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Steroid-C^{14} Metabolism in Bile Acid and Cholesterol-Treated Mice after Injection of Mevalonic Acid-2-C^{14}.* (26994)

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In the study of cholesterol and bile acid metabolism, use of single injections of isotopically labeled cholesterol or cholesterol precursors coupled with fecal steroid analysis has been incompletely investigated. Siperstein and coworkers(1,2) gave single intravenous and oral doses of cholesterol-4-C^{14} to rats and found that in a 2-week interval from 50 to 90% of metabolized cholesterol was excreted in the fecal bile acid fraction. Similar results were obtained by Danielsson(3), using intraperitoneal injections of cholesterol-4-C^{14} emulsions. Kummerow *et al*(4) demonstrated that the pattern of steroid metabolism in the chicken differs from that in the rat only in respect to rates of synthesis and excretion. On the other hand, Frantz *et al*(5) could not confirm these findings in human studies in which, following a single injection of cholesterol-C^{14}, 80% of the fecal C^{14} steroids were excreted in the non-saponifiable fraction.

In the present experiment, we have determined the pattern of fecal steroid-C^{14} excretion as a function of time after single intraperitoneal injections of mevalonic acid-2-C^{14} in normal, bile acid and cholesterol treated mice. The results demonstrate some of the advantages and limitations of such an approach in the study of cholesterol metabolism under various conditions.

Methods. Forty Webster strain female albino mice were placed on a cholesterol free diet(6) for 1 week, then divided into 5 groups and supplemented as shown in Table I. After 3 weeks, each animal received intraperitoneally 1 μc of mevalonic acid-2-C^{14} in saline (1.31×10^6 counts/min.). Each mouse was housed in a metabolism cage and feces collected at 24 hour intervals for the 7-day ex-

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TABLE I. Fecal Steroid-C^{14} Excretion as a Time Function, Following Injection of Mevalonic Acid-2-C^{14}

	Control		Cholic Acid*		Cholesterol + Cholic†		Litho-cholic acid*		Hyodeoxy-cholic acid*	
Days	$\alpha + \beta$ Sterols	Bile acid	$\alpha + \beta$ Sterols	Bile acid	$\alpha + \beta$ Sterols	Bile acid	$\alpha + \beta$ Sterols	Bile acid	$\alpha + \beta$ Sterols	Bile acid
	Counts/min./mouse/24 hr									
1	7264	19810	10643	10705	14920	7522	9295	7025	20665	11381
2	5755	2187	5600	3248	6134	1278	7403	3257	15211	2055
3	3326	1683	5004	1492	4034	843	5980	2236	10698	1494
4	3061	1818	3755	1044	3732	158	5584	1670	9171	1340
5-7	2572	2459	1846	2030	3127	261	4993	1877	7621	1148
Total Excretion	21978	27957	26848	18519	31947	10062	33255	16065	63366	17418

* Diet supplemented with .5% of bile acid.
† " " " .5% cholic acid + 1% cholesterol.

perimental period, after which the animals were sacrificed, livers removed, and both livers and carcasses quick frozen.

The feces samples were continuously extracted with hot ethanol for 48 hours. Aliquots of the ethanol extract containing the total steroids, were made strongly basic by addition of 7 N NaOH and heated in an autoclave at 15 lb pressure for 3 hours. The nonsaponifiable fraction was extracted with petroleum ether, and aliquots removed for determination of total β sterols and C^{14} activity of the $\alpha + \beta$ sterols. The aqueous residue was acidified and fatty acids extracted with petroleum ether and discarded. The remaining acidified aqueous fraction was extracted with ethyl ether. Aliquots of this bile acid containing extract, were plated and counted.

Liver and carcass cholesterol and β-sterol-C^{14} were determined by saponification of the respective tissues in alcoholic KOH, followed by petroleum ether extraction. Aliquots of the nonsaponifiable fraction were evaporated to dryness, taken up in alcohol-acetone (1:1) and precipitated with digitonin. β-Sterol-C^{14} was determined by filtration and washing of the digitonide precipitate on a filtration tower. All C^{14} determinations were made in an automatic windowless flow counter. All cholesterol assays were done according to the method of Sperry and Webb(7).

Results and discussion. Control. In normal mice the distribution of excreted fecal-C^{14} activity in the first 24 hours was 73% bile acids, 27% $\alpha + \beta$ sterols (Table I). This distribution was reversed in the 2nd 24-hour period (28% bile acids, 72% neutral sterols), and remained more or less fixed during the following collection periods. Thus, the overall pattern of excreted steroid-C^{14} activity in mice during the 7-day experimental period was 56% bile acids, 44% neutral sterols. Since there was a definite reversal in the excretory pattern after the first 24-hour collection, it is possible that shortly after injection most of the activity appears in the bile acid fraction. Although no experimental data are available, it seems likely that this phenomenon may be explained by the rapid turnover rate in liver cholesterol coupled with the relatively slow rate in extrahepatic tissues, since in normal animals the neutral fecal sterols are mainly extrahepatic in origin(3,8). Of the injected mevalonic acid-2-C^{14} activity, some 3.8% was incorporated in fecal C^{14}-steroids excreted during the 7-day period (Table II).

Cholic acid. A strong deterrent effect of cholic acid and its conjugates on the conversion of cholesterol to bile acids, in mice with accumulated liver cholesterol, has been demonstrated previously(8,9). Experiments of others have shown a reduction in production of bile acids in bile fistula rats receiving taurochenodeoxycholic acid(10). Both of these approaches utilized an abnormal situation, while the present experiment was designed to test the effect of cholic acid on bile acid formation in the intact mouse. The time-pattern of steroid-C^{14} excretion in cholic acid-treated mice was similar to that in untreated animals, but total bile acid synthesis

TABLE II. Effect of Bile Acids and Cholesterol on Fecal and Tissue Steroid and Steroid-C^{14} Levels.

	Control	Cholic acid	Cholesterol + cholic acid	Lithocholic acid	Hyodeoxycholic acid
			Counts/min./mouse/24 hr		
Total steroid-C^{14} excreted in feces	49935	45367	42009	49320	80784
β-Sterol-C^{14} remaining in liver	9399	21090	38954	8747	5989
β-Sterol-C^{14} remaining in carcass*	45539	44864	32621	44260	46978
Total C^{14}-activity accounted for	104873	111321	113584	102327	133751
			mg/24 hours		
Fecal β-sterol	1.93 ± .50	2.79 ± .54	—	4.34 ± .70	10.3 ± 1.90
			mg/g		
Liver cholesterol	4.52 ± 1.56	7.53 ± 2.54	48.0 ± 11.4	4.35 ± 1.44	2.64 ± .44
Carcass* "	2.16 ± .50	2.30 ± .39	2.93 ± .25	1.80 ± .43	2.00 ± .28

* Carcass weight = Body weight minus liver weight.
± numbers are standard deviations.

during the 7-day experimental period was less (Table I). There was a significant inhibition (50%) of bile acid excretion in the first 24 hours, but in subsequent periods there was no inhibition. Analysis of liver and carcass β-sterol-C^{14} activity at the end of 7 days revealed a significant retention of β-sterol-C^{14} activity in livers of cholic acid-treated animals (Table II). No significant difference in carcass activity was observed, therefore the deterrent effect of cholic acid must be confined to the liver. Total steroid-C^{14} synthesized was similar in control and cholic acid-treated mice. Since cholic acid retards hepatic cholesterol synthesis 75% in mice(9), it is impossible to assess the effect of cholic acid on cholesterol synthesis rates from an experiment of this type.

Cholesterol plus cholic acid. Cholesterol plus cholic acid increased the output of C^{14}-labeled $\alpha + \beta$ sterols during the 7-day collection period, and especially enhanced the output during the initial 24 hours. The rate of appearance of C^{14}-activity in the fecal bile acid fraction was greatly retarded throughout the collection periods (Table I). Because of this retarded bile acid-C^{14} synthesis, mice in this group retained much more of the liver β-sterol-C^{14} activity at the end of 7 days (Table II) than the controls, yet their total fecal steroid-C^{14} excretion was *not* significantly lower. This is explained by the fact that in this group a greater proportion of the fecal C^{14}-activity appeared in the $\alpha + \beta$ sterol fraction, which compensated for the loss in excreted bile acid-C^{14} (Table I).

The enhanced loss of carcass β-sterol activity, which appeared in the fecal $\alpha + \beta$ sterol fraction, can not be explained by the data of the present experiment. However, it seems probable that this loss was due to an increased rate of carcass endogenous-exogenous cholesterol turnover caused by dietary cholesterol.

Hyodeoxycholic acid. Hyodeoxycholic acid altered the time-pattern of fecal C^{14} activity (Table I). There was a large absolute increase in $\alpha + \beta$ sterol-C^{14} excretion at all time intervals, reflecting the increased fecal β-sterol excretion (Table II). A similar increase has been shown to occur in the golden hamster on the first day of hyodeoxycholic acid feeding(11). The present data (Table II) show that at termination the livers of hyodeoxycholic acid-treated mice contained much less β-sterol-C^{14} than livers of controls, while carcass β-sterol-C^{14} was about the same in these 2 groups; therefore, it may be concluded that fecal sterols in hyodeoxycholic acid-treated mice originate in the liver and are excreted via the bile duct. It is conceivable that liver cholesterol could first be transferred to extrahepatic tissues and then be excreted; however, one would expect a definite change in carcass cholesterol-C^{14} if this were true. Direct proof awaits bile duct

cannulation experiments in a larger species. If one considers that hepatic cholesterol synthesis is increased some 7-fold in hyodeoxycholic acid-treated mice(12) and that there is a 5-fold increase in β-sterol excretion (Table II), the inadequacy of using single injections of mevalonic acid for detection of changes in cholesterol synthesis rates is apparent (hyodeoxycholic acid-treated: 133,751 counts/min.; controls: 104,873 counts/min.). Some investigations may have failed to reveal changes in synthesis rates from mevalonic acid, while demonstrating large increases or decreases in cholesterol synthesis from acetate-1-C^{14}, because of the use of limiting concentrations of mevalonic acid.

Lithocholic acid. The effects of lithocholic acid on mouse liver cholesterol levels, synthesis rates(12), and blood cholesterol levels(13) were similar to, but less extensive than, the effects of hyodeoxycholic acid. The data in Tables I and II indicate that the mechanism of action of these bile acids is similar, although the detailed and overall effects of lithocholic acid are smaller.

Considering the results of the foregoing experiments *in toto,* it is evident that under normal circumstances mobilization of mouse liver cholesterol depends upon the ability of this organ to convert cholesterol to bile acids. However, hyodeoxycholic and lithocholic acids in some unknown way enable the mouse liver to bypass this requirement and excrete cholesterol directly. Direct excretion was shown to apply not only to accumulated liver cholesterol(8) but also to normal hepatic cholesterol (Table II).

This study makes it clear that the time-pattern of fecal steroid-C^{14} excretion is greatly influenced by the experimental conditions. In almost all cases observed differences were due to alterations in hepatic cholesterol metabolism.

Summary. The effects of bile acid and cholesterol on the time-pattern of fecal steroid-C^{14} elimination was investigated in mice following a single injection of mevalonic acid-2-C^{14}. In controls, excreted bile acid-C^{14} assayed 3 times sterol-C^{14} during the first 24 hours. This ratio reversed during the 2nd 24-hour period, and remained constant for the remainder of the 7-day experiment. Cholic acid depressed bile acid-C^{14} excretion, but failed to alter the time pattern of fecal sterol-C^{14} elimination. Cholesterol plus cholic acid depressed bile acid-C^{14} and increased $\alpha + \beta$ sterol-C^{14} excretion throughout the experiment. Hyodeoxycholic acid increased sterol-C^{14} excretion (shown to be of hepatic origin), had little effect on bile acid-C^{14} excretion, and increased total steroid-C^{14} eliminated. Lithocholic acid had a similar though smaller effect. After sacrifice, the livers of cholic acid-treated mice had twice the β-sterol-C^{14} concentration found in livers of controls, while those of mice treated with cholesterol plus cholic acid had β-sterol-C^{14} levels 4 times as high. Livers of lithocholic and hyodeoxycholic acid-treated animals showed reduced β-sterol-C^{14} levels. Carcass β-sterol-C^{14} levels were unaffected by any of the bile acids, but were lowered by the combination of cholesterol and cholic acid. The total amount of sterol-C^{14} synthesized from injected mevalonic acid was similar in all cases.

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