Actions of Cholecystokinin/Pancreozymin, Secretin and Gastrin on Extra-hepatic Biliary Tract Motility *in Vitro*

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To the duodenal hormone cholecystokinin/pancreozymin is ascribed the dominant role in the regulation of the motor activity of the extra-hepatic biliary tract. However, it is not clear whether the other gastroduodenal polypeptide hormones gastrin and secretin have any direct action on the biliary musculature.

In a previous report we described the distribution of smooth muscle, the *in vitro* motility of the common bile duct of man and the dog and the *in vitro* motility of the gallbladder and sphincter choledochus in the dog.[15] In both man and the dog, the wall of the common bile duct was found to contain small quantities of smooth muscle, which was in greater amount at the lower end and orientated chiefly in a longitudinal direction. Longitudinally suspended specimens of the common bile duct were found to exhibit spontaneous contractions in characteristic wave forms *in vitro* (Fig. 1). Furthermore, the common bile duct, gallbladder and sphincter choledochus responded to the stimulus of both sympathomimetic and parasympathomimetic drugs.

The purpose of this study was to investigate the effects of the gastrointestinal hormones cholecystokinin/pancreozymin, secretin and gastrin, on the motility of the common bile duct, gallbladder and sphincter choledochus of the dog and on the motility of the human common bile duct *in vitro*.

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Material

Canine Specimens

Thirty specimens of the extra-duodenal common bile duct, ten specimens of the sphincter choledochus and four gallbladders were obtained at laparotomy from healthy anesthetized dogs.

Human Specimens

One specimen of the whole human extrahepatic biliary tract was obtained from a cadaver kidney donor 30 minutes after death. Four longitudinally cut strips 2–3 mm. in width and 1–2 cm. in length were obtained at operation from the common bile duct in four patients undergoing supraduodenal exploration of dilated ducts.

Methods

Immediately after removal all specimens were placed in Earle's solution at room temperature.

Common bile duct specimens were studied for changes in length in the longitudinal axis by suspending the duct by threads attached to each end of the duct.

The canine sphincter choledochus was studied by excision of all duodenal muscle around the intra-duodenal segment of the common bile duct, leaving a band of mus-

* Composition of Earle's solution:

NaCl 6.80 Gm.; KCl 0.40 Gm.; $CaCl_2$ 0.20 Gm.; $MgSO_4 \cdot 7H_2O$ 0.20 Gm.; $NaH_2PO_4 \cdot H_2O$ 0.14 Gm.; $NaHCO_3$ 2.20 Gm.; Glucose 1.00 Gm.; Water 1 liter

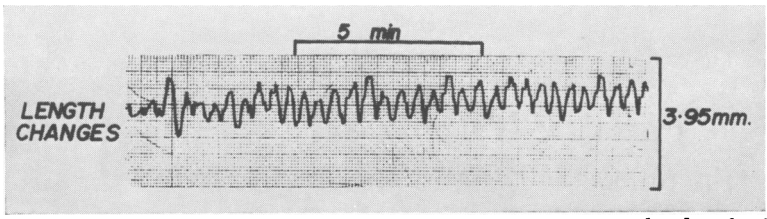

FIG. 1. Spontaneous activity of the canine common bile duct *in vitro*. The characteristic wave pattern is demonstrated by changes in the length of the duct. Two types of waves are shown: Spiked waves of 5 to 15 seconds duration and of a frequency of 3 to 4 per minute and waves of greater amplitude of 20 to 30 seconds duration and frequency of 2 to 3 per minute.

cle approximately 2 mm. in thickness around the duct. The motility of the circular muscle of the sphincter was studied by suspension of the sphincter between two looped threads passed through the duct lumen. Motility of the longitudinal muscle of the sphincter was studied by suspending the sphincter between two threads, one attached at the entrance of the common bile duct into the duodenum, the other at its exit on the duodenal papilla.

In vitro motility of the canine gallbladder was studied by preparing full thickness strips approximately 1 cm. in width and 4 cm. in length, cut obliquely from the wall. These specimens were suspended by threads attached to each end of the specimen.

All specimens were suspended from an isotonic lever with a load of 3 Gm. in Earle's solution at 37° C. and a mixture of 95% O_2 and 5% CO_2 was bubbled through the solution. Under these conditions the pH was 7.4, P_{O_2} 140 mm. Hg and P_{CO_2} 44 mm. Hg. Recordings of length changes were made using a linear differential transducer placed in series with the thread atttaching the specimens to the isotonic lever, and recordings were made on a pen chart recorder.

At the start of each experiment time was allowed for each specimen to exhibit spontaneous motility. The effect of addition of the following gastrointestinal hormones was then studied on all preparations:

Cholecystokinin/pancreozymin (G.I. Hormone, Lab, Karolinska Institute, Stockholm) in concentrations ranging from 0.001 to 0.1 Ivy Dog units/ml.

Secretin (Boots) in concentrations ranging from 0.0004 to 0.3 Crick Harper units/ml.

Synthetic gastrin I and gastrin-pentapeptide (ICI.10123 'Peptavlon,' ICI) in concentrations ranging from 10^{-8} to 10^{-4} Gm./ml.

In addition, the effects of the cholinergic blocker atropine sulphate and the adrenergic blockers phentolamine hydrochloride and propranalol hydrochloride on the actions of these hormones were examined.

Results

Canine Common Bile Duct

Spontaneous Activity

Specimens of canine gallbladder, common bile duct and sphincter choledochus and human common bile duct usually exhibited a spontaneous motility comprised of three wave types (Fig. 1).

There were small spiked waves superimposed on waves of greater amplitude and longer duration; their frequency was 3–4 per minute duration between 5 and 15 seconds and amplitude between 0.05 mm. to 0.5 mm.

There were more prominent waves of duration between 20 and 30 seconds, frequency 2–3 per minute and amplitude which varied from 0.5 mm. to 12 mm.

Slow, tonal waves also were identified; these were of approximately 8 minutes duration and with an amplitude varying from 0.5 to 2 mm.

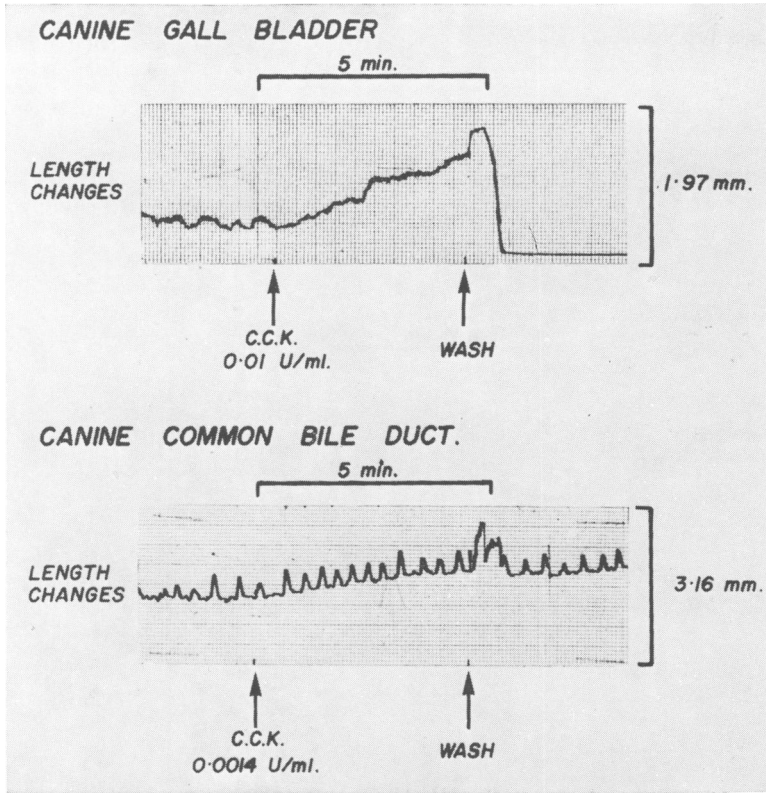

FIG. 2a. Effect of cholecystokinin/pancreozymin on the canine ballbladder and common bile duct *in vitro*. There is an immediate contraction of the gallbladder specimen in a concentration of 0.1 units/ml. The common bile duct contracts and a regular wave form is produced in a concentration of 0.0014 units/ml.

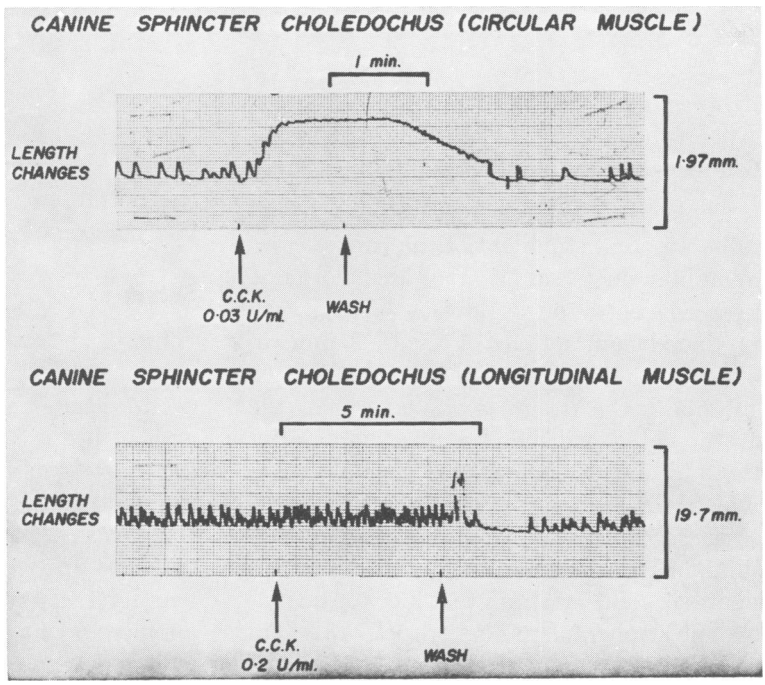

FIG. 2b. Effect of cholecystokinin/pancreozymin on the circular and longitudinal muscle of the sphincter choledochus. There is an immediate contraction of the circular muscle in a concentration of 0.03 units/ml. and an increased wave frequency in the longitudinal muscle in a concentration of 0.2 units/ml.

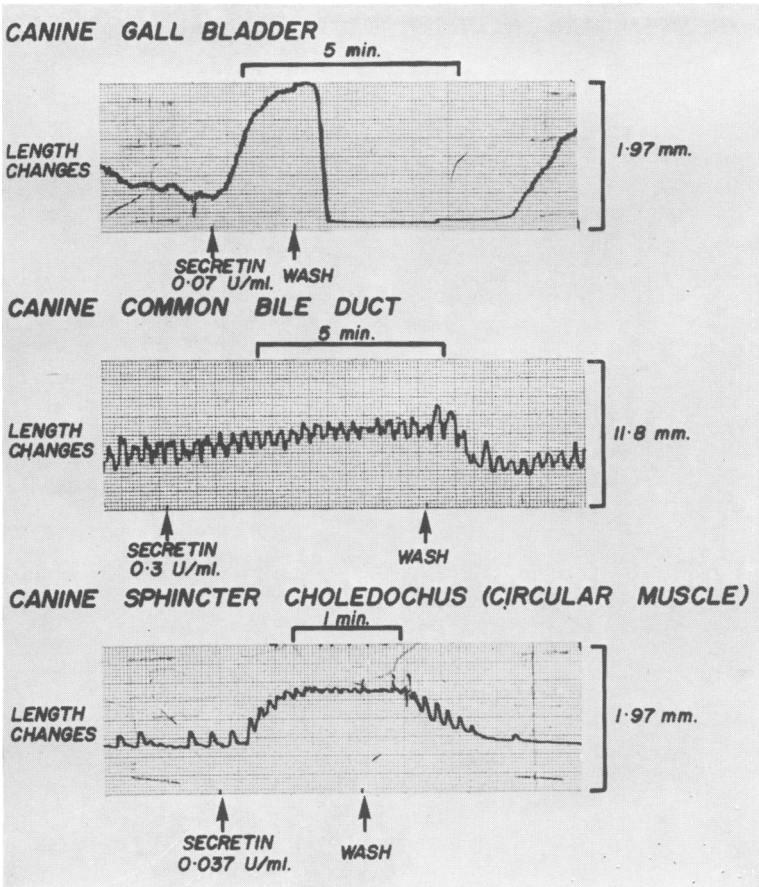

FIG. 3. Effect of secretin (Boots) on the canine gallbladder, common bile duct and sphincter choledochus (circular muscle).

There is an immediate contraction of the gallbladder in a concentration of 0.07 units/ml., a slow contraction of the common bile duct in a concentration of 0.3 units/ml. and an immediate contraction of the circular muscle of the sphincter choledochus with a change to a regular wave form in a concentration of 0.037 units/ml.

Cholecystokinin/pancreozymin

Cholecystokinin/pancreozymin was found to be a potent stimulant of the canine common bile duct. Addition of this hormone in a concentration as low as 0.001 u./ml. produced contractions of up to 3 mm. in eight of nine specimens (Fig. 2a). In two specimens the irregular wave form of the unstimulated specimen became regular after the addition of cholecystokinin/pancreozymin to the bath (Fig. 2a).

These contraction responses to cholecystokinin were not blocked by atropine sulphate (in concentrations that blocked acetylcholine), phentolamine hydrochloride (in concentrations that blocked adrenaline tartrate) or propranalol hydrochloride (in concentrations that blocked isoprenaline hydrochloride).

Secretin

In six of seven specimens this hormone, in concentrations as low as 0.004 u./ml., produced an immediate contraction of the duct of up to 3 mm. and a modification of the wave form; in those specimens in which the spontaneous activity was prominent there was a decrease in its amplitude, while in those specimens in which the spontaneous activity was not prominent there was an increase in amplitude (Fig. 3). These contraction responses were not blocked by

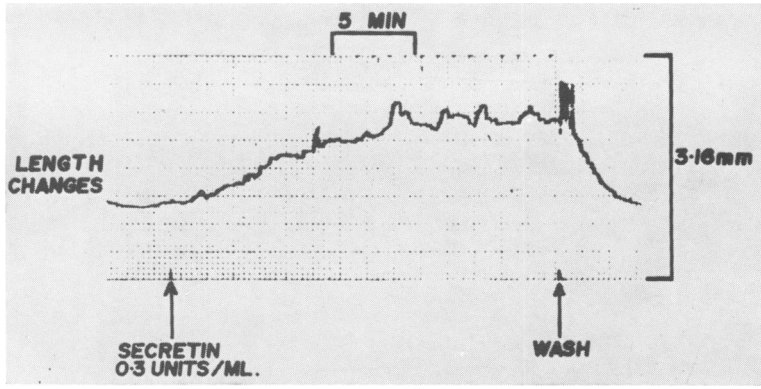

FIG. 4. Effect of secretin (Boots) on the human common bile duct. There is a contraction of the duct and an appearance of a wave form in a concentration of 0.3 units/ml.

atropine sulphate, phentolamine hydrochloride or propranalol hydrochloride.

Gastrin I and Gastrin Pentapeptide

Synthetic gastrin I and gastrin pentapeptidde (ICI.10123) were found to have identical actions in any single specimen but variable responses were obtained in different specimens. In four specimens, contractions of up to 3 mm. without change in the wave form were produced (Fig. 5); in two specimens there was a relaxation of up to 1 mm. which was associated with an increase in the amplitude of the wave form; in two specimens there was an initial relaxation of approximately 1 mm. followed by contractions of up to 3 mm. with no change in the wave form. In two specimens there was no response.

Atropine sulphate, phentolamine hydrochloride and propranalol hydrochloride did not block these responses.

Human Common Bile Duct

Cholecystokinin/pancreozymin

Four of the five human common bile duct specimens responded to the addition of cholecystokinin/pancreozymin by contractions of up to 0.2 mms. There was no effect on the wave form during these contractions.

Secretin

Secretin produced contractions of up to 0.5 mm. in all three specimens of common bile duct tested (Fig. 4). There was an associated increase in the amplitude of the spontaneous activity.

Gastrin I

Two human common bile duct specimens were tested; in each gastrin produced contractions of up to 0.2 mm. without change in the wave form.

Canine Sphincter Choledochus

Cholecystokinin/pancreozymin

(a) *Circular Muscle.* In three of seven specimens, contractions of up to 2 mm. occurred with a simultaneous decrease in the amplitude of the wave form (Fig. 2b). In one specimen there was no change in length but there was a decrease in the amplitude of the wave form. There was no response in the other three specimens.

(b) *Longitudinal Muscle.* Two specimens were tested. In one specimen there was no contraction. However, there was an increase in the wave frequency over that of the unstimulated specimen (Fig. 2b). There was no response in the other specimen.

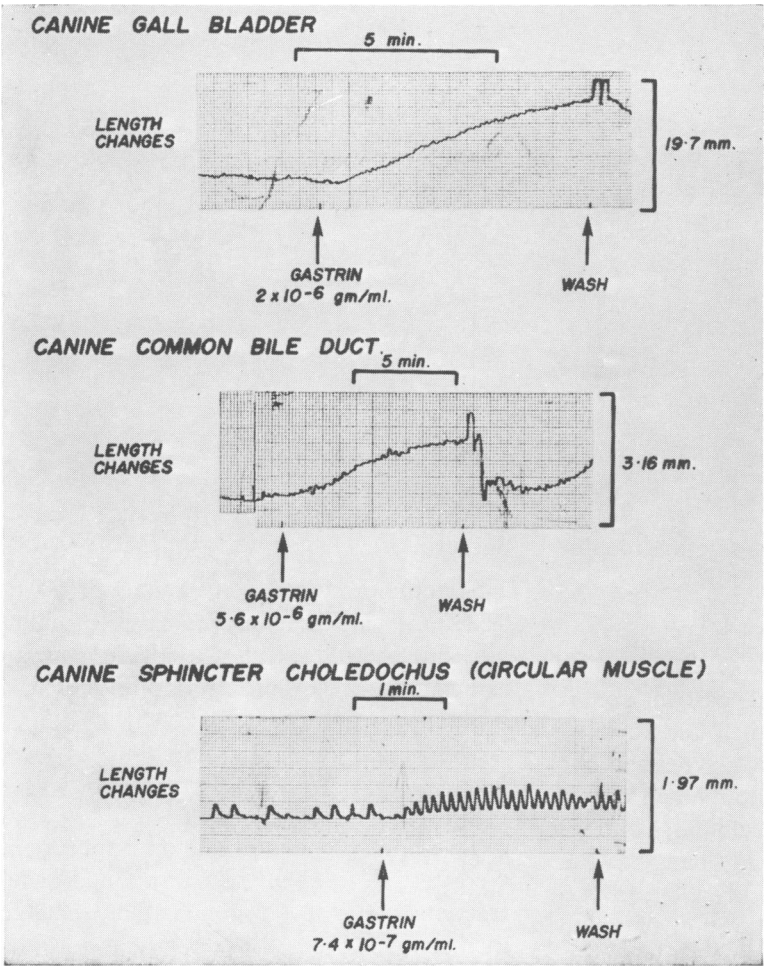

FIG. 5. Effect of gastrin I on the canine gallbladder, common bile duct and sphincter choledochus (circular muscle).

There is an immediate contraction of the gallbladder in a concentration of 2×10^{-6} Gm./ml., of the common bile duct in a concentration of 5.6×10^{-6} Gm./ml. and a contraction of the circular muscle of the sphincter choledochus with a change to a regular wave form of increased amplitude in a concentration of 7.4×10^{-7} Gm./ml.

Secretin

(a) *Circular Muscle.* Contractions of up to 1 mm. were observed in three of five specimens, with a decrease in the amplitude of the wave form (Fig. 3). There was no response in two specimens.

(b) *Longitudinal Muscle.* There was no response in the two specimens tested.

Gastrin

(a) *Circular Muscle.* Of four canine specimens there was a response in two. There was a contraction and an increase in the frequency and amplitude of the wave form over that observed in the unstimulated specimen (Fig. 5). No response was obtained in the other two specimens.

(b) *Longitudinal Muscle.* There was no response in the two specimens tested.

Canine Gallbladder

Cholecystokinin/pancreozymin

In three gallbladder strips cholecystokinin/pancreozymin produced immediate contractions of up to 4 mm. without any change in the wave form (Fig. 2a). Atro-

pine sulphate, phentolamine hydrochloride and propranalol hydrochloride did not block the action of cholecystokinin on the gallbladder.

Secretin

In the two canine gallbladder specimens tested, immediate contractions of up to 4 mm. were elicited without any change in the wave form (Fig. 3).

Gastrin

There were contractions of up to 8 mm. in two of three specimens tested. There was no change in the wave form (Fig. 5).

Discussion

The conventional physiological view of the action of cholecystokinin/pancreozymin is that this hormone stimulates contraction of the gallbladder and, at the same time, relaxes the sphincter of Oddi, thereby facilitating the flow of gallbladder and hepatic bile into the duodenum. There is no dispute about the action of cholecystokinin/pancreozymin on the smooth muscle of the gallbladder and there is experimental data from infusion studies [13] that the resistance to flow through the sphincteric region at the lower end of the common bile duct is decreased when this hormone is administered. On this evidence the postulated dual action of cholecystokinin on the smooth muscle at either end of the extra-hepatic biliary tract rests.

However, now there is evidence, both from the *in vivo* studies of the canine extra-hepatic biliary tract [18] and from the *in vitro* experiments reported in this paper that cholecystokinin/pancreozymin produces a rhythmical contraction of the smooth muscle of the entire extra-hepatic biliary tract, although the evidence for a rhythmical contraction of the sphincter at the lower end of the common bile duct rests on experiments in the dog alone. Watts and Dunphy [18] demonstrated that cholecystokinin/pancreozymin produced a contraction of the canine gallbladder and closed segments of the common bile duct, both *in vivo* and *in vitro*. They also showed that at the time of the apparent relaxation of the sphincter of Oddi (that is a decrease in resistance to infusion through its lumen), pressure recordings from within the sphincter *in vivo* demonstrated an increased level of rhythmical activity. *In vitro* experiments reported in this paper also demonstrate that cholecystokinin/pancreozymin stimulates a contraction of the canine gallbladder and of the longitudinally suspended common bile duct of both man and the dog. It also produced an increase in activity in both circularly and longitudinally orientated smooth muscle in the sphincteric region at the lower end of the duct.

Cholecystokinin/pancreozymin is therefore stimulatory to all smooth muscle throughout the canine extra-hepatic biliary tract and it is suggested that the flow of bile into the duodenum is increased by the action of this hormone in three ways:

(1) Contraction of the gallbladder.
(2) Shortening and lengthening (and possibly a primitive form of peristalsis) of the common bile duct.
(3) An increase in activity in the sphincteric region, whereby a peristaltic or milking action actively expels bile from the common bile duct into the duodenum.

Secretin and gastrin were found to have similar actions to cholecystokinin/pancreozymin on the smooth muscle of the extra-hepatic biliary tract *in vitro*. However, it is difficult to interpret our results with these hormones as the preparations used were impure.

The studies of Ravdin and Morrison [12] and Ivy [8] demonstrated that intravenous secretin produced a contraction of the gall-

bladder in dogs, but this was of lesser magnitude than that produced when cholecystokinin was administered. Mack and Todd [9] demonstrated that secretin had a contractile action on isolated human gallbladder strips suspended in a Ringer's solution.

In our experiments, secretin produced contraction of the canine gallbladder without change in the wave form and a contraction of the common bile duct with a modification of the wave form. In the circular muscle of the canine sphincter choledochus, secretin produced a contraction, with a decrease in the amplitude of the wave form, but we were unable to demonstrate any effect on the longitudinal muscle in the sphincteric area. The effect of secretin on the human common bile duct was similar to that of the dog; there was a contraction with an increase in the amplitude of the wave form (Fig. 4).

These actions of secretin on isolated segments of the extra-hepatic biliary tract were usually obtained in fairly high concentrations and using a preparation which contains small amounts of CCK/PZ.[2] The responses could therefore be produced by this contaminant and further experiments using pure natural or synthetic secretin are essential before ascribing any physiological role to this hormone on the smooth muscle of the biliary tract. The studies of Dinoso, Chey and Lorber [6] and Vagne, Stening, Brooks and Grossman [17] demonstrated that secretin had an inhibitory effect on intestinal and gastric motility. It would therefore be of great interest if pure secretin stimulated contraction of the smooth muscle of the biliary tract as indicated by these experiments.

Both gastrin I and gastrin pentapeptide have been shown to have a stimulatory effect on gastric and intestinal smooth muscle in a number of animal species and in man.[1, 3, 4, 7, 10 and 14] Mack and Todd [9] were unable to obtain any effect with gastrin pentapeptide on human gallbladder preparations *in vitro*. However, these experiments demonstrate that gastrin I and gastrin pentapeptide usually, but not invariably, produce contractions of the smooth muscle of all parts of the canine extra-hepatic biliary tract and of the common bile duct in man. These responses, particularly those on the canine common bile duct, were variable and the action of gastrin *in vitro* was not as predictable as that of cholecystokinin/pancreozymin and secretin. The same five C-terminal amino acids are shared by cholecystokinin/pancreozymin and gastrin,[11] so that a similarity of action of these two substances on the biliary smooth muscle might be expected.

Vagne and Grossman [16] experimented with dogs with cannulated gallbladders and found that gastrin II produced gallbladder contraction. However, after quantitative comparison of its effect with that of cholecystokinin/pancreozymin, they concluded that endogenous gastrin probably does not participate significantly in the regulation of gallbladder contraction. However, threshold concentration of gastrin which produced contraction of the biliary smooth muscle in these experiments was of the same order as the threshold concentration for acid secretion by the bullfrog gastric mucosa in the studies of Davidson, Lemmi and Thompson.[5] It is possible therefore that gastrin is a physiological rather than a pharmacological stimulant to biliary smooth muscle.

Summary

The actions of the gastrointestinal hormones cholecystokinin/pancreozymin, secretin and gastrin on the extrahepatic biliary tract of man and the dog were examined *in vitro*.

Cholecystokinin/pancreozymin and secretin (Boots) were found to have similar actions on the canine gallbladder, common bile duct and sphincter choledochus and the human common bile duct. They usually produced a contraction and an increase in the amplitude and frequency of the sponta-

neous activity. The effect of gastrin was similar but its responses were more variable.

Acknowledgments

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