The Effect of Gastric Antral Stimulation upon the Secretion of Hepatic Bile* (33537)

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It has been suggested that both gastrin and secretin contribute to the augmentation of biliary secretion after a meal (1). The physiologic importance of endogenous secretin as a choleretic has been questioned however (2), and the choleretic effect of antral stimulation has only been demonstrated in dogs during interruption of the enterohepatic circulation of bile salts (3, 4). These experimental conditions produce a rapid diminution of hepatic bile flow resulting in an abnormally low basal bile volume and bile salt output (1). In order to assess the physiologic importance of the gastric antrum in the regulation of hepatic bile secretion, the effect of antral stimulation was studied in conscious dogs with an artificially maintained enterohepatic circulation of bile salts.

Methods. Ten mongrel dogs, weighing between 12 and 18 kg were used. Each dog underwent cholecystectomy, ligation of the minor pancreatic duct, and gastrojejunostomy 12–18 cm distal to the ligament of Treitz. A Thomas cannula (5) was placed in the duodenum opposite the opening of the common bile duct. In 7 of the dogs a vagally denervated antral pouch was fashioned, the pyloric end was exteriorized as a mucocutaneous fistula and the duodenal stump was oversewn. In 3 of these 7 dogs with antral pouches a Heidenhain pouch was also prepared, and in the other 4 animals a gastric cannula was placed in the ventral wall of the stomach about 5 cm proximal to the gastrojejunostomy. The remaining 3 dogs had an antrectomy with reconstruction by gastrojejunostomy at least 45 cm distal to the ligament of Treitz.

Tests were commenced 3 weeks after operation. Each animal was fasted, except for water *ad libitum*, for 24 hr prior to an experiment. A solution of taurocholic acid (Matheson, Coleman, and Bell) in 0.9% NaCl (2 g/100 ml) was infused intravenously throughout each experiment at a constant rate of 0.5 ml/min using a constant infusion pump (Harvard Apparatus Company). The duodenal cannula was opened and the common bile duct cannulated with a no. 10 Fr. ureteral catheter inserted approximately 5 cm into the duct. The antral pouch was perfused by means of a Foley catheter at a rate of 90 ml/hr. The perfusate consisted of 0.15 *N* HCl during the control periods, or choline chloride in 0.9% NaCl (4 g/100 ml) buffered to pH 7.5 with Sorensens' phosphate buffer during the test periods.

The control period commenced 60 min after the beginning of the taurocholate infusion at which time the bile flow rate was relatively constant. Samples of bile and gastric juice were collected by gravity drainage at 15-min intervals during the 1-hr control period and during the 90-min test period. In the experiments in dogs with gastric cannulas, the cannulas were kept open during the 1-hr control period. This control period was followed by two consecutive test periods of 90-min during which the gastric cannula was alternately open or closed. The order of these two test periods was reversed in half of the experiments. In the 4 experiments on the dogs with antrectomy, the enterohepatic circulation was maintained as before, and bile was collected at 30-min intervals for 180 min without any additional stimulus.

The volume of each sample of bile was recorded and the concentration of bicarbonate determined by adding 0.5 ml of bile to 1.0 ml of 0.1 *N* HCl, boiling the mixture for 4 min and back-titrating to pH 7.0 with 0.05 *N* NaOH using an automatic titrator and pH meter (Radiometer, Copenhagen). Sodium and potassium determinations were performed with a flame photometer (Instru-

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TABLE I. Bile Flow and Composition in the Dogs with Gastric Pouches.[a]

Bile	Baseline (mean ± SEM)	Test period (mean ± SEM)	p
Volume (ml/15 min)	2.5 ± 0.3	4.8 ± 0.4	$p < 0.01$
Bicarbonate conc (meq/liter)	24.1 ± 2.4	42.3 ± 4.5	$p < 0.01$
Bicarbonate output (μeq/min)	3.9 ± 0.6	12.6 ± 0.9	$p < 0.01$
Chloride conc (meq/liter)	69.4 ± 5.1	81.7 ± 4.3	$p < 0.01$
Chloride output (μeq/min)	11.3 ± 2.5	26.0 ± 3.1	$p < 0.005$
Bile salt conc (meq/liter)	100.9 ± 6.2	60.1 ± 4.4	$p < 0.001$
Bile salt output (μeq/min)	13.7 ± 0.5	19.3 ± 2.1	$p < 0.02$
Bile solid conc (g/100 ml)	8.6 ± 0.5	5.5 ± 0.3	$p < 0.005$
Bile solid output (mg/min)	12.4 ± 0.8	19.0 ± 2.3	$p < 0.02$
Sodium conc (meq/liter)	182.8 ± 2.4	175.8 ± 2.1	$p > 0.1$
Sodium output (μeq/min)	26.4 ± 1.3	56.0 ± 4.6	$p < 0.01$
Potassium conc (meq/liter)	7.3 ± 0.5	6.3 ± 0.3	$p > 0.1$
Potassium output (μeq/min)	1.03 ± 0.03	2.00 ± 0.1	$p < 0.01$

[a] Volume and composition of bile during antral perfusion with 4% choline chloride or 0.15 *N* HCl in 3 dogs with antral pouches, Heidenhain pouches and duodenal cannulas (5 experiments). The baseline values represent the mean of the 4 samples during antral perfusion with 0.15 *N* HCl and the test values represent the mean of the last 4 samples obtained during antral perfusion with 4% choline chloride. The p values refer to the statistical significance of the observed changes derived from the Student's t test as applied to paired samples.

mentation Laboratory, Boston) and chloride determinations were carried out with a chloridometer (Buchler Instrument Company, New Jersey). Bile salt concentation was calculated by the method of Wheeler and Ramos (6) as the difference between the total estimated cation concentration (sodium and potassium) and the total estimated anion concentration (chloride and bicarbonate). Total bile solids were determined by repeated weighings after evaporation of 1-ml bile samples at 100° in an incubator. The volume of gastric juice was recorded and the acid concentation was determined by titration to pH 7.0 with 0.1 *N* NaOH using the automatic titrator.

Alterations of biliary flow and composition after antral stimulation were assessed by comparing the mean of the 4 control values with the mean of the values from the last 4 samples of the test period in each experiment. The statistical significance of the difference of the mean values in each experiment using each animal as its own control was estimated by Students' t test for paired samples (7).

Results. Bile. A. Dogs with Heidenhain pouches (Table I). Antral stimulation with choline chloride produced significant increases in biliary volume and rises in concentration and output of both bicarbonate and chloride. There were significant decreases in the concentrations of bile salts and bile solids but the output of bile salts and solids was nevertheless significantly increased because of the increased biliary volume. There were no significant changes in sodium and potassium concentrations but the output of these cations was significantly increased as a result of the increased biliary volume.

B. Dogs with gastric fistulae (Table II). When the gastric fistula was open, antral stimulation produced no significant alterations from the control values for biliary flow or for the concentrations and outputs of the measured biliary constituents. Antral stimulation with the gastric cannula closed produced changes in biliary flow and composition similar to those occurring in the experiments on the dogs with Heidenhain pouches. Paired comparison of the results of experiments in which the gastric cannula was closed versus those in which it remained open showed that the changes in biliary flow and composition were of similar magnitude to the paired comparison between the experiments with gastric

TABLE II. Bile Flow and Composition in the Dogs with Gastric Fistulas.[a]

Bile	Baseline (mean ± SEM)	p_1	Antral stimulation (mean ± SEM) Gastric fistula closed	p_2	Gastric fistula open
Volume (ml/15 min)	2.4 ± 0.3	$p < 0.001$	3.8 ± 0.2	$p < 0.001$	2.5 ± 0.3
Bicarbonate conc (meq/liter)	30.6 ± 4.5	$p < 0.001$	46.0 ± 2.9	$p < 0.02$	35.5 ± 3.5
Bicarbonate output (μeq/min)	5.4 ± 1.1	$p < 0.001$	11.3 ± 1.0	$p < 0.001$	6.2 ± 0.9
Chloride conc (meq/liter)	65.1 ± 5.4	$p < 0.01$	74.6 ± 4.8	$p < 0.02$	66.7 ± 5.2
Chloride output (μeq/min)	11.1 ± 1.8	$p < 0.001$	18.9 ± 2.0	$p < 0.001$	11.8 ± 2.0
Bile salt conc (meq/liter)	105.0 ±11.8	$p < 0.01$	70.7 ± 5.0	$p < 0.02$	96.5 ±10.7
Bile salt output (μeq/min)	15.4 ± 0.4	$p < 0.02$	17.8 ± 0.7	$p < 0.001$	14.5 ± 0.7
Bile solid conc (g/100 ml)	9.2 ± 0.1	$p < 0.01$	6.7 ± 0.3	$p > 0.1$	7.8 ± 0.7
Bile solid output (mg/min)	13.7 ± 0.5	$p < 0.01$	16.5 ± 0.5	$p < 0.005$	12.6 ± 1.1
Sodium conc (meq/liter)	193.5 ± 6.1	$p > 0.05$	185 ± 2.8	$p > 0.05$	192.4 ± 5.8
Sodium output (μeq/min)	30.7 ± 2.5	$p < 0.001$	46.6 ± 1.9	$p < 0.001$	32.0 ± 2.7
			Antral perfusion (mean ± SEM)		
Potassium conc (meq/liter)	6.9 ± 0.5	$p > 0.05$	5.9 ± 0.2	$p > 0.05$	6.1 ± 0.3
Potassium output (μeq/min)	1.05 ± 0.04	$p < 0.001$	1.49 ± 0.07	$p < 0.001$	1.02 ± 0.07

[a] Volume and composition of bile during antral perfusion with 4% choline chloride or 0.15 *N* HCl in 4 dogs with antral pouches, gastric, and duodenal cannulas (8 experiments). The baseline values represent the mean of the four 15-min samples during antral perfusion with 0.15 *N* HCl and the test values represent the mean of the last 4 samples obtained during the antral perfusion with 4% choline chloride. The *p* values refer to the statistical significance of the observed changes derived from Student's *t* test as applied to paired samples. The p_1 refers to the comparison between control values and those obtained when the gastric fistula was closed; p_2 refers to the comparison between values obtained when the gastric fistula was closed and when it was open.

cannulas closed and the control values.

C. Dogs with antrectomy (Fig. 1). The concentration and outputs of the measured bile constituents remained stable over the experimental period of 3 hr.

Acid. Antral perfusion with choline chloride caused a significant increase in acid secretion from both the Heidenhain pouches and the gastric fistulas (Table III).

Discussion. Wheeler and Ramos (6) have established that there is a linear relationship between the quantity of bile salt presented to the liver and the hepatic output of bile salt and bile volume. Our experiments on the

TABLE III. Acid Output from the Gastric Pouches and the Gastric Fistulas.[a]

Preparation	Acid output (meq/hr ± SEM) Basal	Antral stimulation	*p*
Heidenhain pouch	0.08 ± 0.03	3.94 ± 0.9	$p < 0.01$
Gastric fistula	0.58 ± 0.2	13.5 ± 2.3	$p < 0.01$

[a] Acid outputs from the Heidenhain pouches (3 dogs) and gastric fistulas (4 dogs) during antral perfusion with 4% choline chloride or 0.15 *N* HCl (13 experiments). The baseline values represent the mean of the sum of the four 15-min samples during antral perfusion with 0.15 *N* HCl and the test values represent the mean of the sum of the last 4 samples obtained during perfusion with 4% choline chloride. The *p* values refer to the statistical significance of the observed changes derived from Student's *t* test as applied to paired samples.

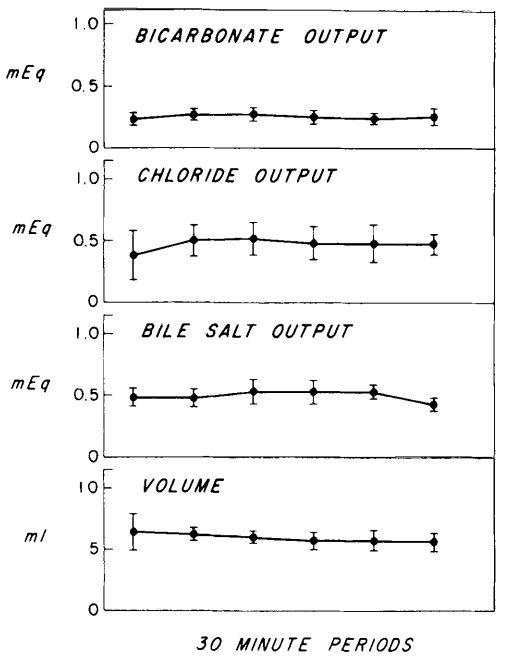

FIG. 1. Biliary volume, bile salt, chloride, and bicarbonate 30-min outputs in response to intravenous infusion of sodium taurocholate without any additional stimulus. Means and SE of 4 experiments in 3 dogs.

dogs with antrectomy demonstrate that the intravenous infusion of 2% sodium taurocholate at a constant rate gives rise to a steady state of biliary output enabling the choleretic effect of antral stimulation to be tested. The rate of infusion of taurcholic acid, about 13.5 μmoles/min was well below the "transport maximum" of the liver (110–140 μmoles/min) (8). Also, our observed steady state biliary volume averaging about 2.5 ml/15 min is within the normal range measured in dogs with an intact enterohepatic circulation (9). Wheeler and Ramos (6) suggested that the intermittent entrance of acid into the duodenum with the resultant release of secretin was probably responsible for the spontaneous change in the output of bile in their experiments during the constant infusion of taurocholate. Our findings support this view since the remarkably constant biliary output was observed in our animals subjected to previous antrectomy with gastrojejunostomy at least 45 cm from the ligament of Treitz.

The experiments fail to demonstrate a choleretic effect of endogenous antral gastrin when the gastric contents were prevented from entering the intestine. Conversely, a marked choleresis followed antral stimulation when the gastric sections were not diverted, demonstrating that the liver was capable of responding to a choleretic stimulus. It is probable that the choleresis observed in animals with an intact stomach or with gastric cannula closed resulted from the entry of acid into the duodenum or jejunum. That antral stimulation did release gastrin is indicated by the acid secretory response from the Heidenhain pouches and from the stomachs with fistulas.

The effect of gastrin on bile flow is uncertain. Intravenous gastrin administered to dogs with an interrupted enterohepatic circulation was stated to have a weak choleretic action (10) but others found no effect (11). When the enterohepatic circulation was artificially maintained, crude porcine gastrin had a marked cholerectic effect (12) but pure gastrin and pentagastrin had only a slight effect, suggesting that perhaps gastrin itself was not responsible for the choleresis produced by the crude extract. It is also possible that the entry of acid into the jejunum during those experiments might have produced some of the observed increase in bile flow (12).

Antral stimulation wtih acetylcholine solution (3) or 5% peptone (4) causes a choleretic effect in the dog with an interrupted enterohepatic circulation of bile salts. Choline chloride was used in our experiments because unlike acetylcholine, it is stable in solution and is also a powerful stimulus for the release of gastrin when applied locally to the antral mucosa (13). It is of interest that there was a significant increase in the output of bile solids and volume when antral stimulation was produced with 5% peptone (4) whereas only an increase in volume and not in bile salts was observed during antral perfusion with acetylcholine (3). It is possible that these experimental discrepancies can be explained by the presence in the gastric antrum of a choleretic substance other than gastrin

which is not released by perfusion with choline chloride, only slightly by local acetylcholine, and released even more by 5% peptone.

If gastrin itself is the choleretic agent produced by antral stimulation then it has a choleretic effect when the enterohepatic circulation is interrupted (3, 4) but none when it is intact as in the above experiments. The choleretic effect of intravenous secretin was shown to be independent of the bile volume output when the latter was varied by infusing taurocholate at different rates (8). If gastrin were similar to secretin in this respect then its effect would be more apparent at lower outputs of hepatic bile which occur when the enterohepatic circulation is interrupted whereas the effect may be masked when the enterohepatic circulation is intact. Alternatively, gastrin may have no effect on the liver which is actively transporting bile salts and yet may stimulate biliary electrolyte secretion when the hepatic cells are deprived of bile salts.

The choleresis with elevation of biliary bicarbonate and chloride concentrations that occurred in our experiments in which the gastric contents were permitted to enter the jejunum, resemble that produced by exogenous and endogenous secretin (6). The observed increased output of bile salts and bile solids which is not characteristic of the effects of secretin may be due to a "washout effect" or possibly the release of a choleretic besides secretin. Crude pancreozymin has been reported to increase bile solid output (14) but this has not been confirmed (15). Endogenous glucagon was shown recently to have a choleretic action in the dogs with an artificial intact enterohepatic circulation but it has a secretin-like effect (16).

Our results support the concept that secretagogues arising in the small intestine, namely secretin and possibly pancreozymin are probably of greater physiologic importance than gastrin in increasing the output of hepatic bile.

Summary. Seven dogs were prepared with completely separated vagally denervated antral pouches, gastrojejunostomy, and cholecystectomy. A duodenal cannula was placed opposite the opening of the common bile duct in each animal. In 3 of these dogs, a Heidenhain pouch was also constructed whereas in the other 4 animals a gastric cannula was placed in the ventral wall of the stomach. Biliary volume and composition were studied by catheterization of the common bile duct during constant i.v. infusion of 2% sodium taurocholate to maintain an intact enterohepatic circulation of bile salts. In the dogs with Heidenhain pouches or when the gastric cannula was closed, perfusion of the antral pouch with buffered 4% choline chloride solution at pH 7.5, caused highly significant increases in biliary volume, HCO_3 concentration and output, Cl concentration and output and total outputs of Na, K, bile salts, and bile solids. The concentration of Na and K was unchanged but the concentration of bile salts and solids was decreased. When the gastric cannula was opened, none of these effects were observed and there were no significant changes from the control values in biliary volume or composition. These studies indicate that the effect of endogenous gastrin on hepatic bile flow during artificial maintenance of the enterohepatic circulation of bile salts is predominantly an indirect one resulting from the entrance of acid into the small intestine.

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Serological and Immunofluorescent Study of Soluble Collagen in Tissue Culture of Fibroblasts (33538)

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The soluble fraction of collagen fibers appears before the insoluble fraction. Electron microscopic studies suggested that anticollagen antibodies inhibit the development of an intracellular substance, a probable precursor of collagen (1), in tissue culture of fibriblasts. According to morphological and biochemical studies the endoplasmic reticulum and microsomes are the sites of collagen synthesis (2, 3). The pericellular or extracellular organization of collagen fibers, however, is controversial (4, 5). It was previously suggested (6, 7), on the basis of immunofluorescence investigations, that soluble collagen is found in the fibroblasts of embryonic connective tissue, while the insoluble fraction develops extracellularly. The present study attempts to determine more precisely the specificity and the histological localization of soluble collagen antibodies and their relation of cell fibrillogenesis in cultures of fibroblasts.

Material and Methods. Preparation of collagen. Purified neutral salt-soluble, citrate-soluble, and insoluble fractions of collagen were prepared from leg tendons of adult chickens, according to Jackson (8). Other antigens, such as neutral salt-soluble collagen of bovine sclera and an acetic acid extract of carp swimming bladder prepared, following Jackson (8) and Gallop (9), respectively, served as control. All antigens were lyophilized. In the neutral salt-soluble collagen of chicken tendon, total sugars were determined by the anthrone method (10), proline by the Chinard technique (11), and hydroxyproline according to Logan (12), after collagen had been hydrolyzed with 6 *N* HCl for 24 hr at 100°.

Immunization. Four adult rabbits were immunized with the neutral salt-soluble collagen of chicken tendon. The total dose of collagen given in 45 days was 21 mg/animal. The antigen was first injected intracutaneously with complete Freund's adjuvant; after 2 weeks' interval a similar dose without adjuvant was injected subcutaneously. Ten days later, three successive daily injections were given by intraperitoneal, subcutaneous, and intravenous routes and repeated after a 1-week interval. The animals were bled 12 days after the final injection.

Serological tests. Complement fixation (13), agar double diffusion (14), tanned red cell agglutination (15), and immunoelectrophoresis (16) were utilized. Direct and cross reactions were used to verify: (a) the serological specificity of the neutral salt-soluble collagen by comparing it with the citrate-soluble and insoluble fraction of chicken tendon; (b) the species specificity of the same antigen, by comparing it with the neutral salt collagen-soluble fraction of bovine sclera and with an acetic acid extract of ichthyocol;