ful genetic marker, since at selective AG concentrations (a wide plateau at 1-10 μg/ml) colony formation by resistant cells is not impaired and sensitive cells are destroyed. A method for protein determination is described, based on measurement of eluted bromphenol blue from $HgCl_2$-fixed, glass-attached cells.

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Received May 22, 1959. P.S.E.B.M., 1959, v101.

Mechanism of Inhibition of Gastric Secretion in the Rat Following Bile Duct Ligation.* (25054)

RENÉ MENGUY† AND EDWARD KOGER (Introduced by Gilbert S. Campbell)
Dept. of Surgery, University of Oklahoma Medical Center and Vet. Admin. Hospital, Oklahoma City

Madden *et al.*(1) noted that simultaneous bile duct ligation in the Shay rat preparation prevented formation of rumenal ulcers. Ambrus(2) found that gastric secretory output from gastric fistula rats was slightly decreased when the bile duct was ligated. To date, this interesting phenomenon has not been adequately explained. On the basis of some evidence(3) that bile, when present in the intestine, is a gastric secretagogue, it was thought that removal of bile from the intestine could lead to decrease in gastric secretory activity. Actually, it has been postulated on the basis of a possible secretagogue effect of bile that continuous entrance of bile into the intestine in the rat, by virtue of absence of gall bladder, could explain the large interdigestive gastric secretion so characteristic of the rat(4). Our data show that gastric secretion in the rat is inhibited by bile duct ligation. This inhibition is due, not to removal of bile from the intestine, but to inhibitory effects of bile salts on gastric secretion.

Materials and methods. Gastric secretion was measured by pylorus ligation method(5) in 151 male Holtzman rats weighing from 160 to 220 g. Prior to experiments, the rats were fasted for 48 hours in individual cages with wide mesh wire bottoms to prevent coprophagia. Water was given *ad lib.* during fasting period. Following the 48 hour fast, the rats were operated under light ether anesthesia and under clean conditions and the pylorus gently ligated with a 4-0 silk ligature. Simultaneous ancillary procedures were done and will be described in relation to specific test groups. Exactly 4 hours later (6 hours in one group) the animals were killed by exsanguination, the stomachs removed and gastric content studied individually for volume and free acidity. The rats were divided into 5 groups.

Results. Group 1. Simultaneously with pyloric ligation, the proximal portion of bile duct was divided between 2 ligatures. In random controls, the duct was merely exposed. There was inhibition in volume and free HCl concentration of gastric content in bile duct ligated rats (Table I). There was relatively greater inhibition in volume than in free HCl concentration.

Group 2. An external biliary fistula was created simultaneously with pyloric ligation. This was accomplished by catheterizing proximal portion of bile duct with a fine polyethyl-

* The authors wish to acknowledge the valuable technical assistance of Mr. Howard Guiles.

† Markle Scholar in Medical Sciences.

TABLE I. Effect of Bile Duct Ligation, External Biliary Drainage, Internal Diversion of Bile from Small Intestine and Parenteral Administration of Bile Salts on Gastric Secretion in Pylorus Ligated Rats.

Group		No. of rats	Wt (g)*	Vol (ml)*	Free HCl (meq/l)*
1	Controls	12	208 ± 12.68	7.20 ± 1.90	112 ± 13.07
	Bile duct ligation	14	209 ± 16.55	1.25 ± .68 p <.001	62.5 ± 19.0 p <.001
2	Controls	10	204 ± 9.64	2.63 ± .96 p <.001	83 ± 13.6 p <.001
	Ext. biliary fistula	13	213 ± 8.60	3.46 ± 1.47 p <.001	104 ± 15.45 p <.001
3†	Controls	12	165 ± 10.48	9.80 ± 1.90 p <.001	107 ± 9.38 p <.001
	Bile shunt	10	160 ± 10.77	9.14 ± 1.60 p <.001	106 ± 10.0 p <.001
4	Controls	12	220 ± 23.70	7.44 ± 1.50	109 ± 12.80
	20% sodium dehydrocholate, 150 mg/200 g by I.V. infusion	12	224 ± 28.10	.65 ± .90 p <.001	36 ± 41.94 p <.001
5	Controls	19	182 ± 18.30	6.59 ± 1.59	110 ± 10.44
	Ox bile salts, 20 mg/200 g intraper. inj.	18	174 ± 16.50	2.77 ± .87 p <.001	99 ± 13.71 p <.01
	Ox bile salts, 30 mg/200 g intraper. inj.	19	175 ± 21.70	1.10 ± .59 p <.001	59 ± 26.55 p <.001

* Mean ± stand. dev. † 6 hr collection of gastric juice in Group 3. p = Probability of significance of t value.

ene tube and leading the tube to the outside *via* a stab wound. The duct was ligated below point of insertion of the tube. In controls the tube was implanted into the duodenum. In rats with external biliary drainage, the 4 hour gastric output was a little higher than in controls (Table I).

Group 3. Because of longer duration of anesthesia in Group 2, removal of bile from the intestine was achieved in another manner. Twenty-four hours prior to pyloric ligation, the bile duct was shunted to the rectum (Fig. 1). A sham operation was done in controls. There was no difference in gastric secretion between bile shunted rats and controls (Table I).

Group 4. Simultaneously with pyloric ligation, a fine polyethylene tube was placed in the tail vein and the latter infused continuously during 4 hour post operative period with 20% solution of sodium dehydrocholate. The infusion speed was adjusted so that a total dose of 150 mg/200 g of sodium dehydrocholate was given. Controls received an equal volume of normal saline. There was inhibition of volume and free HCl concentration of gastric content in the treated rats (Table I). Lower doses of sodium dehydrocholate or of ox bile salts administered by this route produced only inconsistent inhibition.

Group 5. Treated rats received 20 or 30 mg of ox bile salts in 2 ml of normal saline as single intraperitoneal dose at time of pyloric ligation. Controls received the same volume of normal saline. Consistent inhibition of same parameters occurred at these dosage levels (Table I). Doses higher than 30 mg/200 g resulted in total inhibition whereas doses lower than 20 mg/200 g resulted in inconsistent inhibition and only of volume of gastric content.

Discussion. These data suggest that gastric inhibition produced by bile duct ligation in the rat can be attributed to increase in level of circulating bile salts. This is known to be one of the effects of bile duct obstruction. The lesser inhibitory activity of intravenously administered bile salts may be explained by the fact that it is difficult to increase the level of circulating bile salts by intravenous injection unless huge doses are given. This appears to be due to rapid uptake by liver and adsorption by tissues and blood vessel walls(6). Presumably, this inhibitory effect of bile salts on

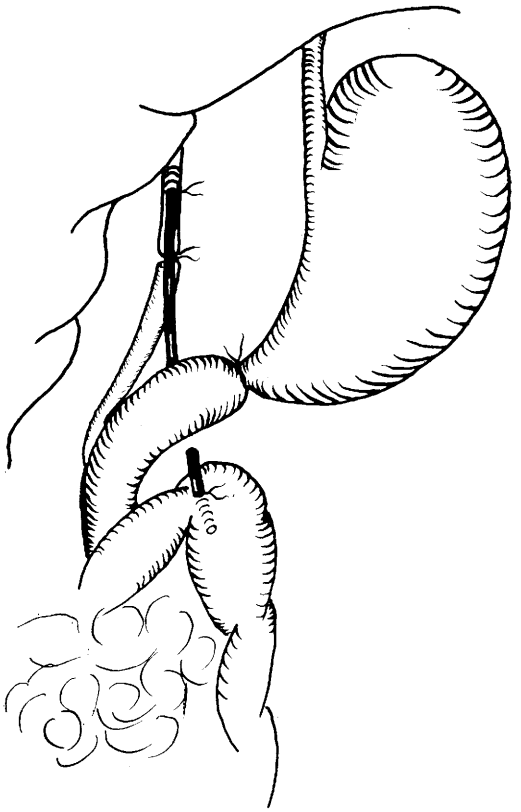

FIG. 1. Proximal portion of bile duct catheterized with fine polyethylene tube. Duct is ligated below point of insertion of tube. Free end of cannula is lead through mesentery of duodenum and implanted into rectum. With this technic entire bile output is diverted from small intestine and coecum. Pancreatic secretions reaching intestine via distal segment of bile duct are left undisturbed.

gastric secretion is related to increase in level of bile salts circulating in the pool intestine-portal blood-liver, and intraperitoneal administration should therefore be more effective. Amounts administered (23-30 mg/200 g) do not appear excessive in view of the fact that the rat excretes approximately 10-17 mg of cholic acid/hour(7) in the bile. At present, the mechanism by which parenterally administered bile salts inhibit gastric secretion in the rat cannot be explained. That this does occur is interesting since bile salts are naturally occurring compounds. Increased resorption of bile salts during digestion and absorption of food in the intestine may play a part in the physiological mechanisms of gastric inhibition following gastric emptying.

Summary. Gastric secretory activity (volume and free HCl concentration) in the pylorus ligated rat is inhibited by bile duct ligation. This is not due to removal of bile from the intestine but appears to be due to increase in level of circulating bile constituents, particularly bile salts, resulting from obstruction to the outflow of bile from the liver.

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Received May 25, 1959. P.S.E.B.M., 1959, v101.

Urinary Metabolites of Chlorpropamide in Dogs, Rabbits, and Man.* (25055)

J. S. WELLES, M. A. ROOT AND R. C. ANDERSON
Lilly Research Labs., Eli Lilly and Co., Indianapolis, Ind.

During pharmacological investigation of chlorpropamide [1-(*p*-chlorobenzenesulfonyl)-3-n-propylurea](1), a hypoglycemic agent, urinary excretion of the drug was measured by the method of Spingler(2). From 30 to 40% of orally administered chlorpropamide was detected in dog urine. This suggested that more than 50% of the ingested dose was metabolized. This report is concerned with identification of chlorpropamide metabolites in dog, rabbit, and human urine.

Methods. Twenty-four hour urine speci-

* The authors are deeply indebted to D. O. Woolf for infrared absorption curves, to Ann VanCamp for x-ray diffraction patterns, and to Dr. R. E. McMahon for sublimations.