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Increased Bile Acid Synthesis after Interruption of the Enterohepatic Circulation: Evidence against Hormonal Mediation[1] (39601)

NEVILLE E. HOFFMAN[2] DAVID E. DONALD[3] AND ALAN F. HOFMANN[4]

Gastroenterology Unit, Division of Gastroenterology and Internal Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901; [3]Department of Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Interruption of the enterohepatic circulation by creation of a bile fistula or ileal resection causes a fall in serum bile acid levels (1, 2) and a five- to tenfold increase in bile acid synthesis in the experimental animal (3–5) or man (6–10). To explain this observation, several authors have suggested that bile acid synthesis is regulated by the amount of bile acids returning to the liver (4, 11).

We speculated that another explanation might be possible. The concentration of bile acids in portal or systemic blood might influence the release of a hormone which in turn would regulate hepatic bile acid synthesis. To test this, we have carried out experiments using the *in situ*-isolated perfused canine liver and compared the effect of blood from normal dogs with that of dogs with increased bile acid synthesis on the rate of bile acid synthesis by the perfused liver. As a control, we also perfused livers from dogs with increased bile acid synthesis with blood from normal dogs.

Materials and methods. The experimental technique featured the isolated perfused liver as described by Urquhart (12) in which the liver of a small dog is perfused *in situ* by arterial blood from a large dog. Details of this perfusion technique have been published previously (13). The experimental design was a 2-by-2 Latin Square: livers from small dogs with or without ileal resection were perfused by large dogs with or without ileal resection.

The animals used were mongrel dogs who had been treated for intestinal worms, were vaccinated against rabies and canine distemper, and had been on regular kennel diet for at least 3 weeks. Twelve days prior to study, all animals underwent laparotomy under pentobarbital anesthesia, and a modified Thomas cannula was positioned in the third part of the duodenum to serve as a sham operation in the animals not resected. Continuity of the intestine was reestablished with an end-to-end anastomosis in the larger dogs and a side-to-side anastomosis in the small dogs. The extent of resection was confirmed by weighing the excised gut at the time of surgery and the remainder of the gut at the conclusion of the study. All animals used made an uneventful recovery from surgery and were within 15% of their preoperative weight at the time of the study.

In each experiment a dog of 20–30 kg (pump dog) was used to provide the circulatory system to support the isolated liver of a second dog (donor liver dog) of 4–8 kg. The isolated liver was left *in situ* and perfused via the portal vein with blood from the femoral artery of the pump dog. The inferior vena cava of the donor liver dog was cannulated and liver venous outflow was returned to the femoral vein of the pump dog. The hepatic artery of the donor liver dog was ligated. Blood flow to the perfused liver was maintained at a constant value in the range of 0.8–1.5 ml/g liver or 30 ml/kg body weight donor liver dog, by adjusting portal pressure in the range of 5–20 cm H_2O. The cystic duct was ligated and a cannula placed in the common bile duct for bile collection.

Bile was collected for half-hour periods for at least 7 hr in each study. Portal inflow pressure and portal blood flow were moni-

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[2] Present address: Department of Medicine, Austin Hospital, University fo Melbourne, Melbourne, Australia. Dr. Hoffman was a FA Hadley Travelling Fellow from the University of Western Australia and an Australian-American Educational Foundation Scholar.

[4] Address reprint requests to Dr. Alan F. Hofmann, Gastroenterology Unit, Mayo Foundation, Rochester, Minnesota 55901.

tored continuously and recorded every 15 min, as was systemic blood pressure in the pump dog. An intravenous infusion of dextran was used as required to maintain systemic blood pressure. Oxygen tension, Hb, and O_2 saturation was measured in the inflow and outflow blood of the perfused liver on three occasions, 2 hr apart. This allowed calculation of hepatic oxygen utilization which was always in the range of 1.5–4 mmol/kg liver/min. Tissue was taken for histologic examination at the end of each experiment and all tissue was within normal limits.

Bile was analyzed for total bile acid concentration in each sample by a modification of the 3α-steroid dehydrogenase technique of Talalay (14).

The bile acid pool of the pump dog was labeled with [^{14}C]cholyltaurine 24 hr prior to the experiment on two occasions. By estimation of the specific activity of gallbadder bile acid from the pump dog and the bile acid secretion by the donor liver dog, we were able to show that the pump dog contributed less than 5% of the secreted bile. Therefore, we considered the bile acid secreted after the first hour to be newly synthesized bile acid.

The four possible combinations of resected and sham-operated dogs were tested: 10 experiments were performed using two sham-operated dogs; 2 experiments with a sham-operated donor liver dog and an ileal-resected pump dog; 2 experiments with an ileal-resected donor liver dog, and a sham-operated pump dog; and 2 experiments in which both pump and donor liver dogs underwent ileal resection.

The perfused liver was weighed at the end of the experiment and the rate of bile acid secretion is expressed as micromoles per kilogram of liver per minute to allow comparison of livers of differing weights.

Results. In the control experiments, the rate of bile acid secretion was quite steady by the end of the first hour of perfusion, but a slow decline occurred during the next 7 hr (Fig. 1). Essentially the same pattern of bile acid secretion was seen when the liver from a sham-operated dog was perfused by the blood of an animal with an ileal resection (Fig. 2). However, if the donor liver dog had had an ileal resection, the rate of bile acid secretion was considerably elevated above base line whether the pump dog had an ileal resection (Fig. 3) or was sham-operated (Fig. 4). In all studies, the rate of bile acid secretion beyond the first hour was considered to be an estimate of the rate of bile acid synthesis, since in an animal with a gallbladder such as the dog, most of the bile acid pool is excluded when the liver is prepared for perfusion.

Discussion. These experiments lend no support to the concept of a hormonal substance controlling bile acid synthesis. Previous work with this model has demonstrated a response to glucagon within 2 hr (12), and an isolated perfused rat liver has been shown to respond to cortisol growth

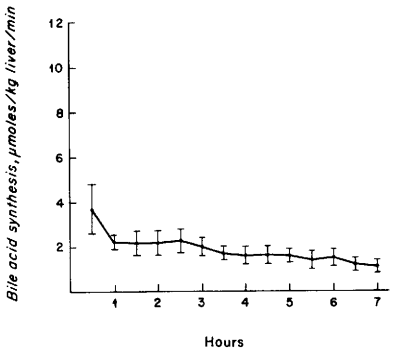

FIG. 1. Time course of bile acid synthesis (M ± SE) in the *in situ*-perfused canine liver in 10 dogs. Both the donor and pump dog had undergone a sham ileal resection.

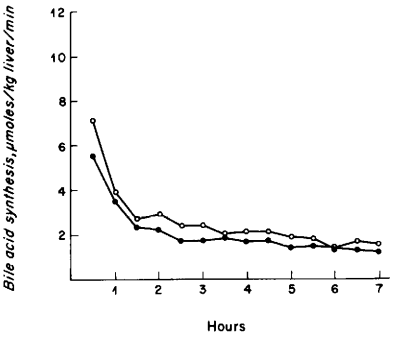

FIG. 2. Time course of bile acid synthesis in the *in situ*-perfused canine liver of two dogs each of whom had undergone a sham ileal resection; the pump dog had undergone ilial resection. Each curve represents a single study.

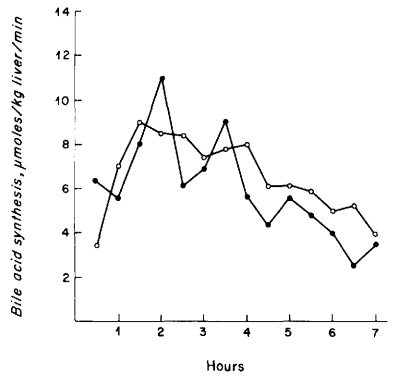

FIG. 3. Time course of bile acid synthesis in the *in situ*-perfused canine liver of two dogs each of whom had undergone ileal resection; the pump dog had also undergone ileal resection. *Each curve represents a single study.*

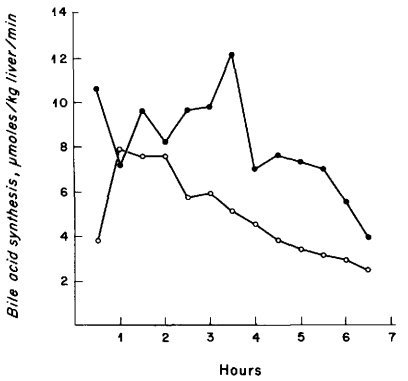

FIG. 4. Time course of bile acid synthesis in the *in situ*-perfused canine liver of two dogs, each of whom had undergone ileal resection; the pump dogs had a sham ileal resection. *Each curve represents a single study.*

hormone and triiodothyronine within 2 hr (15). Thus, a response by the perfused liver in these experiments might have been expected within 7 hr although the possibility of a substance of longer latent period has not been excluded. We think it unlikely that the failure to observe a response by the perfused liver is attributable to loss of capacity for bile acid secretion, since we have shown previously that this preparation will secrete a physiological bile acid load (0.22 mmole/hr) quantitatively even after 7 hr of perfusion (13).

Other workers (16) have implied that it is more likely that it is the concentration of bile acids within the hepatocyte that determines the rate of bile acid synthesis. Thus, bile acid synthesis falls during fasting (17) despite a decreased return of bile acids to the liver, perhaps because of increased concentrations of bile acids in the liver cell in association with decreased bile acid secretion.

Summary. Experiments were carried out using an *in situ*-perfused canine liver to test whether a hormone is responsible for the striking increase in bile acid synthesis occurring after interruption of the enterohepatic circulation by ileal resection. Large dogs with ileal resections or sham operations were used as pump dogs to perfuse the livers from small dogs with sham surgery or ileal resection. Bile acid synthesis during hours of observation was not influenced by the type of pump dog. Since most known hormones act rapidly on the isolated perfused liver, the experiments suggest that a hormonal substance is not responsible for the striking increase in bile acid synthesis observed when the enterohepatic circulation is interruped by removal of the ileum.

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