Effects of Cholesterol on Bile Acid Metabolism in the Rat[1] (34841)

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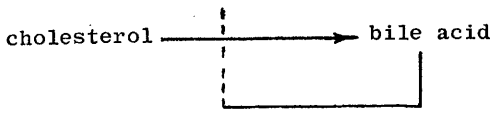
There are two major pathways for mobilization of tissue cholesterol. Cholesterol may be metabolized to bile acids by liver mitochondria and then eliminated in the feces, or free cholesterol itself may be eliminated in the feces. It has been shown that the rate of mobilization by the bile acid pathway is controlled by a negative feed-back mechanism:



by which changes in the concentration of bile acids in their recirculating pool have an inverse effect on the rate of conversion of cholesterol to bile acids (1, 2). On the other hand, the factors responsible for changes in rates of excretion of neutral sterols are not clear. The relative importance of the two pathways varies from species to species (3–5), and under different conditions in the same species (6–8).

Recently Wilson (9, 10), in a series of investigations in rats, has shown that a major factor influencing the relative contributions of the two pathways is dietary cholesterol. When diets supplemented with cholesterol were fed to rats, the relative proportion and rate of cholesterol excreted via the bile acid pathway increased. Wilson calculated that the increased amount of cholesterol metabolized to bile acids in cholesterol-fed rats was sufficient to prevent accumulation of tissue cholesterol.

The present experiments were designed to study the mechanism of the effects of dietary and of accumulated tissue cholesterol on the rate of conversion of cholesterol to bile acids by determining bile acid turnover, synthesis, and excretion rates, as well as bile acid pool sizes and spectra. It was also of interest to determine whether the effects of accumulated tissue cholesterol on bile acid turnover are due to the accumulated tissue cholesterol *per se* or due to the presence of elevated β-sterols in the gastrointestinal tract.

Exptl. Protocol. Section A. Effects of cholesterol and corn oil on bile acid metabolism. Forty-eight 6-month-old, female, Sprague-Dawley albino rats were divided into six equal groups and fed *ad libitum* throughout the experiment. The basal diet was Rockland Rat Diet (RRD) containing (%): protein, 24.27; fat, 4.15; fiber, 4.86; carbohydrate, 56.23; and ash 7.78. Two untreated control groups were fed this diet unsupplemented. For the other groups RRD was supplemented as follows: the third group with 3% corn oil; the fourth group with 1.0% cholesterol; and the fifth and sixth groups with 1.0% cholesterol plus 3% corn oil. After 3 weeks on these diets, each rat of one of the untreated control groups and of one of the groups fed 1.0% cholesterol and 3% corn oil supplement received an intraperitoneal injection containing 5 μCi of chenodeoxycholic acid-24-^{14}C. Each rat in the remaining groups received a 5-μCi intraperitoneal injection of cholic acid-24-^{14}C. Bile acid half-lives and pool sizes were determined by *Methods a* and *b* below.

Exptl. Protocol. Section B. Effects of accumulated tissue cholesterol. Three aspects of steroid metabolism were investigated in rats with accumulated tissue cholesterol: (i) the rate of regression of accumulated blood and liver cholesterol, along with the size of the β-sterol pool in small intestine plus contents and cecum plus contents; (ii) the bile acid

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pool half-life, size, and spectrum; and (iii) the rate of fecal sterol excretion.

For the tissue cholesterol regression study (i), seven groups of six female albino, Sprague-Dawley rats (6-month-old) were used. One group, the untreated control, was fed Rockland Rat Diet for a 1-month observation period and then killed. The remaining groups were fed RRD supplemented with 1% cholesterol plus 0.5% cholic acid for 3 weeks to effect blood and liver cholesterol accumulation. A second group of rats was killed (0 regression time), and the remaining groups were returned to unsupplemented Rockland Rat Diet to effect cholesterol regression. These five groups were killed after 1, 2, 3, 4, and 6 days. Blood samples, livers, ceca, and small intestines plus contents were removed from all animals when sacrificed, and assayed for β-sterols (*Methods c, d,* and *e*).

For the bile acid metabolism study (ii) three groups of six rats were utilized—controls maintained on Rockland Rat Diet throughout the experiment; a cholesterol-accumulation group fed a 1% cholesterol plus 0.5% cholic acid supplement during a 3-week build-up period; and a group fed 0.5% cholic acid for this same period. After 3 weeks on these diets, the two experimental groups were returned to unsupplemented RRD. One day was allowed for clearance of accumulated cholesterol from the intestinal tract of the experimental animals. All three groups were then injected with ^{14}C bile acid for determination of bile acid pool half-lives, sizes, and spectra by *Methods a* and *b* below.

Twelve rats were used in the fecal sterol excretion study (iii). Six control animals were fed Rockland Rat Ration throughout the experiment; while the diet of the remaining animals was supplemented for 3 weeks with 1% cholesterol and 0.5% cholic acid to effect tissue cholesterol accumulation. After returning the six experimental animals to unsupplemented rat ration, 1 day was allowed for intestinal clearance. Feces were then collected for 8 days from both control and experimental animals for β-sterol determination (*Method c*).

Method a. Bile acid half-life determination. Rats received single 5-μCi intraperitoneal injections of either cholic acid-24-^{14}C (4.03 mCi/mmole) or chenodeoxycholic acid-24-^{14}C (1 mCi/mmole). The rats were placed in metabolism cages and feces collected daily for 8 days. The animals were then killed and ceca, small, and large intestines (all plus contents) and livers removed. All samples were dried by lyophylization. Aliquots of each feces and tissue sample were combusted in oxygen, and the resulting $^{14}CO_2$ absorbed in 40% ethanolamine in ethanol (11). The ^{14}C content of the resulting solution was determined by liquid-scintillation counting, using a scintillation solution consisting of 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene, dissolved in 1 liter of toluene and 133 ml of ethanol. Bile acid half-lives and turnover times were calculated according to Lindstedt and Norman (12).

Method b. Determination of bile acid pool sizes and spectra. The lipid fraction of the small intestine plus contents was extracted with chloroform:methanol, 2:1. An aliquot was removed for ^{14}C assay. The remaining solution was evaporated to dryness and saponified for 3 hr at 15 lb pressure with 7 *N* NaOH. The neutral sterols were extracted from the hydrolyzate with petroleum ether. The alkaline residue was acidified and extracted with petroleum ether to remove fatty acids, and exhaustively extracted with ethyl ether to recover the "acidic fraction" containing the bile acids. An aliquot of the ether fraction was removed for ^{14}C assay. The ^{14}C assay on this fraction was compared with that on the aliquot taken before fractionation of the lipids, and a factor was determined to correct the bile acid assays for losses during extraction. The "acidic fraction" was fractionated by thin-layer chromatography and quantitated by densitometry of the thin-layer chromatograms (13). The identity of the acids was established using standards and color sprays (14). Bile acid pool sizes were calculated according to Strand (15).

Method c. Fecal and cecal β-sterol assay. Aliquots of feces or of cecum plus contents were heated with alcoholic potassium hydroxide for 2 hr. The nonsaponifiable fraction of the resulting suspension was extracted with

petroleum ether. Aliquots of the petroleum ether extract were evaporated to dryness. The residue was dissolved in ethanol–acetone and the β-sterols precipitated with 5% digitonin in 50% ethanol. The precipitation was allowed to proceed overnight. The amount of digitonide formed was determined gravimetrically, and the β-sterol content was calculated.

Method d. Small intestine and liver cholesterol assay. Aliquots of the small intestines (plus contents) or livers were saponified and the nonsaponifiable fraction isolated as outlined above. Aliquots of the petroleum ether fraction were evaporated to dryness. The residue was dissolved in acetic acid and cholesterol determined colorimetrically by application of the Lieberman-Burchard reaction.

Method e. Serum cholesterol assay. Serum samples were analyzed for cholesterol by the method of Sperry and Webb (16).

Results and Discussion. Figure 1 shows the effects of supplementing a commercial rat ration with cholesterol and/or corn oil on cholic and chenodeoxycholic acid turnover rates in normal rats. The time in days is plotted on the ordinate against $-\log(1 - U^t/U^{max})$ on the abscissa. Here, U^t equals the fecal bile acid-24-^{14}C excretion (cpm) up to and including a given day; U^{max} equals the total bile acid-24-^{14}C recovered in the tissues and feces of a given animal. The straight lines obtained show that bile acid excretion is governed by first-order kinetics in each case. The half-life of the pools, the point at which $U^t/U^{max} = 0.5$, is shown by the dotted vertical line. The half-life of the cholic acid pool was 3.0 days in the controls as compared with 1.4 days in the cholesterol–corn oil-fed rats. Clearly the increased bile acid turnover

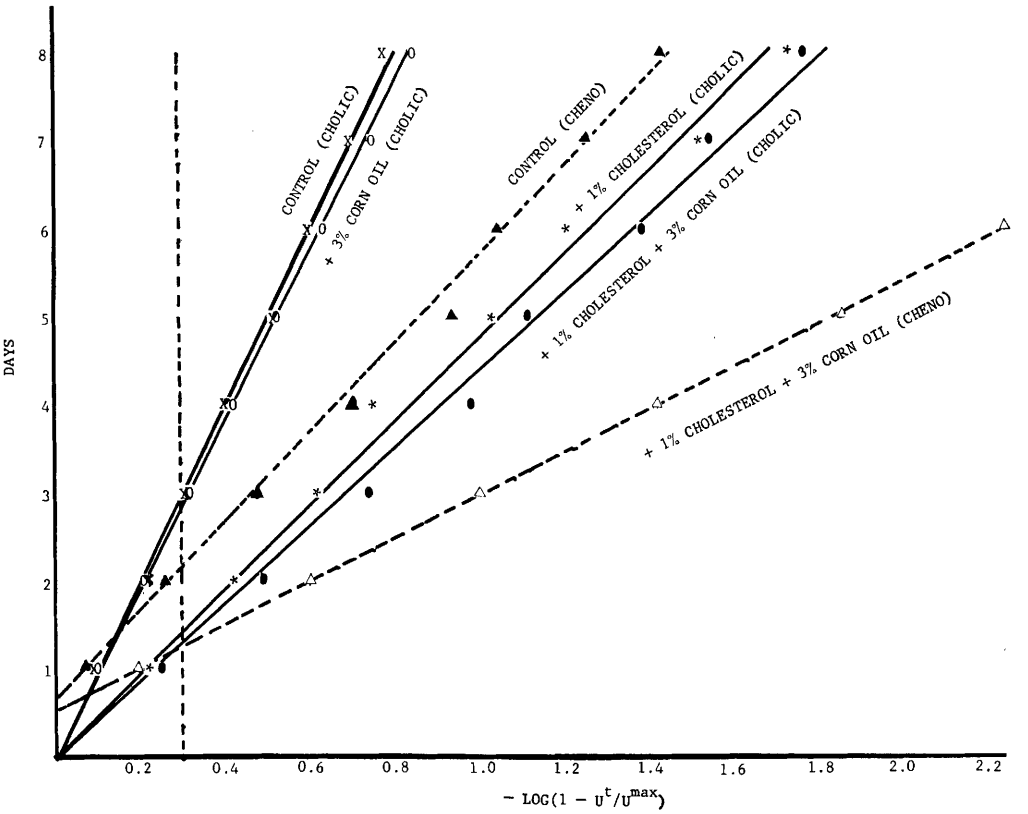

FIG. 1. Rate of elimination of pool bile acids in control rats and rats fed a diet supplemented with 1% cholesterol and/or 3% corn oil. The bile acid in parentheses is the pool acid studied. The vertical line cuts the curves at the half-lives of the bile acids.

TABLE I. Effects of Dietary Cholesterol and Corn Oil on Bile Acid Pool Sizes of Rats.[a]

Group	Cholic acid	Chenodeoxycholic acid	Total bile acids
		mg/100 g rat	
Control	5.40 ± 0.26	0.83 ± 0.12	6.23
Corn oil, 3%	5.79 ± 0.37	0.69 ± 0.20	6.48
Cholesterol, 1.0%	3.48 ± 1.20	1.77 ± 0.35	5.25
Cholesterol, 1.0% + corn oil, 3%	4.71 ± 0.35	1.86 ± 0.14	6.57

[a] ± Values are standard deviations.

rate could have been due to cholesterol alone, to corn oil alone, or to the combination of cholesterol and corn oil. Therefore, diets supplemented with either corn oil or cholesterol alone were fed, and the cholic acid half-lives determined. It can be seen (Fig. 1) that a diet supplemented with cholesterol alone effected an increase in cholic acid turnover equal to that observed with the cholesterol-plus-corn oil supplement. A diet supplemented with corn oil alone had no effect on the cholic acid turnover rate. It is, therefore, apparent that the increase in the bile acid turnover rate is due to the cholesterol supplement alone, and not to the presence of corn oil. Figure 1 also shows that the cholesterol–corn oil-supplemented ration decreased the chenodeoxycholic acid pool half-life from 2.2 to 1.3 days.

The effects of the diets on bile acid pool concentrations are shown in Table I. Here we see that the dietary supplements had little

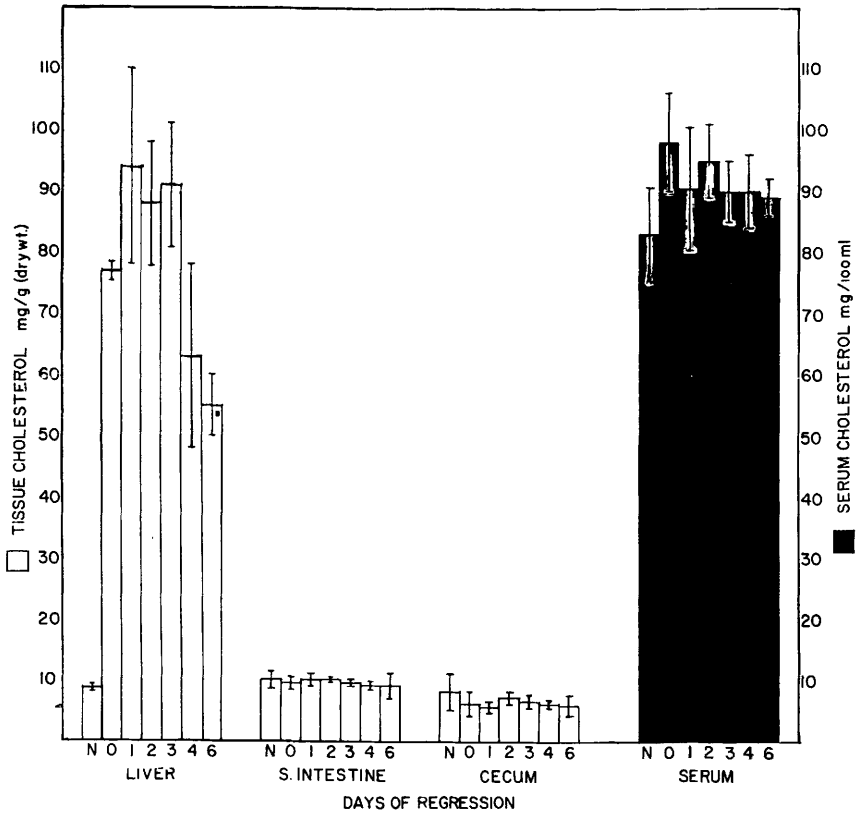

FIG. 2. The accumulation and regression of tissue and gastrointestinal sterols in rats. All rats were fed a basal diet supplemented with 1% cholesterol and 0.5% cholic acid during the accumulation phase. During the regression phase the unsupplemented basal diet was fed.

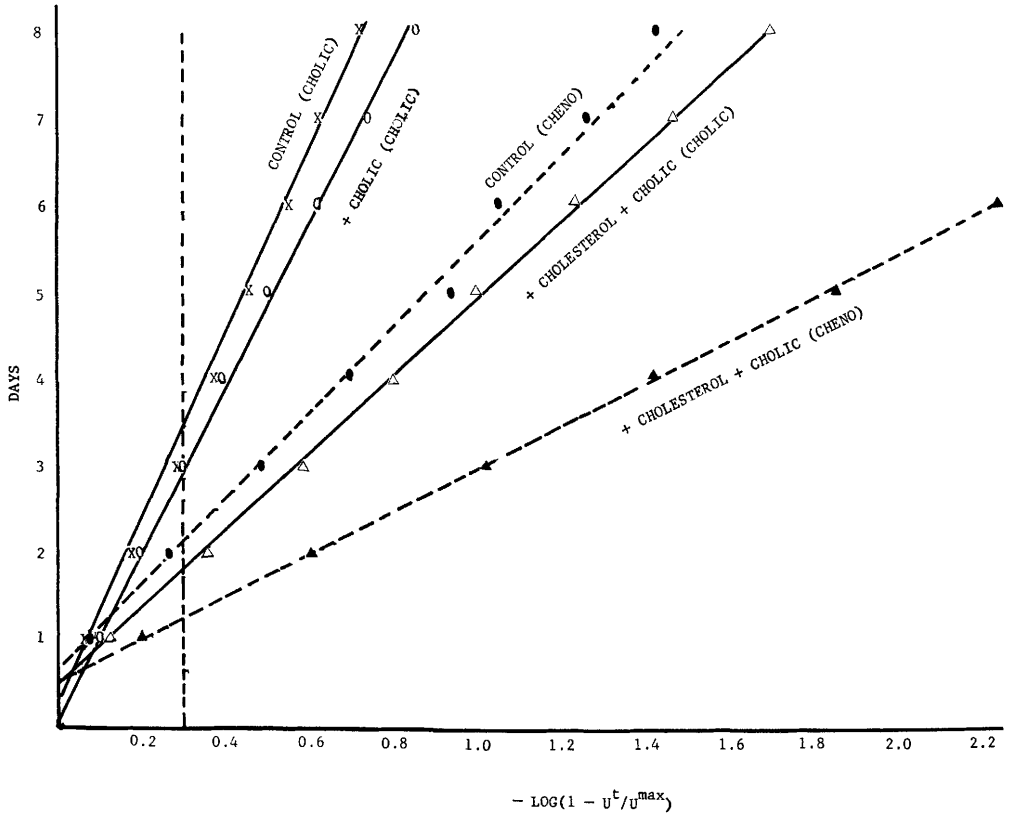

FIG. 3. Rate of elimination of pool bile acids in control rats and rats with elevated tissue cholesterol. After elevating tissue cholesterol by supplementing the diet with 1% cholesterol and/or 0.5% cholic acid for 3 weeks, the rats were returned to an unsupplemented diet during the experimental period. The bile acid in parentheses is the pool acid studied. The vertical line cuts the curves at the half-lives of the bile acids.

or no effect on the total bile acid pool size; however, they effected important changes in the relative concentrations of cholic and chenodeoxycholic acids. When the basal ration was supplemented with cholesterol plus corn oil or cholesterol alone, there was a decrease in the cholic acid concentration and a compensating increase in the chenodeoxycholic acid concentration of the pool. These changes in the relative bile acid concentrations of the pool are important since the chenodeoxycholic pool turns over at a much faster rate than the cholic acid pool (Fig. 1). It is apparent that the increased bile acid excretion in rats demonstrated by Wilson (9, 10) is due to two factors: decreases in the half-lives of the bile acids in their metabolic pool; and a shift in the relative concentrations of the pool acids toward the bile acid with the more rapid turnover rate, namely, chenodeoxycholic acid. It is interesting that corn oil had no effect on either the half-life of the pool or the concentrations of the pool bile acids. It is clear that corn oil does not affect the rate of bile acid excretion in rats, since bile acid excretion is directly proportional to the pool size and inversely proportional to the half-life of the pool. These results confirm and extend the finding of Siperstein (6) that unsaturated oils do not affect the rate of bile acid excretion in this species.

In the investigations reported above and in the studies of other groups (3, 4, 9, 10), cholesterol-supplemented diets were fed to the animals throughout the experiments. It is, therefore, impossible to determine whether this sterol's effects on bile acid metabolism

are triggered by elevated tissue cholesterol concentrations *per se* or by the presence of excess sterols in the intestinal lumen. To distinguish between these possibilities, the studies on the effects of elevated tissue cholesterol concentrations were initiated (Section B).

Figure 2 shows the results of the tissue cholesterol regression studies. In rats in which tissue cholesterol had been build up, liver cholesterol remained elevated throughout a 7-day regression period on an unsupplemented diet. Blood cholesterol was slightly elevated at the end of the build-up period and remained elevated throughout the regression period.

The results of the bile acid turnover studies are shown in Fig. 3. The half-lives of cholic and chenodeoxycholic acids decreased from 3.5 ± 0.4 and 2.2 ± 0.2, respectively, in control rats to 1.9 ± 0.2 and 1.3 ± 0.1 in rats with elevated tissue cholesterol. A comparison of these results (Fig. 3) with those for the dietary cholesterol experiments (Fig 1) shows that bile acid turnover was affected similarly by dietary cholesterol and by elevated tissue cholesterol. The effects of elevated tissue cholesterol concentrations on the relative concentrations of bile acids in the small intestinal pool are shown in Table II. As in the dietary experiment where cholesterol was fed continuously (Table I), the total bile acid concentration in the pool remained constant and there was a shift in the relative concentrations of the individual bile acids. The cholic acid concentration decreased and there was a compensating increase in the chenodeoxycholic concentration. Since cholic acid was used along with cholesterol to effect tissue cholesterol accumulation, it was necessary to determine if feeding this bile acid during the build-up period had any effect on bile acid turnover rates and pool sizes. Examination of Fig. 3 and Table II shows that this substance had little effect on bile acid metabolism, since only slight increases in cholic acid levels were observed.

The effect of elevated tissue cholesterol on the excretion of bile acid, calculated from the pool turnover rates and the total pool chenodeoxycholic and cholic acid concentrations, is shown in Table III. Elevated tissue cholesterol concentrations effected a 2-fold increase in the bile acid excretion rate.

Although the diet consumed during the 7 days of the turnover studies was unsupplemented with cholesterol, it was still possible that the cholesterol concentrations in various sections of the gastrointestinal tract had become elevated during the build-up period, along with the blood and liver cholesterol levels. Under these conditions, there could be increased cholesterol concentrations in the enterohepatic recirculating pool. To check this, the small intestine and cecum (both plus contents) were analyzed for β-sterol concentrations daily during the 7-day period after return to the unsupplemented diet (Section B.i). The results are shown in Fig. 2. It is apparent that there was no elevation of β-sterol concentrations in either section of the gastrointestinal tract.

TABLE II. Effects of Elevated Tissue Cholesterol and Cholic Acid on Bile Acid Pool Sizes of Rats.

Group	Cholic acid	Chenodeoxycholic	α-Muricholic	β-Muricholic	Total bile acids
			mg/100 g rat		
Control	5.54 ± 0.91	1.22 ± 0.20	1.58 ± 0.50	0.55 ± 0.09	8.89
Cholic acid[a]	7.00 ± 0.37	1.74 ± 0.16	1.91 ± 0.26	0.34 ± 0.04	10.99
Elevated tissue cholesterol[b]	3.37 ± 0.55	2.81 ± 0.56	2.21 ± 0.05	0.78 ± 0.09	9.17

[a] Diet supplemented with 0.5% cholic acid for 3 weeks and then unsupplemented during the experimental period.

[b] Diet supplemented with 1% cholesterol + 0.5% cholic acid for a 3-week build-up period and then unsupplemented during the experimental period.

± Values are standard deviations.

TABLE III. Bile Acid Pool Half-Lives, Turnover Times, and Synthesis Rates in Control Rats and Rats with Elevated Tissue Cholesterol.

Group	Half-lives (days)		Turnover times[a] (days)		Synthesis rates (mg/day/100 g of rat)		
	Cholic acid	Chenodeoxy-cholic	Cholic acid	Chenodeoxy-cholic	Cholic acid	Chenodeoxy-cholic	Total
Controls	3.5 ± 0.4	2.2 ± 0.2	5.0	3.2	0.6	0.2	0.8
Elevated tissue cholesterol[b]	1.9 ± 0.2	1.3 ± 0.1	2.7	1.8	0.6	0.8	1.4

[a] Turnover time = half-life/(ln 2).

[b] Diet supplemented with 1.0% cholesterol + 0.5% cholic acid for a 3-week build-up period, and then returned to an unsupplemented diet for the experimental period.

To further check on the possibility that elevated gastrointestinal β-sterols might have been a factor influencing bile acid turnover in rats with elevated tissue cholesterol, the fecal excretion experiments were carried out. During an 8-day collection period, fecal β-sterol excretion averaged 8.18 ± 1.3 mg/day/100 g animal in rats with elevated tissue cholesterol as compared to 6.66 ± 0.84 mg/day/100 g animal in controls. It is, therefore, obvious that in the presence of increased tissue cholesterol there is no increase in cholesterol mobilization via the fecal sterol pathway, once again confirming the results of earlier experiments (17, 18).

It is clear that there are two factors responsible for the increased elimination of accumulated tissue cholesterol in rats: 1. The increased bile acid turnover rate increases the rate of conversion of cholesterol to bile acids by decreasing the negative feed-back inhibition illustrated in the introduction; and 2. The bile acid pool spectrum shifts toward acids with faster turnover rates. Just how elevated tissue cholesterol concentrations effect these changes is not clear. The changes in the relative concentrations of the pool acids—a decrease in the cholic acid pool and an increase in the chenodeoxycholic acid pool—probably reflect changes in the relative synthesis rates of the primary bile acids by the liver mitochondria since the half-lives of both these acids decreased. The changes in bile acid half-lives would appear to be due to decreases in the rates of active and/or passive transport of the bile acids across the small intestinal and cecal walls. Further experiments are necessary if the mechanisms of the effects are to be determined.

Summary. The effects of cholesterol feeding and accumulated tissue cholesterol on bile acid metabolism in normal female rats were studied. Feeding diets supplemented with cholesterol and corn oil over the entire experimental period resulted in decreases in the cholic and chenodeoxycholic acid pool half-lives of 1.6 and 0.9 days, respectively. In experiments on rats with accumulated tissue cholesterol fed unsupplemented basal rations during the experimental period, the half-lives of these acids also decreased 1.6 and 0.9 days, respectively, from the values for normal control rats. In both the dietary and accumulated tissue cholesterol experiments, the total bile acid pool concentration was unchanged but there was an important decrease in cholic acid concentration accompanied by a compensating increase in chenodeoxycholic concentration. Studies in the animals with elevated tissue cholesterol showed that the effects on bile acid turnover were not due to increased levels of sterols in the gastrointestinal tract but were due *per se* to the increased tissue cholesterol levels. Dietary corn oil by itself had no effect on either turnover rates or pool sizes of the bile acids.

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