The origin of cholesterol in the mesenteric lymph of the rat

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Abstract These studies were performed to quantitate the amounts of newly synthesized cholesterol secreted in the mesenteric lymph of the rat and to define the origin of this cholesterol. In control animals receiving no dietary fat, the amount of newly synthesized sterol entering the lymph increased linearly with respect to time over 24 hr. When a continuous intravenous infusion of chylomicrons was given or when the animals were prefed a diet containing 2.0% cholesterol to inhibit hepatic, but not intestinal or peripheral, cholesterol synthesis, the secretion of newly synthesized sterol in lymph was markedly suppressed, suggesting that the liver was its ultimate site of origin. When the animals were subjected to either blockade of intestinal cholesterol absorption or biliary diversion, there was a decrease in both the newly synthesized and total mass of cholesterol in lymph by approximately 60%, indicating that the majority was normally derived from the absorption of luminal (primarily biliary) sterol. In the absence of dietary cholesterol, the remainder was probably derived from plasma lipoproteins that were filtered through the intestinal capillaries into the lymph. In contrast, when lymph was collected during active fat absorption, the intestine was found to secrete sterol newly synthesized by the epithelium. Such newly synthesized cholesterol was found predominantly in the unesterified fraction and accounted for approximately 27% of the total sterol found in lymph at the end of the experiment. From these studies it was concluded that in the absence of fat absorption, sterol synthesized in the intestinal mucosa was incorporated predominantly into cell membranes and did not enter intestinal lymph to any significant degree. However, during fat absorption, a fraction of this newly synthesized sterol pool was incorporated into lipoproteins and so was delivered through the intestinal lymph to the body pools of cholesterol. — **Stange, E. F., and J. M. Dietschy.** The origin of cholesterol in the mesenteric lymph of the rat. *J. Lipid Res.* 1985. **26:** 175–184.

Supplementary key words [^{3}H]water • small intestine • fat absorption

The small intestinal mucosa plays a key role in mammalian cholesterol metabolism both because it is the site for the uptake of sterol present in the intestinal contents and because it is one of the most active tissues in the body for the de novo synthesis of cholesterol (1, 2). The sterol that is found within the intestinal lumen is derived from the diet, from biliary secretion, and from the mucosa itself via desquamation of epithelial cells or direct secretion (3). A fraction of the luminal unesterified cholesterol enters mixed micelles consisting of bile acids, monoglycerides, fatty acids, and phospholipids (3). These water-soluble micelles then penetrate the mucosal unstirred water layers (4), thus bridging the step of highest resistance during the uptake process, and release the sterol in monomolecular form for passive diffusion through the enterocyte brush border (4). The majority of the cholesterol is apparently absorbed in the villus cells of the proximal intestine (5) and is nearly quantitatively esterified in the mucosa by one of two enzyme systems: the cholesterol esterase of pancreatic origin (6) or an acyl coenzyme A:cholesterol acyltransferase (7).

It has also been recognized for years that the intestinal mucosa actively synthesizes cholesterol at a rate that is second only to the liver in the rat (1, 8) and which may be equal to or even exceed the liver in other species such as the rabbit, hamster, guinea pig, and, possibly, man (2, 9–13). Using ^3H_2O as a substrate in vitro and in vivo, the majority of newly synthesized digitonin-precipitable sterols (DPS) was found in the lower villus and crypt cell fractions (14), although active sterol synthesis has been reported to occur all along the villus in both the jejunum and ileum (14–17). The rate of cholesterol synthesis in these various cells is apparently under complex regulation that is dictated by the amount of sterol taken up across the brush border (9–11, 14, 17–20), by the uptake of lipoprotein cholesterol (18, 21, 22), and by the cholesterol demand generated during fat absorption (18, 19) or cellular proliferation (23). However, it is unclear whether, and to what extent, this locally formed sterol supplies structural cholesterol for the assembly of chylomicrons and other intestinal lipoproteins, and so gains access to the body pools of cholesterol. Such a contribution of the intestine to the miscible sterol pools cannot be assumed to occur merely because the synthesis of cholesterol (14) and lipoproteins (24) takes place in the same cell population since

Abbreviations: DPS, digitonin-precipitable sterols.

it is now clear that intracellular compartmentalization plays a critical role in many aspects of cholesterol metabolism (13, 19).

The quantitative contribution of locally synthesized cholesterol to mesenteric lymph sterol, and thus the contribution of intestinal synthesis to body cholesterol pools, has been the subject of a number of previous investigations. Early work based on lymph cholesterol specific activity measurements after the administration of ^{14}C-labeled acetate or cholesterol to lymph fistula animals unequivocally demonstrated that radiolabeled sterol formed in the mucosa of the rat and baboon was delivered into the intestinal lymph (25–27). These studies, however, gave little information on the quantitative importance of this process. More recent data have also suggested a significant flux of intestinally synthesized sterol to the liver and plasma compartments in animals receiving ^3H_2O during a 1-hr period (1). However, even these estimates were derived indirectly by observing the effects of chylomicron administration or enterectomy on the appearance of ^3H-labeled digitonin-precipitable sterol ([^3H]DPS) in the liver and plasma of the experimental animals.

Thus, in order to further and more directly delineate the importance of the intestine as a contributor to the total body sterol pools, we measured in the present studies the amount of total and newly synthesized cholesterol appearing in intestinal lymph. The interpretation of these data in quantitative terms was made possible by two recent experimental developments. First, under both in vitro and in vivo conditions it has become possible to calculate the mass of cholesterol that is newly synthesized from the observed rates of incorporation of ^3H_2O into DPS (28–30). This calculation is based upon the finding that approximately 1.45 μg-atoms of carbon enter the cholesterol molecule from acetyl CoA for each μg-atom of ^3H that is found in the [^3H]DPS fraction. Second, various physiological and pharmacological manipulations are now available that make it possible to alter the rates of sterol synthesis in the intestinal epithelial cells and to distinguish that portion of the [^3H]DPS found in lymph that ultimately is synthesized in the intestine from that which is synthesized in other organs such as the liver, and only passes through the intestine to reach the mesenteric lymph.

MATERIALS AND METHODS

Animal preparations and diets

Female Sprague-Dawley-derived rats (CD$^{(R)}$(SD)BR, Charles River Breeding Laboratories, Inc., Wilmington, MA) were housed in colony cages in a room with alternating 12-hr periods of light (3:00 PM–3:00 AM) and darkness (3:00 AM–3:00 PM). After an adjustment period of 5 days, during which all animals received a low cholesterol diet and water ad libitum, the rats were either continued on the same diet (Wayne Laboratory Animal Diets, Allied Mills, Inc., Chicago, IL) or were fed the various experimental diets. The experimental diets used in this study contained either cholesterol (2% w/w, Byron Chemical Company, Inc., Long Island City, NY), surfomer[1] (2% w/w, Monsanto Company, St. Louis, MO), or cholesterol-free corn oil (10% w/w, Mazola Corn Oil, Englewood Cliffs, NJ), and were fed for 2 weeks, 1 week, and overnight, respectively. The cholesterol was dissolved in ethanol, which was then allowed to evaporate after mixing with the ground diet, whereas both surfomer and corn oil were directly mixed with the ground chow. After the dietary period all animals were in the 175–200-g weight range.

After receiving the corn oil diet overnight, each animal was operated upon and the main mesenteric lymph duct draining the small intestine was cannulated with PE-50 polyethylene tubing while the animal was under ether anesthesia (31). At the same time a PE-90 catheter was inserted into the stomach and advanced into the lower duodenum. Some animals were also fitted with a femoral vein catheter for chylomicron infusion or with a catheter in the common bile duct. All catheters were exteriorized and the animals were placed in restraining cages with free access to water. Subsequently, all animals were infused intraduodenally with a solution made up of equal volumes of FreAmine III (McGaw Lab., Irvine, CA) and an electrolyte/glucose solution (NaCl, 77.1 mM; KCl, 5.0 mM; K_2HPO_4, 10.3 mM; and glucose, 1100 mM, pH 7.4) for 44 to 48 hr at a rate of 2.4 ml/hr. During the overnight period of recovery the lymph cleared, indicating wash-out of the remaining corn oil. Lymph was collected into tubes containing 2 ml of 0.9% saline and 20 mg of EDTA or into beakers containing 10 ml of 0.9% saline and 100 mg of EDTA.

The lymph fistula animals used as chylomicron donors were infused intraduodenally with an egg yolk/saline mixture and the lymph chylomicrons were isolated as described previously (31). Bile donor animals were fitted with a biliary fistula and bile was collected for up to 24 hr. In one experiment [^3H]cholesterol was mixed with fresh rat bile obtained during a 2-hr bile diversion and 0.5 ml of this labeled bile containing $7 \cdot 10^6$ dpm was injected intraduodenally into lymph fistula animals. Cholesterol absorption was then determined from the recovery of radioactivity during the subsequent 24-hr lymph collection.

[1] In studies to be published separately, it has been shown that surfomer nearly completely blocks the uptake of nonpolar sterols like cholesterol into the mucosa both in vitro and in vivo, whereas the absorption of bile acids is unaffected. The nonabsorbable polymer reduces the passive permeability coefficient for cholesterol by increasing the hydrophilicity of the brush border membrane.

Assay of newly synthesized sterol

At 9:00 AM, 20–24 hr after the mesenteric lymph fistulae were placed, the restrained rats were administered 100 mCi of ^3H_2O in 1 ml of 0.9% saline intraperitoneally. Access to drinking water was discontinued and the intraduodenal infusate was supplemented with 70 mCi of ^3H_2O/100 ml to maintain the specific activity of the pool of body water constant during the experiment. Using this regimen the lymph water specific activity was 94.7 ± 7.0% of the final plasma water specific activity at 2 hr, 102.3 ± 1.2% at 4 hr, 101.4 ± 1.9% at 6 hr, 100.1 ± 4.4% at 8 hr, and remained stable thereafter. The infusate water specific activity was always within 5% of the mean plasma water specific activity of an experimental group. Lymph was collected at 2, 4, 6, 8, 12, 22, and 24 hr after injection of the ^3H_2O. The specific activity of plasma, lymph and infusate water and the ^3H-labeled digitonin-precipitable sterol content of plasma, lymph, and tissues were determined as described previously (28, 30).

The synthesis rates of DPS were assayed in vitro in liver and intestinal slices using [1-^{14}C]octanoate (1.5 mM). At this concentration the incorporation of octanoate equals the absolute rate of DPS synthesis measured with ^3H_2O in the intestine (14). Isolation of ^{14}C-labeled DPS was performed as described (32).

Assay of total and unesterified sterol

The content of cholesterol in lymph, plasma, and tissues was determined colorimetrically (33). For estimation of the free and esterified fractions internal standards were added and the samples were extracted in chloroform–methanol 2:1 (vol/vol) and then separated on silicic acid/celite columns using ^{14}C-labeled cholesterol and cholesteryl oleate as internal standards. The fractions were saponified and [^{3}H]DPS content and cholesterol mass were measured (28, 33).

Calculations

The equation for calculating the mass (nmol) of newly synthesized DPS per ml of lymph is as follows and includes the assumption that the ^3H/C incorporation ratio equals 0.69 (14, 29):

$$\frac{\text{(dpm [}^3\text{H]DPS/ml lymph)}}{\text{(sp act of plasma water)(18)(0.69).}}$$

The specific activity of lymph, plasma, and infusate water was calculated from the following expression (the factors of 0.92 or 0.98 apply to plasma (1) or lymph, respectively:

$$\frac{\text{(dpm }^3\text{H/ml)}}{\text{(nmol water/ml water)(0.92 ml water/ml plasma or 0.98 ml water/ml lymph).}}$$

The in vitro rates of sterol synthesis were calculated as described previously in detail (29) and were expressed as the nmol of acetyl CoA incorporated into DPS per g of tissue per hr (nmol/g per hr).

In all instances the data are given as means ± 1 SEM. Differences in mean values were tested for significance using the Student-Neuman-Keuls test for multiple comparisons. The linear regressions were calculated with a TI 59 calculator using the applied statistics module.

RESULTS

In the course of these studies groups of animals were subjected to a variety of manipulations that are known to alter the rates of cholesterol synthesis in liver and intestine. To assess the effects of these manipulations under the exact experimental conditions used in this investigation, preliminary studies were carried out in which rates of sterol synthesis were measured in the liver and intestine using both direct in vitro and indirect in vivo techniques. As summarized in **Table 1**, groups of animals were pretreated with various diets, subjected to several operative procedures, and infused intraduodenally with nutrients and ^3H_2O. The experimental protocol used in these studies was identical to that used in later experiments designed to measure the contribution of newly synthesized DPS to mesenteric lymph cholesterol. At the termination of the 44–48-hr period of lymphatic diversion, the animals were killed and tissue slices were prepared from the intestine and liver. As shown in Table 1, both the prefeeding of a high cholesterol diet (Group C) and the infusion of chylomicrons (Group D) inhibited hepatic sterol synthesis without significantly affecting the rate of sterol synthesis in the intestine (compared to Group B). Surfomer (Group E), which blocks the uptake of cholesterol across the epithelial cell brush border, increased both intestinal and hepatic cholesterol synthesis, as did biliary diversion in animals also receiving whole bile intraduodenally (Group F). A much more pronounced stimulation of intestinal synthesis was observed after the intraduodenal infusion of triglyceride (corn oil) whereas the rate of hepatic synthesis was unaltered by this treatment (Group G). It should be noted that these changes in rates of cholesterol synthesis in the intestine and liver occurred under circumstances where the incorporation of [1-^{14}C]octanoate into CO_2 was unaltered.

The in vitro measurements shown in Table 1, however, were obtained only at one time point in the experiment and this was at the end of the 44- to 48-hr protocol. A second measurement was also made in these animals that, indirectly, better reflected what was happening to the rate of hepatic cholesterol synthesis throughout the last 24 hr of the experiment. As has been previously shown (2, 28), and as was especially true in these animals with external diversion of the intestinal lymph, the amount of newly synthesized sterol appearing in the plasma in vivo was

TABLE 1. Rates of cholesterol synthesis and CO_2 production by the intestine and liver in vitro and the content of [^{3}H]digitonin-precipitable sterols (DPS) in the plasma in vivo[a]

Experimental Group	n	Intestine		Liver		Plasma content of [^{3}H]DPS (nmol/ml)
		DPS (nmol/g per hr)	CO_2 (μmol/g per hr)	DPS (nmol/g per hr)	CO_2 (μmol/g per hr)	
A. Control, intact	5	79.8 ± 22.6	16.7 ± 0.8	1067.8 ± 93.6	8.8 ± 0.4	
B. Control, lymph fistula	5	96.1 ± 24.3	18.1 ± 2.2	1005.3 ± 103.9	8.0 ± 0.7	465.2 ± 50.9
C. Cholesterol-fed	4	89.1 ± 13.9	19.4 ± 1.5	475.7 ± 137.8[b]	6.9 ± 0.3	215.5 ± 101.2
D. Chylomicron-infused						
(High dose)	5	102.9 ± 20.9	17.2 ± 1.9	429.7 ± 52.4[b]	7.2 ± 0.3	162.2 ± 48.5
(Low dose)	6	71.5 ± 4.2	17.7 ± 1.2	785.6 ± 110.4	8.8 ± 0.5	516.3 ± 71.3
E. Surfomer-fed	4	136.4 ± 22.0	22.4 ± 1.4	1385.7 ± 103.5[b]	8.7 ± 0.2	688.9 ± 100.0
F. Biliary diversion	6	140.0 ± 36.8	20.4 ± 0.7	1588.6 ± 100.3[b]	8.4 ± 0.6	908.9 ± 109.7[b]
G. Corn oil-fed	5	386.1 ± 53.2[b]	18.9 ± 1.2	775.1 ± 116.8	9.8 ± 0.4	411.2 ± 106.1

[a]Groups of rats were fed a diet containing 10% corn oil overnight and fitted with a duodenal catheter (groups A–G) and a mesenteric lymph fistula (groups B–G) the following morning. Subsequently all animals were infused with a mixture of 50% FreAmine and 50% electrolyte/glucose solution (NaCl 77.1 mM, KCl 5.0 mM, K_2HPO_4 10.3 mM, and glucose 1100 mM, pH 7.4) for 44 to 48 hr at a rate of 2.4 ml/hr. Twenty-four hr after the surgery all animals were also begun on their infusions of ^3H_2O as described in the Materials and Methods section. Group C received a diet containing 2.0% cholesterol for 14 days before the start of the experiment. Group D was treated with a continuous infusion of chylomicrons which delivered a total of 66 mg (low dose) or 105 mg (high dose) of cholesterol through a femoral vein catheter over the 44- to 48-hr experimental period. Group E was fed 2.0% surfomer for 7 days before the surgery and received 0.54 g of this drug per day after the operation. Group F was fitted with an exteriorized biliary fistula and infused intraduodenally with whole bile (0.9 ml/hr). Group G was fitted with an additional duodenal catheter to allow separate infusion of 0.2 ml of corn oil per hr. At the termination of the 44- to 48-hr experimental period, blood was obtained from each animal and the plasma was assayed for its content of newly synthesized sterol, i.e., [^{3}H]DPS. Liver and intestinal slices were also prepared and incubated in vitro with [1-^{14}C]octanoate. The first four columns show the rates of incorporation of this substrate into DPS and CO_2 by the intestine and liver under these in vitro conditions. The last column gives the amount of DPS that was ultimately synthesized in the liver and then moved into the plasma under in vivo conditions. The data represent means ± 1 SEM for the number of animals shown.

[b]Significantly different from the control animals with a lymph fistula alone (group B) at the $P < 0.05$ level.

directly related to the rate of hepatic cholesterol synthesis (Table 1). As is apparent, the relative differences in the plasma concentration of [^{3}H]DPS found in vivo reflected very closely the relative differences in rates of hepatic cholesterol synthesis measured in vitro at the termination of the experiment.

The initial experiment designed to measure the contribution of newly synthesized sterol to the pool of cholesterol secreted in the intestinal lymph under control conditions is shown in **Fig. 1**. In this and all subsequent studies, animals were given ^3H_2O 20–24 hr after initiating intestinal lymphatic diversion, and the specific activity of the plasma water was kept constant throughout the following 24-hr period. During this interval lymph was collected at seven time points and the samples were analyzed for both total and newly synthesized sterol content (panel A), and the specific activity of lymphatic DPS was calculated (panel B). Except for a slight rise during the last 2 hr, the hourly rate of secretion of DPS in the lymph of control animals was essentially constant at approximately 650 nmol/hr (0.25 mg/hr). In contrast, the hourly secretion of newly synthesized DPS increased as a linear function of time throughout the experimental period ($y = 33 + 15x$, $r = 0.98$). As a consequence of this, fully one-third of the sterol present in the lymph at 24 hr was newly synthesized. When such studies were carried out for 72 hr (data not shown), the proportion of newly synthesized sterol in the lymph continued to increase until it essentially equalled the total content of cholesterol, i.e., essentially all sterol in the lymph was newly synthesized. As would be expected from these results, the specific activity of the lymph DPS also increased throughout the period of observation (panel B).

Under the conditions of this experiment, the [^{3}H]DPS in the lymph could have been *1*) synthesized in the intestine; *2*) synthesized in the liver, secreted in the bile, and absorbed by the intestine; or *3*) synthesized in the liver, exchanged into plasma lipoproteins, and filtered across the capillary bed of the intestine into the lymph. To discriminate among these various possibilities, the rate of appearance of newly synthesized sterol in intestinal lymph was measured in animals in which the rate of hepatic cholesterol synthesis was systematically suppressed by chylomicron infusion or by prefeeding dietary cholesterol.

Although it was not possible to completely suppress hepatic sterol synthesis (Table 1), a high dose of chylomicrons infused at a constant rate during the experiment did reduce the slope of the hourly secretion rate for lymphatic [^{3}H]DPS by 79% ($y = -8.3 + 3.0x$, $r = 0.99$). Thus, chylomicron infusion reduced the rate of increase of [^{3}H]DPS in lymph from approximately 15 nmol/hr per hr to only 3 nmol/hr per hr (Fig. 1). In contrast, the total mass of DPS secreted in lymph was not significantly different in the two groups.

Similar results were obtained in animals prefed a high cholesterol diet. Although the rate of sterol synthesis in the liver had already partially recovered 48 hr after stopping the cholesterol diet (Table 1), the rate of appearance of [^{3}H]DPS in the lymph was still markedly reduced ($y = -14 + 5.0x$, $r = 0.95$) below that seen in the control

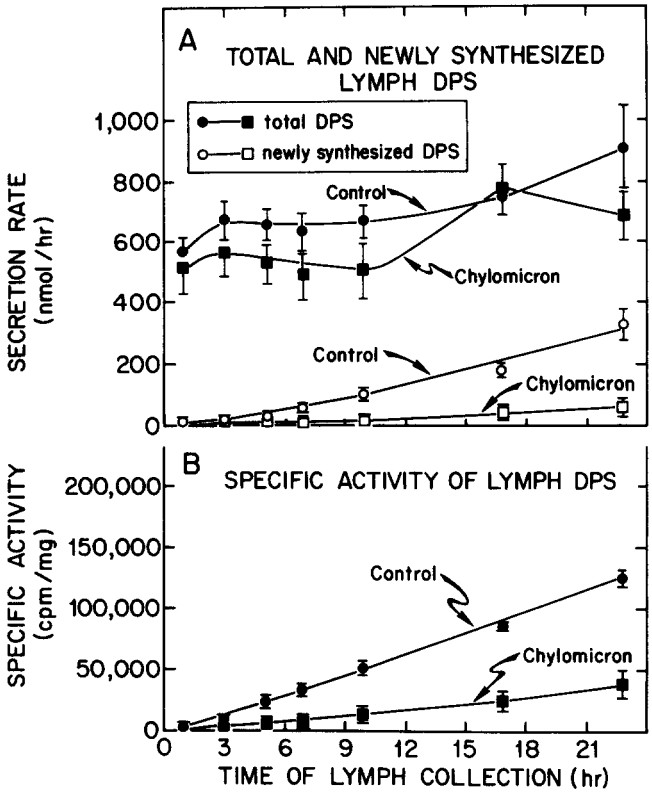

Fig. 1 Secretion of total and newly synthesized DPS (panel A) and the specific activity of DPS (panel B) in the mesenteric lymph of control animals and animals infused intravenously with chylomicrons. All animals received ^3H_2O as a bolus at time 0 and an intraduodenal infusion of amino acids and glucose as detailed in Materials and Methods. The chylomicrons were infused into the femoral vein at a constant rate for about 20 hr before the injection of the ^3H_2O and during the 24-hr lymph collection period. Each animal received a total of 105 mg of chylomicron cholesterol during the 44–48-hr infusion. The intravenous and intraduodenal infusates all contained ^3H_2O at the same specific activity as the plasma beginning at time 0. Lymph was collected at 2, 4, 6, 8, 12, 22, and 24 hr. The values represent the average hourly output at each time interval (panel A) or the specific activities of the DPS in the same samples (panel B). The data are given as the mean ± 1 SEM in seven animals (control group) or four animals (chylomicron group).

animals. Since both of these manipulations inhibited hepatic but not intestinal cholesterol synthesis, these results suggested that the liver, rather than the intestine, was the ultimate source for much of the newly synthesized sterol that was found in the intestinal lymph.

In order to give this observation more quantitative significance, the rate of increase in the appearance of [^{3}H]DPS in the lymph was plotted as a function of the amount of [^{3}H]DPS in the plasma of each animal in the control, chylomicron-infused, and cholesterol-fed experimental groups. It should again be emphasized that under the conditions of these experiments essentially all [^{3}H]DPS in the plasma was derived from de novo synthesis in the liver (2, 28). As shown in **Fig. 2**, the rate of increase of the appearance of [^{3}H]DPS in lymph was a linear function of the content of [^{3}H]DPS in the plasma and was completely independent of the rate of sterol synthesis in the intestine of these animals (Table 1). The correlation of the two parameters was highly significant and, therefore, consistent with two conclusions. First, the increment in appearance of [^{3}H]DPS in lymph was determined primarily by the rate of cholesterol synthesis in the liver and not in the intestine. Second, since the linear regression curve extrapolated essentially to zero when the content of [^{3}H]DPS in the plasma was zero, it could be further concluded that under these experimental conditions (where essentially no fat absorption was taking place), virtually no newly synthesized sterol would appear in the intestinal lymph if the rate of cholesterol synthesis in the liver were fully suppressed, even though sterol synthesis in the intestine was still taking place at very high levels.

If, as these results indicated, the [^{3}H]DPS in lymph was nearly all ultimately derived from the liver, then the newly synthesized cholesterol could have reached the intestine either through the bile or, alternatively, by filtration of plasma lipoproteins through the villus core. To better define its route into lymph, we took advantage of a newly developed, potent inhibitor of cholesterol absorption called surfomer. In preliminary experiments it was established that under the exact conditions of the current studies (see legend to Table 1) the drug reduced the recovery of intraduodenally administered [^{3}H]cholesterol during a 24-hr lymph collection from 48.3 ± 4.0% of the injected dose in controls to 11.2 ± 1.8% in surfomer treated rats ($n = 4$). As shown in **Fig. 3**, the drug also significantly suppressed the cumulative secretion of total lymph DPS at all times during the first 22 hr of the experiment by about 60% (panel A). There was a similar decrease in the rate at which [^{3}H]DPS appeared in the mesenteric lymph ($y = -14 + 6.7x$, $r = 0.94$). As is also apparent in Fig. 3, this effect tended to disappear during the last collection period since the last dose of surfomer was administered at the 8 hr time point. Since this drug decreased both the mass of sterol and the amount of [^{3}H]DPS in the lymph to about the same degree, the specific activity of mesenteric lymph cholesterol remained approximately the same as in control animals (panel B).

In order to more directly evaluate the importance of bile as a source of mesenteric lymph cholesterol, another group of animals was fitted with both biliary and mesenteric lymph fistulae. The animals also received 0.9 ml of unlabeled bile each hour, which contained 306 nmol of cholesterol, through a duodenal catheter. Diversion of the radiolabeled biliary sterol away from the intestinal lumen led to a similar decrease in the rate of appearance of [^{3}H]DPS in mesenteric lymph ($y = 4.3 + 5.5x$, $r = 0.98$) as did the administration of surfomer. It was concluded, therefore, that 63% of the newly synthesized sterol that appeared in lymph in the control animals was derived from the intestinal uptake of [^{3}H]DPS in the bile. This was consistent with the above finding that surfomer administration nearly completely blocked the absorption of

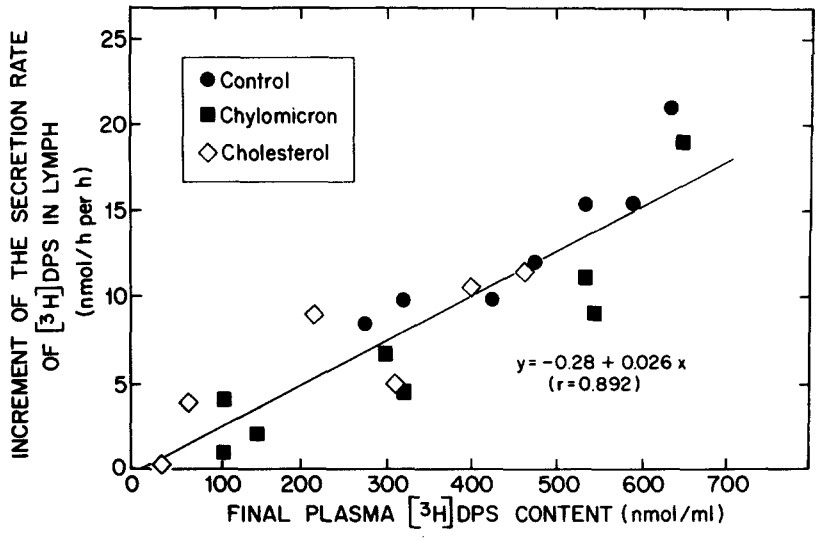

Fig. 2 Increment of the secretion rate of newly synthesized DPS in mesenteric lymph as a function of the final plasma content of newly synthesized DPS. The data were obtained in groups of animals under control conditions or after partial suppression of hepatic sterol synthesis by the infusion of chylomicrons (group D in Table 1) or the feeding of cholesterol prior to the experiment (group C in Table 1). The increment of the [^{3}H]DPS secretion rate is given as the slope of the linear rise during the 24-hr period as shown in Fig. 1. The final plasma [^{3}H]DPS content was determined at the end of the lymph collection period (24 hr after injection of the ^3H_2O) and is nearly all derived from the liver (28).

biliary cholesterol since the rate of increase in the appearance of [^{3}H]DPS in the lymph of the drug-treated and biliary-diverted animals was not significantly different (6.7 nmol/hr per hr and 5.5 nmol/hr per hr, respectively).

We next undertook studies to examine this transfer process under conditions where active triglyceride absorption and chylomicron formation were occurring. In addition to the lymph fistula and duodenal catheter for the infusion of the FreAmine/electrolyte solution, these animals were also fitted with a separate catheter for the intraduodenal infusion of corn oil at the rate of 0.2 ml per hr throughout the 44–48-hr experimental period. Intestinal sterol synthesis in these rats was increased 4-fold whereas liver synthesis and plasma [^{3}H]DPS content were essentially unaltered (Table 1). Under these experimental conditions the rate of appearance of [^{3}H]DPS (**Fig. 4**, panel A) as well as its specific activity (panel B) in mesenteric lymph were significantly higher than in the control group of animals during the 2- to 22-hr period (Fig. 4). During the 2–12-hr interval the average difference between the two groups was constant at 41 ± 2.7 nmol/hr. Since the plasma [^{3}H]DPS content was unchanged (Table 1) and total sterol absorption was certainly not increased (Fig. 4), this value very likely represented a minimal estimate of the contribution of sterol locally synthesized in the intestine to lymphatic cholesterol. As has been previously observed (34), the administration of this large amount of triglyceride into the intestinal lumen was associated with a decrease in the rate of appearance of total DPS in the mesenteric lymph (panel A).

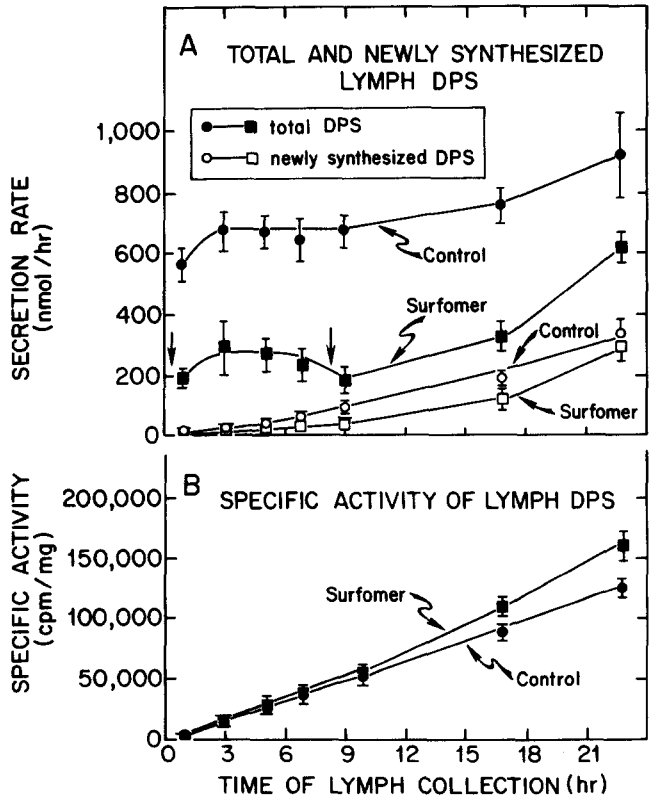

Fig. 3 Secretion of total and newly synthesized DPS (panel A) and the specific activity of DPS (panel B) in the mesenteric lymph of control animals and animals receiving surfomer (as detailed in Table 1). The two arrows indicate the time of administration of the surfomer emulsion. The experiment was performed exactly as described in the legend of Fig. 1. The surfomer group had four animals.

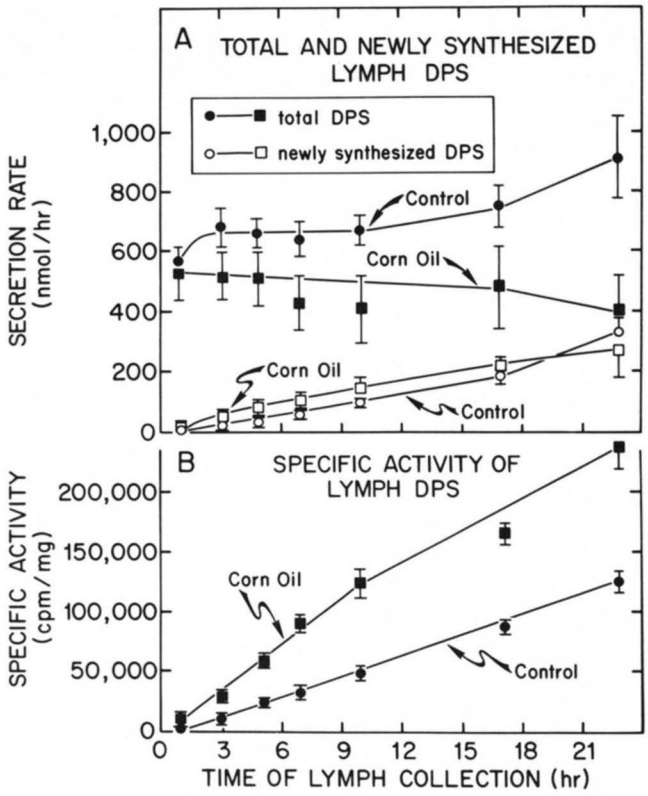

Fig. 4 Secretion of total and newly synthesized DPS (panel A) and the specific activity of DPS (panel B) in the mesenteric lymph of control animals and animals receiving intraduodenal corn oil at a rate of 0.2 ml/hr. The experiment was performed exactly as described as in Fig. 1. The corn oil group had six animals.

Finally, the fraction of lymph cholesterol secreted in the unesterified and esterified forms was determined both for total DPS and [^{3}H]DPS during the 12–22-hr interval. As shown in **Fig. 5**, and as previously reported (6), the majority of lymph DPS was found in the esterified fraction under most experimental conditions, although after the administration of corn oil the relative proportion of unesterified sterol increased considerably (34). As shown in panel B, the newly synthesized DPS present in lymph was esterified to essentially the same degree as total DPS in the various experimental situations.

DISCUSSION

It is now recognized that the intestinal mucosa is one of the major sites for cholesterol synthesis in most animal species (2) including man (12, 13). The highest rates of synthesis are found in the cells of the crypts and lower villi where the newly synthesized sterol presumably is required for the formation and differentiation of cell membranes (14). It is unclear, however, to what extent such newly synthesized cholesterol is incorporated into the lipoproteins of intestinal lymph (24, 35) and so reaches the miscible pools of body cholesterol. This question was addressed in the present study by administering to the experimental animals a priming dose of [^{3}H]water followed by a constant infusion, so that the specific activity of the pool of body water was constant over the period of observation. In this circumstance, the amount of newly synthesized cholesterol present in each organ increases essentially as a linear function of time (28, 30). Under these in vivo conditions [^{3}H]DPS synthesized in one organ may move into another compartment, either by the secretion of cholesterol-containing lipoproteins or by monomolecular flux. Depending upon the magnitude of the rate of cholesterol movement in the opposite direction, either of these processes can result in the net movement of [^{3}H]DPS mass across the cell membrane or, alternatively, only the exchange of a radiolabeled molecule for an unlabeled one.

Under the conditions of the present experiments, the [^{3}H]DPS in intestinal lymph could have been synthesized in the intestine itself or could have been synthesized elsewhere and reached the lymph only after being secreted or exchanged into the plasma or into the intestinal lumen. It was clearly possible to differentiate between these two possibilities, however, since, under these experimental

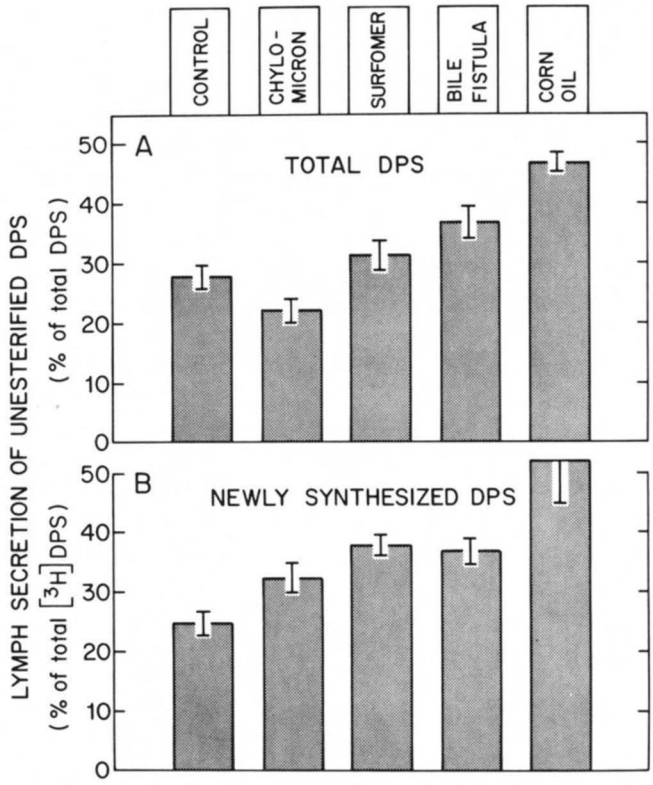

Fig. 5 Lymphatic secretion of unesterified DPS. The percentage of unesterified DPS is given with respect to total DPS mass (panel A) and to total newly synthesized DPS (panel B) in the 12–22-hr sample of mesenteric lymph from the animals treated as detailed in the legend of Table 1. The bars represent the mean ± 1 SEM.

conditions, essentially all [^{3}H]DPS appearing in the plasma and bile is derived from the liver (28, 30). The rate of secretion or exchange of radiolabeled cholesterol from the other extrahepatic organs into the plasma is very slow. Hence, by systematically altering the rates of hepatic cholesterol synthesis, it was possible to identify the principal source of the [^{3}H]DPS appearing in intestinal lymph under various experimental circumstances.

The first major point derived from these studies that should be emphasized concerns the kinetics of the appearance of newly formed DPS in the lymph and the dissociation of this rate of appearance from the rate of intestinal sterol synthesis under most experimental conditions. In control animals, the rate of [^{3}H]DPS appearance in lymph increased linearly with respect to time (Fig. 1) over the entire experimental period of 24 hr, whereas biliary secretion of newly formed sterol reaches an apparent plateau between 4 and 8 hr under similar experimental conditions (30). This latter finding was interpreted as evidence for rapid equilibration of newly synthesized cholesterol in the liver cell with a distinct subpool of sterol that supplies cholesterol for secretion in bile (30). Because of the difference in the kinetics of appearance, such a subpool could not be identified in the enterocyte, although this alone obviously does not rule out a contribution of the intestine to the newly synthesized cholesterol found in intestinal lymph.

However, when experimental interventions were used that directly altered the rates of cholesterol synthesis in either the liver or the small intestine, the appearance of newly formed sterol in lymph generally reflected the changes in the rates induced in the liver, but not in the intestine. For instance, selective inhibition of hepatic synthesis by dietary cholesterol or intravenous administration of chylomicrons (Table 1) significantly depressed the secretion of [^{3}H]DPS in lymph even though rates of synthesis in the intestine and other extrahepatic tissues (36, 37) were not affected. Alternatively, after administration of surfomer or after biliary diversion, secretion of [^{3}H]DPS in the lymph was again inhibited, even though in these instances the rates of intestinal sterol synthesis were actually marginally increased. Notably, the only instance in which there was a parallel change in the rate of mucosal sterol synthesis and the rate of appearance of [^{3}H]DPS in the lymph was after the intraduodenal infusion of corn oil. With this exception, these various findings indicated that the rate of sterol synthesis in the intestinal mucosa was not a major determinant of the rate of movement of [^{3}H]DPS into mesenteric lymph.

The second conclusion drawn from the present studies was that, in the absence of fat absorption, the liver was the only significant source of the [^{3}H]DPS appearing in lymph. This took place both through the uptake of biliary [^{3}H]DPS from the intestinal lumen and through transfer of [^{3}H]DPS from plasma lipoproteins to the lipoproteins in lymph. This conclusion is based upon the very close correlation that existed between the increment in the rate of secretion of [^{3}H]DPS in lymph and the final plasma [^{3}H]DPS content in the individual lymph fistula animals (Fig. 2) where nearly all plasma [^{3}H]DPS must have been derived from the liver (28). When the linear regression curve describing this correlation was extrapolated to the y-axis, the intercept was not significantly different from 0, suggesting that in the absence of plasma [^{3}H]DPS and, consequently, after total inhibition of hepatic, but not intestinal or peripheral, sterol synthesis, virtually no newly synthesized sterol appears in the lymph. Thus, even though the intestinal epithelium was actively synthesizing sterol, there was essentially no net contribution, or even exchange, of the [^{3}H]DPS into the intestinal lymph. This finding is consistent with the view that much of the sterol that is synthesized in the differentiating cells of the intestinal crypts is utilized for new membrane synthesis and is not readily incorporated into the miscible pool of cholesterol secreted into intestinal lymph (19).

Although these data essentially ruled out a significant contribution of the intestine (or peripheral tissues) to the newly formed sterol appearing in lymph under the circumstances of a fat-free diet, the route by which [^{3}H]DPS moved from the liver to the lymph was unclear. To evaluate quantitatively the respective contributions from the two possible pathways, we either blocked the uptake of luminal cholesterol by the administration of surfomer (Fig. 3) or diverted biliary [^{3}H]DPS from the intestinal lumen. Both procedures gave essentially the same result and suggested that more than 60% of the [^{3}H]DPS in lymph was derived from luminal cholesterol (primarily from bile), while the remainder presumably came directly from plasma lipoproteins (35). Such movement of [^{3}H]DPS could have represented net cholesterol uptake or molecular exchange into the lymph.

A third aspect of these studies that warrants emphasis is the specific role of dietary triglyceride in triggering increased synthesis and increased secretion of cholesterol into lymph. Feeding corn oil to rats has recently been shown to result in a pronounced stimulation of sterol synthesis in the mid and lower villus of the jejunum (19). The infusion of corn oil used in the present study has an even greater effect upon local synthesis (Table 1) and significantly enhanced the rate of appearance of [^{3}H]DPS in lymph above the background secretion rates observed in the control animals (Fig. 4). This increase was apparently not of hepatic origin since both liver sterol synthesis and plasma [^{3}H]DPS content were not changed, and the high dose of corn oil suppressed, rather than enhanced, the absorption of luminal cholesterol. Therefore, it seemed very unlikely that this increased rate of appearance of [^{3}H]DPS in lymph resulted from an increased rate of absorption of biliary cholesterol. Interestingly, the rate of [^{3}H]DPS secretion apparently reached a

plateau, relative to the background levels of [^{3}H]DPS in controls, suggesting rapid equilibration of locally formed sterol with the pool designated for lymph lipoprotein formation during active fat absorption. Taking into account the apparent decrease in the rate of absorption of biliary [^{3}H]DPS during the infusion of corn oil, the contribution of cholesterol newly synthesized in the small intestine to lymph cholesterol could be calculated to be about 0.043 mg/hr, which accounts for about 27% of the sterol in the final lymph sample in the corn oil-fed animals. Nevertheless, this amount is only a small proportion of the total sterol synthesized in the gut (0.26 mg/hr) (1), particularly when the nearly 5-fold stimulation of the rate of sterol synthesis is taken into account (Table 1).

Another interesting aspect of the secretion of locally formed sterol during fat absorption is that a high proportion of the [^{3}H]DPS was present in lymph in the unesterified form (Fig. 5). In all other instances, both newly synthesized and total cholesterol were secreted predominantly in the esterified form. However, in the presence of active triglyceride absorption and chylomicron formation, the need for surface (unesterified) cholesterol was apparently met by the increased rate of sterol synthesis induced in the villus cells (19).

Finally, the last point to be made concerns the sources of the sterol in the lymph under conditions where no fat absorption is taking place. Not surprisingly, manipulations of the rate of entry of biliary cholesterol into the gut lumen and the rate of sterol absorption across the intestinal mucosa gave comparable results in reducing the appearance of both total and newly synthesized DPS in the intestinal lymph. The administration of surfomer reduced the content of total sterols in the lymph by 62%; however, it was further shown that while this dose of the polymer markedly reduced the rate of absorption of biliary cholesterol (77%), this inhibition was not complete. Thus, from these data it can be further calculated that if cholesterol uptake from the intestine were totally blocked, the content of sterol in the intestinal lymph would fall by fully 80%. Thus, under circumstances where no dietary triglyceride or cholesterol entered the intestine and where only glucose and amino acid absorption were taking place, approximately 80% of the cholesterol in lymph presumably was derived from the intestinal lumen. The remainder of the lymphatic cholesterol probably came from plasma lipoproteins (35). We made no attempt, however, to further identify which lipoproteins in lymph carried the radioactive and unlabeled DPS because of the very large amounts of ^3H_2O that were in the samples and because of the rapid exchange of free cholesterol that is known to occur between different lipoprotein classes.

Thus, taken together, these studies confirm that much of the cholesterol that is synthesized in the intestine is apparently used for cell division and cell membrane differentiation (14, 19, 23). Under circumstances where there is no triglyceride absorption taking place, little newly synthesized sterol of intestinal origin can be detected in the intestinal lymphatic outflow. During active triglyceride absorption, however, the rate of sterol synthesis increases markedly in the intestinal absorptive cells and a portion of this newly synthesized sterol is incorporated into chylomicrons and other intestinal lipoproteins and delivered into the lymph. Thus, both the rate of sterol synthesis by the intestine and the rate of entry of this cholesterol into the body pools of sterols are partially dictated by the rate of triglyceride absorption. ■

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