Effect of Mixed Micellar Lipid on the Absorption of Cholesterol and Vitamin D_3 into Lymph

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ABSTRACT The absorption of endogenous cholesterol, labeled with tracer doses of cholesterol ^{14}C or cholesterol-^{3}H and of near physiological doses of vitamin D_3-^{3}H was studied in rats with cannulated intestinal lymphatics. The effects of administering mixed micellar solutions of fatty acid, monoglyceride, and bile salt on the absorption of these labeled sterols was determined. It was observed that the specific activity of free cholesterol and the amounts of vitamin D_3 appearing in lymph were significantly increased during the intraduodenal administration of mixed micellar solutions of either linoleic or palmitic acid, in contrast to control rats receiving a micellar solution of taurocholate. These increases were related linearly to the lymph triglyceride level. In addition it was observed that when the linoleic acid solution was administered there was a more marked increase in the ratio of the specific activities of free and esterified cholesterol in lymph than with either the palmitic acid or taurocholate solutions.

Additional studies in rats with intact lymphatics showed that the uptake of labeled cholesterol and vitamin D_3 from the intestinal lumen into the wall was similar whether the sterols were administered in taurocholate or in mixed micellar solution.

These findings suggest that mixed micellar lipid increased the rate of appearance of labeled free cholesterol and vitamin D_3 in lymph by enhancing their transport out of the intestinal mucosa, rather than by an effect on uptake.

INTRODUCTION

Cholesterol and vitamin D_3 are sterols of similar molecular structure, and both are absorbed from the small intestine primarily via the lymphatic route (1, 2). Their absorption differs, however, in that cholesterol is largely reesterified before entering the lymph (3), whereas vitamin D is absorbed mainly as the free sterol (2, 4). Although several groups of investigators have reported that the absorption of cholesterol is increased by dietary fat (5–8), these studies have often involved administration of unphysiologically large amounts of both fat and cholesterol. There is also some indirect evidence that dietary fat may enhance the absorption of vitamin D (9).

The means by which dietary fats influence sterol absorption have not been clearly determined. However, it has been suggested (10) that fat may enhance cholesterol absorption either by (*a*) a stimulatory effect on bile flow; (*b*) providing an increased supply of fatty acids for cholesterol esterification; or (*c*) promoting the formation of mixed micelles in the intestinal lumen, thus increasing the solubility of the sterol (11). The first and third of these possible mechanisms might also apply to vitamin D. However, to date there has been no clear definition of their relative importance.

The present study, therefore, was primarily designed to determine the effect of lipid absorption on the transport of labeled cholesterol and vitamin D_3 into intestinal lymph, under conditions such that the mucosal uptake of these sterols would not be affected by differences in their solubilities within the intestinal lumen. Trace quantities of the radioactive sterols, dissolved in solutions of either bile salt micelles or mixed micelles (fatty acid–monoglyceride-bile salt) were administered intraduodenally to rats with cannulated intestinal lymphatics. By this means intraluminal cholesterol was labeled, and the effect of exogenous fat upon the transport of endogenous cholesterol and exogenous vitamin D could be determined.

METHODS

Materials. Cholesterol-4-^{14}C, cholesterol-1,2-^{3}H, vitamin D_3-1,2-^{3}H, palmitic acid-1-^{14}C and linoleic acid-1-^{14}C were

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obtained from a commercial source.[1] All were found to be greater than 99% pure on thin-layer chromatography except for vitamin D_3-1,2-^{3}H which was 90–94% pure. When the purity fell below 90% it was repurified before use by methods previously described (12).

The following unlabeled materials were obtained commercially: palmitic acid,[2] oleic acid and linoleic acid,[3] monopalmitin,[4] monoolein,[3] and sodium taurocholate.[3, 5] The fatty acids were all more than 99% pure. The monoolein (glyceryl-1-monooleate) was only 90% pure, the remaining 10% consisting mainly of fatty acid and diglyceride on thin-layer chromatography. The sodium taurocholate contained traces of cholic acid but no deoxycholic acid on thin-layer chromatography.

Preparation of micellar solutions. Mixed micellar solutions were prepared by drying down benzene solutions of fatty acid and monoglyceride on a rotary evaporator and redissolving the lipid in 40 mM taurocholate as described by Johnston and Borgström (13). This solution was then diluted with an equal volume of 0.15 M saline–0.1 M phosphate buffer pH 7.0 (5:1, v/v), and the pH brought to 7.4 by the addition of 1 N sodium hydroxide. The resulting solutions contained 9.6 μmoles of fatty acid, equivalent to 2.46 mg of palmitic acid or 2.69 mg of linoleic acid, and 4.8 μmoles of monoglyceride per ml of 20 mM taurocholate. Labeled sterols were added to the benzene solution of fatty acid and monoglyceride in amounts sufficient to provide a single dose in 5 ml of micellar solution. Micellar solutions of taurocholate were prepared by a 1:1 v/v dilution of 40 mM taurocholate with buffered saline, pH 7.4. The taurocholate was presumed to be largely in the form of bile salt micelles, since 20 mM is considerably above the critical micellar concentration of 2–5 mM (14).

Characteristics of micellar solutions. All mixed micellar solutions were initially clear and the linoleic acid–monoolein solution remained so at 37°C. However, as the solutions containing the palmitic acid micelles cooled, they became turbid, presumably because of partial transition from a micellar to a crystalline phase (14). This was slightly more pronounced when monopalmitin was the monoglyceride in the system, and therefore monoolein was substituted for monopalmitin in some of the later experiments. Although the administered palmitic acid mixtures thus contained some crystalline material, they will be designated as "mixed micelles" for the sake of brevity. For convenience linoleic acid–monoolein mixed micelles will henceforth be referred to as linoleic acid mixed micelles, and palmitic acid–monopalmitin and palmitic acid-monoolein mixed micelles will be referred to collectively as palmitic acid mixed micelles.

To assess the distribution of sterol and fatty acid between the micellar and crystalline phases, linoleic acid mixed micelles and palmitic acid mixed micelles were prepared containing tracer amounts of cholesterol-1-2-^{3}H and the respective fatty acid-^{14}C. These solutions were allowed to equilibrate at 37°C, and 1 ml amounts were then passed through 10 mμ and 50 mμ Millipore filters,[6] each in triplicate. The filters were washed with 2 ml of 5 mM taurocholate, transferred to counting vials, and dissolved in 12 ml of a dioxane scintillation solution for radioassay (see below). The results showed that in the case of the linoleic acid mixed micelles 83.3 and 86.7% of the labeled linoleic acid and 82.1 and 90.0% of the cholesterol passed through 10 and 50 mμ filters, respectively. By contrast, only 58.0 and 60.1% of the labeled palmitic acid in palmitic acid mixed micelles passed through these filters. This finding suggested that over 40% of the palmitic acid was in a nonmicellar form, since mixed micelles are approximately 5–10 mμ in diameter (15). However, there was no hold-up in the passage of the labeled cholesterol in palmitic acid micelles, since 90.5 and 92.5% passed through the 10 mμ and 50 mμ filters, respectively. This finding suggested that the labeled cholesterol had a greater affinity for the micellar phase than for the crystalline phase under the present conditions.

Doses of labeled sterols. Cholesterol-4-^{14}C (0.1μc) was used in tracer amounts to label the endogenous cholesterol within the intestinal lumen. Vitamin D_3-1,2-^{3}H (0.2 μc) was given in a dose of 5 mμmoles, equivalent to approximately 2 μg or 80 IU of vitamin D_3. These two isotopes were usually given together. On occasion cholesterol-1-2-^{3}H (0.5 μc) was given alone to rats who had received cholesterol-4-^{14}C the previous day. In some instances the absorption of fatty acid from the mixed micellar solutions was measured using tracer amounts of palmitic and linoleic acids-^{14}C (0.1 μc).

Operative procedures. Nonfasted adult male rats of the CD strain,[7] weighing 300–350 g, having previously been on a standard diet,[8] were anesthetized with intraperitoneal pentobarbital. The intestinal lymphatic duct was cannulated before its entry into the cisterna chyli by a modification of the technique of Bollman, Cain, and Grindlay (16). Postoperatively the rats were placed in restraining cages (17) and allowed free access to 5% dextrose in isotonic saline. Lymph was collected overnight and the volume recorded. The majority of rats undergoing studies of sterol absorption were used on the 1st postoperative day. In four rats studies were carried out on the 2 consecutive days; the results did not differ significantly between the 1st and 2nd postoperative days.

In some studies, rats were prepared with intraduodenal catheters but *without* lymph fistulas. In all other respects handling of the animals was identical.

Absorption studies. Lymph fistula rats were allowed access to dextrose-saline during the basal 1 hr collection of lymph on the morning of the study. At the conclusion of this hour all oral fluids were temporarily withdrawn.

Labeled sterols were administered in 5 ml of 20 mM taurocholate or mixed micelles. These were given by intraduodenal infusion during the 30 min period after the basal hour and then washed in with 0.5 ml of 5 mM taurocholate. 30 min later a second infusion of 5 ml of taurocholate or mixed micelles, containing no labeled sterols, was commenced and maintained slowly over the remainder of the 3-hr period. In a few early experiments, the labeled sterols were administered in 1 ml of 5 mM taurocholate after the conclusion of an initial 5 ml infusion of unlabeled micelles. Sterol absorption data obtained with these two experimental techniques was similar.

Animals without lymph fistulas but with intraduodenal catheters received only the initial 5 ml infusion over a 30 min period. 30 min after the end of the infusion, the animals were sacrificed, the small intestine was excised and washed with 40 ml saline, and then divided into proximal and distal halves. After homogenization lipid was extracted with chloroform: methanol (2:1, v/v) by the method of Folch,

[1] New England Nuclear Corp., Boston, Mass.
[2] Hormel Institute, Austin, Minn.
[3] Calbiochem, Los Angeles, Calif.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] K&K Laboratories, Plainview, N. Y.
[6] Millipore Filter Corp., Bedford, Mass.
[7] Charles River Breeding Laboratories, North Wilmington, Mass.
[8] Purina Lab Chow, Ralston-Purina Co., Davenport, Iowa.

Lees, and Sloane-Stanley (18). The chloroform phase was dried, redissolved in 5 ml toluene or hexane, and 0.5 ml taken for radioassay.

Collection of samples. Lymph was collected on ice in heparinized containers during the 3 hr period after the dose of labeled sterol. At the end of this period rats were allowed access to fluid ad lib. Further collections of lymphs were made from 3 to 6 hr and from 6 to 24 hr. (The lymph flow during these experiments averaged about 2–3 ml/hr.)

In some instances lymph-fistula rats were then sacrificed, their intestinal tract removed, and its radioactivity determined, together with that of its contents and feces, as was described above. The small and large intestinal contents were homogenized separately with saline in a Waring blendor. The total volume of each was recorded and 0.5 ml taken for radioassay.

Measurement of radioactivity. Radioactivity was measured in a Beckman LS-200 liquid scintillation counter using an external standard ratio to correct for quenching. Lymph samples were assayed for radioactivity by adding 0.2–0.5 ml to 12 ml of a scintillation solution consisting of 2,5-diphenyloxazole (PPO), 7.0 g, *p*-bis[2-(5-phenyloxazolyl]benzene (POPOP), 0.05 g, naphthalene, 50 g, and 95% ethanol, 154 ml/liter of dioxane. This procedure gave identical results in terms of disintegrations per minute per milliliter of lymph with those obtained by extracting 1 ml of lymph with chloroform: methanol and then counting the dried chloroform phase in 15 ml of a liquid scintillation solution consisting of PPO, 4 g, and POPOP, 0.05 g/liter of toluene. The degree of quenching with lymph in the dioxane system was similar to that of an equal volume of water. Doses of labeled sterol were counted routinely by adding 0.5 ml to the dioxane scintillation solution, and this procedure was also used to count intestinal content. Any other lipid-soluble radioactivity was counted in the toluene scintillation solution.

Nature of radioactivity in lymph. After administration of labeled cholesterol and vitamin D, most radioactivity appeared in lymph between 0 and 6 hr. Lymph samples taken during this period were extracted and then chromatographed on thin-layer plates of silicic acid, with chloroform as the solvent. Markers of free and ester cholesterol and vitamin D_3 were run at the same time. At the conclusion the marker strips were sprayed with 30% sulphuric acid and heated. The plate was divided horizontally into 1 cm strips, and the silica gel was scraped into counting vials and counted in 15 ml of toluene scintillation solution.

With cholesterol-4-^{14}C, it was found that 96.7% of the radioactivity recovered had moved similarly to either free cholesterol (19.4%) or cholesterol ester (77.3%), the remaining 2.4% staying at the origin. With vitamin D_3-1,2-^{3}H, 77.8% of the radioactivity recovered had moved as vitamin D_3, 5.9% was located in the sterol ester region, 5.3% remained at the origin, and 8.7% ran between the origin and the free sterol zone, i.e., was more polar than vitamin D.

Analytical techniques. Lymph triglycerides were determined by the method of van Handel and Zilversmit (19). Lymph total cholesterol was determined by a modification of the method of Zlatkis, Zak, and Boyle (20). Samples of whole lymph were first saponified for 1 hr in potassium hydroxide in 50% ethanol, v/v at 60°C; the sterols were subsequently extracted with hexane, dried, and dissolved in glacial acetic acid for assay. By this technique saponifiable lipids which interfered with the color determination were removed.

Lymph free and ester cholesetrol were determined as follows: 1-ml samples of lymph were extracted with chloroform: methanol (18). The chloroform phase was separated, dried, and redissolved in hexane. 2 g of silicic acid, Bio-Sil BH 100-200 mesh,[9] were slurried in hexane and poured into 12 × 0.5 inch glass columns. The lymph lipid extract was then loaded onto the column and eluted first with 30 ml of 4% diethyl ether in hexane (fraction 1), followed by 30 ml of 100% diethyl ether (fraction 2). By use of markers of cholesterol-4-^{14}C and cholesterol-7α-^{3}H palmitate,[1] the latter being purified by thin-layer chromatography beforehand, it could be shown that fraction 1 contained 98.6% of the cholesterol ester recovered, the total recovery being 98.6%, and that fraction 2 contained 99.1% of the free cholesterol recovered, the total recovery being 98.8%.

Each fraction was dried down on a rotary evaporator and redissolved in 10 ml hexane, of which 5 ml were taken for chemical determination of cholesterol. The remaining 5 ml were dried and dissolved in 15 ml of toluene scintillation solution for radioassay. Chemical determination of the free and ester cholesterol fractions were then carried out as previously described.

Statistical methods. Differences between the three groups of lymph fistula rats have been calculated by the analysis of variance, using the statistic F.

$$F = \frac{\text{variance between groups}}{\text{variance within groups}}$$

The P value which corresponds to the value of F indicates the probability that the groups were drawn from one homogeneous population. Where the value of P is low this suggests that one or more of the groups differs significantly from the others; which groups differed could usually be determined by inspection of the means.

RESULTS

Uptake of labeled cholesterol. The effect of taurocholate micelles or mixed micellar solutions on the uptake of a tracer dose of cholesterol-^{14}C in rats without lymph fistulas was studied. In Table I it will be seen that the amount of radioactivity recovered from the intestinal contents at 1 hr was least in rats given taurocholate (7.9%) and was slightly higher in both groups given mixed micelles (11.4 and 11.0%). The amount of radioactivity in the small intestinal wall was similar in all three groups, mean values ranging between 69.4 and 76.5%. The over-all mean recovery of radioactivity was also similar in the three groups, ranging from 80.8 to 84.8%. These results indicate that at the end of 1 hr the uptake of labeled cholesterol from the intestinal lumen was no greater from mixed micelles than from taurocholate micelles. The distribution of radioactivity between proximal and distal halves of intestine was also similar in all three groups, 90.2–92.1% of the radioactivity in the wall being in the proximal half.

Uptake of labeled vitamin D_3. As indicated in Table II, the amount of radioactivity remaining within the intestinal lumen at 1 hr was similar in the three groups, mean values ranging between 16.1 and 20.9%. These values were higher than those for cholesterol.

[9] Bio-Rad Laboratories, Richmond, Calif.

TABLE I

Effect of Taurocholate Micelles or Mixed Micelles on the Uptake of a Tracer Dose of Cholesterol ^{14}C at 1 hr

Micelles‡	Per cent of administered radioactivity*		
	Small and large intestinal contents	Small intestinal wall	Total recovery
Taurocholate	7.9 (6.5– 9.2)	76.5 (75.5–78.5)	84.4 (82.1–87.7)
Linoleic acid	11.4 (9.4–12.8)	69.4 (64.3–75.8)	80.8 (77.1–85.2)
Palmitic acid	11.0 (10.5–11.8)	73.8 (72.1–75.6)	84.8 (83.9–86.3)

* Mean and range. There were three rats in each group.

‡ Taurocholate micelles contained 20 mM taurocholate. Linoleic acid mixed micelles contained linoleic acid and monoglyceride in 20 mM taurocholate. Palmitic acid mixed micelles contained palmitic acid and monoglyceride in 20 mM taurocholate.

Both the over-all recovery of radioactivity and that recovered from the intestinal wall at 1 hr were highest in the taurocholate group. Luminal radioactivity was similar in all three groups. These results suggest that although uptake of vitamin D_3 was similar among the three groups, there was an increased transport of vitamin D out of the intestinal wall in rats given mixed micelles, as compared to those which received taurocholate micelles. The distribution of radioactivity between proximal and distal halves of intestine was also similar in the three groups, 76.0–78.5% of the radioactivity in the wall being in the proximal half.

Lymph triglycerides. The effect of the intraduodenal infusion of taurocholate micellar or mixed micellar solutions on lymph triglycerides is shown in Table III. The amount of triglyceride present during the basal hour varied somewhat, the mean values for the three groups of rats being 3.6, 6.4, and 7.2 mg/hr.

The administration of 10 ml of 20 mM taurocholate over 3 hr had no significant effect on the level of the lymph triglycerides. However, the administration of mixed micellar solutions caused the expected increase in triglycerides during the 0–3 hr and 3–6 hr periods. In the case of linoleic acid mixed micelles the level rose to 12.7 mg/hr between 0 to 3 hr, decreasing to 9.0 mg/hr between 3 and 6 hr. The levels after the administration of palmitic acid mixed micelles were not as high, being 10.0 mg/hr between 0 to 3 hr and 6.3 mg/hr between 3 to 6 hr. This apparent difference between the latter two groups may have been due in part to the lower basal triglyceride level in rats given palmitic acid mixed micelles. In all three groups the lymph triglycerides returned to basal levels between 6 and 24 hr.

Absorption of labeled fatty acid into lymph. As shown in Table III the administration of palmitic acid mixed micelles resulted in lower lymph triglyceride levels than did administration of linoleic acid mixed micelles. In an attempt to further examine these differences 5 ml of mixed micellar solutions labeled with tracer doses of fatty acids-^{14}C were administered intraduodenally to lymph fistula rats. In two rats given linoleic acid-^{14}C mixed micelles 82.3 and 83.3% of the linoleic acid was absorbed into the lymph in 3 hr. By comparison, in four rats given palmitic acid-^{14}C mixed micelles the absorption of palmitic acid ranged from 46.4% (palmitic acid–monoolein) to 57.4% (palmitic acid–monopalmitin). These results indicate that palmitic acid was less completely absorbed into lymph than linoleic acid during

TABLE II

Effect of Taurocholate Micelles or Mixed Micelles on the Uptake of a 5 mμmole Dose of Vitamin D_3-^3H at 1 hr

Micelles	Per cent of administered radioactivity*		
	Small and large intestinal contents	Small intestinal wall	Total recovery
Taurocholate	19.9 (16.2–23.0)	59.2 (57.1–62.3)	79.2 (78.5–80.1)
Linoleic acid	20.9 (17.7–23.2)	45.8 (42.0–52.6)	66.8 (63.9–70.3)
Palmitic acid	16.1 (12.9–20.3)	52.9 (47.9–56.4)	69.0 (68.2–69.8)

* Mean and range. There were three rats in each group.

TABLE III

Effect of Intraduodenal Infusion of Taurocholate Micelles or Mixed Micelles on Lymph Triglycerides

Micelles	Lymph triglycerides*			
	Basal hr‡	0–3 hr	3–6 hr	6–24 hr
	mg/hr			
Taurocholate (n = 7)§	6.4 ±5.8	5.2 ±2.2	4.1 ±1.9	4.5 ±0.9
Linoleic acid (n = 7)	7.2 ±2.0	12.7 ±3.4	9.0 ±3.1	4.2 ±1.0
Palmitic acid (n = 11)	3.6 ±1.8	10.0 ±2.5	6.3 ±1.5	5.0 ±1.5

* Mean ±standard deviation.

‡ Hour preceding start of experiment. The large SD in the taurocholate group was due to one rat whose basal triglyceride was 19.2 mg in 1 hr.

§ n = number of rats in each group.

TABLE IV

Effect of Taurocholate Micelles or Mixed Micelles on the Total Cholesterol of Intestinal Lymph

Micelles	Lymph total cholesterol*			
	Basal hr	0–3 hr	3–6 hr	6–24 hr
			mg/hr	
Taurocholate (n = 4)‡	0.51 (0.34–0.61)	0.54 (0.42–0.64)	0.51 (0.41–0.61)	0.43 (0.34–0.50)
Linoleic acid (n = 4)	0.51 (0.38–0.57)	0.59 (0.51–0.69)	0.59 (0.50–0.77)	0.49 (0.33–0.60)
Palmitic acid (n = 5)	0.48 (0.37–0.53)	0.59 (0.53–0.76)	0.59 (0.51–0.76)	0.55 (0.43–0.71)

* Mean and range.
‡ n = number of rats in each group.

the 3 hr after their administration in mixed micelles. Furthermore, although the substitution of monoolein for monopalmitin as the monoglyceride in palmitic acid mixed micelles resulted in a clearer solution, this did not enhance absorption of the fatty acid.

Lymph total cholesterol. The levels of total cholesterol in lymph, determined *chemically,* are shown in Table IV. The mean values ranged from 0.43 to 0.59 mg/hr. Although administration of linoleic acid and palmitic acid mixed micelles resulted in slightly higher cholesterol levels between 0 and 6 hr than did the administration of taurocholate micelles, these differences were not significant.

The effect of intraduodenally administered micelles on the amount of *labeled* cholesterol appearing in lymph is shown in Table V. Analysis of variance showed that there were no significant differences among the three groups during any of the time periods studied.

TABLE V

Effect of Taurocholate Micelles or Mixed Micelles on the Appearance of Labeled Cholesterol in Lymph

Micelles	Per cent of administered radioactivity			
	Lymph*			Small intestinal wall‡
	0–3 hr	3–6 hr	0–24 hr	24 hr
Taurocholate	15.7 ±4.4 (n = 7)§	13.1 ±2.1 (n = 7)	55.8 ±9.9 (n = 7)	12.6 (9.6–19.7) (n = 4)
Linoleic acid	15.1 ±2.4 (n = 9)	12.9 ±3.4 (n = 7)	54.3 ±5.5 (n = 7)	10.5 (6.7–16.5) (n = 4)
Palmitic acid	17.3 ±2.0 (n = 11)	15.0 ±2.3 (n = 10)	59.3 ±4.4 (n = 9)	13.8 (9.4–17.2) (n = 3)
Analysis of variance	$F = 1.61$ $P > 0.05$	$F = 1.69$ $P > 0.05$	$F = 1.26$ $P > 0.05$	—

* Mean ± standard deviation.
‡ Mean and range. Mean total recovery of radioactivity at 24 hr (including large intestinal contents and feces) was 83.1% (n = 7).
§ n = number of rats.

TABLE VI

*Specific Activity of Lymph Free and Esterified Cholesterol**

Micelles	Per cent of administered radioactivity per milligram of cholesterol‡			
	0–3 hr		3–6 hr	
	Free	Ester	Free	Ester
Taurocholate (n = 4)§	4.9 ±1.4	10.9 ±3.0	4.7 ±1.5	10.2 ±2.2
Linoleic acid (n = 4)	8.3 ±0.4	9.5 ±1.0	6.2 ±1.3	10.5 ±1.0
Palmitic acid (n = 5)	6.8 ±1.7	11.3 ±2.6	5.5 ±0.4	10.1 ±1.9
Analysis of variance	$F = 4.51$ $P < 0.05$	$F = 0.48$ $P > 0.05$		

* n = number of rats.
‡ Mean ±standard deviation.
§ The specific activity of each fraction has been calculated as:

$$\frac{\text{lymph radioactivity (free or esterified cholesterol)}}{\text{total administered radioactivity}} \times 100$$

$$\frac{}{\text{lymph chemical cholesterol (free or esterified) milligram}}$$

= percent of administered dose per milligram.

The amount of radioactivity remaining in the wall of the small intestine at 24 hr after the dose was also similar in the three groups, mean values varying between 10.5 to 13.8% of the administered dose. The mean overall recovery of radioactivity at 24 hr from lymph, intestinal wall, and intestinal contents was 83.1%.

Specific activity of lymph free and esterified cholesterol. The specific activities of free and esterified cholesterol in lymph between 0 to 3 hr and 3–6 hr are shown in Table VI. The mean values for free cholesterol between 0 to 3 hr were 4.9, 8.3, and 6.8%/mg respectively, in rats given taurocholate, linoleic acid, and palmitic acid micelles. These differences were significant ($P < 0.05$). The corresponding values for the 3–6 hr period were 4.7, 6.2, and 5.5%/mg, these differences being insignificant.

The specific activity of cholesterol ester between 0 to 3 hr was 10.9, 9.5, and 11.3%/mg in rats given taurocholate, linoleic acid, and palmitic acid micelles. These differences were not significant. The corresponding values for the 3–6 hour period were 10.2, 10.5, and 10.1%/mg.

The relationship between the lymph triglyceride content and the specific activity of lymph free cholesterol between 0 to 3 hr is shown in Fig. 1. It will be seen that there was a significant degree of correlation between the two variables ($r = 0.64$, $P < 0.02$). This relationship was independent of changes in lymph flow, since the slope of the regression of the ratio free cholesterol: tri-

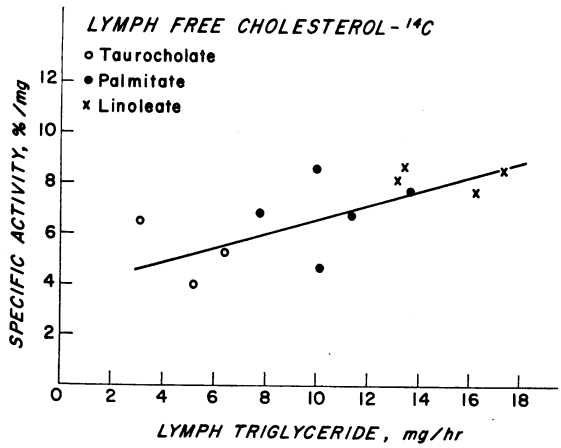

FIGURE 1 Effect of intraduodenal micelles on the specific activity of free cholesterol-^{14}C in lymph (per cent of administered radioactivity per milligram of free cholesterol) at 0–3 hr ($r = 0.64$, $P < 0.02$).

glyceride plotted against lymph flow did not differ significantly from zero.

Ratio of specific activity of free: esterified cholesterol. The ratio of the specific activity of free vs. esterified cholesterol in lymph was calculated for each of the three groups of rats (see Fig. 2). The 0–3 hr values were 0.45 ±0.03 for taurocholate, 0.87 ±0.06 for linoleic acid, and 0.60 ±0.02 for palmitic acid. The analysis of variance showed that there were very significant differences between the free: ester ratios in the three groups ($F = 132.5$, $P < 0.01$), with the highest values occurring in the linoleic acid group. These differences were no longer significant during the 3–6 hr period, the corresponding ratios being 0.46 ±0.10, 0.57 ±0.07, and 0.56 ±0.08 ($F = 2.69$, $P > 0.05$).

Transport of vitamin D_3 into lymph. The effects of taurocholate and mixed micelles on vitamin D_3 transport into lymph are shown in Table VII. The rats given

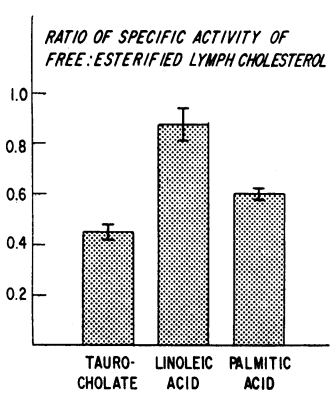

FIGURE 2 Effect of intraduodenal micelles on the ratio of specific activity of free: esterified cholesterol in lymph at 0–3 hr.

TABLE VII

Effect of Taurocholate Micelles or Mixed Micelles on the Appearance of Vitamin D_3-^3H in Lymph

Micelles	Per cent of administered radioactivity: Lymph* 0–3 hr	Lymph* 3–6 hr	Lymph* 0–24 hr	Small intestinal wall‡ 24 hr
Taurocholate (n = 7)§	16.3 ±3.1	11.0 ±1.4	42.9 ± 6.4	3.2 (1.3– 6.3) (n = 4)
Linoelic acid (n = 7)	27.7 ±8.0	12.0 ±2.4	53.9 ±10.8	4.7 (0.8–12.0) (n = 4)
Palmitic acid (n = 8)	26.5 ±5.2	12.3 ±3.1	53.4 ±7.3	2.0 (1.8–2.9) (n = 4)
Analysis of variance	F =8.52 P <0.01	F =0.63 P >0.05	F =3.96 P <0.05	

* Mean ± standard deviation.
‡ Mean and range. Mean total recovery of radioactivity at 24 hr (including large intestinal contents and feces) was 74.2% (n = 8).
§ n = number of rats.

taurocholate absorbed 16.3% of the administered radioactivity into lymph within 3 hr. By contrast those given linoleic acid mixed micelles and palmitic acid mixed micells absorbed 27.7 and 26.5%, respectively; this increase over the taurocholate group was highly significant ($P < 0.01$). This difference had completely disappeared after discontinuing the infusion of micelles. The relationship between the amounts of triglyceride and vitamin D_3 in lymph is shown in Fig. 3. Just as with labeled free cholesterol, there was a significant correlation between lymph triglyceride and the amount of vitamin D_3 appearing in lymph ($r = 0.58$, $P < 0.01$). This relationship was also independent of lymph flow since the slope of the regression of the ratio vitamin D: triglyceride plotted against lymph flow did not differ significantly from zero.

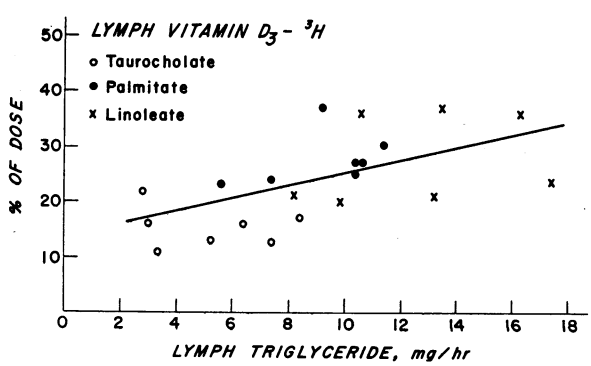

FIGURE 3 Effect of intraduodenal micelles on appearance of vitamin D_3-^3H in lymph at 0–3 hr ($r = 0.58$, $P < 0.01$).

The over-all absorption of vitamin D_3 between 0 and 24 hr was 42.9% for the taurocholate group compared to 53.9 and 53.4% for rats given mixed micelles. Although these differences were significant ($P < 0.05$), they simply reflected the changes which had occurred between 0 and 3 hr.

The amount of lipid-soluble radioactivity remaining in the wall of the small intestine at 24 hr was similar in all three groups, mean values ranging from 2.0 to 4.7% of the administered dose. These values were lower than those for cholesterol. The over-all recovery of radioactivity at 24 hr from lymph, intestine, and intestinal contents in rats given vitamin D_3-^3H was 74.2%.

DISCUSSION

This study demonstrates that the absorption of cholesterol and vitamin D_3 in the rat can be influenced by the presence and simultaneous absorption of relatively small quantities of micellar lipid. It was shown that the intraduodenal administration of mixed micellar solutions of either linoleic acid or palmitic acid resulted in a significant increase in the specific activity of lymph free cholesterol, as compared to controls receiving taurocholate alone. This increase in the specific activity of free cholesterol was linearly correlated with a rise in lymph triglycerides and occurred during the 3 hr period of infusion of mixed micelles. In contrast to lymph free cholesterol there was no significant difference in the specific activity of esterified cholesterol between the three groups of rats.

In all three groups there was a considerable degree of variation in the specific activity of both free and esterified cholesterol in the lymph of individual rats. In the case of free cholesterol, these variations were associated with differences in lymph triglyceride levels. However, there was remarkably little variation among the rats within each group when the specific activities of free and esterified cholesterol were expressed as a ratio, although there were marked differences between each of the three groups. These differences were present only during the actual period of administration of taurocholate or mixed micelles: the highest ratios occurred with linoleic acid micelles. This ratio of free: esterified cholesterol appeared to be independent of any quantitative changes in the lymph triglycerides.

It is well recognized that there is considerable exchange of free cholesterol which occurs across cell membranes, including the intestinal wall (11), and this factor may well have accounted for a significant portion of the radioactivity in lymph. However, the administration of mixed micellar solutions increased the appearance in lymph not only of labeled free cholesterol but also of vitamin D, and both to a similar extent. In the case of vitamin D, this increase almost certainly represented enhanced absorption rather than exchange, since the mucosal pool of vitamin D is relatively small. This suggests that exchange was not the sole explanation for the cholesterol findings, but that increased absorption of labeled free cholesterol probably also occurred. The fact that there was no corresponding increase in the 0–3 hr lymph total cholesterol probably reflects (*a*) the relatively small amount of intraluminal cholesterol labeled during the initial 30 min period of micellar administration; (*b*) the fact that only about one-third of cholesterol in lymph is free; (*c*) that lymph cholesterol is partly derived from sources other than the intestinal lumen (21).

The changes observed among the three groups in the specific activity of lymph free cholesterol were unlikely to have been due to differences in the specific activity of luminal cholesterol, since in other experiments biliary cholesterol, a major component of endogenous luminal cholesterol, was found in similar amounts in animals receiving taurocholate micelles or mixed micelles (unpublished observations). The finding of a higher specific activity of esterified cholesterol in lymph compared to free cholesterol has also been observed by Swell, Trout, Hopper, Field, and Treadwell (22), who found that a similar relationship existed between free and esterified cholesterol within the intestinal wall. These authors considered that the lower specific activity of free cholesterol was due to its greater dilution by endogenous cholesterol within the mucosa. This and other evidence (23) supports the concept that there may be more than one pool of cholesterol within the intestinal wall. During the present study it was found that the ratio of the specific activity of free: esterified cholesterol was higher after the administration of linoleic acid than after taurocholate or palmitic acid. One possible explanation for this effect of linoleic acid is that it somehow increased the proportion of labeled cholesterol which mixed with the free cholesterol pool within the intestinal wall.

The dose of radioactive vitamin D_3 used to study vitamin D absorption was approximately 100 IU, which is somewhat high to be strictly physiological in the rat but is less than has been used in most previous studies of absorption. A similar effect was demonstrated in the case of vitamin D_3 as for free cholesterol, in that both linoleic and palmitic acid mixed micelles caused a significant increase in 0–3 hr lymph radioactivity, this increase again being correlated with lymph triglyceride levels. The over-all increase in the 24 hr absorption of vitamin D_3 in these rats suggested that the rate of transport into lymph during the initial phase of absorption determined the total amount that was eventually absorbed. It is noteworthy that over 70% of the absorbed vitamin D_3 was transported into lymph as unaltered free

sterol, while most of the remaining radioactivity was more polar and presumably represented metabolites (24).

The intraduodenal infusion of 10 ml of 20 mM taurocholate over 3 hr did not cause any change in lymph triglycerides, although it has been shown that larger amounts of taurocholate may cause an increase in lymph triglycerides (25). Although the amount of taurocholate infused was relatively large in comparison with the total bile acid pool of the rat, this was necessary in order to dissolve an adequate amount of lipid in micellar solution. Furthermore, since the control group of rats received the same amount of taurocholate as the rats given mixed micelles it is unlikely that any differences between the groups were due to this factor. The dose of micellar lipid administered to the lymph fistula rats over the course of 3 hr was equivalent to about 40 mg of triglyceride, an amount comparable to that likely to be consumed by adult rats on a normal diet. Lymph triglycerides were found to be higher after linoleic acid mixed micelles than after palmitic acid mixed micelles, although this may well have been due to the higher basal level in the rats given linoleic acid mixed micelles. The fact that tracer doses of linoleic acid were more rapidly absorbed than tracer doses of palmitic acid from their respective mixed micellar solutions could have been due to a lower proportion of the palmitic acid being in micellar form, as suggested by the Millipore experiments (see Methods). However, other workers have found that the monounsaturated oleic acid, even when given as an emulsion, is better absorbed than palmitic acid micelles (26).

In order to determine the role of intestinal uptake in these experiments, rats with intact lymphatics were infused for 30 min with labeled sterol and then killed at 1 hr. This time period was chosen since it has been shown in isolated loop experiments that vitamin D_3 uptake is virtually complete by then, whereas transport of the sterol out of the intestinal wall is still minimal (2). It was clearly shown that the uptake of both cholesterol and vitamin D_3 into the intestinal wall was not significantly affected by the presence of mixed micelles as compared with taurocholate micelles. This implies that the enhanced rate of appearance of free cholesterol and vitamin D_3 in lymph after the administration of mixed micells was probably due to an effect on transport mechanisms within the intestinal wall rather than to an alteration in mucosal uptake.

Studies by Zilversmit (27) have shown that of the free cholesterol in lymph a greater percentage is in chylomicrons, as compared to the other lipoprotein fractions of lymph. This difference is most marked in the chylomicron surface coat, the cholesterol of which is entirely in the free form. Since lymph chylomicrons increase during fat absorption, it seem possible that the increase in radioactive free cholesterol after mixed micells was the result of enhanced chylomicron formation. However, this interpretation would not apply to vitamin D, which is not a known structural component of chylomicrons. It seems more likely that the increase in lymph radioactive free cholesterol and vitamin D reflects, at least in part, their molecular similarity, and is related to the physicochemical effects of triglyceride undergoing transport through the absorptive cell.

Zilversmit (27) found a higher proportion of free cholesterol in the chylomicrons of rats fed corn oil, which contain 50% linoleic acid, than in those fed cream. In the present study the administration of linoleic acid increased the specific activity lymph free cholesterol more than palmitic acid, although it was not possible to determine how much of this was due to enhanced absorption of label and how much to increased exchange. Conceivably, this contrast between the two fatty acids could have been due to a difference in their rate of esterification of cholesterol. However, Karmen, Whyte, and Goodman (28) found that both fatty acids were incorporated into chylomicron cholesterol ester to the same extent. These authors showed that the cholesterol in lymph was preferentially esterified with oleic acid, much of which was of endogenous rather than exogenous origin. Another possibility is that linoleic acid caused a more rapid entry of free cholesterol into lymph, and as a consequence diminished the amount available for esterification in the mucosa.

It has been shown that free cholesterol in chylomicrons equilibrates more rapidly than esterified cholesterol with cholesterol in other tissues, such as the liver (29, 30). Thus it is possible that an increase in the ratio of labeled free: esterified cholesterol in lymph, as demonstrated in the present studies, might influence the subsequent distribution and turnover rate of absorbed cholesterol. These and other recent findings (31) appear to indicate that saturated and unsaturated fatty acids differ in their effects on the transport of cholesterol into lymph.

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