Effects of Age and Sex on Rat Bile Acid Metabolism[1] (35959)

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It is known that tissue cholesterol concentration differs with age and sex in various species of mammals (1, 2). This could be due to a number of factors including variations in (a) the rate of absorption of exogenous cholesterol, (b) the rate of tissue sterol biosynthesis, (c) the rate of exchange of cholesterol among various pools, (d) the size of the sterol pools, and (e) the rate of elimination of cholesterol from the sterol pools.

The present study investigated the effects of age and sex on bile acid metabolism in the rat. Since the elimination of tissue cholesterol via the bile acid pathway is quantitatively important in many species, variation in the rate of bile acid metabolism could be an important factor in determining tissue cholesterol concentration. It is recognized that species differences exist with respect to bile acid metabolism (3), and that our findings may not apply to all animals; however, these experiments should indicate whether or not it is probable that age and/or sex effect important changes in mammalian bile acid metabolism and thus in their cholesterol mobilization.

Methods and Procedures. Animals. All rats used in these experiments were of the Sprague-Dawley strain. The rats were born and raised in our animal quarters, and were maintained *ad libitum* on Rockland Rat Rations from weaning until sacrifice. This diet contains 24% crude protein, 4.0% crude fat, and 6% crude fiber, supplemented with vitamins and minerals. Groups of 5 male and 5 female rats were used in experiments at the following ages (days): 1, 8, 15, 27, 46, 65, 83, 111, 146, and 450.

Bile acid half-lives. Each animal in a group received a 5 μCi intraperitoneal tracer dose of cholic acid-24-^{14}C (52.4 mCi/mmole) or chenodeoxycholic acid-24-^{14}C (30.4 mCi/mmole). Following injection, the rats were placed in individual metabolism cages; and feces were collected daily for 8 days. The rats were killed; and cecums, small intestines, large intestines (all plus contents), and livers were removed. The feces and tissue samples were dried by lyophilization and exhaustively extracted with boiling ethanol in Bailey-Walker extractors. Aliquots of these extracts were added to 17 ml of phosphor solution (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) for determination of ^{14}C activity in a scintillation spectrometer. An automatic external standard was used to correct for quenching. Bile acid half-lives were calculated according to Lindstedt and Norman (4).

Bile acid pool sizes and spectra. The small intestine plus contents of each animal in a group was dried by lyophilization, exhaustively extracted with boiling ethanol, and evaporated to dryness. The lipid residue was saponified at 15 lb pressure with 7 *N* sodium hydroxide for 3 hr; and then extracted with petroleum ether to remove the nonsaponifiable fraction. The alkaline residue was acidified and extracted first with petroleum ether to remove fatty acids and then exhaustively with ethyl ether to recover the acidic fraction containing bile acids. The individual bile acids were separated and quantitated by thin-layer chromatography and densitometry (5).

Results. Figures 1, 2, and 3 illustrate the cholic and chenodeoxycholic acid excretion rates for male and female rats at the various age intervals studied. The feces collection time (days) is plotted on the ordinate against $-\log(1-u^t/u^{max})$ on the abscissa. u^t= the fecal bile acid-24-^{14}C excretion (cpm) up to and including a given day; u^{max}= the total bile acid-24-^{14}C recovered in the tissues and feces of a given animal. The straight lines obtained in each case show that cholic and

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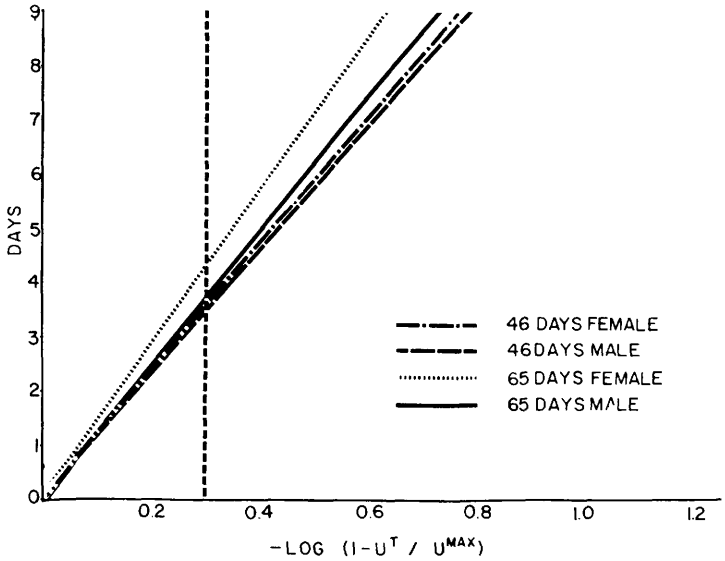

FIG. 1. Cholic acid turnover in male and female rats at different ages.

chenodeoxycholic acid excretion are governed by first order kinetics at all age intervals investigated. The half-life of the bile acid pool can be read from the plots at the point where $u^t/u^{max} = 0.5$. The broken vertical line intersects the rate line at the half-life of the cholic or chenodeoxycholic acid pool.

The data show that: (a) there was no significant sex difference in the cholic or chenodeoxycholic acid pool half-life at any age studied; (b) pool half-lives did not change significantly with age from 46 through 450 days; and (c) the chenodeoxycholic acid half-lives were considerably shorter than the corresponding cholic acid half-lives in all groups investigated.

In contrast to the limited variation found in the half-life studies, the studies of bile acid pool sizes and spectra showed important differences related to both sex and age. Table

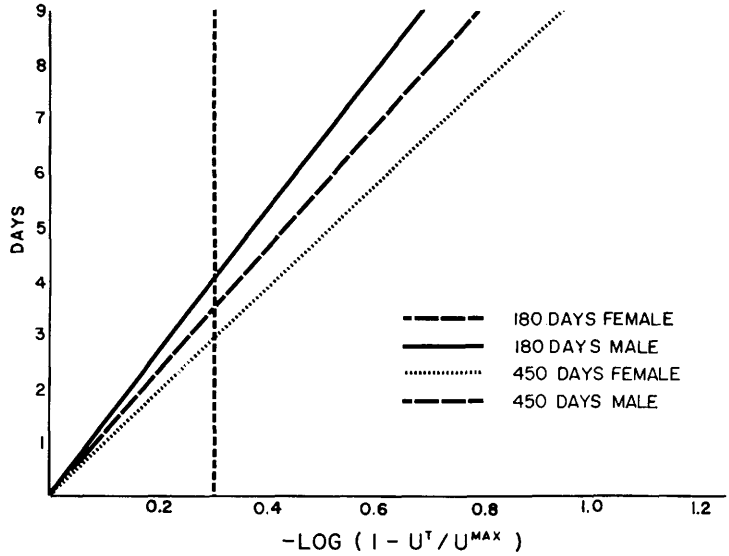

FIG. 2. Cholic acid turnover in male and female rats at different ages.

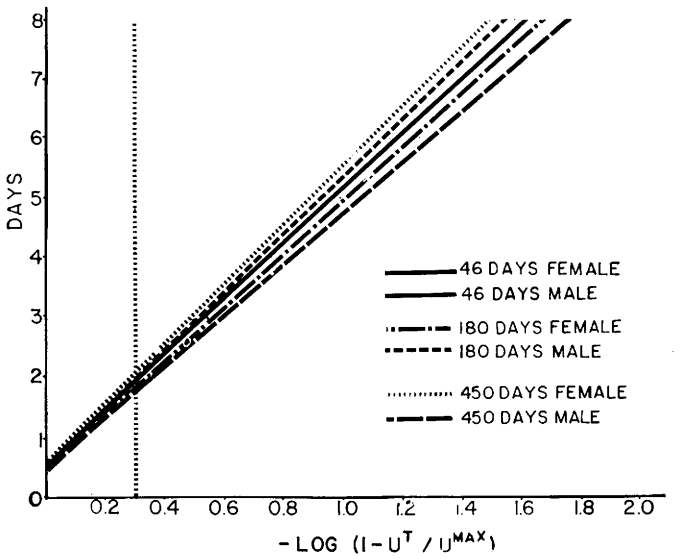

FIG. 3. Chenodeoxycholic acid turnover in male and female rats at different ages.

I presents data on the pool sizes of the primary bile acids (cholic and chenodeoxycholic acid): (a) from 1 through 15 days the bile acid pools of male and female rats were equal in size and contained only cholic acid; (b) from 27 through 450 days the pools of female rats contained major concentrations of both primary bile acids, while the pools of male rats contained only trace amounts of chenodeoxycholic acid; and (c) from 1 day through 450 days, the cholic acid pools were similar for the two sexes. The variations observed within these pools were probably due to differences between the rate at which the gastrointestinal tract and other parts of the body develop.

Table II presents data on the pool sizes of the secondary bile acids (α- and β-muricholic and deoxycholic acids). It shows that: (a) the secondary bile acids did not appear in the bile acid pool until 46 days after birth; (b) from 46 through 450 days, the pools of the two sexes contained similar concentrations of α- and β-muricholic and deoxycholic acids. While there was variation within these pools, there was no consistent pattern of change in the concentration of these acids related to either age or sex.

TABLE I. Primary Bile Acid Pool Sizes of Developing Rats.[a]

	Cholic acid (mg/100 g of body wt)		Chenodeoxycholic acid (mg/100 g of body wt)	
Age (days)	Female	Male	Female	Male
1	9.95 ± 1.86	10.2 ± 1.69	0.0	0.0
8	4.13 ± 1.08	3.69 ± 0.32	0.0	0.0
15	3.41 ± 1.62	3.96 ± 0.47	0.0	0.0
27	13.4 ± 1.02	12.0 ± 1.58	2.45 ± 0.97	2.45 ± 0.86
46	11.4 ± 2.27	12.5 ± 1.85	1.19 ± 0.40	Trace
65	9.03 ± 1.05	8.59 ± 3.39	1.78 ± 0.18	Trace
83	6.18 ± 1.85	4.63 ± 0.67	1.35 ± 0.58	Trace
111	4.47 ± 0.46	4.40 ± 0.84	1.56 ± 0.17	Trace
146	5.76 ± 1.01	3.93 ± 0.65	1.34 ± 0.30	Trace
450	7.80 ± 2.86	6.85 ± 0.93	1.81 ± 0.46	Trace

[a] Trace = <0.10 mg/100 g of body weight; ± values are standard deviations.

TABLE II. Secondary Bile Acid Pool Sizes of Developing Rats.[a]

Age (days)	α-Muricholic acid (mg/100 g of body wt)		β-Muricholic acid (mg/100 g of body wt)		Deoxycholic acid (mg/100 g of body wt)	
	Female	Male	Female	Male	Female	Male
1	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0
46	1.01 ± 0.31	0.90 ± 0.29	0.55 ± 0.20	0.99 ± 0.37	0.0	Trace
65	1.13 ± 0.84	1.00 ± 0.35	0.96 ± 0.27	0.53 ± 0.23	0.19 ± 0.27	Trace
83	0.54 ± 0.60	Trace	1.09 ± 0.87	1.29 ± 0.56	0.14 ± 0.15	0.51 ± 0.10
111	0.46 ± 0.22	0.34 ± 0.20	0.45 ± 0.34	0.34 ± 0.04	0.22 ± 0.11	0.38 ± 0.11
146	0.66 ± 0.15	0.45 ± 0.17	0.40 ± 0.14	0.0	0.15 ± 0.06	0.30 ± 0.17
450	0.64 ± 0.20	0.34 ± 0.09	0.98 ± 0.33	0.24 ± 0.09	0.45 ± 0.23	0.0

[a] Trace = <0.10 mg/100 g of body weight; ± values are standard deviations.

Discussion. The absence of chenodeoxycholic and α- and β-muricholic acids in the pools of female and male rats through 15 days of age is interesting. It appears that the liver enzyme systems—or the balance of these systems—necessary for chenodeoxycholic acid synthesis, may not develop until after day 15. There is evidence that the metabolism of cholesterol to cholic and/or chenodeoxycholic acids plus α- and β-muricholic acids depends on the balance between the activity of liver sterol 26-hydroxylase and that of 12-hydroxylase (6). In the generally accepted scheme for conversion of cholesterol to cholic acid, the nuclear changes—including 7 and 12 hydroxylation—precede side-chain oxidation and cleavage (7). Cholic acid is a major product. However, if this order is reversed and side-chain oxidation of cholesterol precedes the nuclear changes, chenodeoxycholic and α- and β-muricholic acids would be the major products (8–10). Thus it is possible to account for the lack of chenodeoxycholic acid in the bile acid pool of the young rat either by low liver sterol 26-hydroxylase activity or by high liver sterol 12-hydroxylase activity. Further studies will be necessary to determine whether the balance of these enzyme activities is actually responsible for this absence of chenodeoxycholic acid.

Changes in the composition of the bile acid pool spectrum with age have been observed in other species. In contrast to our findings in the rat, Peric-Golia and Jones (11) observed that the guinea pig excretes only chenodeoxycholic acid until it is several months old. This phenomenon is probably related to variations between breeds or individual animals (12). A study by Poley *et al.* (13) demonstrated that cholic acid was the predominant acid in human infant bile, while chenodeoxycholic was predominant in older children (13).

The observation of very low levels of chenodeoxycholic in male rats from 46 through 450 days is also very interesting. It seems probable that this phenomenon is due to the action of androgens or estrogens on the bile acid synthetic pathway. It should be noted that in rats aged 27 days the two sexes had similar chenodeoxycholic acid concentrations, however by 46 days this acid had nearly disappeared from the male pool. Now during this interval it is probable that the testes begin producing male hormones, and this leads to the decline in chenodeoxycholic acid. Further experiments will be necessary to test this hypothesis.

The fact that α- and β-muricholic concentrations of male and female rats 46 to 450 days are almost equal, even though male rat chenodeoxycholic concentrations are vanishingly small, needs further investigation. It is generally thought that the muricholic acids originate by metabolism of chenodeoxycholic acid (14, 15). However, if this is true, it

would be very difficult to explain the presence of α- and β-muricholic acids in the male bile acid pool in the absence of chenodeoxycholic acid. It is of course conceivable that chenodeoxycholic is present in small amounts which are being almost totally converted to the muricholic acids. However, it is more probable that other pathways exist for the synthesis of the muricholic acids which do not involve chenodeoxycholic (16, 9). In that case, if the synthetic pathways leading to chenodeoxycholic are blocked, alternate pathways would be available for muricholic acid synthesis.

The lack of chenodeoxycholic acid in the male bile acid pool, coupled with equal female and male cholic acid concentrations, has important implications with respect to the amount of cholesterol being converted to bile acids by these animals. Because the chenodeoxycholic pool has a shorter half-life than the cholic acid pool, the larger amount of chenodeoxycholic acid in the female bile acid pool could enable the females to turn over more cholesterol per day via the bile acid pathway than males. Since this could be an important factor in the relative ability of males and females to handle body cholesterol, further mechanistic and comparative investigation of this effect is obviously warranted.

With respect to the relative rate at which the two sexes oxidize cholesterol via the bile acid pathway, it should be noted that Kritchevsky *et al.* (17) found a marked difference in the rate of oxidation *in vitro* of cholesterol-26-^{14}C by liver mitochondria isolated from male and female rats. Female rat liver mitochondria were more active than male (17).

Summary. A study was made of the effects of age (1 through 450 days) and sex on bile acid half-lives, pool sizes, and spectra. No significant sex or age differences were found in the cholic or chenodeoxycholic pool half-lives. Chenodeoxycholic acid half-lives were considerably shorter than corresponding cholic acid half-lives in all groups (cholic acid ≅ 3.5 days; chenodeoxycholic acid ≅ 2.0 days). With respect to pool sizes, from 1 through 15 days the pools of females and males were equal in size and contained only cholic acid, possibly because the liver enzyme systems necessary for chenodeoxycholic acid synthesis had not yet developed. From 27 through 450 days, the pools of females contained major concentrations of both cholic and chenodeoxycholic acid. In contrast the male pools from 46 to 450 days contained only traces of chenodeoxycholic acid, along with cholic acid concentrations about equal to those of female rats. This lack of chenodeoxycholic acid in the male bile acid pool, which could result from the action of androgens or estrogens on the bile acid synthetic pathway, has implications with respect to the relative ability of mature male and female rats to handle body cholesterol. The secondary bile acids (α- and β-muricholic and deoxycholic acids) did not appear in the pools until day 46. From then through 450 days, the concentrations of these acids were similar for the two sexes although there were individual variations. The fact that α- and β-muricholic acids are present in the adult male pool, although chenodeoxycholic acid is absent, suggests the possibility that alternate pathways may exist for the synthesis of the muricholic acids which do not involve chenodeoxycholic acid.

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