Turnover of cholesterol-4-^{14}C and cholic acid-24-^{14}C by rabbits fed a diet containing lactose

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ABSTRACT Rabbits fed 0.35% of cholesterol in diets containing either 29.35% of lactose or sucrose were studied for 14 weeks. The rabbits fed lactose had higher plasma and liver cholesterol concentrations than those fed sucrose. The half-life of cholesterol was 19.0 days and 35.0 days for rabbits fed sucrose and lactose, respectively. The half-life, pool size, and daily production of deoxycholic acid were 9.7 days, 1.29 g, and 74.1 mg for rabbits fed sucrose; and 14.2 days, 1.40 g, and 49.1 mg, for those fed lactose. Cholesterol was the major neutral sterol in the feces of the rabbits fed lactose, whereas coprostanol (5β-cholestan-3β-ol) dominated the corresponding fraction in those fed sucrose.

The fecal steroid composition did not vary between day and night collections. No sterol esters were detected in the feces. Urinary elimination of radioactivity was less than 10% of that injected.

The "lactose effect" seems to be due to enhanced retention of steroids, the mechanism of which has not been elucidated.

KEY WORDS rabbit · diet · sucrose · lactose · turnover · cholesterol · deoxycholic acid · biosynthesis · fecal composition · intestinal microorganisms

RABBITS FED A DIET containing 0.35% of cholesterol with nearly 30% of lactose for 8 weeks had serum cholesterol and total hepatic cholesterol concentrations which were consistently higher than those fed the same diet with sucrose instead of lactose (1, 2). Portman (3) reported that rats fed a diet containing lactose had a cholic acid half-life of 6.5 days compared with 4.2 days for sucrose-fed controls. Rats fed lactose readily developed intestinal disorders, and therefore the rabbit was preferred as an experimental animal for studies of the effect of lactose on sterol metabolism (1). Until the advent of gas–liquid chromatographic separations of bile acids and sterols, analysis of these steroids in fecal material was difficult. The effects of dietary lactose on cholesterol and bile acid metabolism in the rabbit have been reinvestigated with the aid of recently developed methods of GLC (4, 5). The present study confirms the original findings (1, 2) and provides evidence in support of a mechanism of steroid retention to account for the "lactose effect."

METHODS

Male albino rabbits of the New Zealand strain (local supplier) weighing between 750 and 1100 g were used. The animals were housed in individual cages and furnished with diet and water ad lib. Feces were collected for 3-day periods on a plastic screen of fine mesh placed a few centimeters above stainless steel trays containing 95% ethanol. Urine was collected daily and pooled for analysis. When indicated, night feces were collected from 9:00 p.m. to 9:00 a.m., and day feces from 9:00 a.m. to 9:00 p.m. over a period of 3 days during which the animals were restrained from coprophagy by means of a harness.

Rabbits were divided into two groups and fed a diet containing sucrose or lactose (1). The sucrose diet consisted of soybean meal 45%, sucrose 29.35%, cellulose 5%, Wesson salt mixture 5%, cottonseed oil 13%, cod liver oil 2%, cholesterol 0.35%, choline chloride 0.2%, vitamin mix 0.1%, and a supplement of α-tocopheryl

Abbreviations: GLC, gas–liquid chromatography; TMS, trimethylsilyl.

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acetate. The experimental diet was identical, with the exception that it contained lactose instead of sucrose. The diet intake was recorded, and animals were weighed weekly. Feces were air-dried, weighed, and ground into a homogeneous powder.

Cholesterol-4-^{14}C with a specific activity of 115 μc/mg was obtained from New England Nuclear Corp., Boston, Mass. Samples of a 0.5 ml solution containing 5 μc of cholesterol-4-^{14}C dissolved in 20% Tween 80 (polyoxyethylene sorbitan monooleate) in water (v/v) were injected intraperitoneally into each of four rabbits from each of the two dietary groups, 8 weeks after initiation of the experiment. Cholic acid-24-^{14}C with a specific activity of 3.3 μc/mg was obtained from Tracerlab Inc., Waltham, Mass., and dissolved in 20% ethanol (10 μc/ml). Of this, 0.5 ml (5 μc) was injected intraperitoneally into each of four animals in each of the dietary groups 8 weeks after ingestion of their respective diets. These diets were continued throughout the succeeding 6 week period. The purity of the radioactive compounds was evaluated by thin-layer chromatography. Only single spots containing radioactivity and possessing R_f values identical with those of standards were found. Feces and urine were collected for each 24 hr interval after injection of the labeled steroids, for 1 week. Subsequently, collections of 48 hr duration were made weekly.

Extraction of Fecal Neutral Sterols and Bile Acids

Pooled samples of feces were weighed and extracted in a Soxhlet apparatus with acetone–absolute ethanol 1:1 (v/v) for 48 hr. The samples were extracted further with 0.1 N HCl in ethanol for 24 hr (6). An aliquot of the combined extracts was evaporated to dryness and the neutral sterols and bile acids were analyzed by GLC as reported previously (4, 5). For some samples the total lipid was separated into neutral and polar fractions by chromatography on brushite ($2CaO \cdot P_2O_5 \cdot 5H_2O$) columns according to the method of Wells, Makita, Wells, and Krutzsch (7). The neutral lipid was chromatographed on silicic acid (Unisil, Clarkson Chemical Company, Williamsport, Pa., 200–325 mesh) by the procedure of Horning, Williams, and Horning (8). The cholesterol esters were eluted from the column with benzene–Skellysolve B 1:4 (Skellysolve B is petroleum ether, bp 60–65°C, Skelly Oil Company, Kansas City, Missouri) and were methanolyzed. The resultant cholesterol was analyzed by GLC. Free cholesterol, eluted from the column with benzene, was also analyzed by GLC.

For measurement of the cholesterol radioactivity, an aliquot of the hydrolyzed ester fraction or the free cholesterol fraction was purified by precipitation with digitonin (9). The washed and dried digitonide was dissolved in methanol; radioactivity was determined by liquid scintillation counting (scintillator, 5 g of 2,5-diphenyloxazole and 300 mg of 1,4-bis-[2-(5-phenyloxazolyl)]-benzene in 1 liter of toluene). Corrections for quenching were made when necessary.

Analysis of Urinary Bile Acids

Aliquots (100 ml) of the pooled urine collections were dried by means of a flash evaporator in 250-ml round bottom flasks. The residue was autoclaved in the presence of 10 ml of 1.25 N NaOH at 15 psi for 3 hr. Bile acids were isolated from the hydrolysate exactly as described for their isolation from feces (5).

Gas–Liquid Chromatography

A modification of a previously described method (4, 5) was adopted for the analysis of sterols from feces or tissue. To each aliquot prepared for trimethylsilylation, a known amount (100–200 μg) of 5α-cholestane (mp 78–82°C, Mann Research Laboratories, New York, N. Y.) dissolved in chloroform was added as an internal standard. After removing the solvent, an accurate volume (200–300 μl) of trimethylsilylating reagent (pyridine–hexamethyldisilazane–trimethylchlorosilane 17:2:1)(10) was added to the tube. After 10 min, 1–2 μl of the reaction mixture was injected into the gas chromatograph (Barber Colman Model 10, equipped with hydrogen flame detector). A borosilicate glass column, 6 ft by 0.25 inch O.D., packed with Gas-Chrom P[1], 80–100 mesh, coated with 0.5% of Hi-Eff 8B (dimethylol cyclohexane succinate polyester)[1] by the solution-coating technique (11) was maintained at 245°C with an argon inlet pressure of 18 psi. The response of cholesterol and coprostanol trimethylsilyl (TMS) ethers over the range 0.1–5.0 μg was linear, and a constant detector response was observed for cholestane and the TMS ethers of cholesterol or coprostanol.

Liver and Plasma Cholesterol

Liver was weighed, and a sample (1 g) was homogenized in a mortar with 10 ml of water. The homogenate was extracted 3 times with 19 volumes of choloroform–methanol 2:1 (v/v). The combined extracts were washed according to Folch, Lees, and Sloane Stanley (12), and the chloroform was removed. The total lipid was weighed and analyzed immediately. The lipid was fractionated as described in an earlier section by means of brushite and silicic acid chromatography, and the cholesterol ester fraction was split by methanolysis. Free and esterified cholesterol were determined by GLC. Plasma was extracted with acetone–absolute ethanol 1:1 (v/v) as described by Sperry and Webb (9). Free and esterified cholesterol from plasma were determined exactly as described for liver cholesterol.

[1] Applied Science Laboratories, Inc., State College, Pa.

TABLE 1 EXCRETION OF FECAL STEROLS AND BILE ACIDS BY RABBITS FED DIETS CONTAINING SUCROSE (S) OR LACTOSE (L)

	4 Days		4 Weeks		9 Weeks		11 Weeks	
	S	L	S	L	S	L	S	L
				mg/24 hr				
Sterols								
Coprostanol	5.4	1.4	86.5	17.3	75.6	13.5	69.2	18.8
Cholesterol	11.1	7.3	34.9	48.8	58.0	23.5	49.9	27.3
Bile Acids								
Deoxycholic	12.9	2.5	29.5	8.2	14.7	10.0	19.4	9.2
Lithocholic	2.2	0.8	10.0	2.5	7.4	6.6	11.4	4.8
12-Ketolithocholic	4.7	1.7	21.5	3.7	9.5	5.0	6.9	5.3
Unknown*	—	—	8.1	1.9	—	—	—	—
Total steroids	36.3	13.7	190.5	82.4	165.2	58.6	156.8	65.4

* The TMS ether, methyl ester of the unknown bile acid has a retention time on GLC equal to that of 7-ketodeoxycholic acid (3α,12α-dihydroxy-7-keto-5β-cholanoic acid).

Determination of Specific Activity of Sterols and Bile Acids

For those animals injected with cholesterol-4-^{14}C, fecal neutral sterol and bile acid fractions were assayed for total radioactivity. The specific activities of cholesterol, coprostanol, deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid (3α-hydroxy-12-keto-5β-cholanic acid) were determined after collection of the corresponding TMS ethers, or TMS ethers of the methyl esters. Collections were made with the aid of an effluent splitter attached to the F & M Model 400 gas chromatograph. A column of SE-30 (methylpolysiloxy gum)[2], 3% on Gas-Chrom P, 80–100 mesh, and packed in a glass U-tube 6 ft × $^1/_8$ inch I.D., was employed. With a stream-splitting ratio of approximately 1:10, it was possible to obtain about 100 μg of pure steroid derivatives from a single injection without appreciably sacrificing resolution. The fractions were collected on the inside surface of Pasteur pipettes inserted into the collection part of the splitter. The pure derivative, collected in a single pipette from several injections, was washed into a small volumetric flask (1 ml) with toluene. The purity of the collected peak was assessed, and impure peaks were rechromatographed. When the peak was considered gas chromatographically pure, aliquots of the solution in toluene were directly assayed for radioactivity (by counting) and mass (by GLC) (4, 5), and the specific activity was determined.

Half-Life, Pool Size, and Daily Production of Cholesterol and Deoxycholic Acid

The specific activities of cholesterol, coprostanol, deoxycholic acid, 12-ketolithocholic acid, and lithocholic acid were determined from feces collected at various times up to 35 days after the administration of labeled cholesterol or cholic acid. The specific activities of cholesterol and deoxycholic acid were plotted against time on semi-logarithmic graph paper. From this we determined the half-life of cholesterol, and the half-life, pool size, and daily production of deoxycholic acid by the method of Lindstedt (13).

RESULTS

At the end of the experiment (14 weeks) each rabbit that had been fed sucrose had consumed an average of 57.0 g of diet per day and weighed 2680 g. In comparison, each rabbit fed lactose ingested an average of 53.5 g of diet per day and weighed 2498 g.

Excretion of Neutral Sterol and Bile Acids in Feces

The quantities of neutral sterols, and of the major fecal bile acids, excreted at various periods after initiation of the dietary regimen are given in Table 1. Although plant sterols were present, they were not analyzed. They do not interfere with the determination of cholesterol and coprostanol by GLC. Lactose in the diet inhibited the formation of coprostanol, in agreement with the findings of others (14–16). Three bile acids (deoxycholic, lithocholic, and 12-ketolithocholic) accounted for most of the bile acid fraction (Fig. 1: peaks 1, 3, and 5, respectively). Small quantities of epilithocholic acid (3β-hydroxy-5β-cholanic acid) were present in most samples (Fig. 1, peak 2); after 4 weeks of sucrose feeding, there appeared in the feces a steroid (Fig. 1, peak 4) (8.1 mg/rabbit per day) corresponding in retention time to 7-ketodeoxycholic acid (3α,12α-dihydroxy-7-keto-5β-cholanoic acid). Smaller amounts of this bile acid were found in the feces of the rabbits fed lactose (1.9 mg/rabbit per day).

The effect of lactose in the diet on the excretion of sterols and bile acids was apparent even after 4 days. The amounts of bile acid excreted daily by rabbits fed sucrose and lactose 4 weeks after the start of the experiment were 69.1 and 16.3 mg, respectively. Although the excretion of total bile acids by rabbits fed lactose was approximately one-fourth that excreted by the group

[2] General Electric Company, Silicone Division, Waterford, N. Y.

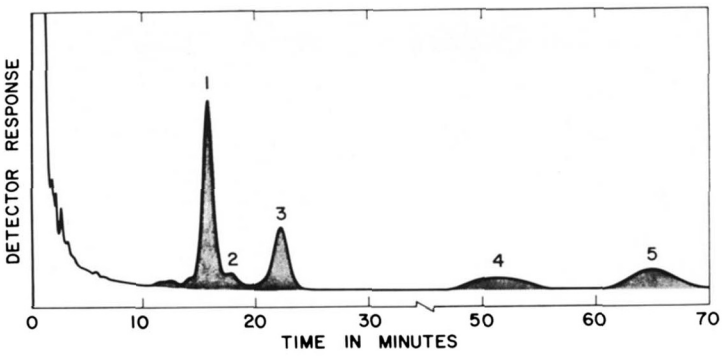

FIG. 1. Gas chromatogram on Hi-Eff 8B of the trimethylsilyl ethers of the methylated bile acid fraction from feces of rabbits fed the sucrose (control) diet. The peaks were tentatively identified as corresponding to: *1*, deoxycholic acid; *2*, epilithocholic acid; *3*, lithocholic acid; *4*, 7-ketodeoxycholic acid; *5*, 12-ketolithocholic acid.

fed sucrose, no major qualitative difference was observed.

An estimate of sterol balance based on dietary intake and fecal excretion at various periods is shown in Table 2. A significant positive balance was observed for both groups of animals 4 days after initial feeding. After 4 weeks and during succeeding periods, however, the animals fed sucrose retained much less sterol than those fed lactose.

Day Versus Night Feces

Animals restrained to prevent coprophagy excreted smaller quantities of feces than when not restrained, and the excretion of steroids was correspondingly low; compare Table 3 with Table 2. The compositions of neutral sterols and bile acids in the night and day feces from each group of animals were similar (Table 3). For the animals fed lactose, the sterol fraction of the night feces consisted of 77% of cholesterol, whereas that of the group fed sucrose contained 63% of coprostanol.

TABLE 2 ESTIMATION OF TOTAL STEROL BALANCE IN RABBITS AT VARIOUS PERIODS DURING THE INGESTION OF DIETS CONTAINING SUCROSE (S) OR LACTOSE (L)

Time after Starting Diet	Diet	Cholesterol Intake (Diet)*	Fecal Excretion		Balance
			Sterols	Bile Acids	
		mg/day			
4 Days	S	129.0	16.5	19.8	+92.7
	L	129.9	8.7	5.0	+115.3
4 Weeks	S	193.5	121.4	69.1	+3.0
	L	175.5	66.1	16.3	+93.1
9 Weeks	S	190.0	133.6	31.6	+24.8
	L	175.0	37.0	21.6	+116.4
11 Weeks	S	198.0	119.1	46.1	+41.2
	L	197.0	37.7	19.3	+131.6

* Based on dietary intake of diets containing 0.35% of cholesterol.

Esterification of Fecal Neutral Sterols

In the present study, essentially all the neutral sterols in the feces were unesterified. The average value amounted to 98% of free sterol.

Urinary Excretion of Neutral Sterols and Bile Acids

The quantity of cholesterol excreted in the urine of all groups was less than 0.3 mg/rabbit per 24 hr. Furthermore, no recognizable bile acids were present. However, several peaks of possible unknown bile acids were observed in quantities no larger than 1.0 mg/rabbit per day. After injection of cholic acid-24-^{14}C, 2.4–3.2% of the injected radioactivity was associated with the bile acid fractions of the urine, whereas 4.0–6.5% was found in the aqueous fraction.

Turnover of Injected Cholesterol-4-^{14}C

The specific activity of fecal cholesterol-4-^{14}C plotted against time (days) on semilogarithmic graph paper

TABLE 3 ANALYSIS OF NEUTRAL STEROLS AND BILE ACIDS FROM DAY AND NIGHT FECES OF RABBITS FED DIETS CONTAINING SUCROSE OR LACTOSE FOR 4 WEEKS

	Sucrose		Lactose	
	Day	Night	Day	Night
Weight of feces, *g/12 hr*	3.7	3.4	3.4	2.7
		mg/12 hr		
Sterols				
Coprostanol	19.4	16.7	6.8	4.9
Cholesterol	9.9	9.8	20.2	16.4
Total	29.3	26.5	27.0	21.3
Bile Acids				
Deoxycholic	3.5	4.4	2.6	1.4
Lithocholic	1.6	2.5	0.9	0.4
12-Ketolithocholic	2.7	3.4	0.8	—
Unknown*	1.4	0.5	1.5	0.2
Total	9.2	10.8	5.8	2.0

* See footnote to Table 1; Fig. 1, Peak 4.

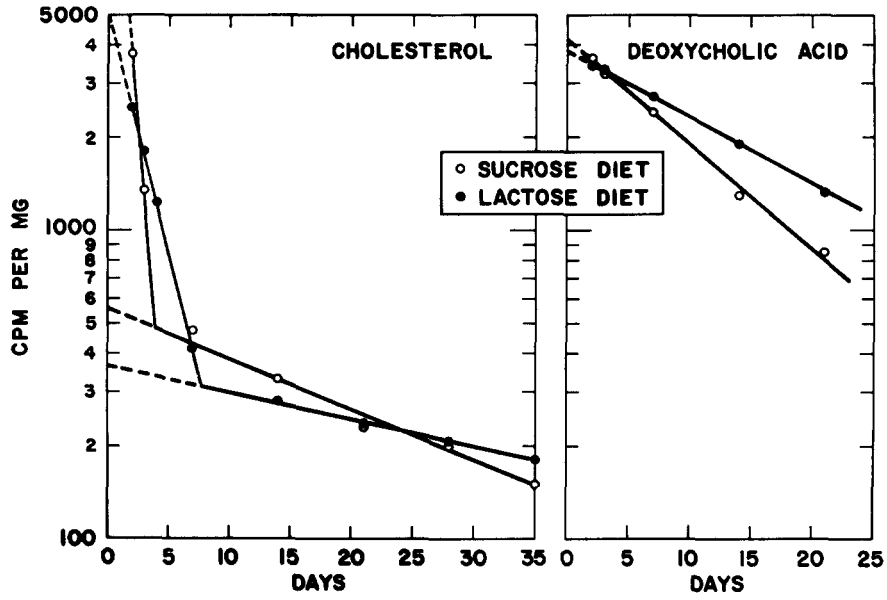

FIG. 2. Semilogarithmic plot of the specific activities of cholesterol and deoxycholic acid from rabbits fed diets containing sucrose (O—O) and lactose (●—●).

gave biphasic curves for rabbits fed both sucrose and lactose (Fig. 2). The first phase of the curve was probably the result of incomplete equilibration of the endogenous cholesterol with the administered isotopic cholesterol. The half-life of cholesterol, calculated from the slope of the second phase of each plot, was 19.0 and 35.0 days for rabbits fed sucrose and lactose, respectively. Similarly, a plot for coprostanol was biphasic, and the half-life values from the second phase closely agreed with those of cholesterol, namely 21.0 and 39.5 days for rabbits fed sucrose and lactose.

Turnover of Injected Cholic Acid-24-^{14}C

Plots of the specific activity of deoxycholic acid against time on semilogarithmic graph paper were linear (Fig. 2). The values for the half-life of deoxycholic acid based on the collected derivatives were 8.7 and 14.2 days for rabbits fed sucrose and lactose, respectively. A similar amount of radioactivity per milligram was associated with 12-ketohthocholic acid from both dietary groups. As expected, only trace amounts of radioactivity were found in the lithocholic acid fractions, since tlihocholic acid in the rabbit originates from chenodeoxycholic acid (17). Pool sizes and daily production of deoxycholic acid given in Table 4 are calculated on the assumption that this acid mixed completely with the endogenous pool.

Plasma and Liver Distribution of Cholesterol-4-^{14}C

The quantities and specific activities of free and esterified cholesterol in plasma and liver 5 weeks after administration of cholesterol-4-^{14}C are summarized in Table 5. In the plasma, the cholesterol concentration was higher in the animals fed lactose than in those fed sucrose, primarily as a result of differences in the ester fraction. The specific activity of the free cholesterol was greater than that in the cholesterol ester fraction in both dietary groups. The ratios of the specific activity of free to esterified cholesterol were 1.8 and 6.0 for the groups fed sucrose and lactose, respectively, and the total radioactivity was likewise higher in the free than in the ester fraction. Rabbits fed lactose had significantly higher liver cholesterol concentrations than those fed sucrose (Table 5). As observed for plasma, the ratio of the specific activity of free to esterified cholesterol in the liver (Table 5, last column) indicated incomplete equilibration of these pools. The total radioactivity in the liver free cholesterol fraction was less than that in the ester fraction for either dietary group. Although the specific activities of the free cholesterol in the plasma and liver of the rabbits fed sucrose were in good agreement (407 ± 241 cpm/mg and 432 ± 186 cpm/mg), the corresponding values of animals fed lactose were not similar (plasma, 734 ± 261 and liver, 264 ± 16 cpm/mg). The specific activities of the cholesterol ester fractions of the

TABLE 4 TURNOVER OF ^{14}C AFTER INJECTION OF CHOLIC ACID-24-^{14}C INTO RABBITS FED DIETS CONTAINING SUCROSE OR LACTOSE

Deoxycholic Acid	Half-Life	Pool Size	Daily Production
	days	*g*	*mg*
Sucrose	8.7	1.290	74.1
Lactose	14.2	1.405	49.1

TABLE 5 DISTRIBUTION OF ^{14}C IN PLASMA AND LIVER CHOLESTEROL 5 WEEKS AFTER ADMINISTRATION OF CHOLESTEROL-4-^{14}C TO RABBITS FED DIETS CONTAINING SUCROSE (S) OR LACTOSE (L)

Tissue	Diet	Free		Ester		Specific Activity Free/Ester
		Concentration	Specific Activity	Concentration	Specific Activity	
		mg/ml	*cpm/mg*	*mg/ml*	*cpm/mg*	
Plasma	S	5.1 ± 1.7*	407 ± 241*	5.4 ± 1.1*	259 ± 202*	1.81
	L	5.5 ± 1.6	734 ± 261	11.3 ± 1.8	123 ± 41	5.96
		mg/g wet wt	*cpm/mg*	*mg/g wet wt*	*cpm/mg*	
Liver	S	3.9 ± 0.9	432 ± 186	18.5 ± 12.2	146 ± 98	2.96
	L	8.0 ± 3.2	264 ± 16	41.5 ± 16.8	163 ± 38	1.62

* SD (n = 4).

plasma and liver were in close agreement in both experimental groups.

DISCUSSION

Cholic acid-24-^{14}C was selected for the study of the turnover of deoxycholic acid on the basis of the report of Hellström, Sjövall, and Wigand (18) that cholic acid-^{14}C and deoxycholic acid-^{14}C gave the same results, presumably because of the rapid conversion of the former to the latter. Lithocholic acid collected by GLC in the present study after administration of cholic acid-24-^{14}C contained only traces of radioactivity, which confirms the established observation that lithocholic acid is derived only from chenodeoxycholic acid (17). Lithocholic acid would be expected to be radioactive after injection of cholesterol-^{14}C, but we did not test this.

In rabbits, as in rats (3, 19), lactose feeding resulted in a larger deoxycholate pool than did sucrose feeding (Table 4). For both groups of animals, the daily production of deoxycholic acid calculated from the pool size and half-life was higher than the amount of deoxycholic and 12-ketolithocholic acids found in the feces. Although the calculation of daily production depends on the assumption that the isotope administered is completely mixed with the pool, a discrepancy in the results of the present study may have been partly affected by a significant increase of both cholesterol and deoxycholic acid pools as the animals grew. In rabbits fed lactose there was a diminished synthesis of deoxycholate (longer half-life) in agreement with a decrease of steroid excretion as compared to those fed sucrose.

The biphasic nature of cholesterol-4-^{14}C turnover (Fig. 2) suggested the presence of at least two metabolic pools. These results might be explained by incomplete equilibration of cholesterol between the free and ester fractions. The administered cholesterol may have been excreted at a more rapid rate before complete distribution throughout the animal occurred. As expected, coprostanol, which is derived by reduction of free cholesterol by intestinal microorganisms, had turnover rates similar to those of cholesterol. There have been few studies of total body cholesterol turnover in the rabbit with which our results can be directly compared. Popják and Beeckmans (20) reported the cholesterol half-life in rabbit liver and intestine to be 3.0 and 1.5 days, respectively.

Although the present study revealed the existence of a larger bile acid pool and slower turnover of both neutral sterols and bile acids in the rabbits fed lactose, the exact mechanism for this effect remains obscure. Rats with lymph duct cannulae were shown previously to absorb greater amounts of radioactive cholesterol when fed lactose than when fed sucrose (19). In the present study, animals fed lactose demonstrated a greater positive balance of cholesterol than the corresponding rabbits fed sucrose. The amount of night feces excreted by both dietary groups was similar, but was probably not equivalent to the quantity of night feces ingested during the unrestrained periods, because the confinement resulted in anorexia and occasional diarrhea. The anal–oral circulation, which is well recognized in rabbits (21, 22), may be important in this study since rabbits excreting predominantly cholesterol (lactose-fed) receive more absorbable sterol per cycle than those excreting chiefly coprostanol (sucrose-fed). [Coprostanol seems not to be absorbed to any significant extent (23) despite some reports to the contrary (24, 25) in which absorption was calculated from the differenence between orally administered coprostanol and that determined in the feces. However, corrections were not made for the fact that coprostanol fails to form a completely insoluble digitonide under the conditions of assay (26). Although minute amounts of coprostanol may be absorbed, to our knowledge no detectable amounts of coprostanol have been found in the blood or tissues of animals practicing coprophagy.] The occurrence of sterol esters in the rabbit feces [sterol esters have been reported in human feces (27)] during this study was negligible. This rules out the possibility of an additional source of unabsorbable sterol in a coprophagic animal. Intestinal tract motility, another possible factor contributing to the "lactose effect," has not been ruled out. In rats fed lactose, the movement of

material along the intestinal tract was reported by Fischer and Sutton (28) to be faster than in those fed sucrose.

Another attractive explanation for the "lactose effect" is that of sterol and bile acid adsorption to microorganisms (29). The amount of bile acids and sterols excreted in the feces would be a function of the number and type of microorganisms in the intestinal tract. Smith (30) has studied the intestinal flora of the rabbit on normal and milk powder diets. Rabbits had relatively few types and numbers of intestinal microorganisms: the dominant ones were bacteroides, with some lactobacilli and streptococci. Dietary milk powder was reported to increase the population of lactobacilli greatly. Through the courtesy of Dr. A. I. Braude, Department of Medicine, University of Pittsburgh, feces from both day and night collections were examined for identity and approximate quantification of bacterial flora. In all animals the predominant bacteria were anaerobes. No marked differences were noted between day and night feces, and the only variation between dietary groups was a slightly heavier population of lactobacilli in the feces of animals fed sucrose. In this study, the major organisms were bacteroides, lactobacilli, and occasionally micrococci. No coliform and only slight amounts of aerobic bacteria were observed in the feces. Thus no satisfactory explanation can be proposed for the retention of steroids in rabbits fed lactose at the present time.

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