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Evidence for an Intestinal Factor Stimulating Hepatic Cholesterogenesis (41496)

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Abstract. External diversion of bile leads to an increase in hepatic cholesterol synthesis. To study the role of the small intestine in this phenomenon we conducted a series of experiments in bile-diverted rats with and without surgical removal of most of the small intestine, its upper half or its lower half. The pancreas was preserved intact in all experiments. Hepatic cholesterol synthesis at the time of surgery and 24 hr later was measured in liver homogenates using [2-^{14}C]acetate as substrate. Hepatic cholesterogenesis increased almost 4-fold 24 hr after biliary diversion in rats with intact intestine, and decreased to 64% of the baseline rate in bile-diverted rats with the jejunum–ileum removed, and to 58% in sham-operated animals. To investigate the possibility that the stimulation seen in the bile-diverted rats with intact intestine was due to a substance absorbed from the diet, the experiments were repeated with animals fed only 10% glucose in water 24 hr prior to surgery. Again the rats with intact small intestine showed an increase in hepatic cholesterogenesis (6.7-fold) while those with the jejunum-ileum removed and the sham operated showed a decrease to 60 and 70% of the baseline rate, respectively. These results support the hypothesis that the small intestine produces a factor(s) that stimulates hepatic cholesterol synthesis in response to a drop in the intraluminal content of bile. To narrow the site of production of the factor(s), the effect on hepatic cholesterogenesis of removal of the upper or lower half of the small intestine in bile diverted animals was studied by comparison to a group of bile-diverted intestine-intact controls. As before, the latter showed a 6-fold increase in hepatic cholesterogenesis. With the upper or lower half of the small intestine removed a similar degree (4- to 5-fold) of stimulation was still observed. These results suggest that the factor(s) is produced along the entire length of the jejunum-ileum.

On the basis of a substantial body of suggestive evidence Krumdieck and Ho (1) have postulated that the intestine plays a role in the regulation of hepatic cholesterogenesis by producing a stimulatory factor whenever the intraluminal concentration of bile acids drops below requirements. The factor is presumably transported to the liver by the portal circulation. According to this hypothesis, the well-documented increase in the rate of hepatic cholesterol synthesis seen in animals in which the flow of bile has been diverted away from the intestinal lumen by means of a biliary fistula (2) would be the result of an increased production of the intestinal stimulatory factor. Conversely, it may be postulated that the intestine produces an inhibitory factor of hepatic cholesterogenesis, the amount of which is decreased when the concentration of bile in the lumen decreases. If this hypothesis is true the functional integrity of both the intestine and the portal circulation would be essential for the elevation of hepatic cholesterogenesis in bile-diverted animals. Consequently, removal of the small intestine from a bile-diverted animal ought to completely eliminate the normally observed elevation of the rate of cholesterol synthesis in the liver. In this article we report the effects on hepatic cholesterogenesis of the simultaneous production of a bile fistula and the removal of nearly all the small intestine (from the ligament of Treitz to the cecum), its upper half, or its lower half, in rats. The results obtained support the existence of the proposed intestinal stimulatory factor.

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Materials and Methods. *Animals and surgical procedures.* Male Sprague–Dawley rats weighing 200 to 250 g were used in all the experiments. The animals were maintained in an air-conditioned room with alternating 12-hr light and dark cycles; the lights were on from 6:00 AM until 6:00 PM. To minimize confounding effects attributable to the circadian rhythm of hepatic cholesterol synthesis all animals were operated and sacrificed between 9:00 and 10:00 AM. The animals were maintained for at least one week prior to surgery on a modified MIT-200 diet (3) containing by weight 20% casein, 65% sucrose, 5% corn oil, 2% agar, and 4% cellulose plus 1% vitamin mix and 3% salt mixture. Under ether anesthesia groups of animals were subjected to one of the following surgical procedures:

(*a*) *Liver sampling and biliary diversion:* After ligating the pedicle, the lateral portion of the medial lobe of the liver was excised and immediately homogenized as described below to determine the baseline rate of hepatic cholesterol synthesis. After removal of the liver sample the common bile duct was cannulated using a polyethylene tube (PE-10; o.d. 0.6 mm) which was externalized through a small orifice in the abdominal wall and securely tied to the skin.

(*b*) *Liver sampling, biliary diversion, and removal of the small intestine:* The animals in this group underwent the same procedures to remove a sample of liver and to establish a biliary fistula as those in group (a). In addition, most of the small intestine was removed along its mesenteric attachment leaving in place the duodenum and approximately 1 cm of the distal ileum. The duodenum and terminal ileum were connected with a short plastic tube (o.d. 4 mm) to prevent the development of gastric distention. This operation leaves the pancreas intact and does not alter the venous drainage of the pancreatic circulation into the portal system.

(*c, d*) *Liver sampling, biliary diversion, and removal of either the upper* (*c*) *or the lower* (*d*) *half of the small intestine:* After sampling the liver and preparing a biliary fistula as in group (a), either the upper or the lower half of the small intestine was excised. When removing the upper half, the duodenum and 1 cm of the proximal jejunum were left in place. The continuity of the intestine was restored as in procedure (b) by means of a plastic tube. The same was done when removing the lower half of the intestine connecting the stump of terminal ileum to the remaining upper half of the intestine.

(*e*) *Liver sampling and sham-operation:* The animals in this group underwent removal of the lateral portion of the medial lobe of the liver followed by manipulation of the viscera to mimic the surgical trauma of the other groups. No bile fistula was prepared in this group.

All animals received a subcutaneous injection of 20 ml of 5% glucose in saline immediately after surgery and were maintained at room temperature and fasted for the next 24 hr following which they were sacrificed and their livers removed for estimation of the rate of cholesterol synthesis.

Determination of the rate of cholesterol synthesis. The samples of liver removed at the time of surgery and at the time of sacrifice were washed in ice-cold Tris–HCl buffer, 0.1 *M* pH 7.8, containing 0.01 *M* K_2HPO_4, 0.03 *M* nicotinamide, 0.1 m*M* EDTA, and 0.6 m*M* $MgCl_2$. The pieces were then blotted dry, weighed, and homogenized in the above buffer (2.5 ml/g of liver) containing also 5.0 m*M* oxidized glutathione. The homogenization was done using a loosely fitting glass–Teflon Potter-Elvehjem homogenizer and was completed in two strokes. The homogenates were centrifuged at 500*g* for 20 min at 4°. Eight-tenths milliliter of supernatant was incubated at 37° in a Dubnoff incubator (140 oscillations/min) for 5 min to allow temperature equilibration and the synthesis was initiated by the addition of 0.2 ml of a solution containing 1.8 m*M* [2-^{14}C]sodium acetate (specific activity, 0.4 mCi per mmole), coenzyme A 50 μM, ATP 1.0 m*M*, NADP 0.5 m*M*, and glucose 6-phosphate 3.0 m*M*. The incubation was continued for 30 min at the end of which 2.0 ml of 95% ethanol and 0.5 ml of 60% KOH (w/v) were added. After saponification for 90 min at 80° the 3-β-hydroxy steriods were precipitated

as the digitonides, washed, and counted as described before (4). Protein determinations in the homogenates were done following the Lowry method with bovine albumin as standard (5). The results are given in terms of nanomoles of acetate incorporated into cholesterol per gram of protein per hour. The percentage change in cholesterol synthesizing activity between the baseline and the 24-hr post-surgery values were calculated for each animal.

Results. *Effect of biliary diversion on the rate of hepatic cholesterogenesis 24 hr after surgery.* Before studying the effect of intestinal removal upon the hepatic cholesterol synthetic activity of bile fistula rats, it was necessary to demonstrate that the stimulation of hepatic cholesterogenesis after bile diversion occurred within a period short enough to assure the survival of the animals with the small intestine removed. Penhos *et al.* (6) demonstrated that eviscerated rats with a functional liver in which the stomach, small and large intestines, pancreas, mesentery and spleen had been excised and in which a bile fistula had been produced to allow continuity of bile secretion, survived for more than 72 hr provided that dehydration was prevented by the administration of saline. It was therefore safe to assume that rats subjected to the much less traumatic procedure (b), i.e., liver sampling, biliary diversion, and excision of the small intestine, would easily survive for 24 hr. It remained to be demonstrated that 24 hr after biliary diversion there was already a detectable increase in the rate of hepatic cholesterogenesis. To this effect three rats, fed the maintenance diet until the time of the experiment, were subjected to procedure (a), i.e., liver sampling and biliary diversion, and the rate of hepatic cholesterol synthesis was determined at the time of surgery and 24 hr later. In these animals the incorporation of acetate into cholesterol increased significantly from 198 ± 12 (mean ± SE) nmole/g of protein/hr to 552 ± 8 nmole/g protein/hr, or to 280 ± 30% of the baseline value ($P < 0.001$).

Effect of removal of the small intestine on the rate of hepatic cholesterol synthesis of rats 24 hr after total biliary diversion: Experiment I. Six animals were subjected to procedure (a), liver sampling and biliary diversion with intact intestine; five to procedure (b), liver sampling, biliary diversion, and nearly total removal of the small intestine; and four to procedure (e), liver sampling and sham operation. All these animals were fed the maintenance diet until the day of the experiment. The results are summarized in Table I. As in the preliminary experiment, biliary diversion markedly increased the rate of hepatic cholesterol synthesis to 377 ± 65% of the baseline value ($P < 0.01$). Removal of the small intestine in the bile-fistula rats completely eliminated this response resulting instead, in a small but significant drop of hepatic cholesterol synthetic activity to 64 ± 10% of the baseline rate ($P < 0.01$). The sham-operated animals showed also a modest but significant ($P < 0.05$) decrease in the rate of hepatic cholesterogenesis (Table I).

Experiment II. To investigate the possibility that the stimulation seen in the bile-diverted rats with intact intestine was due to some substance absorbed from the diet, the experiment was repeated with animals that consumed the maintenance diet until 24 hr prior to surgery and then given 10% glucose in water *ad libitum* as their only food. The results are shown in Table I. As in experiment I, cholesterol synthesis increased very significantly (to 670 ± 79% of baseline, $P < 0.01$) in the bile-diverted rats and decreased slightly (to 60 ± 8% of the baseline ($P < 0.01$) in the bile-diverted animals with the jejunum and ileum removed. The sham-operated group also showed a small decrease in hepatic cholesterogenesis. The most striking difference between experiments I and II was in the absolute rate of cholesterol synthesis which was many times higher in the diet-fed than in the glucose-fed group.

Effect of partial removal of the small intestine on the rate of hepatic cholesterol synthesis of rats 24 hr after total biliary diversion. Experiments I and II support the existence of a stimulatory factor of hepatic cholesterol synthesis produced by the jejunum-ileum. In an attempt to determine more precisely the portion of the small in-

TABLE I. EFFECT OF REMOVAL OF THE SMALL INTESTINE[a] ON THE RATE OF HEPATIC CHOLESTEROL SYNTHESIS OF RATS 24 HR AFTER TOTAL BILIARY DIVERSION

Surgical procedure	Experiment I (fed maintenance diet until surgery)			Experiment II (fed 10% glucose 24 hr prior to surgery)		
	Baseline rate	24 hr after surgery		Baseline rate	24 hr after surgery	
		Rate[b]	% of baseline rate		Rate[b]	% of baseline rate
Biliary diversion with intact intestine	88 ± 35 (n = 6)[c]	293 ± 120*	377 ± 65†	3.2 ± 0.7 (n = 6)	21.4 ± 5.5†	670 ± 79†
Biliary diversion and removal of the small intestine	124 ± 64 (n = 5)	75 ± 37*	64 ± 10†‡	3.5 ± 1.3 (n = 5)	2.4 ± 0.8*‡	60 ± 8†‡
Sham operation. Intact biliary tract and intestine	101 ± 56 (n = 4)	68 ± 51*	58 ± 12*‡	2.0 ± 0.9 (n = 4)	1.4 ± 0.9*‡	70 ± 8†‡

Note. No difference found between the group with biliary diversion and removal of the small intestine and the sham-operated group.

[a] From the ligament of Treitz to the cecum.

[b] Mean ± SE nmole of [2-^{14}C]acetate incorporated into cholesterol/g of protein/hr.

[c] Number of animals.

*,† Significant difference from the baseline rate by paired t test at $P < 0.05$ (*) or $P < 0.01$ (†).

‡ Significant difference from the group with biliary diversion and intact intestine by Student's t test at $P < 0.01$.

testine involved in its production, biliary-diverted rats with either the upper half or the lower half of the small intestine removed were prepared and the rate of cholesterol synthesis of their livers before and after surgery was compared to that of biliary-diverted animals with the small intestine intact. All the animals used in this experiment were fed nothing but 10% glucose *ad libitum* 12 hr prior to surgery. The results are shown in Table II. It can be seen that preserving either the upper or the lower half of the small intestine was sufficient to elicit the increase in the rate of hepatic cholesterogenesis characteristic of bile-fistula rats with intact small intestine (Table II). Although the extent of the stimulation found in the animals with partial removal of the small intestine seemed to be lower than in the intact animals, no statistically significant difference could be demonstrated among the three groups.

Discussion. It is well established that removal of bile from the intestinal lumen, by whatever means, results in increased rates of hepatic cholesterol synthesis. Thus, ligation of the bile duct (7), creation of a bile fistula (2), cholestyramine administration (8), or ileal bypass (9), all produce significant elevations in hepatic cholesterogenesis. We have postulated (1) that these responses follow a homeostatic mechanism, mediated by a stimulatory factor produced by the intestine, and serving to restore to normal the intraluminal concentration of bile acids whenever it drops below requirements. The stimulation of *de novo* hepatic bile acids synthesis implies a stimulation of the biosynthesis of cholesterol, their obligatory precursor. This hypothesis is supported by the results presented in Table I, experiment I. The complete disappearance of the stimulation of liver cholesterogenesis in the bile-fistula rats when the jejunum-ileum was removed argues strongly for the loss of a stimulatory factor or factors contributed by the small intestine.

The presence in portal blood of a substance or substances capable of promoting higher rates of hepatic cholesterol synthesis has been indicated before by the elegant experiments of Starzl *et al.* involving partial transposition of portal and vena caval blood in dogs (10, 11). In these studies, portal blood was supplied to one portal branch of the liver while the other portal branch was supplied with blood from the inferior vena cava. The lobes receiving the portal blood had higher cholesterol synthesizing activity than the lobes irrigated with systemic blood. Starzl and his co-workers attributed this difference primarily, but not solely, to the much higher concentration of insulin in portal than in caval blood. Although the role of insulin in maintaining the trophism

TABLE II. EFFECT OF PARTIAL REMOVAL OF THE SMALL INTESTINE ON THE RATE OF HEPATIC CHOLESTEROL SYNTHESIS OF RATS 24 HR AFTER TOTAL BILIARY DIVERSION

Surgical procedure	Baseline rate	24 hr after surgery	
		Rate[a]	% of baseline rate
Biliary diversion with intact intestine ($n = 4$)[b]	42 ± 6	258 ± 49*	615 ± 78†
Biliary diversion and removal of the upper half of the small intestine ($n = 4$)	45 ± 11	182 ± 39*	409 ± 57†
Biliary diversion and removal of the lower half of the small intestine ($n = 5$)	59 ± 10	303 ± 56†	511 ± 36†

Note. No significant differences found between the three groups of rats either in baseline rates or rates after surgery.

[a] Mean ± SE nmole of [2-^{14}C]acetate incorporated into cholesterol/g of protein/hr.

[b] Number of animals.

*,† Significant difference from the baseline rate by paired t test at $P < 0.01$ (*) or $P < 0.001$ (†).

of the liver cannot be denied, it is difficult to attribute our findings to differences in the supply of insulin to the liver of the bile-diverted animals with and without small intestine since in all of our groups the pancreas and its venous drainage into the portal vein were preserved intact. Furthermore, all animals were fasted after surgery which should have reduced the concentration of insulin in the portal blood to very low levels in all of our groups. Schneider *et al.* (12) have also provided experimental support for the existence of a factor in blood capable of stimulating liver cholesterol synthesis. By cross-circulation studies they showed that the blood of ileal bypassed rats stimulated hepatic cholesterol synthesis in their intact parabiosed partners. The ileal bypass procedure employed by these authors diverts the flow of bile from the distal half of the small intestine which, according to our hypothesis, would then respond by producing the postulated stimulatory factor.

A slight, but significant decrease of hepatic cholesterol synthetic activity was observed in the sham-operated animals and in the group with biliary diversion and jejunum-ileum removal. This effect is attributed to the period of fasting (13) after surgery. The most important difference, however, was the total disappearance of the stimulation of hepatic cholesterogenesis in the bile-diverted animals with simultaneous removal of the jejunum-ileum. These results are compatible with the postulate that a stimulatory factor is produced by the intestine in response to decreased intraluminal concentrations of bile. The alternative hypothesis, that the intestine generates an inhibitor of liver cholesterol synthesis which would be produced in lesser quantities in the bile-diverted animals, can safely be discarded since this explanation implies a maximal rate of cholesterol synthesis in the animals with the small intestine removed in which production of the inhibitory factor would have dropped to near zero.

Experiment II was an attempt to answer the question of whether or not the intestinal factor(s) is produced by the intestine or is absorbed from the lumen where it may be present as a dietary component or as a constituent of the secretions of the digestive apparatus. We reasoned that if a similar stimulatory response to that observed in experiment I was obtained when the animals had received nothing but 10% glucose in water for 24 hr prior to surgery, it could not be attributed to something absorbed from the diet. Although our results show comparable percentage increases in the rates of cholesterol synthesis of the bile-diverted intestine-intact animals in both experiments I and II, and the total abolition of this response by intestinal removal, the baseline rates of cholesterogenesis in experiment II were strikingly lower than those of experiment I. We attribute this to the effect of food deprivation in experiment II. Fasting markedly inhibits the rate of hepatic cholesterol synthesis (13) and we have calculated that the rats fed 10% glucose as their sole food for 24 hr prior to surgery (experiment II) consumed during that period less than 10% of the calories ingested by the animals fed the maintenance diet until the time of surgey (experiment I). Furthermore, the rats subjected to partial intestinal removal (vide infra) which were fed nothing but 10% glucose for 12 hr prior to surgery had intermediate baseline rates of liver cholesterogenesis, as would be expected given their shorter period of food restriction. Taken together the results of experiments I and II indicate that the baseline rate of hepatic cholesterogenesis, set as a function of recent food intake, can be accelerated about three- to sevenfold following bile diversion. The intestinal stimulatory factor appears therefore capable of altering the baseline rate but is certainly not its sole determinant. Furthermore, the results of experiment II support the contention that the factor is produced by the intestine and not merely absorbed from the luminal content.

Removal of either the upper or lower half of the jejunum-ileum failed to prevent the stimulation of hepatic cholesterogenesis after creation of a bile fistula (Table II). These results indicate that the small intestine

has a considerable reserve capacity for production of the stimulatory factor, and that this function resides in both the lower and upper halves of the jejunum-ileum. It seems clear also that the duodenum lacks the capacity to produce the stimulatory factor. These experiments also indicate that the absence of stimulation of cholesterogenesis observed in the bile-fistula animals with removal of the entire jejunum-ileum cannot be attributed to the surgical trauma per se. There is little difference in the trauma inflicted to the animals undergoing removal of half or the whole of the jejunum-ileum.

Based on the data provided by our experiments we conclude that removal of bile (or more precisely of bile acids) from the intestinal lumen leads to the production by both the upper and lower halves of the jejunum-ileum of a stimulatory factor or hormone which reaches the liver via the portal circulation and accelerates the rate of hepatic cholesterogenesis. The isolation and characterization of such a hormone should advance our understanding of the physiological regulatory mechanisms of cholesterol homeostasis.

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1. Krumdieck CL, Ho KJ. Intestinal regulation of hepatic cholesterol synthesis: an hypothesis. Amer J Clin Nutr 30:255–261, 1977.
2. Myant NB, Eder HA. The effect of biliary drainage upon the synthesis of cholesterol in the liver. J Lipid Res 2:363–368, 1961.
3. Navia JM, Lopez H, Harris RS. Purified diet for dental caries research with rats. J Nutr 97:133–140, 1969.
4. Siperstein MD, Guest MJ. Studies on the site of the feedback control of cholesterol synthesis. J Clin Invest 39:642–652, 1960.
5. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
6. Penhos JC, Woodbury C, Tizabi Y, Ramey ER. Metabolic studies in eviscerated rats with functional livers. Proc Soc Exp Biol Med 148:1159–1163, 1975.
7. Fredrickson DS, Loud AV, Hinkelman BT, Schneider HS, Frantz Jr ID. The effect of ligation of the common bile duct on cholesterol synthesis in the rat. J Exp Med 99:43–53, 1954.
8. Huff JW, Gilfillan JL, Hunt VM. Effect of cholestyramine, a bile acid binding polymer on plasma cholesterol and fecal bile acid excretion in the rat. Proc Soc Exp Biol Med 114:352–355, 1963.
9. Moutafis CD, Myant NB. Increased hepatic synthesis of cholesterol after ileal by-pass in monkeys. Clin Sci 34:541–548, 1968.
10. Starzl TE, Lee IY, Porter KA, Putnam CW. The influence of portal blood upon lipid metabolism in normal and diabetic dogs and baboons. Surg Gynecol Obstet 140:381–396, 1975.
11. Starzl TE, Porter KA, Francavilla JA, Benichou J, Putnam CW. A hundred years of the hepatotrophic controversy. In: Hepatotrophic Factors. Ciba Foundation Symposium 55 (new series). Amsterdam, Elsevier, pp111–129, 1978.
12. Schneider PD, Guzman IJ, Rucker RD, Stocks TG, Varco RL, Buchwald H. Increased hepatic cholesterol synthesis in normal rats by cross-circulation with ileal bypassed partners. Atherosclerosis 34:383–389, 1979.
13. White LW, Rudney H. Regulation of 3-hydroxy-3-methylglutarate and mevalonate biosynthesis by rat liver homogenates. Effects of fasting, cholesterol feeding, and Triton administration. Biochemistry 9:2725–2730, 1970.

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