. The test strips were treated in the same way except that secretin (1 or 10 units/100 ml), gastrin (1 or 10 μg/ml), or glucagon (1 or 10 μg/100 ml) was added for the three periods. A standard strip was also run in CCK-PZ (10 units/100 ml) for 20 min so that the effects of the other hormones could be compared with a known stimulus (Fig. 5).

The values (B—A), (C—A), and (D—A) for the controls (Fig. 5) were subtracted from the equivalent values for the test strips. From these calculations, stimulation or

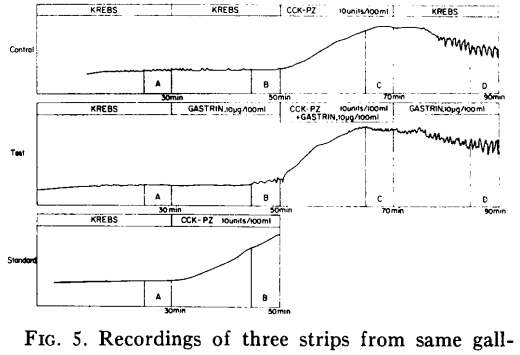

FIG. 5. Recordings of three strips from same gallbladder: CCK-PZ caused shortening of each strip; Gastrin had no direct effect and did not significantly affect CCK-PZ-induced contraction; Changes in length of each strip were compared by planimetry of areas A, B, C, and D (see text).

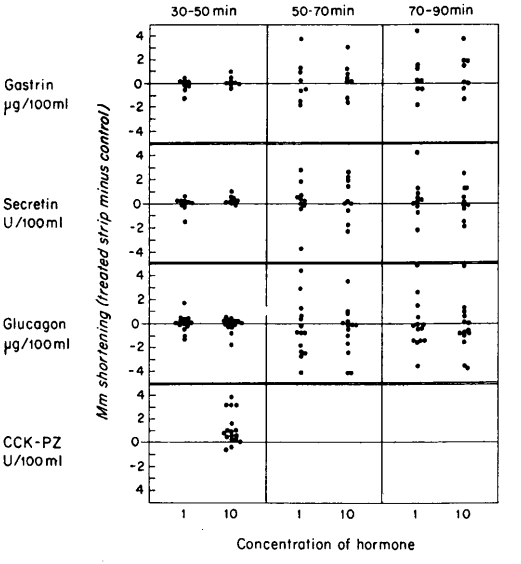

FIG. 6. Effect of gastrin, secretin, and glucagon on gallbladder strip shortening: positive values indicate that test strip contracted more than control; negative values indicate less contraction than control. In first 20-min period, secretin (10 units/100 ml) had significant effect but smaller than that of standard strips in CCK-PZ.

inhibition of the strips as well as potentiation or inhibition of CCK-PZ-induced contraction could be assessed. In the first 20-min period (Fig. 6), strips bathed in secretin (10 units/100 ml) shortened slightly but significantly ($p < 0.02$, $n = 10$) in comparison controls from the same gallbladder. Mean strip shortening (0.26 mm) was less ($p < 0.005$) than the mean shortening (1.43 mm) of standard strips from the same gallbladders treated with CCK-PZ (10 units/100 ml). Secretin, in similar concentration, had no apparent effect during or after stimulation with CCK-PZ. Neither secretin at 1 unit/100 ml, gastrin, nor glucagon had demonstrable stimulatory or inhibitory action during any of

the three periods, and no effect of these hormones on the regular or irregular contractions was observed.

Discussion. These studies supplement the data, obtained by other methods, that show that CCK-PZ has a direct action on the muscle of the human gallbladder (3). The principal effect *in vitro* was to cause progressive strip shortening which parallels the progressive emptying of the organ *in vivo*. CCK-PZ also was found to increase the rate but not the amplitude of superimposed waves. After CCK-PZ-induced contraction, the human gallbladder slowly refills *in vivo* (2) and the time required for this is comparable to the relaxation of the gallbladder strips found *in vitro*.

Despite these similarities between the behavior of gallbladder strips *in vitro* and the whole organ *in vivo*, the strips were much less sensitive to CCK-PZ in comparison with the effects of intravenous injection of the hormone on contraction of the human gallbladder (17). The lowest concentration found active *in vitro* was calculated to be approximately 25 times as great as that active *in vivo*. Although the muscle was histologically well preserved, deterioration of the tissue after its removal from the body may have occurred. However, strips stored in cold Krebs solution for 6 to 8 hr seemed to respond as well as those used within 30 min of surgery and not cooled below room temperature. Loss of vagal innervation may also be important. Thus, when the common bile duct is divided or infiltrated with procaine in dogs, gallbladder emptying in response to CCK-PZ is imparied (18) whereas vagal stimulation enhances the effect of CCK-PZ on gallbladder tone in cats (19). The relative insensitivity of the tissue could also reflect the fact that the hormone was not reaching the core of the strips through an intact blood supply.

Secretin (10 units/100 ml; 7.3×10^{-9} *M*), when used alone, had approximately one sixth of the potency of CCK-PZ (10 units/100 ml; 8.6×10^{-9} *M*). However, secretin did not potentiate CCK-PZ-induced contraction, and the physiologic importance of the secretin effect is uncertain, especially since assays of natural secretin have stressed lack of action on the gallbladder as a measure of purity (20). The lowest effective concentration of CCK-PZ was 4 units/100 ml (3.4×10^{-9} *M*). This hormone probably shares the C-terminal tetrapeptide sequence of gastrin (4, 5), but gastrin (10 μg/100 ml; 47.3×10^{-9} *M*) had no effect on the gallbladder strips. It was recently shown (21) that gastrin had about 1/22 of the cholecystokinetic effect of CCK-PZ on a molar basis, *in vivo* in the dog. Higher concentrations of gastrin than those used possibly would have had an effect *in vitro*. Glucagon, which shares part of the amino acid sequence of the secretin molecule, had no effect even in a concentration of 10 μg/100 ml (28.8×10^{-9} *M*).

Summary. A method was devised to study the action of gastrointestinal hormones on the motility of strips of human gallbladder muscle *in vitro*. Cholecystokinin-pancreozymin (CCK-PZ) in concentrations of 4 units/100 ml and greater caused progressive shortening. With increased concentrations, the latent period before contraction was briefer. CCK-PZ also caused an increase in the frequency, but not the amplitude, of superimposed contraction waves. The mean duration of action of CCK-PZ was 18 min. Synthetic secretin at 10 units/100 ml had about one sixth of the activity of a similar concentration of CCK-PZ; gastrin and glucagon had no effect.

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Macroamylasemia: Observations on the Nature of the Macroamylase* (33827)

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Evidence has been advanced indicating that prolonged elevation of serum amylase activity may result from the existence in the serum of a macromolecule of amylase that is too large to be excreted by the kidneys (1–4). Certain observations made on the nature of this large amylase structure constitute the basis of the present report.

Materials and Methods. Serum samples were obtained from 12 patients with so-called "macroamylasemia." The existence of a macromolecular serum amylase component was established in each case by dextran gel (Sephadex G 200[1]) filtration as previously described (2). This was confirmed in one case (Case 5) by filtration through a column of polyacrilamide gel (Bio-Gel P-300[2]). Additional verification was obtained in 2 cases (Cases 1 and 8) by sucrose density-gradient ultracentrifugation using essentially the same method as that employed by Levitt and Cooperband (3) except that the buffer was 0.1 *M* Tris at pH 7.4 instead of 0.01 *M* phosphate at pH 7.0. The sedimentation coefficient of the large amylase component in each case was roughly estimated from its Sephadex G200 elution position as compared with the elution positions of the major protein fractions (5).

To test the effect of dissociation of possible amylase–protein complexes on the amylase elution pattern (6), urea was added to serum specimens from each of the 12 patients to a final concentration of 6 *M*. The urea–serum mixtures were allowed to stand at room temperature for 30 min and were then applied to a dextran gel column prepared by exposure to 6 *M* urea in 0.85% sodium chloride. The same urea–saline solution was used as the eluant.

The Michaelis constant for starch using

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[1] Sephadex G 200, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

[2] Bio-Gel P-300, Bio-Rad Laboratories, Richmond, Calif.