Transfer of locally synthesized cholesterol from intestinal wall to intestinal lymph

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ABSTRACT The cholesterol-fed rat subjected to cannulation of the intestinal lymph duct and injected with acetate-2-^{14}C has been utilized for a study of the mechanism by which cholesterol synthesized in the intestinal wall gains access to the circulation. It has been concluded that locally synthesized cholesterol is excreted bidirectionally, approximately half going into the lymph and half into the lumen. Furthermore, under the conditions of these experiments, little of the luminal cholesterol appears to be reabsorbed, which suggests that direct transfer from wall to lymph is the principal route for the entry of this endogenously derived cholesterol pool into the lymph and ultimately into the blood stream. Finally, it has been demonstrated that bile is required for this transfer of cholesterol from wall to lymph as well as for the absorption of dietary cholesterol.

KEY WORDS cholesterol · lymph · intestinal wall · lymph duct cannulation · rat · bile · bile duct cannulation

ALTHOUGH CHOLESTEROL is synthesized by virtually every tissue in the mammal (1), the quantitative significance of the extrahepatic tissues as biosynthetic sources of plasma cholesterol has never been resolved. Indeed, after the studies of Hotta and Chaikoff in 1955 (2), it was assumed that circulating cholesterol is either derived from dietary sources or synthesized by the liver.

However, during the past few years considerable evidence has been accumulated to indicate that a portion of serum cholesterol is synthesized by extrahepatic tissues. First, Morris, Chaikoff, Felts, Abraham, and Fansah demonstrated (3) that 10–18% of serum cholesterol in the rat was of endogenous origin under circumstances of cholesterol feeding in which hepatic synthesis was suppressed. Subsequently, it has been well established that in man the major fraction of circulating cholesterol may be of endogenous origin under circumstances in which hepatic synthesis is inhibited by cholesterol feeding (4, 5). In addition, Lindsey and Wilson have reported (6) that cholesterol synthesized in the intestinal wall enters into the circulating cholesterol pool. Although it was not possible in the last-mentioned studies to assess with certainty the quantitative significance of the intestinal wall as a source of circulating cholesterol, no radioactive cholesterol appeared in the blood stream of the cholesterol-fed, lymph duct-cannulated rat for many hours after the feeding of acetate^{-14}C (6). Thus, it is possible that the intestine is a significant source of the circulating pool.

Again, while it has been established that virtually all of the intestinal cholesterol, whether of exogenous (7) or endogenous (6) origin, reaches the circulation via the intestinal lymph, the mechanism of the transfer of cholesterol from wall into lymph is poorly understood. Cholesterol synthesized in the intestinal wall could reach the lymph in at least three different ways: by direct movement into lymph, by movement into the intestinal lumen followed by reabsorption along with the dietary cholesterol, or by a combination of these two mechanisms. The experiments to be described in the present report have been interpreted as furnishing evidence that while a bidirectional flux of cholesterol does exist, involving a movement into both lymph and lumen, the reabsorption pathway appears to play only a minor role in the absorption of endogenous cholesterol into lymph. In addition, evidence has been obtained that bile plays a major role in the movement of cholesterol from the intestinal wall to the lymph.

PROCEDURE

Male rats, of both the Sprague-Dawley and Long Evans strains and weighing 200–300 g, were allowed free access

to a diet containing 0.5% cholesterol[1] for periods of 1–3 wk. These animals, in which hepatic cholesterol synthesis was inhibited (6), were utilized for two types of experiments.

Intestinal Perfusion Studies

Intestinal lymph duct cannulations were performed under ether or Nembutal anesthesia by a modification of the procedure of Bollman, Cain, and Grindlay (8). At the time of operation a polyethylene cannula (Intramedic, Clay-Adams, Inc., New York, cat. no. PE 50) was inserted into the duodenum and secured by a purse string suture, and a terminal ileostomy was performed. The animals were placed in restraining cages and allowed to recover overnight. A solution containing 2.5% mannitol, 2.5% glucose, 0.08 N NaCl, and 0.004 N KCl was infused into the duodenal cannula at a rate of 1–2 ml/hr via a Braun infusion pump. The animals were not allowed access either to food or water. In two experiments 1 ml of oleic acid was introduced into the duodenal cannula every 3–4 hr in addition to the mannitol infusion, and in two experiments egg yolk was added to the infusate at a concentration of 16 g/100 ml, as the result of which the measured cholesterol concentration of the infusion was 1 mg/ml. In these animals lymph (estimated flow 0.3–0.8 ml/hr) and intestinal contents (average flow 1–2 ml/hr) were easily collected. The measured intestinal transit time in these animals was approximately 1.5 hr.

After the animals had been allowed to recover overnight, acetate-2-^{14}C (5×10^7 cpm) in normal saline was injected intravenously, and samples of lymph and intestinal contents were collected at intervals for 48 hr. At the end of the experimental period, the animals were killed, and the intestine and the washed intestinal contents were separated for further processing.

Tube-Feeding Studies

In a second series of experiments lymph duct cannulations were performed as described above, and polyethylene cannulae (PE 90) were placed in the stomach. In addition, high bile duct cannulations were also performed in some animals at the time of operation, and bile samples were collected along with the lymph. To prevent major acceleration in the rate of intestinal cholesterol biosynthesis in the bile-duct cannulated rats (9), we allowed the animals in these studies to recover for only 1–3 hr prior to the injection of the acetate-2-^{14}C (5×10^7 cpm). One of two diets was administered through the stomach tube every 8 hr—either a low cholesterol diet, consisting of 2.5 ml of 50% Lactum modified milk powder (Mead Johnson & Company, Evansville, Indiana) and containing 10 μg of cholesterol per g of diet, or a high cholesterol diet, consisting of 2.5 ml of 50% Lactum in which we dissolved 1.0 g of cholesterol per 100 ml of diet. The animals were allowed free access to drinking water containing 0.6% NaCl in order to enhance lymph flow. During the 48 hr period of study, all feces were saved from each animal, and at the end of the study the intestine and the washed intestinal contents plus all the feces collected were separated for cholesterol-^{14}C analysis.

[1] To a prepared diet obtained from General Biochemicals. (Chagrin Falls, Ohio), we added cholesterol dissolved in oleic acid so that the final composition of the diet was as follows: casein, 20%; sucrose, 56%; nonnutritive fiber, 3.8%; USP XIV salt mix, 3.0%; oleic acid, 5%; cholesterol, 0.5%; and vitamins.

METHODS AND MATERIALS

The methodology for these studies has been previously described (6). In brief, the samples were saponified in an excess of KOH (1 ml of 10 N KOH for each 5 ml of liquid sample and 0.5 g of KOH for each g of tissue) at 150°C and 15 psi for 30 min. An equal volume of ethanol was added, and the samples were extracted with pentane. The pentane layer was washed with water and taken to dryness by heating on a steam bath. Cholesterol digitonides were precipitated from the residue and washed by the method of Sperry and Webb (10). The digitonide was dissolved in methanol; one aliquot was assayed for ^{14}C in a Packard Tri-Carb liquid scintillation spectrometer, and cholesterol content was determined on another portion by the Liebermann-Burchard reaction (10). In these studies the internal quenching of the digitonide was less than 5% under circumstances in which the counting efficiency was 45%. In one experiment esterified and nonesterified cholesterol from chloroform–methanol extracts of lymph were separated on silicic acid columns as previously described (11).

RESULTS

In the preparation utilized for these studies, the cholesterol of intestinal lymph and the intestinal contents can be collected and analyzed after the injection of acetate-^{14}C under circumstances in which hepatic synthesis is suppressed and in which lymphatic cholesterol-^{14}C is derived solely from that synthesized in the intestinal wall (6). Under such circumstances, theoretical predictions can be made for the specific activity–time curves for each of the postulated mechanisms of cholesterol transfer into lymph. If the locally synthesized cholesterol were only transported directly into lymph, the specific activity of lymph would be higher than that of the intestinal contents. If the cholesterol were excreted into the lumen and subsequently reabsorbed, the specific activity of intestinal contents would initially be higher than that of lymph. And if both direct transfer and excretion and reabsorption take place, the initial specific activities of lymph and intestinal contents would probably be similar.

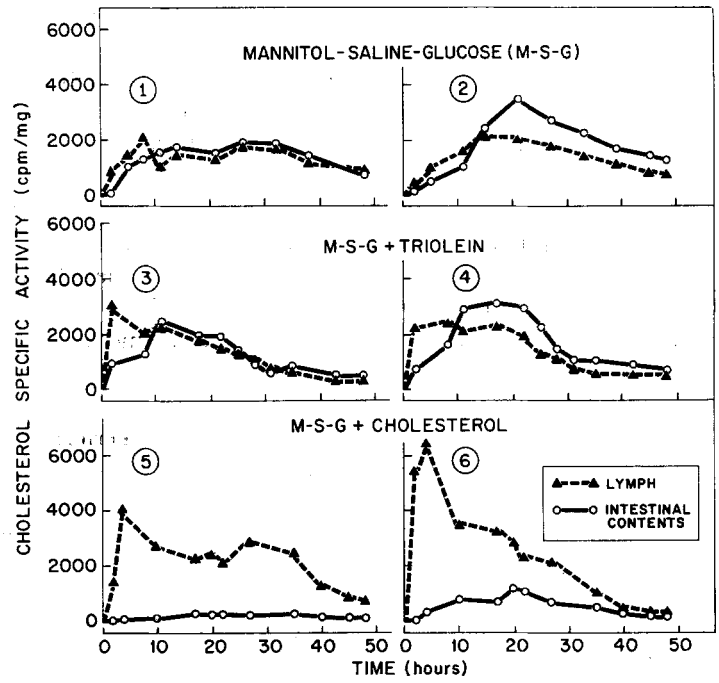

FIG. 1. Appearance of cholesterol-^{14}C in the intestinal lymph and intestinal contents following the injection of acetate-2-^{14}C.

Six male rats were allowed free access to diets containing 0.5% cholesterol for 7–21 days. After cannulation of the intestinal lymph duct and duodenum and ileostomy, each animal was placed in a restraining cage, and a solution of mannitol, saline, and glucose or this solution plus triolein or cholesterol was infused into the duodenum at a rate of 1–2 ml/hr. After an overnight period to allow for recovery of the animals and the establishment of uniform flows of lymph and intestinal washings, acetate-2-^{14}C (5.0×10^7 cpm) was injected intravenously. Lymph and intestinal contents were continuously collected for the next 48 hr period and analyzed for cholesterol specific activity. The cumulative excretion data for these animals are listed in Table 1.

The results of six such experiments in which the change in the specific activity of cholesterol-^{14}C in intestinal lymph and lumen was plotted against the time after administration of acetate-2-^{14}C are shown in Fig. 1. The specific activity of lymph cholesterol is shown by the dotted lines, and the cholesterol specific activity of the intestinal contents is shown by the solid line. In the two experiments shown in the upper parts (rats 1 and 2) the specific activity of cholesterol in lymph was somewhat higher than that of the lumen only during the first 8–11 hr after acetate-^{14}C administration, which suggests that cholesterol synthesized in the intestinal wall moves both into the lymph and lumen. In the next experiments (rats 3 and 4) triolein was administered in order to enhance chylomicron formation and, consequently, the rate of transfer of cholesterol into lymph; in these experiments the differences between the specific activities of cholesterol in lymph and in the lumen were somewhat accentuated during the first 8 hr of the experiment, but, again, during the remainder of the experiment the specific activity of the luminal cholesterol was as high or higher than that of lymph. These experiments were interpreted as furnishing evidence for a bidirectional movement of cholesterol from the wall both to the lymph and to the lumen. Cholesterol clearly moved into the lumen, where it could be reabsorbed, and the early discrepancy between lymph and lumen specific activities indicates that direct secretion into lymph can occur since this component could not have been derived from the lumen.

In order to clarify this question further, we assessed the influence of cholesterol perfusion on the transfer of intestinal and cholesterol-^{14}C into lumen and lymph (rats 5 and 6, Fig. 1). Cholesterol (in the form of egg lipoprotein) was added to the solution perfused into the duodenum (1 mg/ml). Thus, since the cholesterol content of the lumen was high at all times, any cholesterol-^{14}C which was excreted from the wall to the lumen would be diluted and would be prevented from contributing significantly to the lymph cholesterol specific activity. In these two experiments, the specific activity of lymph cholesterol greatly exceeded that of the lumen for the first 34 hr of the experiment. Although in these two animals the total counts recovered in the intestine were somewhat lower than in the previous studies (rats 1–4), the percentage recoveries in lymph and lumen at the end of the experiment were similar. Thus, when the lumen cholesterol-^{14}C is diluted, the rate of delivery of cholesterol into lymph remains unimpaired. These experiments have been inter-

preted as substantiating further the view that cholesterol synthesized in the intestinal wall can be transferred directly into lymph. Furthermore, since the specific activity of lymph cholesterol was initially as high when cholesterol was perfused and when cholesterol-^{14}C reabsorption from the lumen was minimized as when cholesterol-free solutions were perfused (animals 1–4), it seems reasonable to conclude that the reabsorption pathway must be small in comparison with the quantity of lymphatic cholesterol-^{14}C derived from direct transfer from wall to lymph (at least under circumstances in which mannitol solution is constantly perfusing the gastrointestinal tract of animals which have been fed a high cholesterol intake for several days).

This conclusion is supported by the cumulative excretion data summarized in Table 1. During the 48 hr following acetate-2-^{14}C administration, 20.9–45.3% of the total intestinal sterol-^{14}C was recovered in lymph and 31.9–63.2% in the lumen of animals perfused with cholesterol-free perfusions. These percentages are similar for the animals perfused with the high cholesterol solution (21.2 and 36.4% for lumen and 29.7 and 35.4% for lymph). Thus, under circumstances in which cholesterol reabsorption would probably be minimized by the very rapid transit time induced by mannitol, approximately half the cholesterol-^{14}C leaving the intestinal wall during the first 48 hr after acetate-^{14}C administration appears to be directly transferred into the lymph, and about half appears in the lumen. It was not possible from these studies to determine whether the reabsorption of desquamated cholesterol would, under circumstances of a normal intestinal transit time, form a significant pathway for the transfer of locally synthesized cholesterol from wall to lymph.

Therefore, we performed a series of experiments in which we tube-fed the animals diets either high or low in cholesterol content, instead of perfusing the gastrointestinal tract after lymph duct cannulation (Fig. 2). In these studies, the feeding of a high cholesterol diet during the 48 hr experiment (rats 7–9) did not depress either the specific activity or the cumulative appearance of cholesterol-^{14}C in lymph in comparison with the animals fed the

TABLE 1 ANALYSIS OF THE CUMULATIVE APPEARANCE OF CHOLESTEROL-^{14}C IN THE LYMPH OF ANIMALS FED 0.5% CHOLESTEROL AND INJECTED WITH ACETATE-2-^{14}C

Figure	Rat	Days on Diet Prior to Operation	Operation	Time Between Operation and Injection of Acetate-^{14}C	Duodenal Infusate	Tube-Feeding During Experiment	Average Lymph Flow*	Digitonin-Precipitable Sterol-^{14}C Recovered in: Lymph	Intestinal Contents	Intestinal Wall	Bile	Total Intestinal Sterol	Total Intestinal Sterol-^{14}C Recovered in 48 hr in: Lymph	Lumen
				hr			*ml/hr*	*cpm/48 hr*	*cpm/48 hr*	*cpm*	*cpm/48 hr*	*cpm*	%	%
1	1	7	Lymph duct cannulation plus duodenal intubation plus ileostomy	15	Mannitol–saline–glucose (M–S–G)	None	—	11,832	20,484	10,500	—	42,816	27.6	47.8
	2	14		15			—	21,168	14,910	10,650	—	46,728	45.3	31.9
	3	14		15	M–S–G plus triolein		—	18,240	55,263	13,875	—	87,378	20.9	63.2
	4	14		15			—	17,529	27,984	4,800	—	50,313	34.8	55.6
	5	21		15	M–S–G plus cholesterol		—	9,864	7,000	16,300	—	33,164	29.7	21.2
	6	21		15			—	9,144	9,360	7,020	—	25,704	35.4	36.4
2	7	10	Lymph duct cannulation	2	None	High cholesterol diet	1.2	17,756	26,000	10,200	—	58,396	30.4	44.5
	8	7		3			1.1	15,941	10,400	25,600	—	51,944	30.7	20.0
	9	7		2			0.9	27,044	20,800	12,300	—	60,144	45.0	34.6
	10	10		3		Low cholesterol diet	0.9	13,599	11,700	8,875	—	34,174	39.8	34.2
	11	7		3			0.9	15,190	15,400	13,900	—	44,490	34.1	34.6
	12	7		2			0.9	21,780	19,200	14,600	—	55,580	39.2	34.5
3	13	10	Lymph duct cannulation plus bile duct cannulation	2	None	High cholesterol diet	0.6	4,400	43,600	13,000	60	61,040	7.3	71.4
	14	7		2			0.5	6,880	53,600	17,800	330	78,280	8.8	68.5
	15	10		3		Low cholesterol diet	1.3	4,225	35,520	12,375	39	52,149	8.1	68.1
	16	7		2			0.4	6,737	34,720	12,700	420	54,156	12.4	64.1
4	17	7	Lymph duct cannulation	2	None	Low cholesterol diet	0.7	16,384	8,680	17,600	—	42,664	38.4	20.3
	18	7		2			0.6	25,616	19,920	24,800	—	70,336	36.4	28.3
	19	7	Lymph duct cannulation plus bile duct cannulation	2			0.3	3,540	22,240	26,100	520	51,880	6.8	42.9
	20	7		2			0.2	3,968	20,400	23,000	440	47,368	8.4	43.1
	21	7	Lymph duct cannulation	2		Low cholesterol diet plus cholestyramine	0.4	10,168	23,120	20,700	—	53,988	18.8	42.8
	22	7		2			0.4	4,936	24,720	20,700	—	50,356	9.8	49.1

* The lymph duct cannulae in rats 1–6 were washed periodically with heparin, and consequently precise measurements of lymph volume could not be obtained; flows were estimated to vary from 0.3 to 0.8 ml/hr.

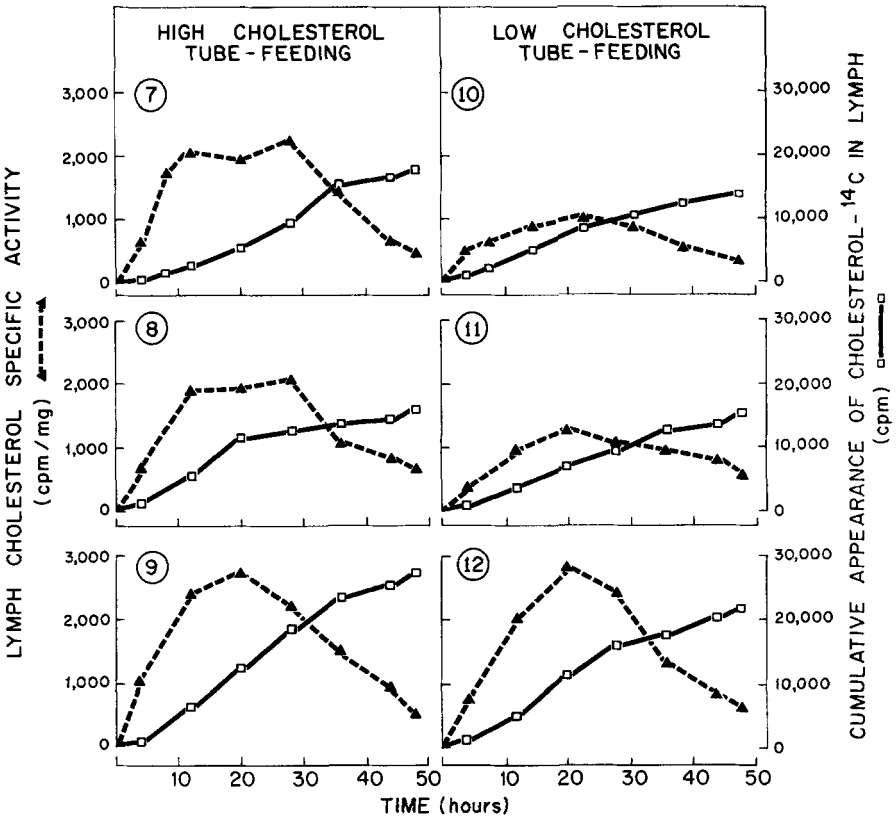

FIG. 2. Specific activity–time curves and cumulative appearance of cholesterol-^{14}C in the intestinal lymph of rats injected with acetate-2-^{14}C and subsequently fed diets either low or high in cholesterol content.

Six male rats were allowed free access to diets containing 0.5% cholesterol for 7–10 days. After cannulation of the lymph duct and intubation of the stomach, the animals were fed by stomach tube 2.5 ml of diet containing either 0.02 mg (low diet) or 25 mg (high diet) of cholesterol every 8 hr. After a 2–3 hr recovery period, acetate-2-^{14}C was injected intravenously and lymph and feces were collected continuously for 48 hr and analyzed for cholesterol-^{14}C and cholesterol content. The cumulative data for these animals are listed in Table 1.

low diet (rats 10–12). Furthermore, the feces were collected during these studies and analyzed along with the intestinal contents at the time of death for cholesterol-^{14}C content (Table 1). As before, in all of these animals, approximately half of the cholesterol-^{14}C which left the intestinal wall during the 48 hr experiment appeared in the lymph, and an equal amount was recovered in the lumen. Thus, even under circumstances in which transit time approximated the normal values, we could find no evidence of a significant role for the reabsorption pathway for cholesterol movement from wall to lymph.

The next experiments were designed to determine whether bile, which is required for optimal cholesterol absorption, is also required for the transfer of cholesterol from wall to lymph. Four rats which had been fed 0.5% cholesterol diets for 7–10 days were subjected to cannulation of the bile ducts, as well as intubation of the stomach and intestinal lymph duct (Fig. 3). These animals were injected with acetate-2-^{14}C within 2–3 hr after the operation and subsequently treated as in the previous experiment, two animals (rats 13 and 14) being fed high cholesterol and two rats (15 and 16) the low cholesterol diets. While bile duct cannulation had no consistent effect on the specific activity of the cholesterol-^{14}C appearing in lymph during the 48 hr collection period, the cumulative appearance of cholesterol-^{14}C was uniformly depressed in comparison to the animals described in Fig. 2. Again, the cumulative excretion data are tabulated in Table 1. In these animals only 7.3–12.4% of the total intestinal sterol-^{14}C was recovered in lymph in comparison to 30.7–45% in the animals with an intact biliary circulation. In contrast, the amount recovered from the lumen plus that in the lymph was approximately equal to that of the control animals. Clearly, the rate of movement from wall to lumen was not inhibited by bile duct cannulation.

The mechanism of this effect of bile on lymphatic absorption of endogenous cholesterol is not clear from these studies. On an average, the flow of intestinal lymph was decreased in the bile duct-cannulated group (0.4–1.3 ml/hr in contrast to the control values of 0.9–1.2 ml/hr). However, an effect on lymph flow cannot be the sole mechanism by which bile accelerates the movement from

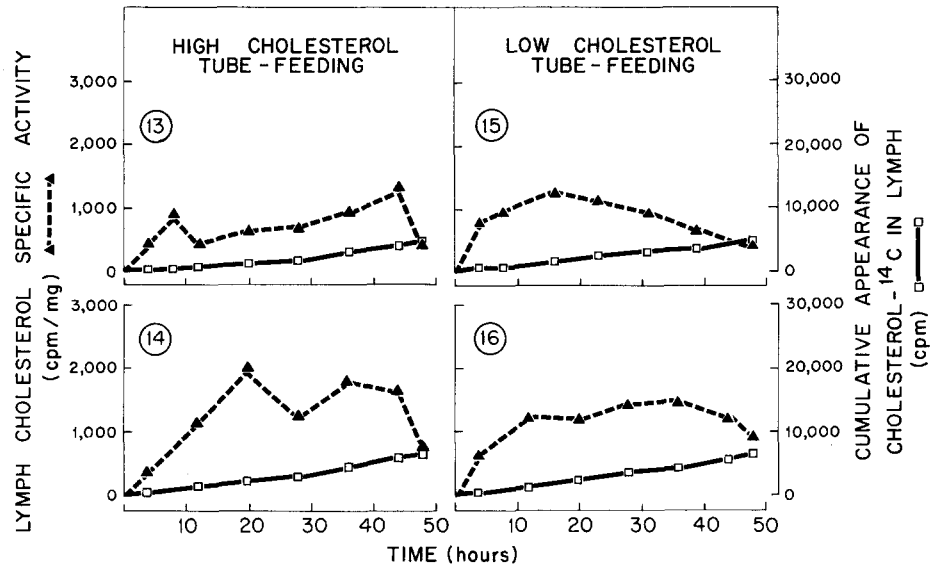

FIG. 3. Influence of bile duct cannulation on the appearance of cholesterol-^{14}C in the intestinal lymph of rats injected with acetate-^{14}C and subsequently fed diets either low or high in cholesterol content.

Four male rats were allowed free access to diets containing 0.5% cholesterol for 7–10 days. After cannulation of the intestinal lymph duct and common bile duct and intubation of the stomach the animals were treated as described in Fig. 2.

wall into lymph since one of the bile duct-cannulated group (rat 15) had a higher average lymph flow (1.3 ml/hr) than was attained by any of the control animals. That the animals in this study did in fact have effective suppression of hepatic cholesterol synthesis is demonstrated by the cumulative excretion data for bile; in each instance less than 1% of the intestinal sterol-^{14}C could have been derived from the bile.

In order to investigate further the mechanism by which bile might accelerate the transfer of cholesterol from intestinal wall to lymph, we studied the effect of bile duct cannulation and of cholestyramine administration on the appearance of esterified and nonesterified cholesterol-^{14}C in lymph (Fig. 4). In the control animals (17 and 18), approximately 60% of the lymphatic cholesterol-^{14}C was found to be in the esterified form; this observation is in agreement with previous findings in this laboratory (6). Bile duct cannulation (rats 19 and 20) resulted in a depression of the appearance both of esterified and of nonesterified cholesterol, but this inhibition was most marked in the case of esterified cholesterol which almost disappeared from the lymph of the bile duct-cannulated animal. The effects of cholestyramine administration for the 48 hr of study only (rats 21 and 22) were similar, although smaller in magnitude, to the results of bile duct cannulation: nonesterified cholesterol was predominant in lymph, and the depression of the appearance of the esterified form was more marked than that of the nonesterified cholesterol. The cumulative excretory data for these animals are tabulated in Table 1; the results are similar to the previous study in which bile duct cannulation was performed (rats 13–16).

DISCUSSION

The cholesterol-fed, lymph duct-cannulated rat has been utilized for an analysis of the means by which cholesterol synthesized in the intestinal wall gains access to the circulation. In such animals, almost no radioactive cholesterol enters the circulation following acetate-2-^{14}C administration (6), and consequently, time curves for the change in specific activity of the cholesterol of intestinal lumen and intestinal lymph have been determined under a variety of circumstances. The present studies have been interpreted as furnishing evidence that approximately half the cholesterol synthesized in the intestinal wall is transferred directly into the lumen and half is transferred into the lymph. This relationship is apparently true both when the intestinal transit time is made very rapid by infusion of mannitol into the duodenum and when small volumes of food are placed in the stomach. Furthermore, neither the peak specific activity of lymphatic cholesterol-^{14}C nor the cumulative appearance of cholesterol-^{14}C in lymph is consistently affected by feeding further amounts of cholesterol, suggesting that the reabsorption of luminal cholesterol is not a very important means by which the sterol gains entry into lymph.

It is possible, of course, that sufficient cholesterol remains from the feeding of cholesterol prior to the operation so that the subsequent feeding of cholesterol during

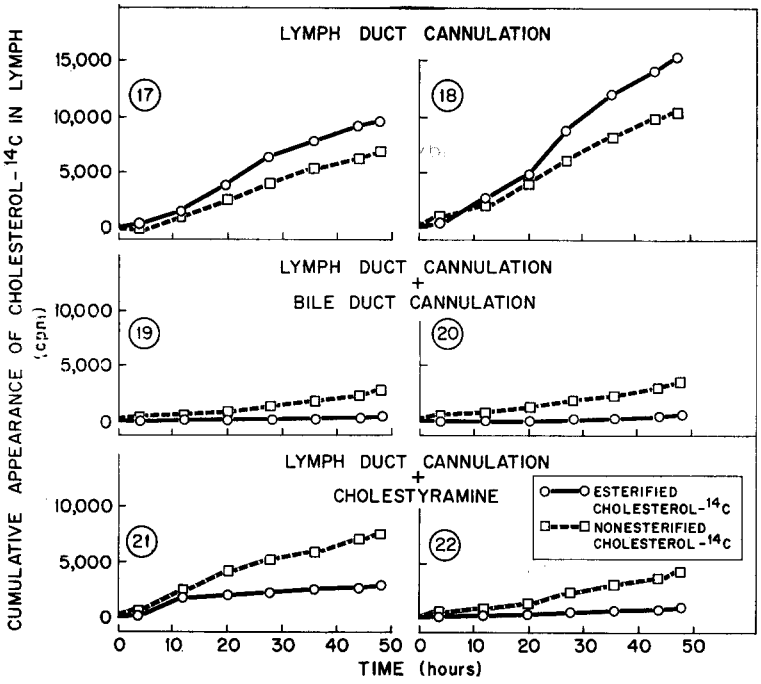

FIG. 4. Effect of bile duct cannulation and cholestyramine administration on the appearance of esterified and nonesterified cholesterol-^{14}C in the intestinal lymph of rats injected with acetate-^{14}C.

Male rats were allowed free access to diets containing 0.5% cholesterol for 7 days. Within 1 hr of intubation of the stomach, the intestinal lymph duct, and in two instances (rats 19 and 20) the bile duct, the animals were given acetate-^{14}C, and lymph, bile, and feces were collected for 48 hr. 2.5 ml of the low cholesterol diet were given to each animal, and, in addition, rats 21 and 22 were given 2.5 ml of 8% cholestyramine in water by stomach tube at 8-hr intervals, the first dose being given at the time of surgery. Prior to saponification of the lymph, esterified and nonesterified cholesterol were separated as described in the text. The cumulative data for these animals are given in Table 1.

the experiment can have no additional effect. This possibility is not likely to be the sole explanation for two reasons: the specific activity of the luminal sterol is further diluted by the cholesterol infusion, and the experiments have been continued for a sufficient period of time to allow for at least one turnover of the cholesterol present in the wall (12). Consequently, it seems reasonable to assume that the reabsorption pathway must be very small in comparison with that entering the lymph directly. The reason for this relationship can be adduced from the studies of Dietschy and Siperstein, who demonstrated (9) that most of the cholesterol synthesis in the small intestine takes place in the terminal ileum, whereas cholesterol absorption in the rat occurs principally in the upper small bowel (13). Thus, any cholesterol excreted from the terminal ileum would be expected to have very little temporal or anatomical access to potential absorptive sites before passing into the colon. Furthermore, the terminal ileum contains many bacteria, and some conversion of cholesterol to coprostanol takes place there (14); any such transformation would further diminish the chance for the reabsorption of luminal cholesterol.

It is, of course, impossible from these experiments to exclude with absolute certainty the possibility that the cholesterol that enters the lymph is excreted into the lumen in some special form such as a lipoprotein which permits selective reabsorption into lymph. However, since neither mannitol infusion nor cholesterol feeding has an effect on this transfer, such a mechanism seems highly unlikely. Even if such were the case, the physiological implication of these studies remains clear, namely that cholesterol synthesized in the intestinal wall is not equivalent to dietary cholesterol in that it never mixes in the lumen with that of the diet as a single pool. It follows from this that since cholesterol synthesis in the intestinal wall is not subject to major feedback control by cholesterol feeding (9, 15) and since the reabsorption pathway is small, cholesterol feeding does not significantly influence the delivery of locally synthesized cholesterol into the intestinal lymph. This conclusion is in accord with the finding in the squirrel monkey, as the result of experiments utilizing a double isotopic technique, that cholesterol feeding does not influence the delivery of locally synthesized cholesterol from intestinal wall to intestinal lymph (16).

The final implication of these studies, namely, that the optimal transfer of cholesterol from wall to lymph requires an intact biliary circulation, deserves some com-

ment. It has, of course, been demonstrated repeatedly that bile is required for cholesterol absorption (17, 18), and evidence has been obtained that bile salts are necessary for the uptake of cholesterol from the lumen into the intestinal mucosa (19). The results of the present study suggest that this requirement of bile for optimal movement of cholesterol from wall to lymph may be due in part to the activation of cholesteryl esterase by bile acids (20) [since cholesterol is known to enter the lymph principally in the esterified form (21)] and in part to inhibition of lymph flow. However, neither of these mechanisms appears adequate to account for the entire effect, which could be the passive consequence of diminished lipoprotein formation, of interference with some other aspect of the transfer process, or of nonspecific effects of the operation with its ensuing electrolyte and volume depletion. The fact that the transfer of nonesterified cholesterol from wall to lymph was not inhibited as strikingly by bile duct cannulation as was that of the esterified fraction may imply that the mechanism of the transfer differs for the two molecular forms, i.e., the nonesterified fraction might exchange with that of the circulation under circumstances in which net movement cannot be determined. It is also clear that if bile is required for maximal transfer, then cholestyramine, which interferes with the absorption of dietary cholesterol by binding bile acids (22), might also act to prevent this source of endogenous cholesterol from reaching the circulation.

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