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The Effect of Abdominal Evisceration or Adrenalectomy on Insulin Choleresis (40386)

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Studies in dogs revealed that insulin administered intravenously caused large increases in bile secretion (1–4). Because anticholinergic drugs or vagotomy reduced the stimulated bile secretion, insulin choleresis was thought to be vagally mediated (1). More recent studies, however, showed that insulin choleresis was not abolished following vagotomy, and there remained a substantial nonvagal component to insulin stimulated bile secretion (2–4). Therefore, although hypoglycemia is a potent vagal stimulant, insulin has another choleretic mechanism.

If insulin does not stimulate bile secretion by vagal mechanisms, then it acts directly upon the liver or stimulates another choleretic mechanism. The following experiments were performed to determine if any of the other hormones secreted by the gastrointestinal tract or adrenal glands are essential to the choleresis that follows insulin administration. Other hormones demonstrated to stimulate bile secretion include gastrin (5), secretin (6), cholecystokinin (5), glucagon (7), and cortisone (8).

Materials and methods. Mongrel dogs were anesthetized with intravenous pentobarbitol and randomly assigned to evisceration, adrenalectomy, or control groups. All dogs underwent cholecystectomy and cannulation of the common bile duct with polyethylene tubing (Intramedic PE 200). Animals in the evisceration group additionally underwent removal of stomach, spleen, pancreas, duodenum, rest of the small intestine, and colon. Controlled mechanical ventilation was provided throughout all experiments, and care was taken to avoid injury to the hepatic artery during evisceration.

Bile was collected by gravity drainage in graduated tubes. Throughout each experiment fifteen hundredths molar NaCl was infused into a leg vein via a polyethylene tube (Intramedic PE 50) at 50 ml/hr by a calibrated peristaltic infusion pump (Harvard Apparatus Co., Dover, MA). At the end of the fourth 15-min period 250 mg/hr sodium taurocholate (Maybridge Chemical Co., Tintage, North Cornwall, United Kingdom) was administered for the remainder of the experiment. After the 8th or 12th 15-min period each dog received an intravenous injection of regular insulin 1 unit/kg dissolved in 10 ml of 0.15 *M* NaCl.

Bile volumes were measured to the nearest 0.1 ml. Bile salt concentrations were measured by a steroid dehydrogenase method (9). Recovery of sodium taurocholate added to dog bile was 98%. Blood sugar concentration were measured with an Ames Blood Analyzer. Statistical comparisons were made using *t* tests for paired or unpaired values (10). *P* values less then 0.05 were considered significant.

Three groups of dogs were studied: (a) Fourteen eviscerated dogs were compared with 14 controls. Each of these dogs received the insulin at the end of the 8th 15-min period. (b) Six additional eviscerated dogs received insulin after 12 15-min periods so that taurocholate was infused for 2 hr prior to insulin administration. (c) Eight adrenalectomized dogs were compared to 8 controls. In these studies, the insulin was administered at the end of the 12th 15-min period.

Results. Evisceration studies. Bile flow. After insulin administration, bile flow increased significantly within 30 min in both the control animals and the eviscerated animals (Fig. 1). All bile flow rates after the 4th 15-min period in the eviscerated animals were significantly lower than controls. Because evisceration resulted in lower bile flow rates the effect of insulin on bile flow was assessed further by expressing post insulin responses as percent of the pre insulin (8th 15-min period) value. Peak post insulin flow in control dogs was 147% of the pre insulin values, while for

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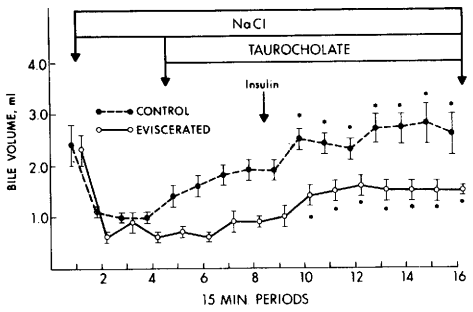

FIG. 1. The effect of evisceration on insulin stimulated bile flow. Each point represents the mean of 14 observations ± SEM. Asterisks indicate $P < 0.05$ compared with the 8th 15-min period.

eviscerated dogs the peak insulin response was 180% of the pre insulin flow.

Bile salt concentration. Pre- and postinsulin bile salt concentrations did not differ significantly in either the control or eviscerated groups (Fig. 2).

Bile salt output. The control animals exhibited no difference between pre insulin bile salt output and postinsulin bile salt output. In the eviscerated animals there were marked and statistically significant differences between the pre insulin and post insulin values (Fig. 3).

Blood glucose concentrations. Mean pre insulin blood sugar concentration in the control animals was 87.5 ± 5.2 mg% and in the first and second hours following insulin the values were 39.5 ± 2.4 and 52.9 ± 9.5 mg% respectively. Pre insulin sugar concentration in the eviscerated animals was 108.2 ± 18.3 mg% and in the first and second hours following insulin the values were 68.0 ± 16.3 and 60.2 ± 13.8 mg% respectively.

Evisceration studies with delayed insulin administration. Bile flow. In the group of eviscerated dogs administered insulin after 2 hr of taurocholate, bile flow increased significantly from 0.9 ± 0.1 ml/15 min to 1.3 ± 0.1 ml/15 min 30 min after insulin. Peak postinsulin flow was 1.4 ± 0.2 ml/15 min, 156% of the pre insulin value, occurring 75 min following insulin.

Bile salt output. In the second group of eviscerated dogs the preinsulin bile salt output was 102.4 ± 10.0 μEq/15 min, which was not significantly different from the values for the first and second postinsulin hours, which were 108.4 ± 18.2 and 111.8 ± 18.6 μEq/15 min respectively.

Adrenalectomy studies. Bile flow. Bile flow of both the control and adrenalectomized dogs increased significantly within 30 min following insulin administration (Fig. 4). Peak post insulin bile flow in the control dogs was 127% of the preinsulin value and peak post insulin bile flow in the adrenalectomized dogs was 145% of the preinsulin value. The responses to insulin of the two groups were not significantly different from each other.

Bile salt concentration. Bile salt concentration did not change significantly after insulin in either the control or the adrenalectomized animals (Fig. 5). There were also no significant differences in bile salt concentrations when the control and adrenalectomized groups were compared to each other.

Bile salt output. Bile salt output, like bile salt concentration, did not change significantly within 2 hr of insulin administration

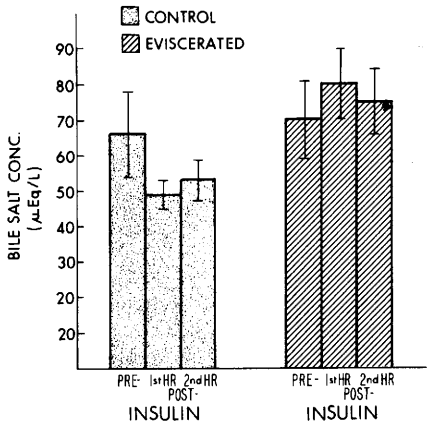

FIG. 2. Bile salt concentration before and after insulin in control and eviscerated groups. The bars represent the mean of 14 observations and the brackets designate SEM.

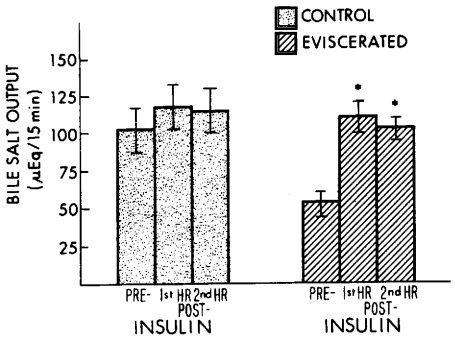

FIG. 3. Bile salt output before and after insulin in control and eviscerated groups.

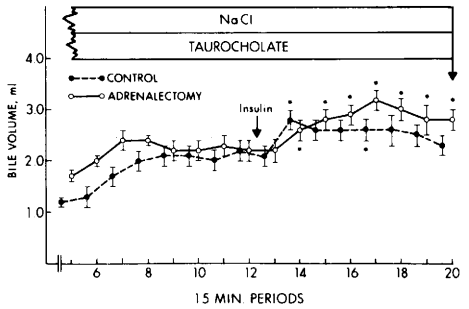

FIG. 4. The effect of bilateral adrenalectomy on insulin stimulated bile flow. Each point represents the mean of eight observations ± SEM. Asterisks denote $P < 0.05$ compared with the 12th 15-min period.

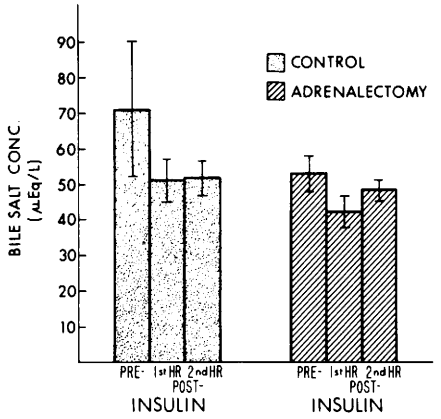

FIG. 5. Bile salt concentration before and after insulin in control and adrenalectomy groups. The bars represent the mean of eight observations and the brackets designate SEM.

in either the control or the adrenalectomized animals (Fig. 6). There were similarly no significant differences in bile salt output when control and adrenalectomized animals were compared for preinsulin, 1 hr postinsulin, and 2-hr postinsulin values.

Serum cortisol. Preinsulin serum cortisol concentration in the control group was 11.2 ± 0.5 μg/100 ml, and 1 hr following insulin was 11.0 ± 0.8 μg/100 ml. In the adrenalectomy group pre insulin serum cortisol concentration was 1.2 ± 0.1 μg/100 ml and one hour following insulin was 0.8 ± 0.2 μg/100 ml.

Discussion. These studies indicate that insulin increased hepatic bile secretion in the absence of the rest of the gastrointestinal tract. Insulin choleresis therefore is not caused by the release of other hormones from the stomach, spleen, pancreas, duodenum, rest of the small intestine, or colon. Previous work demonstrated that insulin choleresis resembles glucagon choleresis; both hormones had a canalicular action, increased chloride output, and had no effect on bicarbonate output (9, 11, 12). Gastrin, CCK, and secretin stimulated bile secretion, but acted predominantly on the ducts or ductules rather than on the canaliculi (5, 6). The present studies suggest that none of the other gastrointestinal hormones explains insulin's action, and that insulin choleresis is due to a direct action of insulin upon the liver.

As in previous studies in dogs, insulin increased bile secretion and did not change bile salt output. Experiments using erythritol clearance techniques showed that insulin also increased canalicular secretion, which suggested that insulin stimulates the bile salt independent canalicular fraction (11). The physiologic roles of insulin choleresis and of the bile salt independent canalicular fraction are not known.

Our earlier studies demonstrated that while insulin did not affect bile salt output, it did reduce bile salt concentration (2). The decreased bile salt concentration during insulin choleresis was explained by dilution of the bile salts by water. The reduction in bile salt concentration in the present studies was not statistically significant, probably because bile volumes in these studies were considerably smaller than in the earlier studies.

The data on bile salt output in the eviscerated animals is of special interest. It is evident in Fig. 3 that the pre insulin bile salt output in the eviscerated group was much lower than

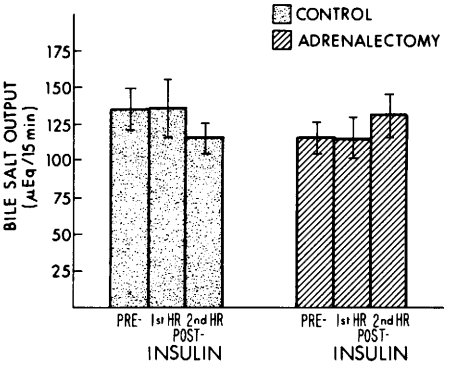

FIG. 6. Bile salt output before and after insulin in control in control and adrenalectomy groups.

the other bile salt output values in both groups. The bile salt infusion rate was approximately 116 μEq/15 min which is in the range of all bile salt outputs except the pre insulin value in the eviscerated group. Although several explanations for this observation are possible, we believe that a larger portion of the bile salt pool was lost in the animals with the excised intestine than was lost in the control preparation. This longer period of bile salt infusion would theoretically be required to achieve steady bile salt outputs in the eviscerated animals. Because the changes in bile salt flow in the eviscerated group were associated with changes in bile salt output, it was possible that the increased bile flow following insulin in the eviscerated group was due to increased bile salt secretion rather than to insulin. To clarify that question the six additional eviscerated dogs in the second part of the study were administered insulin later in the experiment, after bile salt output stabilized. In all six animals insulin increased bile flow again while bile salt outputs were unchanged, and pre insulin bile salt output values were about the same as post insulin values. Therefore, the choleresis was due to insulin and not to changing bile salt output.

It is interesting that evisceration decreased bile flow. In all periods subsequent to the 4th 15-min period, bile flow rates were significantly lower in the eviscerated animals than in control animals (Fig. 1). Removal of a portion of the bile salt pool does not explain this because bile salt outputs of both eviscerated control animals after the second hour of the experiments were comparable. It is tempting to speculate that deprivation of portal blood from the liver caused the reduction in bile flow but previous work by Neistadt *et al.* (13) indicate that is not the case. In that study bile flow was observed in anesthetized dogs before and after end-to-side or side-to-side portocaval shunt. Neither type of shunt changed basal bile secretion or dehydrocholate stimulated choleresis. Another explanation is that removal of the abdominal viscera deprived the liver of certain hormones that regulate bile flow.

Most experiments investigating bile secretion have been conducted in conscious, unanesthetized animals to avoid the effect of the anesthetic agent upon the experimental observation. The present studies were performed acutely because maintaining fluid balance and nutrition in chronic, eviscerated animals would also introduce additional variables into the experiment. Since experimental and control groups responded with significant increases in bile secretion after insulin, comparison between those groups would appear to be valid even though a general anesthetic was employed.

The third portion of these studies was performed to test the hypothesis that cortisone causes insulin choleresis. Previous investigators have demonstrated that hydrocortisone is also a potent stimulator of the bile salt independent canalicular fraction of bile secretion (8). It has also been demonstrated clearly that insulin hypoglycemia caused secretion of cortisone which resulted in increased plasma cortisone concentrations (14). It was therefore possible that insulin stimulated cortisone secretion may have contributed to the choleresis observed following insulin administration. Adrenalectomy produced no alteration in the choleresis following insulin administration. Also, insulin for unclear reasons did not cause a significant increase in serum cortisol concentration in the control dogs. In the adrenalectomy group the serum cortisol concentration was reduced and there was no increase following insulin. Therefore, it is highly unlikely that endogenous cortisone secretion is responsible for insulin choleresis.

The results of these studies strongly suggest that insulin administration influences bile secretory mechanisms by direct action of the hormone on the liver. More specifically, the gastrointestinal tract and adrenal glands are not essential for insulin choleresis. In an earlier study, insulin choleresis appeared to be reduced by antrectomy (15). However, those studies were carried out on dogs with depleted bile salt pools because of an interrupted enterohepatic circulation and no bile salt replacement. Gastrin probably is not primarily responsible for insulin choleresis also because the character of gastrin choleresis is quite different from insulin's (5, 7). Although insulin choleresis is not abolished by removal of the gastrointestinal organs, it is still possible that other gastrointestinal hormones

somehow participate in insulin choleresis.

Summary. In acute experiments performed on anesthetized dogs insulin choleresis was observed in control animals and compared with a group subjected to abdominal evisceration. Other studies compared insulin choleresis in control dogs with a group prepared by bilateral adrenalectomy. These experiments demonstrated that insulin choleresis was not abolished by evisceration or adrenalectomy. Increments in gastrointestinal or adrenal hormones are not necessary for insulin choleresis.

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