The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: the influence of dietary cholesterol

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SUMMARY A means of quantifying the two major pathways of cholesterol elimination from the body, bile acid production and neutral sterol excretion, has been devised for use in the intact rat. Utilizing an isotopic "steady state" for blood cholesterol (which has been attained by the subcutaneous implantation of capsules containing cholesterol-4-C^{14}) and the measurement of C^{14} appearance in the bile acid and sterol fractions of feces, it is possible to measure the amounts of these two excretion products. With this method the increase in bile acid formation following cholesterol feeding has been demonstrated directly; this increase has been shown to be sufficient in magnitude to account for the entire positive balance for neutral sterol which occurs in the cholesterol-fed animal after equilibrium has been attained. Finally, utilizing a double isotopic steady state in animals fed cholesterol-7α-H^3 and implanted with cholesterol-4-C^{14}, it has been possible to demonstrate that the major portion of fecal cholesterol of endogenous origin is derived from or is in equilibrium with that of the blood. In these animals the measurement of dietary cholesterol absorption has also been possible.

CHOLESTEROL IS ELIMINATED from the organism almost entirely by excretion into the feces. Two major pathways of excretion are known, one involving the degradation of cholesterol to bile acids (1–3), and the other involving the excretion of cholesterol and its neutral transformation products, notably coprostanol (4). Quantification of the relative and absolute importance of the two pathways in the intact animal, despite a common channel of excretion, is a difficult problem for a number of reasons. First, after reaching the lumen of the intestine both the bile acids and cholesterol are transformed by bacterial action into a variety of different products (5), and some of these are difficult to measure by ordinary techniques. Second, an enterohepatic circulation exists both for bile acids and to a lesser extent for cholesterol, with the result that major changes in the rates of excretion of these two substances may not be quantitatively reflected in the external balance until a new steady state is attained. Third, in the intestinal lumen endogenous cholesterol is mixed with dietary sterols so that the fecal pool of neutral sterols represents a mixture of derivatives of endogenous and exogenous cholesterol and of dietary sterols other than cholesterol (6). Bile acids, also, presumably may be formed from noncholesterol sterols of dietary origin (7). Fourth, short-term isotopic techniques, because of the varying pool sizes and varying turnover rates of the products involved, do not lend themselves readily to quantitative interpretation. Finally, in addition to its excretion in the bile, endogenous cholesterol can reach the intestinal lumen by direct transfer across the intestinal wall; consequently measurements of fecal excretion do not yield information as to the ultimate site of origin of the sterol fraction even when the animal is on a cholesterol-free diet.

Several attempts have been made to devise methods suitable for the measurement of these pathways in intact animals. Balance techniques utilizing gas–liquid chromatography for the precise measurement of the fecal neutral sterols have been used to approach the problem, but quantification of the bile acid pathway by this method is indirect and does not allow for separation of endogenous from exogenous pathways under circumstances where cholesterol is added to the diet (8). The only technique available at the present time for direct assay of the bile acid pathway is the isotopic method of cholic acid turnover; this technique does not allow for simultaneous quantification of neutral sterol production (9, 10).

The present report describes an isotopic technique for quantifying both parameters of cholesterol metabolism in

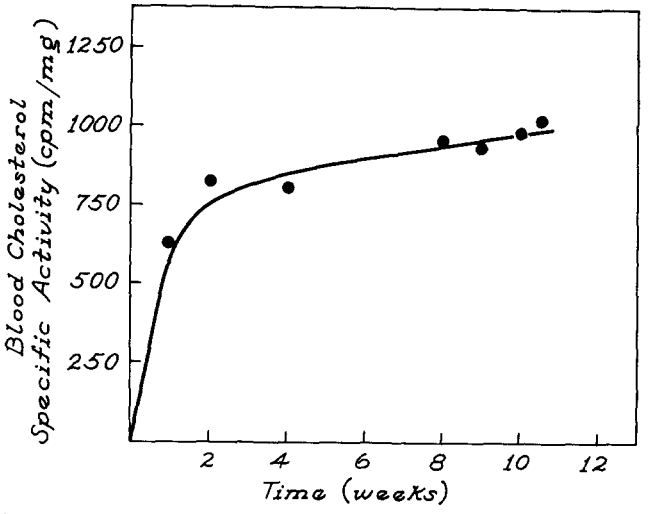

FIG. 1. The change in whole blood cholesterol specific activity with time after the subcutaneous implantation of a capsule containing 104 mg cholesterol-4-C^{14} (55,780 cpm/mg).

the rat. By utilizing animals which have been implanted subcutaneously with capsules of cholesterol-4-C^{14} and fed varying quantities of cholesterol-7α-H^3 until the steady state has been attained for both isotopes, it is possible to quantify bile acid production, neutral sterol excretion, net neutral sterol balance, and the dietary cholesterol absorption per animal per day, regardless of the type or amount of dietary sterol.

METHODS AND PROCEDURE

Male rats of the Sprague-Dawley strain, weighing 100–150 g, were anesthetized with ether, and gelatin capsules containing 75–105 mg of crystalline cholesterol-4-C^{14} (specific activity 56,000 cpm/mg) were implanted subcutaneously through a small dorsal incision. The animals were then allowed free access to Purina rat chow, and blood samples were periodically collected from the tail of each rat. Approximately 1–2 months after implantation the rats were utilized for the feeding studies.

Rats which had previously received subcutaneous implants of cholesterol-4-C^{14} and untreated rats were fasted overnight, placed in individual cages, and fed weighed quantities of diets which contained varying quantities of cholesterol.[1] As described in the individual protocols, the dietary cholesterol was either unlabeled or contained cholesterol-4-C^{14} or cholesterol-7α-H^3.

The feeding periods lasted for 12–16 days, and feces collections were made from each rat for varying periods of time during the experiment. At the end of the experimental period the rats were killed, and the carcasses were stored at −20°. Neutral sterols and bile acids were separated from the feces and assayed for radioactivity as follows. The rat feces were dried for 2 days at room temperature; the samples were then pulverized in a mortar and pestle, and washed into 250-ml Erlenmeyer flasks with 150 ml of chloroform–methanol 2:1. Boiling chips and Celite filter aid (2 g) were added to each sample, and the mixture was boiled on a hot plate for 5 min and filtered into 250-ml Erlenmeyer flasks. The residue was rinsed twice with 25 ml chloroform–methanol. Boiling chips were added to the filtrate, and the sample was taken to dryness under nitrogen on a steam bath. The residue from the filtrate was then suspended in 10 ml of 2 N KOH and autoclaved at 15 psi for 3 hr. The solution was then mixed with ethanol (10 ml), refluxed on a steam bath for 30 min, and extracted twice with 100 ml of petroleum ether by shaking on an International shaking machine for 5 min. The combined petroleum ether extracts (which contained neutral sterols) were backwashed with 5 ml of aqueous ethanol and taken to dryness; one aliquot was assayed for radioactivity in a liquid scintillation counter, and the remainder was utilized for chromatography. The water layers following petroleum ether extraction and backwashing were combined, acidified to pH 3 with 10 N H_2SO_4, and extracted with ethyl ether on a shaking machine; the ethyl ether layer (which contained bile acid) was taken to dryness, the residue was dissolved in methanol, and an aliquot was assayed for radioactivity. In experiments in which C^{14} and H^3 were assayed in the same samples, the assays for radioactivity were performed in a Packard Liquid-Scintillation counter equipped with differential discriminators. Internal standards of H^3 and C^{14} were then added, and corrections for quenching were made when necessary.

Cholesterol and coprostanol were assayed on aliquot portions of the neutral sterol fractions by the previously described modification (6) of the gas chromatographic technique of VandenHeuvel et al. (11). Whole blood cholesterol was precipitated as the digitonide and washed as described by Sperry and Webb (12); aliquots of the digitonide were assayed colorimetrically and for C^{14} content. In experiments in which free and esterified cholesterol were separated in serum and tissue, a previously described modification (13) of the method of Frantz et al. (14) was used.

RESULTS

Experiments with Implanted Cholesterol-4-C^{14}

The changes in specific activity of the whole blood cholesterol with time after the subcutaneous implantation of a capsule containing cholesterol-4-C^{14} is demonstrated in the representative experiment of Fig. 1. The specific

[1] The diet, which was prepared by General Biochemicals, contained 21% vitamin-free casein, 58% sucrose, 16% non-nutritive fibre, 4% USP XIV salt mix, and added vitamins, to which was added oleic acid (5 g per 100 g of diet) in which was dissolved measured amounts of cholesterol.

activity rose rapidly during the first 2 weeks following the implantation and then very slowly for the remainder of the 3 month study. While the specific activities varied slightly from week to week, these variations were usually less than 10% of the total values. The specific activities of the blood cholesterol have not been followed for periods longer than 3 months. However, the animal described in Fig. 1 excreted 6,360 cpm of C^{14} per day from days 60–64 of the study; since the implanted cholesterol contained 56,000 cpm/mg of cholesterol-4-C^{14} it can be calculated that approximately 0.1 mg of cholesterol was absorbed per day from the implanted crystals. If absorption continued at this rate, a relatively steady state might be expected to be maintained for 2 years or more in an animal implanted with 100 mg of cholesterol.

The results of similar experiments on 19 rats implanted with cholesterol-4-C^{14} and subsequently fed 0, 0.05, or 0.5% dietary cholesterol are summarized in Table 1. In each group of rats the average daily intake of food, the average weights of the animals, and the weights of the feces were similar. The specific activities of whole blood cholesterol are listed for each rat for the beginning and the end of a 4-day period during which feces were collected. Since the weights of the implanted capsules in these animals were similar, the variation in the average specific activities 2 months following implantation can probably be attributed to variation among animals in the rate of absorption of the implanted cholesterol. In this group of animals, furthermore, there was a maximal variation of 20% between the specific activities of blood cholesterol in individual rats at the beginning and the end of the collection period; consequently, it can be assumed that the error in the subsequent calculations due to variation in the blood cholesterol specific activity will be no more than 20%.

The bile acid excretion and neutral sterol excretion have been determined for each animal by the following formula:

$$\text{Mg excreted per day} = \frac{\text{C}^{14}\text{ recovered in either the bile acid or neutral sterol fraction (cpm)}}{\text{Specific activity of blood cholesterol (cpm/mg)}} \quad (1)$$

In the animals fed no cholesterol the average bile acid excretion was 3.5 mg/day, and the average neutral sterol excretion was 2.5 mg/day. These values were similar in animals fed 0.05% cholesterol (4.3 mg bile acid per day and 2.1 mg neutral sterol per day). In the animals fed 0.5% cholesterol in the diet, neutral sterol excretion did not increase (2.2 mg/day), but bile acid excretion rose to an average value of 9.8 mg/day. Evidence that the use of the whole blood cholesterol specific activity is valid for this type of calculation is presented in the last four columns of Table 1; here the ratios of whole blood cholesterol specific activity in each animal to the specific activities of esterified and free cholesterol of serum and liver are listed. Although occasional deviations do exist, the average ratio in each instance is close to 1; this has been interpreted as evidence that the animals have achieved a nearly ideal steady state in regard to the equilibration of cholesterol-4-C^{14} throughout these tissues and indicates that the whole blood cholesterol specific activity is a valid index of the serum and liver cholesterol specific activities under the conditions of these experiments.[2]

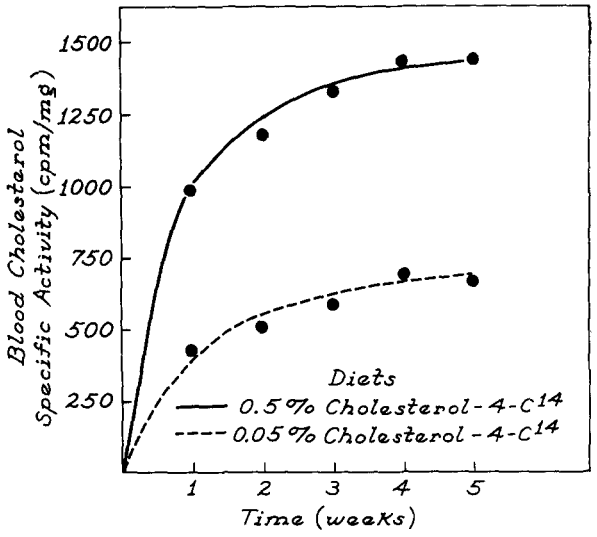

FIG. 2. The change in whole blood cholesterol specific activity with time after feeding 0.05 or 0.5% cholesterol-4-C^{14} (1700 cpm/mg).

Experiments with Dietary Cholesterol-4-C^{14}

The change in blood cholesterol specific activity with time after feeding diets containing either 0.05 or 0.5% cholesterol-4-C^{14} to two rats is shown in Fig. 2. In each of the rats the specific activity rose very rapidly for the 1st week and more slowly thereafter, approaching a plateau by the 5th week. The maximal specific activity in the rat fed the higher amount of cholesterol was approximately twice that of the other rat.

Sixteen feeding experiments identical with those described above are summarized in Table 2. Again the weights, daily food intakes, and feces weights are similar in the two groups. There is considerable variation in the specific activities of the bloods drawn at the beginning and end of the collection period in individual rats fed 0.05% cholesterol; in the group fed 0.5% cholesterol,

[2] In a separate series of experiments the ratio of fecal cholesterol specific activity to blood cholesterol specific activity was found to average 1.11 for animals implanted with cholesterol-C^{14} and 1.08 for animals fed cholesterol-C^{14} until an isotopic steady state was attained.

TABLE 1 THE EFFECT OF DIETARY CHOLESTEROL ON THE EXCRETION OF BILE ACIDS AND NEUTRAL STEROLS BY RATS PREVIOUSLY IMPLANTED WITH CHOLESTEROL-4-C^{14}

Rat No.	Cholesterol in Diet	Average Daily Food Intake	Weight of Rat	Weight of Feces	Whole Blood Cholesterol Specific Activity			Bile Acid Excretion		Neutral Sterol Excretion		Ratio of Final Whole Blood Cholesterol Specific Activity to			
					Beginning	End	Average					Serum Esterified Cholesterol Specific Activity	Serum Free Cholesterol Specific Activity	Liver Esterified Cholesterol Specific Activity	Liver Free Cholesterol Specific Activity
		g	*g*	*g/day*		*cpm/mg*		*cpm/day*	*mg/day*	*cpm/day*	*mg/day*				
1		10	280	1.5	829	868	848	2,550	3.0	1,510	1.8	1.06	0.69	1.09	0.96
2		10	302	1.8	678	678	678	2,060	3.0	1,540	2.3	0.85	0.96	0.92	1.02
3	0	10	301	1.6	1,168	1,217	1,192	5,070	4.3	2,930	2.5	0.89	1.02	1.09	1.34
4		10	361	1.7	803	697	750	2,580	3.4	1,990	2.7	0.81	1.08	0.99	0.86
5		10	332	1.5	1,208	1,067	1,137	2,240	2.9	2,890	2.6	0.88	0.89	1.18	0.90
6		10	327	1.8	755	1,002	879	3,970	4.5	2,710	3.1	0.97	1.07	1.01	0.92
Avg		10	320	1.6					3.5		2.5	0.91	0.95	1.05	1.00
7		10	339	1.6	671	701	686	2,730	4.0	1,400	1.8	0.94	1.09	1.16	0.94
8		10	321	1.8	947	958	952	3,990	4.2	2,320	2.4	0.86	0.98	0.98	0.89
9	0.05%	10	310	1.7	612	608	610	3,300	5.4	1,390	2.3	0.93	1.04	1.17	0.85
10		10	326	1.7	518	591	554	3,010	5.4	1,180	2.1	0.93	0.83	1.23	0.95
11		10	318	1.8	1,288	1,146	1,217	3,890	3.2	2,780	2.3	0.89	1.03		0.91
12		10	314	1.9	987	1,064	1,025	3,580	3.5	1,742	1.7	0.91	0.98	1.44	1.44
Avg		10	321	1.8					4.3		2.1	0.92	0.99	1.20	0.95
13		10	346	1.8	580	685	632	5,800	9.2	1,340	2.1	1.06	1.24	1.16	1.19
14		10	330	1.8	421	383	402	3,240	8.1	830	2.1	0.91		1.01	0.93
15		10	338	1.8	591	612	602	7,670	12.7	1,230	2.1	0.85	0.89	1.07	0.75
16	0.5%	10	307	1.9	727	691	709	7,420	10.5	2,150	3.1	1.09	1.04	1.02	0.77
17		10	304	2.0	848	803	826	7,670	9.3	1,550	1.9	1.02	0.68	1.04	0.77
18		10	296	1.8	800	781	790	8,420	10.7	1,640	2.1	0.94	1.08	1.12	0.84
19		10	329	1.8	592	426	509	4,020	7.9	1,090	2.2	0.95	0.79	0.95	0.72
Avg		10	321	1.8					9.8		2.2	0.97	0.95	1.05	0.85

Feces were collected for days 9–13 after starting the special diets, i.e., 60–64 days after implantation with cholesterol-4-C^{14}. The blood cholesterol specific activities were determined at the beginning and end of the feces collection period.

TABLE 2 THE INFLUENCE OF DIETARY CHOLESTEROL-4-C^{14} ON THE EXCRETION OF BILE ACID BY THE RAT

Rat No.	Cholesterol-4-C^{14} in Diet	Average Daily Food Intake	Weight of Rat	Weight of Feces	Blood Cholesterol Specific Activity			Bile Acid Excretion	
					Beginning	End	Average		
		g	*g*	*g/day*	*cpm/mg*	*cpm/mg*	*cpm/mg*	*cpm/day*	*mg/day*
1		10	186	2.0	923	679	801	4,990	6.2
2		10	209	2.2	976	737	854	4,070	4.8
3		10	198	2.0	1,035	815	925	5,960	6.4
4	0.05%	10	203	1.8	766	755	760	5,270	7.0
5		10	191	1.9	796	783	790	5,200	6.6
6		10	177	2.0	766	742	755	4,220	5.6
7		10	158	2.2	666	672	669	6,330	9.4
Avg		10	189	2.0					6.6
8		10	187	2.2	1,449	1,464	1,456	33,060	22.7
9		10	183	2.0	1,577	1,507	1,542	30,930	20.0
10		10	210	2.0	1,465	1,509	1,487	32,930	22.1
11	0.5%	10	200	2.0	1,389	1,535	1,462	29,930	20.5
12		10	208	2.2	1,575	1,540	1,558	30,120	19.4
13		10	194	2.0	1,533	1,665	1,599	27,860	18.7
14		10	194	2.8	1,472	1,466	1,469	26,790	18.2
15		10	186	2.1	1,264	1,433	1,348	33,060	24.5
Avg		10	195	2.2					20.8

Feces were collected for days 34–38 after starting the diets; the blood cholesterol specific activities were determined at the beginning and end of the feces collection period. The specific activity of the dietary cholesterol-4-C^{14} was 1,842 cpm/mg.

however, the specific activities were much more constant. Bile acid excretion in this experiment averaged 6.6 mg/day in the 0.05% group and 20.8 mg/day in the 0.5% group. It is not clear why bile acid excretion was higher in these animals than in the previous experiment. The animals in this experiment were fed cholesterol for much longer periods of time than before, and it is possible that bile acid excretion may rise slightly in animals fed only 0.05% cholesterol for long periods of time.

Implantation Plus Feeding Experiments

In Table 3 are listed the results of measurements of sterol balance in animals implanted with capsules of cholesterol-4-C^{14} and fed varying quantities of cholesterol-7α-H^3. The weights and daily food intake in these animals are again similar. As in the previous experiment, bile acid excretion was uninfluenced by the feeding of 0.04% cholesterol (4.2 mg/day), but rose to 19.9 mg/day in the group fed 0.3% cholesterol. As before, the sterols excreted were calculated from the C^{14} content of the recovered neutral sterol fraction divided by the specific activity of the blood cholesterol. The neutral sterol excretion was comparable in the rats fed no cholesterol (1.7 mg/day) and 0.04% cholesterol (1.8 mg/day), and was slightly increased in the group fed 0.3% cholesterol (3.7 mg/day).

In these animals careful dietary and total fecal neutral sterol measurements were made so that it was possible to analyze several other aspects of cholesterol metabolism in the isotopic steady state. The net neutral sterol balance, defined as the intake minus the total fecal neutral sterols, was determined. At very small levels of cholesterol feeding (0.04%) the slight positive neutral sterol balance (+1.9 mg/day) was not accompanied by a significant change in either neutral sterol or bile acid excretion. However, in the group fed 0.3% cholesterol the average net neutral sterol balance (+14.0 mg) is almost exactly equal to the bile acid production minus the bile acid production in the group not fed cholesterol (14.4 mg). Therefore, in these animals, the change in net sterol balance after equilibrium has again been attained can be acounted for in toto by increased bile acid production.

It has been suggested by Chevallier (15) and by Danielsson (16) that cholesterol that does not equilibrate with the blood cholesterol is *secreted* into the lumen of the gastrointestinal tract, in addition to the blood cholesterol which is *excreted* across the intestinal wall. If cholesterol not in equilibrium with that of blood is secreted into the intestinal lumen, then in the isotopic steady state total fecal neutral sterol should be greater than the amount of sterol excreted as determined by formula 1. It is quite clear, however, that when no cholesterol is fed the average amount of neutral sterol excreted (1.7 mg/day) equals that of the total fecal sterols (1.6 mg/day) and consequently that no cholesterol, not in equilibrium with that in blood, is secreted into gut. However, it was possible that during cholesterol feeding secretion might occur. At any level of cholesterol feeding the amount of cholesterol secreted (not in equilibrium with blood cholesterol) can be estimated in the following manner:

Cholesterol secreted (mg/day) =

Total fecal neutral sterol − (dietary cholesterol not absorbed + sterol excreted) (2)

TABLE 3 BILE ACID AND NEUTRAL STEROL EXCRETION IN RATS IMPLANTED WITH CHOLESTEROL-4-C^{14} AND FED CHOLESTEROL-7α-H^3

Rat No.	Cholesterol-H^3 in Diet	Weight of Rat	Cholesterol Intake	Blood Cholesterol Specific Activity						Bile Acid Excretion		Neutral Sterol Excretion				
				C^{14}			$H^3 \times 10^{-3}$			C^{14}		C^{14}	$H^3 \times 10^{-5}$	Sterols Excreted	Total Neutral Fecal Sterols	Net Neutral Sterol Balance
				Beginning	End	Average	Beginning	End	Average							
		g	*mg/day*	*cpm/mg*	*cpm/mg*	*cpm/mg*	*cpm/mg*	*cpm/mg*	*cpm/mg*	*cpm/day*	*mg/day*	*cpm/day*	*cpm/day*	*mg/day*	*mg/day*	*mg/day*
1		169	0	5,083	5,051	5,067				17,280	3.4	8,360		1.7	1.8	−1.8
2		190	0	2,291	2,409	2,350				15,380	6.6	5,220		2.2	1.7	−1.7
3	0	193	0	2,796	2,935	2,866				14,460	5.1	5,530		1.9	1.6	−1.6
4		189	0	3,229	3,314	3,272				21,920	6.7	5,740		1.8	1.9	−1.9
5		169	0	3,151	3,083	3,117				7,240	2.3	3,970		1.3	1.0	−1.0
6		188	0	4,083	3,370	3,726				11,220	3.0	4,840		1.3	1.5	−1.5
Avg		183									4.5			1.7	1.6	−1.6
7		163	4.5	3,378	2,911	3,144	10.78	10.67	10.72	13,580	4.3	5,330	1.30	1.7	2.3	+2.2
8		160	4.5	2,677	2,827	2,752	10.22	11.24	10.73	8,920	3.3	3,830	0.95	1.4	1.5	+3.0
9	0.04%	183	4.5	3,762	3,265	3,514	8.57	9.42	9.00	19,020	5.4	8,710	1.44	2.5	3.9	+0.6
10		187	4.5	3,267	3,048	3,158	8.73	11.45	10.09	15,400	4.9	4,240	1.82	1.4	3.3	+1.2
11		170	4.5	3,604	3,480	3,542	9.29	11.73	10.51	10,760	3.1	6,150	1.06	1.8	2.0	+2.5
Avg		173	4.5								4 2			1.8	2.6	+1.9
12		141	30	1,082	1,375	1,228	45.94	67.94	56.94	24,400	19.9	5,210	17.79	4.2	17.4	+12.6
13		186	30	1,797	2,137	1,967	48.03	68.47	58.25	42,460	21.6	6,720	17.96	3.4	17.6	+12.4
14	0.3%	183	30	1,653	1,536	1,594	58.86	60.00	59.43	39,380	25.7	7,460	16.71	4.7	18.4	+11.6
15		170	30	1,681	1,420	1,550	64.64	63.79	64.22	28,560	18.5	4,470	13.07	2.9	12.5	+17.5
16		174	30	1,592	2,021	1,806	45.71	58.88	52.29	26,720	14.8	5,970	16.81	3.3	14.3	+15.7
Avg		171	30								19.9			3.7	16.0	+14.0

Feces were collected for days 13–14 after starting the special diets and days 40–41 after implantation with cholesterol-4-C^{14}. The blood cholesterol specific activites represent average values for samples drawn at the beginning and end of the feces collection periods. The specific activity of the dietary cholesterol-7α-H^3 was 102,957 cpm/mg.

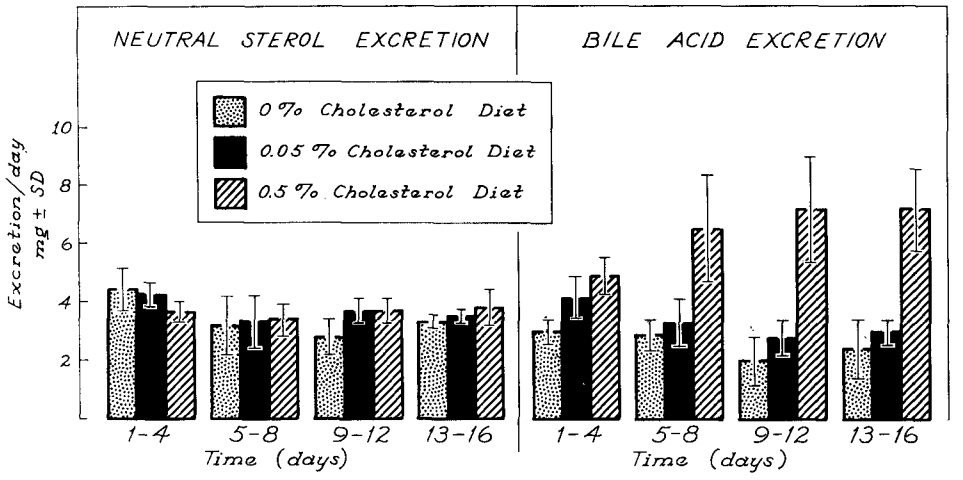

FIG. 3. The change in bile acid excretion with time following the feeding of diets which contained 0, 0.05, or 0.5% cholesterol. The rats had been implanted with capsules of cholesterol-4-C^{14} one month prior to the beginning of the study; each value represents the mean and standard deviation from five animals. The average weight of the rats in the study was 158 g.

where

$$\text{Dietary cholesterol not absorbed (mg/day)} = \frac{\text{H}^3 \text{ in fecal neutral sterols (cpm/day)} - \text{H}^3 \text{ excreted (cpm/day)}}{\text{Specific activity of dietary cholesterol-H}^3 \text{ (cpm/mg)}} \quad (3)$$

and

$$\text{Cpm H}^3 \text{ excreted per day} = \text{Mg cholesterol excreted} \times \text{specific activity of blood cholesterol-H}^3 \quad (4)$$

This expression allows for the correction of the portion of fecal sterol-H^3 which has been absorbed into the animal and reexcreted. When secreted cholesterol is calculated in this manner for the animals in Table 3, it is found to be negligible (-0.3 ± 0.5 mg/day in the group fed 0.04% cholesterol and -1.6 ± 1.4 mg/day in the group fed 0.3% cholesterol). Consequently, within the limits of the present methodology the dietary cholesterol not absorbed plus the excreted cholesterol is sufficient to account for all the fecal neutral sterols. These data do not imply that secretion of cholesterol by the intestinal wall does not occur but only that it may be quantitatively small in comparison with the other sources of fecal cholesterol.

Finally, in addition to the values for net neutral sterol balance which do not take into account the cholesterol excreted it is possible to determine the amount of dietary cholesterol absorbed per animal per day as follows:

$$\text{Cholesterol absorption (mg/day)} = \text{Cholesterol fed (mg/day)} - \text{cholesterol not absorbed (mg/day)} \quad (5)$$

Cholesterol absorption is sufficient to account for most of the cholesterol fed (3.2 out of 4.5 mg) at low levels of feeding, but only about half the dietary cholesterol fed at the higher level (14.6 out of 30 mg); thus, as would be expected, at low levels of cholesterol feeding the net sterol balance underestimates the amount of dietary cholesterol absorbed (1.9 mg/day rather than 3.2 mg/day), whereas at higher levels the net sterol balance (14.0 mg) approaches the value for absolute amount absorbed (14.6 mg).

Effect of Time of Feeding Cholesterol on Cholesterol Excretion and Degradation

In Fig. 3 is shown the change in bile acid excretion with time following the institution of diets containing 0, 0.05, and 0.5% cholesterol. The rats had been implanted with capsules of cholesterol-4-C^{14} one month prior to the beginning of the study. At no time during this 16-day study was any change noted in the neutral sterol excretion, regardless of the amount of dietary cholesterol fed. However, bile acid excretion in the animals fed 0.5% cholesterol rose slightly during the first 4 days of the study and had reached almost maximal levels within the second 4 day study period. Thus, the alterations that occur in the metabolism of cholesterol following the institution of cholesterol feeding appear to occur early and to persist thereafter.

DISCUSSION

A technique has been described for quantification in the intact rat of the two major pathways of cholesterol elimination, namely bile acid formation and the excretion of cholesterol and its neutral transformation products into the feces. This procedure involves the subcutaneous im-

plantation of capsules of crystalline cholesterol containing radioactive cholesterol; following the attainment of an isotopic steady state (as determined from the specific activity of the blood cholesterol), bile acid-C^{14} and neutral sterol-C^{14} are measured in the feces, and the absolute amounts determined as functions of the specific activity of blood cholesterol during the period of feces collection. The fact that the specific activity of the esterified and unesterified pools of cholesterol in the liver were essentially identical with those in the serum indicates that the specific activity of blood cholesterol under these conditions is a valid index of the specific activity of the miscible cholesterol pool.

Accuracy of the method is shown in rats fed diets containing no cholesterol, where the values obtained for neutral sterol excretion using this technique (1.7 mg/day) are virtually the same as obtained by direct measurement in the same animals of the fecal total neutral sterol (cholesterol plus coprostanol) by gas–liquid chromatographic techniques (1.6 mg/day). Furthermore, the values obtained for bile acid excretion in the absence of dietary cholesterol in these experiments (averaging 3.5 and 4.5 mg/day) are very similar to values which have been reported by Strand (10) in rats of comparable size utilizing the techniques of bile acid turnover (4.9 mg/day).

The application of this technique to the problem of quantifying bile acid production in rats fed cholesterol has also been explored. Previous attempts in this laboratory to evaluate the response of bile acid production to cholesterol feeding, utilizing both short-term isotope injections and chemical measurements (8, 17), have demonstrated that the net positive balance of cholesterol was far greater when large quantities of cholesterol were fed than could be accounted for by these indices of bile acid production. In the present experiments when balance techniques were combined with isotopic measurements in the steady state, it has been possible to demonstrate directly that bile acid excretion is sufficient to account for the entire positive balance of cholesterol, once equilibrium has again been attained. These results are in accord with the previous demonstration by Siperstein and Chaikoff that bile acid formation and neutral sterol production are virtually the only routes of excretion of cholesterol-4-C^{14} (2). Furthermore, the data demonstrate directly that the acceleration of bile acid production in the rat is the major compensatory response to cholesterol feeding; this response may explain the relative stability of rat blood cholesterol levels despite the absorption of large amounts of dietary cholesterol.

Two other aspects of cholesterol metabolism have been measured in these studies by utilizing the isotopic steady state in animals implanted with one isotope of cholesterol (C^{14}) and fed another (H^3). Since it is possible in these circumstances to correct the amount of neutral sterol appearing in the feces for the amount which has been absorbed and reexcreted, the amount of dietary cholesterol absorbed can be measured accurately; in these experiments, as would be expected, it has been demonstrated that the amount of cholesterol absorbed in animals fed small amounts of cholesterol is sufficient to account for a major fraction of the cholesterol fed (3.2 of 4.5 mg) and that the percentage absorbed decreases as the amount of dietary cholesterol rises (14.6 out of 30 mg).

In addition, utilizing this type of determination for the measurement of dietary cholesterol not absorbed together with the measurement of sterol excreted, it has been possible to show, at low and high levels of cholesterol intake, that these two sources of fecal sterol are sufficient to account for virtually all the cholesterol and coprostanol of the feces; i.e., in the isotopic steady state little endogenous cholesterol which is not in equilibrium with that of the blood reaches the feces. These results are in contrast to those of Chevallier, who reported evidence that cholesterol is both excreted through the intestinal wall from the blood and synthesized and secreted by the intestinal wall without achieving equilibrium with the cholesterol of blood (15). The most likely reason for the discrepancy between the two studies is that Chevallier's animals were injected daily with cholesterol-4-C^{14}, thus allowing wide fluctuations in blood cholesterol specific activities between injections. The present data indicate that the cholesterol synthesized within the intestinal wall does equilibrate with that of blood.

Finally, it should be pointed out that the present methodology does not allow one to define in the intact animal two important gross aspects of cholesterol metabolism. First, the calculated amount of dietary cholesterol absorbed may not be the same as the absolute amount of cholesterol absorbed. Since the magnitude of the enterohepatic circulation of cholesterol is unknown, it is possible that the figure derived for dietary cholesterol absorbed (although an accurate indication of net dietary cholesterol absorption) may reflect only a small fraction of absolute absorption. Second, it has not been possible to determine which fraction of excreted sterol is derived from the bile and which is derived from excretion across the intestinal wall. While it is well documented that fecal cholesterol arises from both sources (16), data from cannulated or other "prepared" animals cannot readily be applied to the intact animal.

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