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Research Article

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Studies of the Hepatic Excretory Defects in Essential Fatty Acid Deficiency

THEIR POSSIBLE RELATIONSHIP TO THE GENESIS OF CHOLESTEROL GALLSTONES

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ABSTRACT Male hamsters were fed normal and essential fatty acid (EFA)-deficient diets for at least 12 wk before bile duct cannulation. With [^{32}P]phosphate, hepatic synthesis of lecithin was similar, but biliary excretion of newly synthesized lecithin was significantly reduced in EFA-deficient compared to that in normal hamsters. Hepatic uptake of intravenously infused taurocholate (TC) and taurochenodeoxycholate (TCDC) were similar in both groups of animals. However, biliary excretion of intravenously infused TC was significantly reduced in EFA-deficient hamsters, whereas that of TCDC was unchanged. The absolute rate of biliary cholesterol excretion was similar in both groups. Canalicular bile flow, as measured by [^{14}C]erythritol clearance after functional nephrectomy, was significantly lower, with both the bile salt-dependent and independent fractions of this flow being diminished in EFA-deficient hamsters infused with TC.

It is concluded that EFA deficiency leads to impaired biliary excretion of taurocholate, lecithin, and water, while cholesterol transport is unaffected, and thus results in supersaturation of bile with respect to cholesterol and production of lithogenic bile.

INTRODUCTION

Essential fatty acid (EFA)[1]-deficient hamsters form cholesterol stones as a result of excretion of an abnormal bile which is supersaturated in respect to cholesterol (1). Investigations in this and other animal models have led many observers to implicate the liver, rather than the gallbladder as the site of production of lithogenic bile (2–4). This abnormal bile could result from increased cholesterol excretion into bile, while excretion of bile salts and lecithin, the primary solubilizers of cholesterol, remains normal. Alternatively, the primary defect could be reduced excretion of bile salts and lecithin, while that of cholesterol remains unchanged.

The present study was undertaken to examine the effects of EFA deficiency on aspects of biliary and hepatic metabolism of bile salts, lecithin, cholesterol, and water in an attempt to determine the nature of possible derangements of excretion of these biliary constituents, which might lead to the production of lithogenic bile. The results indicate that in EFA deficiency the excretion of taurocholic acid and lecithin is decreased, and canalicular bile flow is diminished, while cholesterol excretion is apparently unchanged.

METHODS

Male weanling golden hamsters (Blue Spruce Farms, Altamont, N. Y) were used in all experiments. For 4 wk the animals were fed regular laboratory chow (Wayne Lab Blox, Chicago, Ill.). They were then placed on various experimental diets for 12 wk as described below. The surgical procedures are described below. Following isotope injection, performed 30 min after recovery from anesthesia, the animals were loosely restrained in Bollman type cages and kept at constant room temperature (24±2°C).

The fat-free diet (FFD) and this diet with 4% additions of safflower oil (control diet) or tripalmitin (EFA-deficient diet) were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. These diets were kept refrigerated when not in use. Taurocholate (TC) and taurochenodeoxycholate (TCDC) were obtained from Calbiochem, La Jolla, Calif. and were shown to be >95% pure by thin-layer chromatography. [^{32}P]orthophosphate and [U-^{14}C]erythritol were obtained from Amersham/Searle Corp., Arlington Heights, Ill. Tritiated taurocholate ([^{3}H]TC)

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Dr. Sarfeh was a Trainee in Gastroenterology during the conduct of these studies.

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[1] *Abbreviations used in this paper:* EFA, essential fatty acids; FFD, fat-free diet; TC, taurocholate; TCDC, taurochenodeoxycholate.

and chenodeoxycholate (both generally labeled) were obtained from New England Nuclear, Boston, Mass.

In all studies lipids of bile and liver were extracted in chloroform: methanol (2:1) as previously described (5). Lecithins were purified by thin-layer and argentation chromatography to obtain the various lecithin fractions (6, 7). Radioactivity in lecithins was determined in a Nuclear-Chicago Mark 1 System (Nuclear-Chicago Corp., Des Plaines, Ill.) as previously described (7). Radioactivity of bile salts and erythritol in bile, plasma, and liver water was determined with a Beckman LS 250 instrument (Beckman Instruments, Inc., Fullerton, Calif.) with window settings adjusted to give <0.5% spill of tritium into the ^{14}C channel and <10% spill of ^{14}C into the tritium channel. Suitable standards were used with each set of samples, and quench correction made by external standard ratios. Aqueous samples were dissolved in a dioxane, naphthalene, 2′,5-diphenyloxazole system (1,000:100:5–vol:wt:wt, Beckman manual).

Cholesterol in bile was determined, after precipitation from the chloroform phase of the extract as the digitonide, by the method of Zak (8). Bile salts were quantified in aliquots of bile in all experiments using a modification (9), of the method of Mosbach (10) as previously described (11). Individual bile salts in bile were separated after hydrolysis by thin-layer chromatography (12, System 11).

Experiment 1: hepatic synthesis and biliary excretion of lecithin

After the 4-wk period on the laboratory chow, 13 of the hamsters were continued on this diet while 16 others were placed on the fat-free diet (FFD hamsters) for 12 wk. At that time, the bile duct and femoral vein of each hamster were cannulated with a PE 10 catheter, and the cystic duct was ligated during ether anesthesia. After 30 min for recovery from anesthesia, each received an intraperitoneal injection of 310 μCi $^{32}PO_4$ in 0.2 ml of 0.15 M NaCl. Six of the 13 fed the chow diet, and seven of the 16 FFD hamsters were immediateiy infused via the femoral vein with 10 mM TC at a rate of 0.3 ml/h with a constant infusion pump. The remaining animals from both groups received intravenous 0.15 M saline at the same rate. Bile was then collected from individual animals at hourly intervals until sacrifice at 2 or 4 h later. Bile flow was measured gravimetrically with tared 1-ml test tubes. The hourly individual bile collections within each diet and infusion group were pooled for analyses. Thus, there were two pools available for analysis at 1 and 2 h for both saline- or TC-infused, chow-fed or FFD hamsters, and one pool for each group at 3 and 4 h. At the time of sacrifice livers were quickly excised, rinsed in cold saline, blotted, weighed, and a measured aliquot extracted as described above.

Eight additional hamsters were fed the chow diet for 16 wk and then studied as described above. Four hamsters were killed at 2 h and the other four at 4 h.

Experiment 2: canalicular bile flow and bile salt excretion studies

After the 4-wk period on a regular diet, 30 hamsters received FFD supplemented with either 4% safflower oil (15 controls) or 4% tripalmitin (15 EFA-deficient animals), so that the fat content of the diet offered was equal in both groups. After 12 wk on these diets in addition to common bile duct cannulation and cystic duct ligation, a PE 10 catheter was placed in the inferior vena cava, and both renal pedicles were ligated during ether anesthesia. Each hamster was then injected immediately with a bolus of 0.2 ml of 10 mM [U-^{14}C]erythritol (2 μCi) via the vena cava so that indirect measurements of canalicular flow could be made as described by Forker (13) and Wheeler, Ross, and Bradley (14). This amount of erythritol gave steady plasma levels of ^{14}C for at least 6 h. 30 min later these hamsters were infused via the vena cava with one of two concentrations of [^3H]TC or with tritiated taurochenodeoxycholate ([^3H]TCDC) in the manner described below. Blood samples (0.05 ml) were obtained from the inferior vena cava cannula at hourly intervals after the start of infusion of these bile salts and plasma obtained by centrifugation. Simultaneous hourly bile collections were made and bile flow measured gravimetrically. Radioactivity in aliquots of each bile and plasma sample was determined as described above. Biliary clearances for erythritol were calculated by the standard clearance formula, and the results expressed in milliliters/hour per gram liver water. The first hour after erythritol injection was excluded to allow for a sufficient period of equilibration. The injected erythritol and that in plasma and bile were analyzed by paper chromatography as described by Forker (13) and all three were found to have identical mobilities.

A. TC infusion. [^3H]TC (10 mM, 0.1 mCi/mmol) was infused via the inferior vena cava at a constant rate in each of a group of ten animals (5 control and 5 EFA-deficient) at 3 μmol/h. Another group of 5 control and 5 EFA-deficient hamsters were similarly infused with 1.67 mM [^3H]TC (0.3 mCi/mmol) at 0.5 μmol/h. Bile was collected at hourly intervals for 4–5 h for each animal. Aliquots of each bile sample were analyzed for bile salt content as well as for [^3H]TC radioactivity. Before use in these experiments an aliquot of [^3H]TC was injected intravenously into a rat equipped with a biliary cannula 48 h earlier. Bile was collected for 8 h. More than 85% of injected radioactivity was recovered as TC in bile. This indicated adequate stability of the label in vivo. Cholesterol and phospholipid determinations for each animal were made on combined 1- and 2-h samples, as well as for the pooled 3-, 4-, and 5-h samples. More than 95% of ^3H radioactivity was shown to be in TC by thin-layer chromatography of aliquots of bile extracts (12). Radioactivity was similarly measured in saline extracts of each liver at the end of each experiment, and expressed as cpm/g liver water. Liver water content was determined by weighing before and after drying to constant weight and was found to be 72±4% (mean±SD) of wet liver weight for both control and EFA-deficient animals.

B. TCDC infusion. [^3H]TCDC was prepared in vivo. A rat which had been previously bile diverted for 48 h to clear the enterohepatic circulation of secondary bile acids was injected intravenously with [^3H]chenodeoxycholate. Bile was collected on ice for 8 h. More than 80% of injected radioactivity was recovered. The [^3H]TCDC was purified from the bile by thin-layer chromatography (15). A group of ten hamsters (5 control and 5 EFA-deficient) was infused via the inferior vena cava with 10 mM (0.05 mCi/mmol) [^3H]TCDC at a constant rate of 3 μmol/h. The rest of the procedures were carried out as described above, except that in every animal cholesterol and phospholipid determinations were made on each of the hourly bile samples.

Statistical methods

Statistical significance of differences between means was tested using Student's *t* test or the U test of Man and Whitney. The latter test was used when hourly comparisons were made in a given group of normal and EFA-deficient animals. Regression lines were fitted to the data points by the method of least squares by using the Basic 69 program on a PDP 12 computer (Digital Equipment Corp., Maynard, Mass.).

RESULTS

The EFA-deficient and FFD animals at the time of study showed clinical evidence of essential fatty acid deficiency (scaly paws and skin) and this was confirmed by the fatty acid analysis of biliary and hepatic lecithins, which showed increased content of palmitoleic, oleic, and eicosatrienoic acids, and marked decreases in, or absence of linoleic and arachidonic acids as previously described (16). Cholesterol gallstones were present in 70% of the EFA-deficient and FFD animals. All hamsters withstood the experimental conditions equally well, and those animals (20% in each group) which did not remain alive for the full time period were excluded from the studies and replaced by others. At the time of these studies normal hamsters weighed 149±18 g, while the FFD and EFA-deficient animals weighed 138±16 g. Respective liver weights were 5.1±0.7 g and 5.0±0.8 g (mean ±SD).

Experiment 1: hepatic synthesis and biliary excretion of lecithin

The pool size of hepatic lecithin was similar in all groups (Table I). The specific activities of hepatic lecithins were higher in FFD hamsters than in comparable controls fed the chow diet, but these differences were not statistically significant (Table I).

Biliary excretion of phospholipid (Table II) decreased to very low levels by 4 h after biliary diversion in saline-infused hamsters from both diet groups. The intravenous infusion of 3 μmol TC/h produced increased biliary lecithin excretion in hamsters from both diets and was much better maintained in chow-fed hamsters. The recovery of infused TC averaged 80% in chow-fed hamsters whereas only 40% was recovered in FFD animals (2.40±0.29 vs. 1.22±0.21 μmol/h per hamster, respectively; mean ±SD).

Excretion of newly synthesized lecithins into bile in chow-fed animals was two to four times greater in TC-infused than in saline-infused animals during the first 2 h (Fig. 1 A). By contrast, in FFD animals no such response to TC infusion was noted (Figure 1 B). Dur-

TABLE I

Hepatic Lecithins: Pool Size and Specific Activity

		Chow-fed	FFD
	h	*dpm/μmol lipid P × 10⁻³*	
Saline-infused	2	13.5±5.1* (3)‡	23.4±14.9 (5)
	4	31.9±11.4 (4)	47.9±13.3 (4)
TC-infused§	2	18.3±3.8 (3)	27.6±1.3 (5)
	4	31.4±8.7 (3)	42.6±15.1 (2)
		μmol lecithin/g wet wt	
Saline-infused		16.5±2.0 (7)	14.2±2.3 (9)
TC-infused§		15.9±2.9 (6)	13.9±2.3 (7)

* Mean±SD.
‡ Number of hamsters in parentheses.
§ At 3 μmol/h per hamster.

TABLE II

Biliary Excretion of Phospholipid

Group	Time	Chow-fed Infusion		FFD Infusion	
		Saline	TC*	Saline	TC*
	h	*Lecithin (μmole lipid P/h per hamster)*			
1 A‡	1	0.32 (3)§	0.67 (3)	0.58 (5)	0.42 (5)
	2	0.37	0.50	0.36	0.25
1 B‖	1	0.43 (4)	0.43 (3)	0.44 (4)	0.35 (2)
	2	0.18	0.60	0.28	0.33
	3	0.13	0.60	0.10	0.20
	4	0.03	0.32	0.04	0.15

* Infused with 10 mM TC at 3 μmol/h.
‡ Group 1 A, animals killed 2 h after isotope injection.
§ Number of animals in parentheses.
‖ Group 1 B, animals killed 4 h after isotope injection.

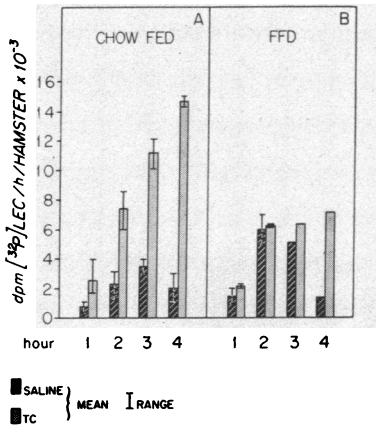


FIGURE 1 Biliary excretion of newly synthesized lecithin. (A) Mean and range of values obtained from three groups of chow-fed hamsters at 1 and 2 h and two groups at 3 and 4 h. (B) Two groups of FFD hamsters at 1 and 2 h and 1 group at 3 and 4 h after injection of [^{32}P]phosphate. Chow-fed, saline-infused hamsters, 11; chow fed, TC infused, 10; FFD fed, saline infused, 9; FFD fed, TC infused, 7.

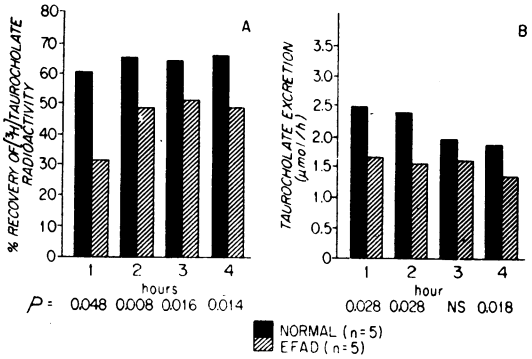


FIGURE 2 Hourly biliary excretion of [^{3}H]TC as percent of infused [^{3}H]TC (A) and as TC micromoles/hour per hamster (B). TC infusion rate of 3 μmol/h per hamster. Solid bars represent data from control animals fed FFD supplemented with 4% safflower oil and hatched bars results from hamsters fed FFD supplemented with 4% tripalmitin (EFA deficient).

ing the third and fourth hours excretion of newly synthesized lecithin continued to increase in TC-infused compared to saline-infused chow-fed hamsters (Fig. 1 A). During the same times, in TC-infused FFD hamsters, excretion remained at a plateau, while in saline-infused FFD animals it declined sharply (Fig. 1 B). Output of radioactivity in lecithin was higher in saline-infused FFD hamsters than in corresponding controls, but this was a reflection of the higher specific activity of hepatic lecithin in the former (Table I).

Experiment 2: bile salt excretion and canalicular bile flow studies

[^{3}H]TC Infusion. During infusion of [^{3}H]TC at 3 μmol/h significantly less [^{3}H]TC was excreted into bile during each hour in EFA-deficient hamsters than in controls (Fig. 2 A). Chemically determined TC excretion was significantly reduced in all but the third hour in EFA-deficient animals (Fig. 2 B). In control hamsters 64±3% (mean ±SD) of infused [^{3}H]TC was recovered in bile over the 4-h infusion period compared to 45±3% in EFA-deficient hamsters ($P < 0.001$). Chemically determined biliary TC excretion rates averaged 2.2±0.4 μmol/h in controls, compared to 1.5±0.2 μmol/h in EFA-deficient animals ($P < 0.001$).

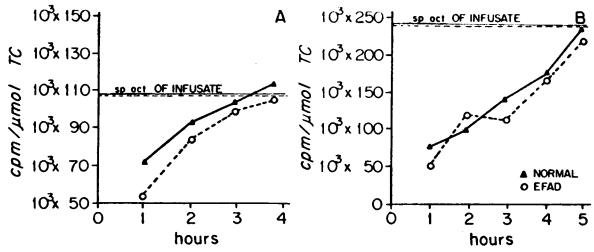


FIGURE 4 Specific radioactivity of infused [^{3}H]TC and of biliary [^{3}H]TC during infusion of either 3 μmol (A) or 0.5 μmol (B) [^{3}H]TC/h per hamster.

In another group of five animals on each diet infused with 1.67 mM [^{3}H]TC (0.5 μmol/h) recovery of radioactivity approached 90% of the infusate at 3, 4, and 5 h in both control and EFA-deficient hamsters, but was only 60–70% at 1 and 2 h in the latter (Fig. 3 A and B) compared to 80–90% in controls, suggesting some delay in excretion of infused TC even at this lower infusion rate.

In these experiments, the plasma levels of [^{3}H]TC reached low and stable levels after the second hour of infusion in both groups of animals. Thus, in control and EFA-deficient animals, at an hourly infusion rate of 3 μmol, 1.2×10^6 cpm of [^{3}H]TC were being infused per hour and total plasma radioactivity, assuming a blood volume of 7% of body weight, was 112,000±3,700 (mean ±SD) cpm. Almost all radioactivity of [^{3}H]TC which was not recovered in bile was accounted for in the liver water in all groups, the EFA-deficient animals exhibiting a greater amount of [^{3}H]TC in the liver (230,000 vs. 144,000 cpm/g liver water). Total recovery of [^{3}H]TC in liver and bile ranged from 94 to 105% of the infused dose in both experiments.

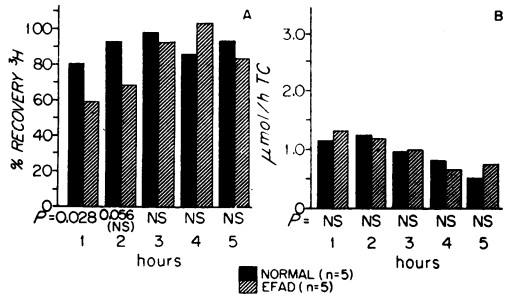


FIGURE 3 Biliary excretion of [^{3}H]TC as percent of infused [^{3}H]TC (A) or as micromoles TC/hour per hamster (B), with TC infusion rate of 0.5 μmol/h per hamster. Symbols as for Fig. 2.

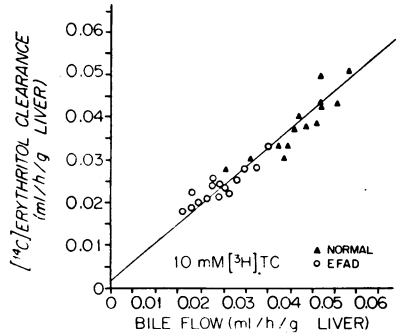


FIGURE 5 Relationship of erythritol clearance to bile flow in normal (▲) and EFA-deficient hamsters (○) infused with TC at 3 μmol/h.

The specific radioactivity of biliary TC was similar in control and EFA-deficient hamsters, and in both groups approached the specific activity of the infused [^{3}H]TC by the end of the experiments (Fig. 4 A and B).

[^{3}H]TCDC Infusion. Average hourly recoveries of [^{3}H]TCDC in bile were 56±5% of infused [^{3}H]TCDC in controls and 58±6% (mean ±SD) in EFA-deficient hamsters. As with [^{3}H]TC infusion, so in these studies with [^{3}H]TCDC plasma levels of radioactivity were 8–9% of the infused dose at all times (0.65×10^6 cpm infused/h vs. $0.05 \pm 0.01 \times 10^6$ cpm in total plasma). All radioactivity not found in bile was shown to be in the liver water. The specific activity of biliary [^{3}H]TCDC reached that of the infused [^{3}H]TCDC by 3 h in both groups of animals. Chemically determined TCDC excretion rates were 2.3±0.5 and 2.0±0.4 μmol/h in control and EFA-deficient hamsters respectively.

Erythritol clearance. Bile:plasma ratios of [^{14}C]-erythritol were close to unity in all experiments and not significantly different in control and EFA-deficient hamsters (0.98±0.2 in controls, vs. 1.02±0.03 in EFA-deficient hamsters). Both bile flow and erythritol clearance were significantly reduced in EFA-deficient animals, as exemplified in Fig. 5. Fig. 5 also demonstrates the linear relationship of bile flow to [^{14}C]erythritol clearance in control and EFA-deficient hamsters infused with 10 mM TC at 3 μmol/h. The values for the EFA-deficient animals are lower than for controls, but fall on the same line with the y-intercept near zero (Fig. 5). Results showed a similar pattern with infusion of 1.67 mM TC at 0.5 μmol/h, and 10 mM TCDC infused at 3 μmol/h.

The bile salt-independent fraction of bile flow was determined by extrapolation of erythritol clearance to zero bile salt excretion rate (13, 14). This fraction was consistently lower in EFA-deficient hamsters with both taurocholate infusion (Fig. 6) and with taurochenodeoxycholate infusion (Fig. 7). The difference between zero-intercepts was significant ($P < 0.01$) for the taurocholate infusion experiments (Fig. 6).

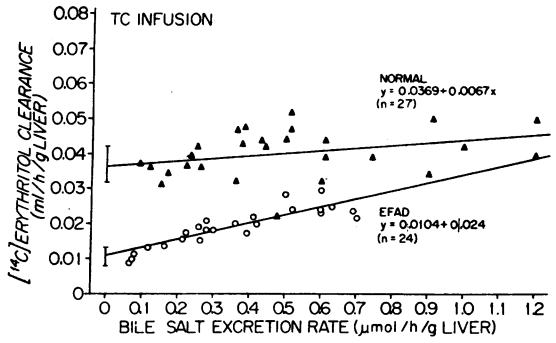


FIGURE 6 Canalicular bile flow and bile salt excretion rate in normal (▲) and EFA-deficient hamsters (○), during intravenous infusion of TC at two different infusion rates (3 μmol and 0.5 μmol/h per hamster). Bars represent ±1 SD of the y-intercept of the calculated regression line. The difference in y-intercept between normal and EFA-deficient hamsters is significant ($P < 0.01$).

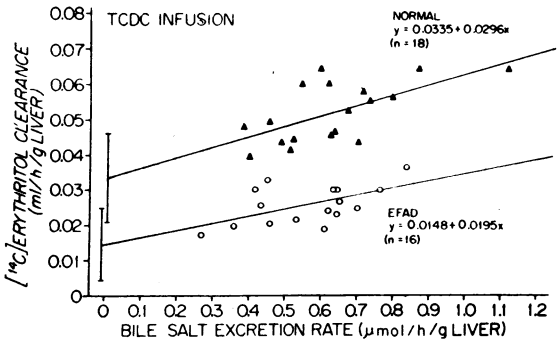


FIGURE 7 Canalicular bile flow and bile salt excretion rate in normal (▲) and EFA-deficient hamsters (○) during intravenous infusion of TCDC at 3 μmol/h per hamster. Bars represent ±1 SD of the y-intercept of the calculated regression lines.

Bile composition. Phospholipid output in bile was significantly ($P < 0.05$) reduced in EFA-deficient animals compared to controls, with both TC and TCDC infusion (Table III). This reduction in lecithin output appeared to be related to reduced bile salt excretion in the TC-infused EFA-deficient hamsters (Table III). With TCDC infusion total bile salt output was similar in the two groups of hamsters, and thus the lower lecithin output in the EFA-deficient group cannot be related to abnormal bile salt excretion alone. Absolute cholesterol outputs were similar in normal and EFA-deficient hamsters (Table III). However, with TC infusion cholesterol output relative to bile salt excretion was 60% higher in EFA-deficient hamsters than in normal controls (0.019 μmol cholesterol/μmol bile salt vs. 0.012

TABLE III

Biliary Excretion of Lecithin, Cholesterol, and Bile Salts

	Normal	EFA-deficient	P
	μmol/h per g liver (Exp. 2) (mean ±SE)		
TC Infused (3 μmol/h)			
Lecithin	0.0191±0.005	0.0125±0.004	<0.05
Cholesterol	0.0095±0.004	0.0095±0.003	NS
TC	0.42±0.05	0.33±0.04	<0.02
Total BS*	0.75±0.09	0.49±0.12	<0.05
Lec:Chol‡	2.01±0.48	1.32±0.44	<0.1, >0.05
TCDC Infused (3 μmol/h)			
Lecithin	0.0383±0.012	0.0272±0.006	<0.05
Cholesterol	0.0146±0.004	0.0120±0.003	NS
TCDC	0.44±0.06	0.43±0.05	NS
Total BS*	0.63±0.04	0.56±0.04	NS
Lec:Chol‡	2.70±0.74	2.26±0.41	<0.1, >0.05

Number of animals = 5 in each group.
* BS, bile salts.
‡ Lec:Chol, lecithin:cholesterol molar ratio.

μmol bile salt). Infusion of TCDC in both control and EFA-deficient hamsters resulted in a two-fold increase in lecithin output compared to results of TC infusion, while cholesterol outputs increased by 50 and 25% in control and EFA-deficient hamsters respectively (Table III). Lecithin : cholesterol molar ratios with infusion of both bile salts remained lower in EFA-deficient than in control hamsters, although the differences in ratios failed to reach statistical significance.

DISCUSSION

The present studies, as well as others (16–18) have confirmed the observation of Dam and co-workers (1) that EFA deficiency in hamsters leads to the production of cholesterol gallstones. Formation of cholesterol gallstones is a consequence of the excretion of bile supersaturated in cholesterol (2–4). Production of such abnormal bile could arise as a result of increased excretion of cholesterol, while that of lecithin and bile salts, the solubilizers of cholesterol (19, 20), remains normal. Alternatively, bile supersaturated with cholesterol could result from diminished outputs of bile salts and lecithins, while that of cholesterol remains unchanged. The studies reported here support the second of these hypotheses.

EFA-deficient hamsters showed significant impairment of their capacity to excrete TC after its intravenous infusion at 3 μmol/h per hamster (Fig. 2). That the reduced rate of TC excretion in EFA-deficient hamsters was not due to impaired hepatic uptake of this bile salt is shown by the observation that plasma levels of [^{3}H]TC were at similarly low levels in both normal and experimental animals. Furthermore, [^{3}H]-TC not recovered in bile was shown to be in the liver in both groups of animals. In both normal and EFA-deficient hamsters the specific radioactivity of biliary TC reached that of the infused [^{3}H]TC, indicating that the residual pool of TC was similar in the two groups of animals. Robins and Fasulo, using an isotope dilution technique, reported that bile salt pool size was not reduced in EFA-deficient hamsters (17). Thus, reduced biliary excretion of TC in our studies could not be due to reduction in available TC. In contrast, hepatic excretion of TCDC infused at 3 μmol/h was similar in control and EFA-deficient hamsters (Fig. 5). This observation suggests that excretion of TC and TCDC may be mediated by two independent transport systems, or one system with different affinities for the two bile salts.

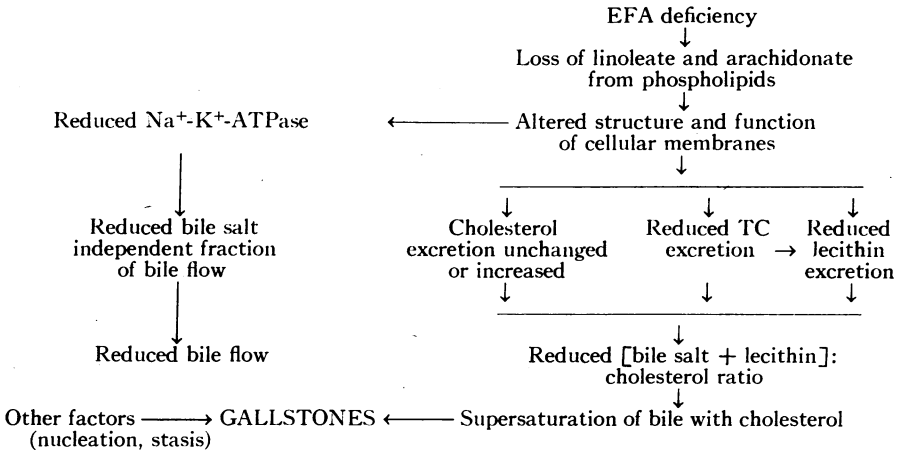
Bile salts have been shown to have a significant effect upon the synthesis and biliary excretion of lecithin in several studies (11, 21–25). Therefore, the reduced biliary excretion of lecithin observed in both experiments in the present studies could have been due to the diminished excretion of TC (Tables II and III, Fig. 1). In both experiments reduced biliary output of lecithin was associated with reduced excretion of TC into bile. That this may not be the whole explanation is, however, suggested by the observation that lecithin excretion into bile was lower than normal in EFA-deficient hamsters infused with TCDC, despite the normal excretion of this bile salt. Since biliary lecithin in EFA-deficient hamsters is esterized with oleic acid instead of linoleic acid at the 2-positions (16), it may be that normal transport of lecithin into bile in association with bile salts is dependent in part upon the structural characteristics of the lecithin. That synthesis of lecithin is not impaired in EFA deficiency is indicated by the fact that the hepatic pool size of lecithins was unchanged, and by the finding that the specific radioactivity of hepatic lecithins was not reduced in this condition (Table 1).

Cholesterol output into bile was similar in control and EFA-deficient hamsters (Table III) despite reduced levels of bile salt and lecithin. Hardison and co-workers (26, 27) and Wheeler and King (28) have recently shown that in normal rats, and dogs, a small fraction of biliary cholesterol is excreted independently of lecithin and bile salts. In the present studies, 60% more cholesterol was excreted into bile per mole of bile salt in EFA-deficient than in normal hamsters infused with TC (Table III). This observation suggests that in EFA deficiency there may be an increase in the bile salt and lecithin-independent fraction of biliary cholesterol. This concept is also supported by the results of other recent studies in EFA-deficient hamsters (17, 18). These reports indicate that increased biliary excretion of cholesterol may be the first abnormality of bile formation in EFA deficiency. However, Wheeler noted significant reduction in biliary excretion of bile salts in EFA-deficient hamsters in the first 4 h after bile duct cannulation (18). Metzger, Adler, Heymsfield, and Grundy (29) have recently demonstrated that cholesterol excretion, especially in the fasting state, is inappropriately high relative to bile salt and lecithin excretion in patients with cholesterol gallstones.

A significant fraction of canalicular bile flow is independent of bile salt excretion rate. This bile salt-independent fraction is believed to be the result of the activity of an active transport system in the canalicular membrane mediated by Na^+-K^+-ATPase (30–32). Recent evidence (33, 34) has indicated that the activity of this enzyme is dependent upon the presence of phospholipid, especially phosphatidyl glycerol and phosphatidyl serine containing linoleate, within the membrane. Therefore, it was of interest to investigate canalicular bile flow in our normal and EFA-deficient animals as a means of seeking further evidence for a pos-

sible membrane transport defect in the latter. Erythritol clearance has been shown to be an accurate measure of canalicular bile flow (13, 14). By these techniques, canalicular bile flow was shown to be diminished in EFA-deficient animals (Fig. 5). Since the ratio of erythritol clearance to bile flow was close to unity in all experiments, ductular secretion can be assumed to make a negligible contribution to total bile flow in hamsters. Calculation of the bile salt-independent fraction of bile flow, by extrapolating erythritol clearance to zero bile salt excretion rate (13, 14, 30) showed a highly significant reduction in this fraction in TC-infused EFA-deficient hamsters compared to normal controls (Fig. 6). A reduction in this fraction in EFA-deficient hamsters was also seen with TCDC infusion; however, the difference between normal and EFA-deficient hamsters failed to attain statistical significance. Nevertheless, these data with TC infusion lend additional support to the concept that in EFA deficiency there exist defects in active transport from hepatocytes to bile across the canalicular membrane.

The data presented here demonstrate impaired transport of TC and lecithin into bile, as well as indirect evidence of reduced activity of Na^+-K^+ATPase in hepatocytes of EFA-deficient animals. Others have observed impaired active transport, or secretion of macromolecules, in tissues of EFA-deficient rats (35–37). EFA deficiency results in loss of linoleic and arachidonic acids from membrane phospholipids (16, 37 38). Evidence has been presented (33, 34) to show that such changes could reduce the activity Na^+-K^+-ATPase, and thus account for the reduced bile flow noted in these studies. Based on these considerations we propose the following scheme as a hypothesis to account for the alterations in bile composition and production in EFA deficiency observed in this study:



We conclude that in EFA deficiency there is alteration in the structure of hepatic cell membranes and possibly of their function. This alteration results in decreased excretion of TC and lecithin, while that of cholesterol remains unchanged. These changes favor the formation of bile supersaturated in cholesterol, thus setting the stage for cholesterol gallstone formation. Our results showing diminution in the bile salt-independent fraction of bile flow lend further support to the concept of altered membrane transport function in EFA deficiency as the cause of abnormal bile formation.

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