Biosynthetic Origin of Serum Cholesterol in the Squirrel Monkey: Evidence for a Contribution by the Intestinal Wall

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ABSTRACT The possibility that the intestinal wall serves as a biosynthetic site for serum cholesterol has been examined in two types of studies in the squirrel monkey. First, animals were fed cholesterol in order to inhibit cholesterol synthesis in the liver, and the intestinal lymph ducts were cannulated. After the administration of acetate-2-^{14}C it was possible to demonstrate that cholesterol synthesized by the intestinal wall enters intestinal lymph and thereby in the intact animal enters the circulating pool. Second, an attempt to quantitate the significance of this intestinal contribution has been made in animals fed cholesterol-3-^{3}H and injected with cholesterol-4-^{14}C for long periods. By an application of the technique of analysis utilizing the isotopic steady state we estimated as a minimal value that in the squirrel monkey 1.5–2.0 mg of cholesterol synthesized in the intestinal wall reaches the circulation each day.

INTRODUCTION

Although cholesterol is synthesized in the adult by virtually all tissues with the exception of the central nervous system (3, 4), it has generally been assumed that serum cholesterol is synthesized predominantly by the liver or derived from the diet (5). Recent experiments in several laboratories, however, have demonstrated that a variable portion of circulating cholesterol is synthesized in the rat, dog, monkey, and man in tissues other than the liver (6–10); this evidence has been obtained from studies in which diets rich in radioactive cholesterol have been fed for long periods until the specific activity of serum cholesterol approaches a plateau or steady state. It is well known that the feeding of dietary cholesterol almost completely inhibits the synthesis of cholesterol by tissue slices of liver in these species (11–15). If liver were the only biosynthetic source for circulating cholesterol, then in the steady state the specific activity of serum cholesterol should equal that of the diet when hepatic synthesis is suppressed. In the dog and rat the specific activity of serum cholesterol approximates 85–90% of that of the diet in the steady state, and hence only about 10–15% of the circulating pool is synthesized within the body when hepatic synthesis is suppressed (6, 7). In man, on the other hand, the specific activity of serum cholesterol never exceeds 40% of that of the fed cholesterol (8, 9). This phenomenon could be the result of an incomplete or absent hepatic cholesterol negative feedback in man, or it could be due to the fact that the extrahepatic tissue(s) of man are quantitatively more important as biosynthetic sites for circulating cholesterol than is the case in lower animals. Since the feeding of a high cholesterol diet has been reported by Bhattathiry and Siperstein (14) to suppress effectively hepatic

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cholesterologenesis in man and since this feedback relationship has been confirmed both by in vivo and in vitro studies in human subjects by Fujiwara and coworkers (16), it is reasonable to conclude that some tissue(s) other than liver must synthesize 60% of the circulating cholesterol in man under these conditions of high dietary cholesterol.

Recent studies in this laboratory have demonstrated that the intestinal wall, which does not possess a cholesterol negative feedback system, serves as a significant biosynthetic source of the circulating cholesterol in the rat (17). However, the biosynthetic site in man of the much larger endogenous contribution to the circulating cholesterol when hepatic synthesis is inhibited has not yet been identified.

Since a dissection of the biosynthetic sources of circulating cholesterol in man is not practical, use has been made of the squirrel monkey, a primate which demonstrates an effective hepatic negative feedback system when cholesterol is fed (18). In the experiments to be described in the present report, evidence has been obtained that the quantitative relation between endogenous and exogenous sources of serum cholesterol in this species resembles that of man when diets high in cholesterol are fed. Furthermore, it has been possible to demonstrate that cholesterol synthesized in the intestine does contribute to the circulating pool and that little endogenously synthesized cholesterol reaches the serum of the cholesterol-fed monkey when the intestinal contribution is diverted from the circulation. Finally, in animals fed cholesterol-3-^{3}H and injected with cholesterol-4-^{14}C the quantitative importance of the intestine as a biosynthetic source for circulating cholesterol has been estimated; under circumstances of both low and high cholesterol intake the minimal value for this contribution is of the order of magnitude of 2 mg/day. It is concluded, therefore, that the intestine is a biosynthetic site of serum cholesterol in this species and may represent a significant source of endogenous cholesterol in the cholesterol-fed animal.

METHODS

Male and female *Samiris* monkeys of a variety of strains, weighing 390–780 g, were used in these experiments. The animals were quarantined for at least 1 wk before the beginning of the experiments, during which time they were allowed free access to Purina monkey chow and drinking water containing tetracycline (100 mg/liter). Intestinal lymph duct cannulations were performed in the animals as a part of two different types of experiments—analyses of the appearance of cholesterol-^{14}C in tissues and body fluids after the single dose administration of acetate-2-^{14}C and studies of the specific activity of tissue and body fluid cholesterol in animals fed and injected with radioactive cholesterol for long periods.

Intestinal lymph duct cannulation. The anatomy of the lymphatic drainage of the intestine of the new-world monkey has been described in some detail by Silvester (19). In brief, a central lymphatic duct empties directly into the inferior vena cava just above the entry of the left renal vein; usually, 1–3 ancillary channels empty into the main duct just before its entry into the vena cava. Occasionally, the ancillary duct(s) independently anastomose into the vena cava, and, rarely, a separate channel can be demonstrated to enter into the left renal vein. Cannulation of the central intestinal lymphatic channel was performed as described by Bollman, Cain, and Grindlay (20) under ether anesthesia; when visible, ancillary lymphatic channels which did not empty into the main duct were ligated. At the time of operation cannulae were also placed in the femoral artery and vein and in some experiments in both the proximal and distal portions of the bile duct. The animals were then placed in Rothman restraining devices and allowed to recover. During the procedure 5% glucose in isotonic saline (0.85%) or Ringer's lactate (20 ml) was administered intravenously; subsequently, 5 ml of the glucose-saline solution was given at 8-hr intervals, and the animals were allowed free access to water. Most animals tolerated the operation and subsequent restraint for the 36–48 hr duration of these experiments; whereas in the early experiments animals frequently died, this could almost invariably be prevented by giving adequate intravenous fluids.

Acetate-2-^{14}C studies. In preparation for these studies animals were fed one of two diets for 3–6 wk. The low cholesterol diet consisted of Purina monkey chow supplemented with 7% triolein; the diet was shown by direct analysis to have a cholesterol content of less than 40 μg/g (corresponding to a cholesterol intake of 0.8–1.4 mg/day in these studies). The high cholesterol diet consisted of Purina monkey chow supplemented with 7 g of triolein and 1 g of cholesterol/100 g of diet (corresponding to a cholesterol intake of 200–500 mg/day). The animals were housed in individual cages, and a record of food consumption for each day was kept. Only animals whose food intake was between 20–50 g/day were subjected to operation.

After lymph duct cannulation the monkeys were allowed to recover for 5–20 hr. 100 μc of sodium acetate-2-^{14}C (New England Nuclear Corp., Boston, Mass. specific activity 1 mc/41 mg) was dissolved in isotonic saline and injected intravenously. Total lymph and intermittent arterial blood samples (2 ml) were collected at intervals for periods of time up to 2 days. In order to enhance the flow of lymph during these studies we administered 5 ml of whole, beaten egg every 8 hr by

stomach tube. In two experiments 15 mM sodium taurocholate was infused into the distal portion of the common bile duct at a rate of 1 ml/hr via a Braun infusion pump, and excreted bile was collected from the proximal bile duct cannula, along with the lymph and blood samples. At the end of the experiments the lymph, serum, bile, and carcass were analyzed for cholesterol-^{14}C and cholesterol content.

Cholesterol-4-^{14}C and cholesterol-3-^{3}H studies. Two formula diets were utilized in these studies. For the experiments in which the cholesterol source was egg yolk, eggs were obtained from laying hens injected with cholesterol-4-^{14}C and mixed in one large batch with fresh eggs as previously described (9); the final cholesterol specific activity was 640 cpm/mg. The formula for this diet consisted of egg mixture, 600 g; sucrose, 45 g; gelatin, 120 g; Hawk-Oser Salt Mix (Nutritional Biochemicals Corporation, Cleveland, Ohio), 4 g; ferrous sulfate, 1 g; and added vitamins. Each animal was given 30 ml of this diet by stomach tube twice daily. Each day's intake contained 120 kcal, (6 g of fat, 8 g of carbohydrate, and 9 g of protein), and 249 mg cholesterol. Because in some animals the egg diet caused a mild but persistent diarrhea, a milk formula was utilized for the later studies. This diet contained Lactum powder (Mead Johnson & Co., Evansville, Ind.), 300 g; water, 300 ml; added vitamins; and ferrous sulfate, 100 mg, in which was homogenized varying amounts of cholesterol-3-^{3}H (New England Nuclear Corp.). Animals were given 20 ml of this mixture twice a day (97 kcal, 4 g of fat, 4 g of protein, and 11 g of carbohydrate/day); in this study 50 μc of cholesterol-4-^{14}C (New England Nuclear Corp.) dissolved with the aid of Tween 40 (polyoxethylene sorbitan monopalmitate) (21) was given to each animal intravenously at the start of the experiment.

After the initiation of these tube feeding studies, 2 ml of blood was drawn from the femoral vein of unanesthetized animals at intervals for periods up to 8 wk, and the serum was separated and stored for subsequent cholesterol analysis. At the end of the experimental period, cannulations of the intestinal lymph duct were performed 2–5 hr after the morning feeding, and lymph was collected for 2-hr intervals.

Cholesterol analysis. Serum, lymph, and bile cholesterol specific activity and content were measured as follows: 0.5 ml aliquots of serum and the entire lymph and bile samples were saponified with 0.5 ml of 10 N KOH in an autoclave for 30 min at 15 lb. pressure. An equal volume of ethanol was added, and the solution was brought to boil on a steam bath. Neutral sterols were then extracted two times with 10 volumes of petroleum ether, and the extracts were taken to dryness. After dissolving the residue in acetone-alcohol (1:1) cholesterol digitonides were then formed, washed, and dried by the method of Sperry and Webb (22). The cholesterol digitonides were dissolved in 4.4 ml of methanol; one ml aliquots were added to 0.4% diphenyloxazole in toluene and assayed for radioactivity in a Packard liquid scintillation spectrometer, and one ml aliquots were assayed for cholesterol content (22). Each determination was performed in duplicate. Samples containing both ^{14}C and ^{3}H were analyzed in a dual channel spectrophotometer under circumstances in which $< 0.3\%$ of the ^{3}H counts observed in the ^{3}H channel were observed in the ^{14}C channel.

For the diet analyses, weighed aliquots of the various diets were homogenized in an excess of chloroform-methanol (2:1) which was subsequently brought to a boil on a steam bath and filtered. Aliquots of the filtrate were saponified and analyzed for cholesterol radioactivity and content as described above. The petroleum ether extracts were also analyzed by gas-liquid chromatography on a 1% neopentyl glycol succinate column (230° C, with an argon flow of 100 ml/min); in the diets containing monkey chow, a variety of plant sterols were shown to be present (estimated at 100 μg/g). Only cholesterol was demonstrated in the case of the egg and milk formulas; consequently, it was concluded that the analysis of specific activity of the digitonides did in fact represent the specific activity of the fed cholesterol in the case of the liquid diets.

In some experiments the animals were dissected, and tissue cholesterol-^{14}C was determined. The liver, washed intestinal wall (stomach, small intestine, and colon), intestinal contents plus all feces excreted during the lymph collection, gall bladder bile plus any bile collected during the experiment, pooled viscera (heart, lung, kidneys, spleen, adrenals, ovaries or testes, and pancreas), and the residual entire carcass (skeleton, muscles, adipose tissue, brain, skin, etc.) were saponified separately in ethanolic KOH (0.5 g of KOH/g of tissue was added to 100% EtOH) on a steam bath. Measured aliquots of the mixture were analyzed for cholesterol-^{14}C and content as described above.

In order to characterize the sterols present in this species, we performed two types of experiments. First, the neutral sterol fractions from 21 different tissues were analyzed by gas-liquid chromatography with 6 ft columns containing 3% QF-1 (methyl fluoroalkyl silicone) on Gas-Chrom Q at a column temperature of 230°C and an argon flow of 100 ml/minute (23). Under these conditions a peak corresponding to cholesterol was the only identifiable sterol peak in all tissues except for skin and hair, in which a second peak was present, corresponding to 0.73 and 0.14 out of 3.25 and 2.44 mg of total sterol/g of tissue; the second peak in these tissues had the chromatographic properties both in this system and on thin-layer chromatography (24) of Δ^{7}-cholestenol. Second, free sterols were regenerated by the method of Sperry (25) from the digitonide precipitates of serum and lymph obtained from a monkey 3 hr after the administration of acetate-2-^{14}C. This free sterol-^{14}C was then analyzed by thin-layer chromatography in two systems. After chromatography on 20 × 40 cm plates of silica gel H with benzene–ethyl acetate (5:1), a system which gives good separation of four major groups of sterols (cholesterol plus cholestanol, lanosterol, Δ^{7}-cholestenol, and Δ^{7}- plus Δ^{8}-methostenols), 77 and 76% of the radioactivity was recoverable in the cholesterol-cholestanol area; the remainder of the radioactivity was present solely in the Δ^{7}-cholestenol area (24). Aliquots of the free sterol-^{14}C

were also chromatographed on 20 × 20 cm plates of silica gel H containing 12% silver nitrate in benzene-hexane (15:85) for 36 hr, a system which separates cholestanol from unsaturated sterols (24); under these circumstances 96 and 97% of the radioactivity from serum and lymph was recovered in the cholesterol area. It was concluded from these studies that cholesterol is the major sterol of serum and lymph and that cholesterol-^{14}C constitutes the principal radioactive sterol in serum and lymph after the administration of acetate-2-^{14}C to this monkey.

RESULTS

The possible significance of the intestinal wall as a source of circulating cholesterol in the squirrel monkey was first examined in the type of experiments shown in Fig. 1. Female squirrel monkeys were fed for 21–42 days diets either low in cholesterol content or containing 1% cholesterol, a concentration known to suppress hepatic synthesis in this species (18). Intestinal lymph duct cannulations were performed, and the animals were placed in restraining devices. Acetate-2-^{14}C was injected intravenously, and lymph and blood samples were collected intermittently for 24–37 hr and subsequently analyzed for cholesterol content and ^{14}C. This type of experiment permits the isolation of any contribution of the intestinal wall to the circulating cholesterol, since, as has been demonstrated by Swell and his associates, the intestinal lymphatic drainage serves as the sole channel for cholesterol egress from the intestine into the circulation (26). The results of four such experiments are illustrated in Fig. 1. Cholesterol specific activity is plotted on the vertical axis, time on the horizontal axis. In the animals fed a high cholesterol diet, and as a consequence whose hepatic synthesis is suppressed, almost no radioactive cholesterol appeared in the blood stream after acetate-^{14}C administration. A marked rise in the specific activity of lymphatic cholesterol occurred, however, reaching a peak after 15–20 hr. The situation is strikingly different in the animals fed a low cholesterol diet and whose hepatic synthesis was unimpaired (the right panels). The specific activity of both serum and lymph cholesterol rose promptly and achieved equilibrium after a few hours. At all time intervals studied in the animals fed the high cholesterol diet the specific activity of lymph cholesterol is greater than that of the serum, excluding the possibility that the lymphatic cholesterol is derived from blood.

That the lymphatic cholesterol of the cholesterol-fed animal could not have been derived from the bile is shown in Fig. 2. In this experiment, in addition to the serial sampling of lymph and blood, cannulae were placed in both the distal and proximal ends of the bile duct, and sodium taurocholate was infused into the distal portion of the duct in order to keep the enterohepatic circulation intact while bile was collected from the proximal end. The change with time in the specific activity of cho-

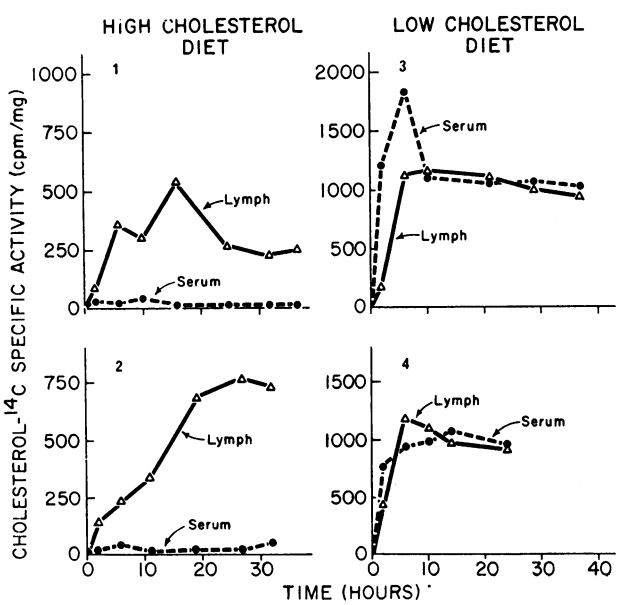

FIGURE 1 Influence of dietary cholesterol on the appearance of cholesterol-^{14}C in lymph and blood after the injection of acetate-2-^{14}C. Female squirrel monkeys (470–780 g) were fed chow diets either low or high in cholesterol content. Lymph duct cannulations were performed; after a recovery period of 5–20 hr, acetate-2-^{14}C was injected intravenously, and blood and lymph samples were collected at intervals and analyzed for cholesterol content and ^{14}C as described in the text. The total body analyses of residual tissue cholesterol-^{14}C are described in Table I. The animal numbers (1–4) correspond to the designations in Table I.

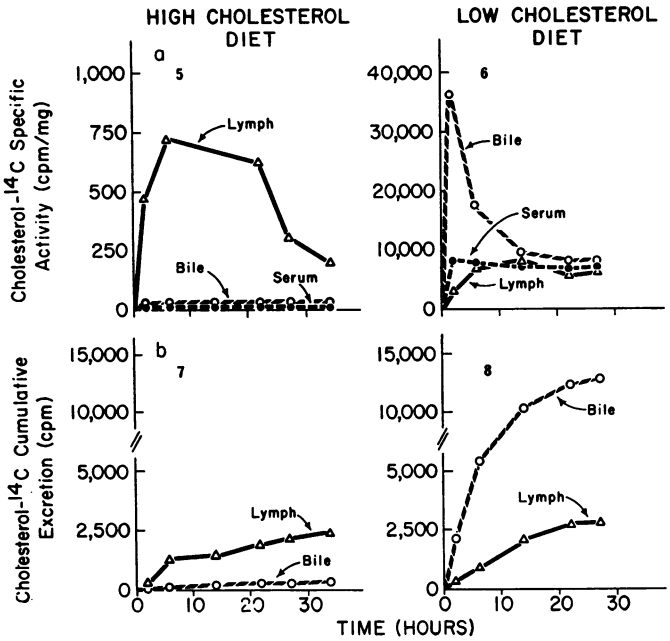

FIGURE 2 Influence of dietary cholesterol on the appearance of cholesterol-^{14}C in lymph, blood, and bile after the administration of acetate-2-^{14}C. *a*, Change in cholesterol specific activity in lymph, blood, and bile with time. *b*, Cumulative excretion of cholesterol-^{14}C into lymph and bile with time. Female, squirrel monkeys (386 and 456 g) were fed either the low or high cholesterol chow diet. Cannulae were then placed in the intestinal lymph duct and both the proximal and distal ends of the common bile duct, and sodium taurocholate was infused into the distal portion of the bile duct. After a recovery period (6 hr) acetate-2-^{14}C was injected intravenously, and samples of lymph, blood, and bile were collected and analyzed as before. The total body analyses of residual tissue cholesterol-^{14}C are described in Table I. The animal numbers (5 and 6) correspond to the designations in Table I.

lesterol in bile, lymph, and serum of two animals fed either high or low cholesterol is plotted in the upper panels (*a*), and the cumulative excretion of cholesterol-^{14}C into lymph and bile by these same animals is shown in the lower panels (*b*). In the animal fed a high cholesterol diet, the specific activity of both blood and bile cholesterol remained almost undetectable, whereas a marked rise occurred in the specific activity of lymphatic cholesterol. In the animal fed a low cholesterol diet, on the other hand, the specific activity of blood, lymphatic, and biliary cholesterol rose promptly within the first 2 hr and reached similar values after 14 hr. The specific activity of lymphatic cholesterol in the animal fed low cholesterol in this experiment (No. 6) as in the previous experiments (Nos. 3 and 4) was considerably higher than in the animals with a high cholesterol intake (Nos. 1, 2, and 5); that this discrepancy is due to expansion of the cholesterol pool of lymph by cholesterol feeding rather than to a partial inhibition of the intestinal synthesis of cholesterol synthesis is suggested by the cumulative cholesterol-^{14}C plot of the data (panel *b*). Little difference could be detected in the total excretion of cholesterol-^{14}C in lymph between the two animals (2374 and 2886 cpm, respectively), despite the marked discrepancy in specific activity. Since blood and bile could not have served as the source of the lymphatic cholesterol in the animal fed the high cholesterol diet, it was concluded from these experiments that the intestine is a site of synthesis for lymphatic cholesterol in the cholesterol-fed monkey and that when this intestinal contribution is excluded from the circulation of the cholesterol-fed animal, virtually no endogenously synthesized cholesterol reaches the circulation during the first 36 hr after acetate-^{14}C administration.

This interpretation of the intestine as a biosynthetic source for serum cholesterol is supported by the type of experiment shown in Fig. 3. In these studies, six animals were fed a high cholesterol diet for 3–6 wk. In three animals (shown on the left) an intestinal lymph duct cannulation was performed, while in the other animals only sham operation was performed. As before, after acetate-2-^{14}C administration, cholesterol-^{14}C was not detected in the serum in significant quantities but appeared promptly in the lymph of cholesterol-fed animals. However, in the sham-operated animals with an intact lymphatic circulation considerable radioactive cholesterol did reach the blood stream of the cholesterol-fed animals. Thus, cholesterol feeding prevents cholesterol of endogenous origin from reaching the blood stream only if the intestinal contribution is simultaneously diverted. Furthermore, these experiments suggest that cholesterol synthesized in the intestine

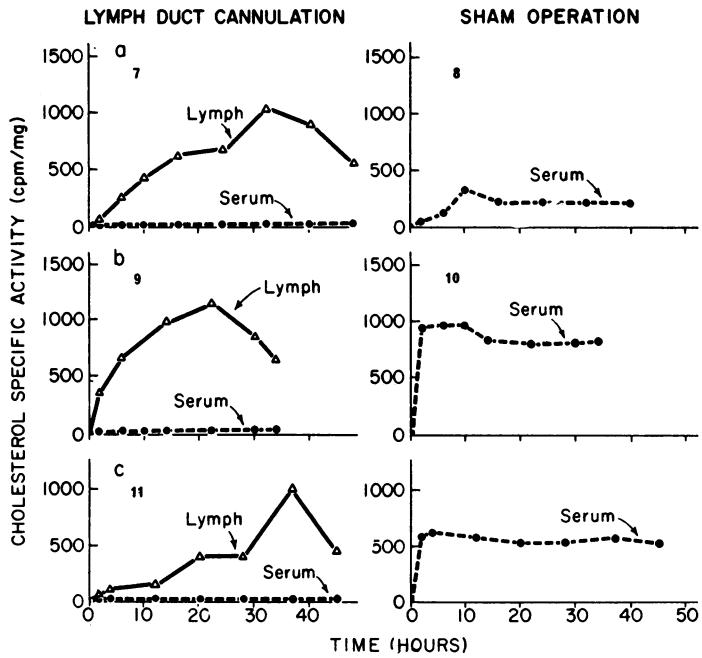

FIGURE 3 Appearance of cholesterol-^{14}C in blood and lymph after the administration of acetate-2-^{14}C to cholesterol-fed monkeys subjected either to cannulation of the intestinal lymph duct or sham operation. Lymph duct cannulation or a sham operation was performed on female squirrel monkeys (486–626 g) which had been fed the cholesterol-supplemented Purina monkey chow. Acetate-2-^{14}C was injected and blood and lymph samples were collected and analyzed for cholesterol-^{14}C and content as before. The groups labeled *a*, *b*, and *c* represent paired experiments in which the sham operated and lymph duct cannulated animals were studied simultaneously. The animal numbers (7–12) correspond to the designations in Table I where the total body analysis of residual cholesterol-^{14}C is described.

not only reaches the circulation but that it might be a significant endogenous source of serum cholesterol in the cholesterol-fed animal.

However, it is difficult to draw conclusions about the quantitative importance of the intestine as a biosynthetic site for cholesterol from this type of experiment for a variety of reasons. Results from single injections of acetate-^{14}C do not necessarily furnish a reliable insight into rates of synthesis because of variations in the pool sizes of the intermediates in cholesterol synthesis and because of different rates of oxidation and turnover of both acetate and cholesterol among various tissues. In addition, the operative and restraint procedures used in these studies doubtlessly influence adversely a variety of parameters. For example, in two of the experiments shown in Fig. 3 (animals 10 and 12) cholesterol-^{14}C reached the blood stream faster than would be expected from the lymph cannulation studies. It is likely that this difference is due to inhibition of lymphatic flow and the necessity of ligating some ancillary lymph channels during the operative procedure. That such an effect may indeed be significant is illustrated by the cumulative data for animals 1–12 summarized in Table I. By the end of the experimental period only a small percentage of the total intestinal cholesterol-^{14}C has appeared in lymph. While the turnover rate of intestinal cholesterol in this species has not been determined, this rather small delivery of cholesterol is probably due, in part at least, to the fact that the lymph flows (0.33–1.13 ml/hour) are smaller than would be expected from animals of this size, that the animals do not always tolerate the period of restraint well, and that the cannulation procedure itself may, by depleting the animals of albumin and other proteins, inhibit cholesterol transfer into lymph. Consequently, it is possible that the significance of intestinal contribution to the circulation might be either under or overestimated by this type of experiment.

For this reason, the experiment shown in Fig. 4 was performed. Four monkeys were fed by stomach tube, twice daily, constant amounts of a formula diet rich in cholesterol-4-^{14}C. The serum cholesterol specific activity was monitored with time and approached plateaus after 20–30 days. At this time only 50–65% of the serum cholesterol was derived from the diet, the remainder coming from endogenous sources. This relation between endogenous and exogenous sources of circulating cholesterol when large amounts of cholesterol are fed is similar to that reported by Mann for the *Lagothrix* and *Cebus* monkeys (10) and is similar to the situation in man (7–9). In the present study, intestinal lymph duct cannulations were performed after 38–49 days of cholesterol-^{14}C feeding, and

TABLE I

Tissue Analyses of Cholesterol-^{14}C in Monkeys Described in Figs. 1–3

Group	Fig. 1				Fig. 2		Fig. 3					
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12
Dietary cholesterol	High	High	Low	Low	Low	High	High	High	High	High	High	High
Days on diet	21	21	21	42	21	21	42	24	35	35	22	35
Operation	lymph duct cannulation (LDC)				LDC plus bile duct cannulation		LDC	Sham	LDC	Sham	LDC	Sham
Hr studied after acetate-2-^{14}C injection	38	32	37	24	28	34	48	40	34	34	45	45
Total body recovery, *cpm*	156,590	308,886	425,934	178,157	1,448,933	376,884	220,318	247,914	160,020	207,525	269,732	347,127
Per cent recovery in tissues												
Intestinal wall	32.6	24.4	7.1	34.6	15.9	48.5	26.0	26.3	20.8	35.1	22.4	22.5
Intestinal lumen	5.7	3.8	4.9	14.9	7.6	21.4	5.6	6.1	12.9	4.1	8.3	4.4
Viscera	3.8	3.5	5.8	10.0	6.1	1.6	2.5	3.9	2.2	5.3	2.9	4.4
Carcass	51.1	66.0	66.2	18.1	50.9	27.5	63.3	58.1	57.2	43.0	63.4	57.0
Liver	5.7	1.7	14.9	20.0	18.4	1.3	1.7	5.4	2.5	12.4	1.9	8.9
Bile	0	0	0.1	0.2	0.9	0.1	0.2	0.1	0.1	0	0.1	0.5
Cumulative lymph	1.0	0.5	0.9	2.1	0.2	0.6	0.7	—	4.4	—	1.1	—
Average lymph flow, *ml/hr*	0.54	0.43	0.52	1.01	0.33	1.13	0.31	—	0.92	—	1.14	—
Average bile flow, *ml/hr*	—	—	—	—	0.31	0.43	—	—	—	—	—	—

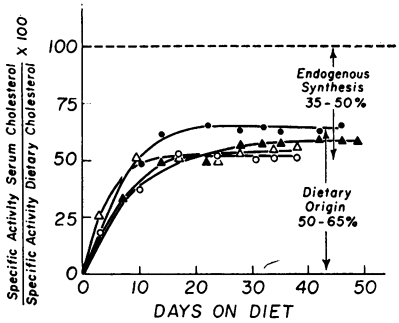

FIGURE 4 Biosynthetic origin of serum cholesterol in the monkey fed diets rich in cholesterol-4-^{14}C. Two male (△ and ○) and two female (▲ and ●) squirrel monkeys were fed by stomach tube twice a day 30 ml of the egg-formula diet containing 249 mg of cholesterol-4-^{14}C (649 cpm/mg) for 38–49 days. Blood samples were drawn from the femoral vessels at intervals, and at the end of the diet the specific activity of serum, intestinal lymph, and dietary cholesterol was determined as described in the text. Average weight increased from 613 g at the beginning to 706 g at the end of the study, and serum cholesterol rose from an average of 131–148 mg/100 ml during the study. The percentage of the lymph cholesterol derived from the diet

[lymph cholesterol-^{14}C (cpm/mg)/
dietary cholesterol-^{14}C (cpm/mg) × 100]

was 61 ± 3% and the percentage of the serum cholesterol derived from the diet

[serum cholesterol-^{14}C (cpm/mg)/
dietary cholesterol-^{14}C (cpm/mg) × 100]

was 65 ± 6%.

intestinal lymph was collected for 2 hr and assayed for cholesterol specific activity. The specific activity of lymphatic cholesterol was 61 ± 3% and that of serum 65 ± 6% of the fed cholesterol-^{14}C. Thus, in the isotopic steady state, sufficient dilution of the dietary cholesterol occurs before the intestinal lymph is reached to account for the entire body dilution.

That the dilution probably occurs between intestinal lumen and intestinal lymph is suggested by the experiment shown in Fig. 5. In this experiment, the cholesterol specific activity was monitored in the isotopic steady state not only in intestinal lymph and serum but at various levels of the lumen of the gastrointestinal tract 5 hr after feeding. It is apparent that at this time relatively little dilution has occurred in the small intestine from biliary cholesterol, the principal drop in specific activity occurring as the intestinal wall is crossed. Thus, dilution of dietary cholesterol-^{14}C with cholesterol in the intestinal wall might be sufficient to account for all of the endogenous cholesterol synthesized in the cholesterol-fed animal.

However, this type of experiment, too, must be interpreted with caution since some other slowly exchanging pool might contribute endogenous cholesterol into the circulation and ultimately into lymph so that in the steady state the drop in specific activity between intestinal lumen and lymph need not be entirely the consequence of locally synthesized cholesterol. Therefore, in hopes of estimating the intestinal contribution more reliably a third type of experiment was performed (Fig. 6). A group of squirrel monkeys was injected with cholesterol-4-^{14}C and fed formula diets with either a high or low content of cholesterol-3-^{3}H for 45–49 days. As before, the serum cholesterol specific activity for the fed isotope approached a plateau after 20–30 days of feeding. The decay in the specific activity of the injected isotope (^{14}C) was relatively linear on a semilog plot during the last 20 days of the studies. Thus, while a steady state is not achieved with the injected isotope, it is entirely reasonable to assume that the rapidly miscible pools are in equilibrium with that of serum after

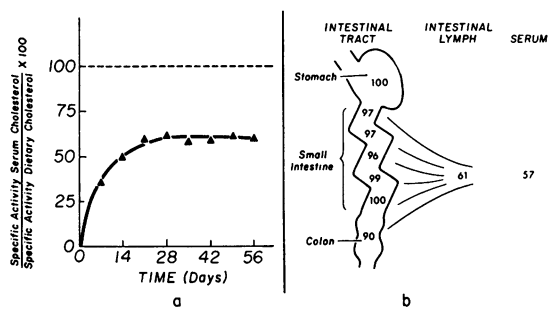

FIGURE 5 The site of dilution of dietary cholesterol with endogenous cholesterol in the isotopic steady state in the monkey. *a*, Change in the specific activity of serum cholesterol with time during the feeding of cholesterol-4-^{14}C for 56 days. *b*, Change in the specific activity of cholesterol-4-^{14}C between the lumen of the gastrointestinal tract, the intestinal lymph, and the serum on day 56. A female monkey, weighing 415 g, was fed the egg-formula diet as described in Fig. 4. On day 56, an intestinal lymph duct cannulation was performed, and lymph was collected for 2½ hr. The animal was then killed, and the specific activity of cholesterol-^{14}C was determined at various levels in the gastrointestinal (GI) tract and in the lymph and serum samples. The sampling of the GI tract was performed 5 hr after the last feeding.

TABLE II

Origin of the Cholesterol of Intestinal Lymph in the Squirrel Monkey Fed Cholesterol-3-^{3}H and Injected with Cholesterol-4-^{14}C

Group	Low cholesterol diet (4)*	High cholesterol diet (3)
	Average (range)	Average (range)
Food intake		
Total, *kcal*	97	97
Fat, % *kcal*	37	37
Carbohydrate, % *kcal*	47	47
Protein, % *kcal*	16	16
Cholesterol, *mg/day*	23	400
Length of feeding period, *days*	45–51	45–51
Weight		
Beginning, *g*	544 (506–565)	553 (547–560)
End, *g*	555 (503–600)	579 (554–600)
Serum analyses		
Cholesterol concentration, end, *mg/100 ml*	150 (128–189)	294 (232–343)
Serum cholesterol-4-^{14}C specific activity at the time of lymph duct cannulation, *cpm/mg*	425 (292–510)	239 (154–301)
Serum cholesterol-3-^{3}H specific activity at the time of lymph duct cannulation, *cpm/mg*	605 (266–788)	640 (476–746)
Dietary cholesterol-3-^{3}H specific activity, *cpm/mg*	5690	867
Serum cholesterol derived from diet in the steady state, end $\left[\frac{\text{serum cholesterol-}^{3}H\text{ (cpm/mg)}}{\text{dietary cholesterol-}^{3}H\text{ (cpm/mg)}} \times 100\right]$, %	8.8 (7.6–9.9)	73.6 (57.0–83.9)
Lymph analyses		
A Lymph cholesterol (mg/2 hrs × 12), *mg/day*	12.0 (8.5–14.4)	31.0 (27.0–34.0)
B Lymph cholesterol derived from serum $\left[\frac{\text{lymph cholesterol-}^{14}C\text{ (cpm/mg)}}{\text{serum cholesterol-}^{14}C\text{ (cpm/mg)}} \times 100\right]$, %	85.8 (79.7–91.8)	76.9 (72.8–79.8)
C Lymph cholesterol derived ultimately from diet $\left[\frac{\text{lymph cholesterol-}^{3}H\text{ (cpm/mg)}}{\text{dietary cholesterol-}^{3}H\text{ (cpm/mg)}} \times 100\right]$, %	11.4 (7.1–13.9)	70.5 (54.3–79.2)
D Lymph cholesterol newly derived from endogenous sources (100 − 100[B + C − BC]), %	12.4 (7.2–17.7)	6.7 (4.2–10.0)
E Absolute amount of lymph cholesterol newly derived from endogenous sources (D × A), *mg/day*	1.5 (0.7–2.4)	2.0 (1.3–2.7)
F Lymph cholesterol newly derived from the diet (C − BC), %	1.8 (0.7–2.7)	16.4 (11.8–21.2)
G Absolute amount of lymph cholesterol newly derived from diet (FA), *mg/day*	0.2 (0.1–0.4)	5.1 (3.2–7.2)
H Absolute amount of lymph cholesterol derived from serum [A −(E + G)], *mg/day*	10.2 (7.5–13.0)	23.5 (21.1–24.8)

The treatment of the animals has been described in the text and in Fig. 6.

* No. of animals.

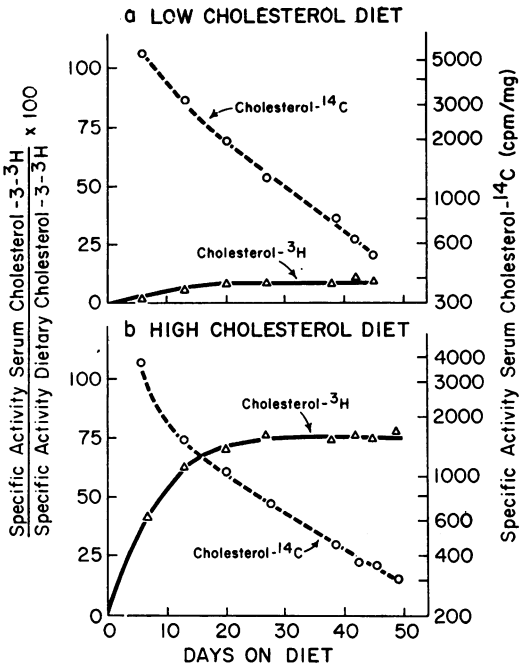

FIGURE 6 Change in specific activity of serum cholesterol with time in monkeys fed cholesterol-3-^{3}H and injected with cholesterol-4-^{14}C. *a*, Low cholesterol diet. *b*, High cholesterol diet. Squirrel monkeys (5 female and 2 male) were tube-fed milk-formula diets with either a low or high content of cholesterol-3-^{3}H for 45–49 days. Cholesterol-4-^{14}C (50 μc) was injected at the start of the experiment. Blood was collected at intervals and analyzed for the specific activity of both the ^{3}H and ^{14}C isotopes. At the end of the experiment, lymph duct cannulation was performed, and a variety of analyses were made as described in Table II.

the first 20 days (*27, 28*) and that for the purposes of the subsequent calculations of serum contributions to the lymph the decay of the two pools can be assumed to be parallel. At the end of the 45–51 day period of study, cannulation of the intestinal lymph duct was performed, and the specific activity of the two isotopes of cholesterol determined.

The results of the seven double isotope experiments in which the lymph duct cannulation was successful are summarized in Table II. In this study, in which the high cholesterol diet contained 400 rather than 250 mg of cholesterol/day the average serum cholesterol rose from 150 to 294 mg/100 ml, and the per cent of cholesterol derived from the diet averaged 8.8 and 73.6% for the low and high diets, respectively. From the lymph analyses, a variety of estimations were possible. When calculated on a 24 hr basis the cholesterol content of lymph rose from 12 mg to 31 mg (line A). The per cent of lymph cholesterol derived from serum

[lymph cholesterol-^{14}C (cpm/mg)/
serum cholesterol-^{14}C (cpm/mg) × 100]

was similar in the animals fed the two diets, 85.8 and 76.9% (line B) whereas the per cent of lymph cholesterol derived ultimately from the diet

[lymph cholesterol-^{3}H (cpm/mg)/
dietary cholesterol-^{3}H (cpm/mg) × 100]

rose from 11.4 to 70.5 (line C). When these figures (B and C) are utilized to determine the percentage of lymph cholesterol newly derived from the intestinal wall [100 − 100 (B + C − BC)], i.e. the total amount of lymph cholesterol minus that derived from diet and serum, corrected for that which has previously been absorbed from the diet and recirculated with the serum, the percentage fell from an average of 12.4–6.7 (line D). However, if one multiplies this percentage figure times the total cholesterol content of lymph (D times A) then it is quite apparent that the intestinal contribution to the circulating cholesterol does not change to a great extent (an average of 1.5 and 2.0 mg/day) as one moves from a low to a high cholesterol diet (line E). This finding, which is of course an estimate based on several assumptions, has been interpreted as evidence in favor of a continuing contribution of newly synthesized cholesterol by the intestine into the circulation regardless of the content of dietary cholesterol. However, it should be emphasized that the quantities of cholesterol estimated by this means should be regarded as minimal figures only, since, in contrast to the estimation of percentages by this technique, the lymph collection (and hence the total cholesterol values) may in fact not be representative of the 2 hr flow in the intact animal.

Two other similar formulations of the data of Table II are of interest. Since in the steady state lymphatic cholesterol of dietary origin could be freshly derived from the diet or have recirculated from the serum, it is possible to estimate the percentage of lymph cholesterol newly derived from the diet, i.e., the total amount from the diet minus the amount which has recirculated (C − BC). As a percentage figure, the amount rose from 1.8 to 16.4% (line F), or in absolute terms from 0.2 to

5.1 mg/day (line G). Nevertheless, even when a high cholesterol diet is fed, the major contribution to lymphatic cholesterol (the total minus the newly derived endogenous and newly absorbed components) is still the recirculated fraction from the serum, 10.2/12.0 and 23.5/31.0 mg/day (line H). Whether this contribution of serum cholesterol to the lymph occurs by direct transfer from serum to lymph or indirectly via the intestinal wall and/or bile or by more than one mechanism cannot be determined from these experiments.

DISCUSSION

The present studies were designed to explore the possibility that the intestine may serve as a significant biosynthetic source for circulating cholesterol in a primate. First, advantage was taken of two factors, namely that the feeding of cholesterol to the squirrel monkey markedly inhibits hepatic cholesterologenesis but does not influence significantly cholesterol synthesis in the 14 other tissues which have been tested (18) and that the intestinal lymph represents the sole means by which the intestine contributes cholesterol into the circulation (26). Therefore, the cholesterol-fed, lymph-cannulated animal was utilized for a study of the conversion of acetate-^{14}C to circulating cholesterol-^{14}C. The results obtained in these studies, as has been demonstrated previously in the rat (17), demonstrate that cholesterol-^{14}C does indeed appear in the intestinal lymph of cholesterol-fed monkeys injected with acetate-^{14}C. This cholesterol-^{14}C does not reach the lymph either via blood or bile and therefore, must have arisen from the gut wall. Furthermore, in the cholesterol-fed, acetate-^{14}C-injected animal, with an intact intestinal lymphatic circulation, but not in the lymph-cannulated animal cholesterol-^{14}C does reach the circulating pool. Therefore, it can be concluded that, under the circumstances of these experiments, cholesterol synthesized in the intestinal wall contributes to the circulating pool. We have also made an attempt to estimate the quantitative significance of the intestine as a biosynthetic site for cholesterol production by utilizing squirrel monkeys fed radioactive cholesterol for long periods of time. In this animal, as in the rat, dog, man, and other new-world monkeys (6–10), an isotopic steady state is approached after a few weeks in which the radioactivity of serum cholesterol never equals that of the diet. In such animals it was possible to demonstrate that, when hepatic synthesis is suppressed, very little dilution of the fed cholesterol occurs in the lumen of the gastrointestinal tract but that on an average sufficient dilution of the ingested cholesterol occurs between lumen and lymph to account for the entire difference between the specific activities of dietary and serum cholesterol. Since, however, dilution at the level of the intestinal wall could be due either to locally synthesized cholesterol or to cholesterol from some other pool that has equilibrated with serum and ultimately with the cholesterol of the intestinal wall, animals fed one isotope and injected with another isotope of cholesterol were utilized for an approximation of the intestinal contribution. Under circumstances of both low and high cholesterol intake a minimal value for the intestinal contribution has been estimated at 1.5–2.0 mg/day. On the basis of these various types of evidence it seems reasonable to conclude that the intestinal wall is in fact a significant biosynthetic site for cholesterol synthesis in this primate. It should be noted in this regard that this conclusion is in keeping with the demonstration that the rate of cholesterol synthesis by in vitro slices of intestine is second only to that of the liver of the 15 tissues studied in the monkey (18).

It is not possible, however, to determine from the studies to date whether the intestine is either the major or the sole extrahepatic, biosynthetic site for serum cholesterol. For, although very little cholesterol-^{14}C reaches the circulation of the cholesterol-fed, lymph duct–cannulated monkey during the first 36 hr after acetate-^{14}C administration, it is entirely likely that in the steady state other slowly exchangeable pools may contribute endogenously synthesized cholesterol into the blood stream. Such an assessment could be made if figures for endogenous production of sterols were available from balance studies under circumstances of high cholesterol feeding; a comparison of the value for total endogenous production with that of the intestinal wall would reveal whether the intestinal wall is a major or minor biosynthetic source when hepatic synthesis is suppressed.

It should be noted in this regard that there are definite limitations to the information which can be drawn concerning the quantitative significance of "endogenous" versus "exogenous" sources from

steady state studies in which diets high in cholesterol-^{14}C are fed for long periods of time. The fact that 60% or more of serum cholesterol in man (7–9) is derived from endogenous sources in comparison with only 15% in the rat when hepatic synthesis is suppressed (6) does not imply that the liver of the rat is a more significant source for cholesterol biosynthesis than that of man when cholesterol-poor diets are fed. Indeed such a difference in the steady state equilibrations under circumstances of high cholesterol intake could result if the only difference in cholesterol metabolism between two species were in the rate of cholesterol absorption. If cholesterol absorption were high in relation to total cholesterol turnover, the endogenous contribution would tend to be diluted whether or not endogenous synthesis were suppressed. Therefore, it is entirely conceivable that extrahepatic sites for cholesterol biosynthesis may be of similar importance under circumstances of low cholesterol intake in all species. Clearly, the intestinal contribution to the lymphatic cholesterol, as estimated in these studies, was not significantly influenced by cholesterol feeding.

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