Absorption and lymphatic transport of cholesterol in the rat

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ABSTRACT Rats with thoracic duct fistulae were fed triolein and triolein containing various amounts of labeled cholesterol. The analysis of the lymph lipids gave the following results.

In the fasting state the cholesterol transported via the thoracic duct was 0.87 μmole/hr. Feeding 800 μmoles of triolein gave a maximum rate of transport of cholesterol of 1.65 μmoles/hr. Addition of cholesterol to the triolein further increased the cholesterol transport to a maximal rate of almost 5 μmoles/hr when 50 μmoles of cholesterol were fed per 800 μmoles of triolein.

The exogenous fraction of the cholesterol transported increased linearly with increasing cholesterol load, constituting at the highest dose almost 90% of the total cholesterol transported.

An almost constant fraction (about 0.4) of the dietary cholesterol was recovered in the thoracic duct lymph in 24 hr irrespective of the dose fed, from a trace up to 100 μmoles in 800 μmoles of triolein.

Cholesterol absorption has the characteristics of a passive diffusion process.

KEY WORDS rat · cholesterol · quantitative · absorption · lymphatic transport · thoracic duct fistulae · triolein · oleic acid · micelles · diffusion

In a detailed study of the quantitative aspects of cholesterol absorption in the rat (1) it was found that the fraction of dietary cholesterol absorbed was almost constant over a wide range of dose fed. The absorption data were based on fecal recoveries after a single isotope feeding. The interpretation of the results obtained from this kind of experiment might not, however, be straightforward, especially for compounds known to undergo an enterohepatic circulation. As it is well established that the lymphatic pathway is the exclusive transport route for absorbed cholesterol (2), it seemed important to relate the fecal recovery data to the lymphatic transport of dietary sterol.

METHODS

White male rats weighing around 250 g were fasted for 24 hr with free access to water. The thoracic duct was cannulated essentially according to Bollman and Flock (3). The stomach was also cannulated to allow the easy administration of test meals. The rats were kept in restraining cages in a cabinet at 28°C with free access to water containing 0.64% NaCl and 0.4% KCl. If the lymph flow was satisfactory the first test sample was given 24 hr after the operation. The test meal, in the form of an oil (triolein or oleic acid) containing cholesterol, was injected through the stomach cannulae and was followed by 2 ml of saline solution.

Lymph was collected into graduated centrifuge tubes holding 200 IU of heparin. Sampling was done for 1 hr periods during the first 8 hr after feeding; thereafter, lymph was collected in one sample for the next 16 hr.

Rats that were in good condition (adequate lymph flow) were used the next day for another test in which cholesterol labeled with a different isotope was used. Cholesterol-4-^{14}C and cholesterol-^{3}H were obtained from The Radiochemical Centre, Amersham, Bucks., England. Their radiopurity, checked by thin-layer chromatography, was found to be better than 98%. The numbers of rats fed the different test meals are given in Table 1. The test meals were given in amounts of 0.4, 0.8, and 1.6 ml of oil, roughly corresponding to 400, 800, and 1600 μmoles of triolein. In a few experiments, 1200 and 2400 μmoles of oleic acid were used. In no instance did the animals develop diarrhea.

Cholesterol was dissolved in triolein or oleic acid by warming at 37°C and the oil was fed at this temperature. With the 100:800 μmoles dose (see Table 1), the oil had

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TABLE 1 NUMBERS OF RATS GIVEN TEST MEALS OF DIFFERENT COMPOSITIONS

Amount of Triolein (TO) or Oleic Acid (OA)	Amount of Cholesterol (μmoles)					
	0	12.5	25	50	100	200
μmoles						
400 TO	5			10		
800 TO	13	6	7	10	10	
1600 TO					8	
1200 OA						6

to be further warmed to bring the cholesterol in solution and probably a supersaturated solution was used.

The lymph samples were centrifuged for 10 min at 2500 rpm to sediment corpuscular elements. 0.5 or 1 ml samples were taken for determination of cholesterol as described by Abell, Levy, Brodie, and Kendall (4). 2 or 4 ml of the petroleum ether supernate used for cholesterol determination was taken into vials for radioactivity determination. After evaporation of the solvent, 15 ml of toluene-based scintillation solution was added and radioactivity was counted to a confidence level of at least 95% in a Packard spectrometer of the 4000 series.

The fraction of the lymph cholesterol derived from dietary sources (exogenous) was calculated from the radioactivity of the lymph cholesterol by means of differential specific activities. Endogenous cholesterol was obtained as the difference between total cholesterol, chemically measured, and the exogenous fraction as calculated above.

The test meals were weighed directly into counting vials in amounts not exceeding 100 mg.

Total ester bonds of the lymph were determined by the method of Snyder and Stephens (5).

The testing of the significance of the difference of the means of two groups in this investigation was done with the Student's "*t*"-test. A difference is taken as significant when P was <0.05.

RESULTS

The amount of fat that appeared in the thoracic duct lymph of rats in this investigation was determined as total lipid ester bonds. Fig. 1 shows the cumulative transport of ester bonds after the feeding of 0.4, 0.8, and 1.6 ml of triolein (including cholesterol), approximately corresponding to 400, 800, and 1600 μmoles of triolein.

During the first hours after feeding the ester bond transport is approximately the same for these three doses. The time during which a constant transport rate occurs, however, is dependent on the size of the dose fed. The 800 μmoles triolein dose gave the highest and most reproducible transport figures for total lipid ester bonds to the

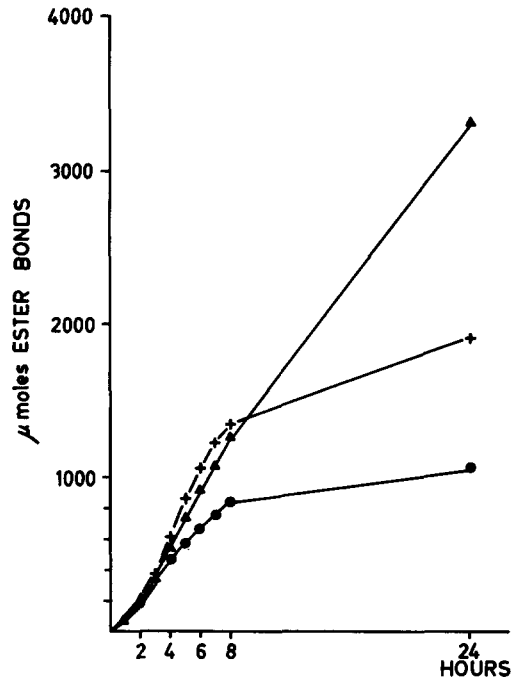

FIG. 1. Cumulative appearance in thoracic duct lymph of total ester bonds after feeding rats: ●, 400 μmoles of triolein (with 50 μmoles of cholesterol); ×, 800 μmoles of triolein (with 50 μmoles of cholesterol); ▲, 1600 μmoles of triolein (with 100 μmoles of cholesterol).

lymph in 8 hr. The maximal rate for all doses seems to be around 200 μmoles/hr, corresponding to 67 μmoles/hr of triolein. The total transport of ester bonds for 24 hr is clearly related to the dose fed.

When the amount of cholesterol in lymph was determined under similar conditions, with cholesterol added to the triolein fat fed, the results given in Fig. 2 were obtained. In these experiments triolein with cholesterol in the molar proportions 8:1 and 16:1 was fed, each mixture at two different levels. Again the initial rates of transport were similar. The cumulative transport of cholesterol to the lymph in 8 hr was significantly higher ($P < 0.02$) when 50 μmoles of cholesterol was fed with 800 μmoles of triolein compared to 400 μmoles. Increasing the dose fed from 50:800 to 100:1600 μmoles resulted in a decrease in the initial rate of cholesterol transport, but this rate remained constant for almost 24 hr.

Because of these results we studied further the transport of cholesterol in the thoracic duct lymph after feeding a standard dose of 800 μmoles of triolein (with or without added cholesterol) and the cholesterol transport was calculated either as the *transport rate* in μmoles per hour between the 3rd and 6th hr after feeding or as the *total transport* in μmoles of cholesterol during 8 or 24 hr following the feeding.

In the fasting state the lymphatic transport of cholesterol was found to be 0.87 μmole/hr. Feeding 800 μmoles

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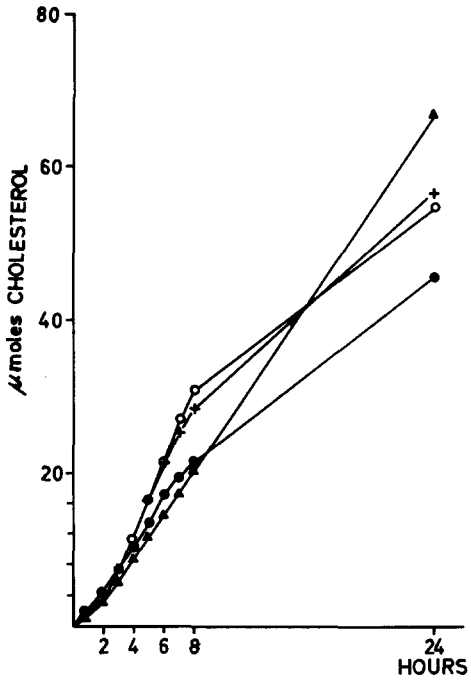

FIG. 2. Cumulative transport in rat thoracic duct lymph of cholesterol after feeding cholesterol in triolein as follows: ●, 50 μmoles of cholesterol with 400 μmoles of triolein; ×, 50 μmoles of cholesterol with 800 μmoles of triolein; ○, 100 μmoles of cholesterol with 800 μmoles of triolein; ▲, 100 μmoles of cholesterol with 1600 μmoles of triolein.

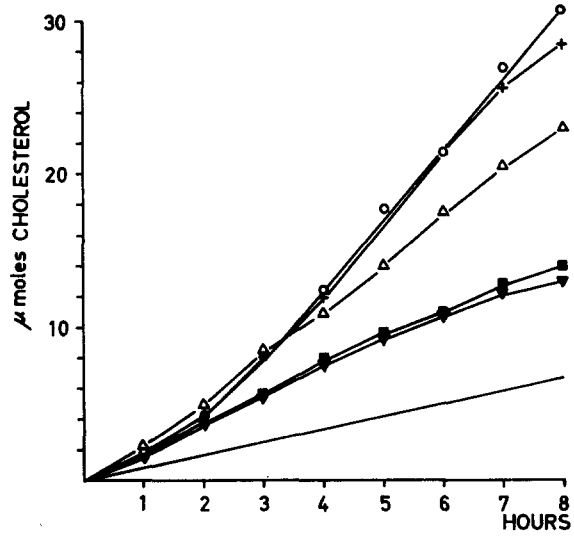

FIG. 3. Cumulative transport of cholesterol in thoracic duct lymph of rats in the fasting state (solid line) or fed 800 μmoles of triolein alone (▼) or with 12.5 (■), 25 (△), 50 (×), or 100 (○) μmoles of cholesterol. Each point is the mean from the number of experiments given for each type of experiment in Table 1.

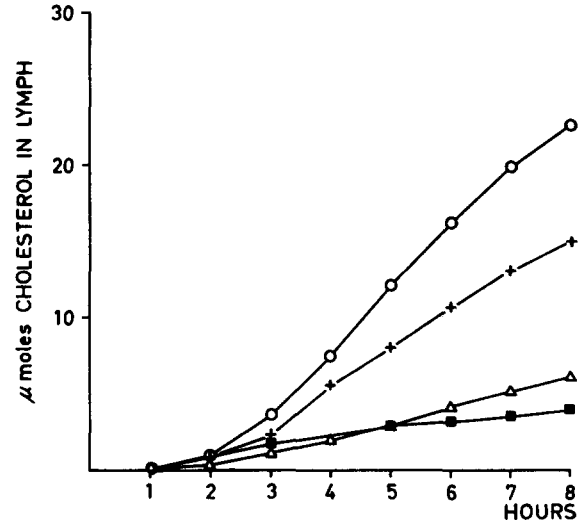

FIG. 4. Cumulative transport in thoracic duct lymph of exogenous cholesterol of rats fed 800 μmoles of triolein with 12.5 (■), 25 (△), 50 (×), and 100 (○) μmoles of cholesterol. The number of rats in each group is given in Table 1.

of triolein increased the rate to 1.65 μmoles/hr during the period of maximal transport, i.e. between the 3rd and 6th hr after feeding.

The effect on cholesterol transport of adding increasing amounts of cholesterol to 800 μmoles of triolein is seen in Fig. 3, which gives the cumulative transport of total lymph cholesterol in μmoles during the first 8 hr after feeding, and in Fig. 5, which gives the maximal transport rate of cholesterol in μmoles per hour for each dose, calculated as a mean for the 3rd to the 6th hr. Addition of 12.5 μmoles of cholesterol to the triolein fed does not significantly influence the cholesterol transport, but further addition results in an increased rate of transport to almost 5 μmoles/hr when 50 μmoles are fed. Still further increase, to 100 μmoles of cholesterol, does not increase the transport rate. The concentration of cholesterol that can be used is limited by the solubility of cholesterol in this oil. It is given as 3.77% at 37°C (6) (about 10 μmoles per 100 μmoles of triolein).

The solubility of cholesterol in oleic acid, however, is four to five times that in triolein (6). Feeding 200 μmoles of cholesterol in 1200 μmoles of oleic acid resulted in a cholesterol transport rate that was no higher than those obtained with 50 μmoles of cholesterol in triolein.

The cumulative transport of *dietary* cholesterol—measured after the feeding of labeled cholesterol—in these experiments is given in Fig. 4. After a lag period of almost 2 hr the dietary cholesterol appeared in the lymph at an approximately constant rate. The rate increased with increase in the dose fed, and was greatest when 100 μmoles was fed in 800 μmoles of triolein.

Fig. 5 gives the rates of transport of the exogenous and endogenous cholesterol of the lymph as related to the different doses of cholesterol fed in the same amount of triolein. The endogenous fraction shows a significant decrease with the highest dose of exogenous cholesterol ($P < 0.005$ compared to the 0:800 μmoles dose). The

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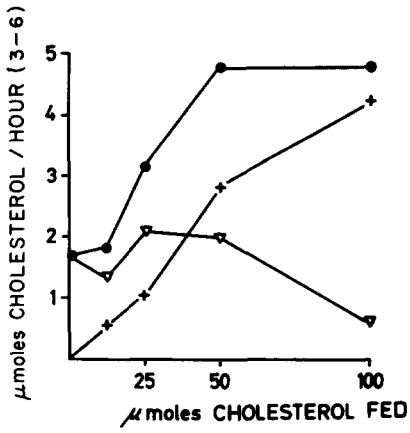

FIG. 5. Rate of transport of cholesterol to lymph in thoracic duct-cannulated rats fed 800 μmoles of triolein with different levels of labeled cholesterol. The rates of transport have been calculated as the mean rate of transport per hour between the 3rd and 6th hr after feeding the test meals for total (●) and exogenous (×) cholesterol. The endogenous (▽) fraction has been calculated as the difference.

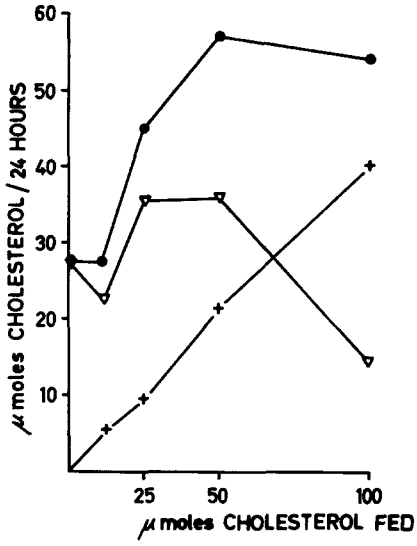

FIG. 6. Transport in lymph of total (●), exogenous (×), and endogenous (▽) cholesterol for 24 hr after feeding 800 μmoles of triolein with different levels of cholesterol.

exogenous fraction shows an almost linear increase with the dose of cholesterol fed and reaches a value that corresponds to 90% of that of the total cholesterol transport per hour.

Fig. 6 shows the transport of dietary and endogenous cholesterol in the lymph for 24 hr after feeding as influenced by the dose of cholesterol. The amount of total cholesterol transported again reached a maximum when 50 μmoles was fed. The exogenous fraction shows a continuous increase and does not seem to reach a maximal value even with the highest dose fed. The endogenous fraction shows a significant decrease for the highest dose ($P < 0.005$ compared to the 0:800 μmoles dose).

The figures for endogenous 24 hr transport for the 25- and 50-μmoles doses are higher than for any of the other in the series (compared to 0:800, $0.01 < P < 0.02$ for 25:800 and $0.15 < P < 0.20$ for 50:800).

If the labeled or dietary cholesterol transported in the lymph is calculated as a percentage of that fed in the different experiments, the curves shown in Fig. 7 are obtained. The values at zero cholesterol refer to experiments in which trace amounts of labeled cholesterol were added to the triolein fed. It appears from Fig. 7 that only a fraction—between 0.40 and 0.50—of the dietary cholesterol is transported in the lymph during 24 hr after feeding. Furthermore, the fraction transported is the same and almost independent of the cholesterol dose when this is increased from traces to 100 μmoles per 800 μmoles of triolein. Thus from 1 μmole fed, approximately 0.4 μmoles will be transported, and from 100 μmoles, almost 42 μmoles. Fig. 7 also shows that approximately 60% of the dietary cholesterol transported during 24 hr appears during the first 8 hr after feeding.

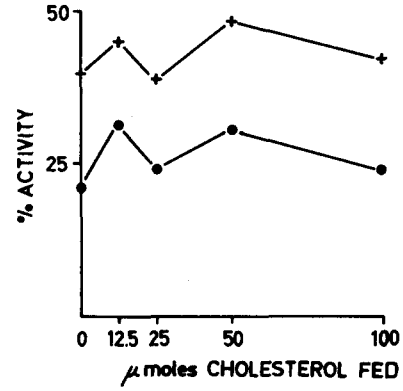

FIG. 7. Recovery in thoracic duct lymph of labeled dietary cholesterol fed in different doses in 800 μmoles of triolein. The figures are given as percentages of the amount fed for 8- (●) and 24- (×) hr samples. The zero figures refer to values obtained when tracer doses of labeled cholesterol were added to the oil.

DISCUSSION

The information obtained in this paper is related to the rate of transport of cholesterol via the thoracic duct lymph in the rat and the influence of dietary cholesterol upon it. It was found that cholesterol transport via the lymphatics is influenced by the feeding of triglyceride (triolein) and even more so by feeding triolein plus cholesterol. In these respects the results confirm the results of other experiments [for references see (7)], but the experiments constitute the first systematic study of the effect of different doses of cholesterol fed on these parameters.

The rate of transport of fat from the intestine to the thoracic duct lymph is a complex function which is a

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result of several interrelated processes, one of which is the rate of transit of the dietary fat from the stomach to the intestine. This process is affected by the amount of fat fed but cannot be otherwise experimentally changed in the intact animal; this factor therefore sets one limit on the amount of cholesterol that can be made available to the absorbing surface of the intestinal mucosa. Another limit is set by the solubility of cholesterol in the fat fed.

The dose of triolein fed as standard in the present experiments, 0.8 ml (800 μmoles), gave, after an initial lag period of 1–2 hr, a constant rate of transport of ester bonds and of cholesterol at least up to 7 hr after feeding. The rate of transport of cholesterol for a given dose was measured as the mean transport per hour between the 3rd and 6th hour. Further increase in the amount of fat fed did not increase the maximal rate of transport, although the time under which the transport occurred at this rate was extended. We therefore believe that the rates of transport obtained in this investigation are close to the maximum for the type of fat used.

In line with earlier work (7) it was found that small doses of dietary cholesterol did not influence the mass transport of cholesterol in the lymph. The 1–3 mg of cholesterol used by Vahouny, Fawal, and Treadwell (8) corresponds to the lowest dose used in this investigation, 12.5 μmoles (4.8 mg). With larger doses we found an increase in the cholesterol transport with a maximum of close to 5 μmoles/hr reached at 50 μmoles. The only comparable figures are those of Swell, Trout, Hopper, Field, and Treadwell (9), who fed 40–44 mg of cholesterol (a little over 100 μmoles) to rats with lymph fistulae. They obtained an increase in the transport of cholesterol with a maximum figure of around 2 mg (5 μmoles)/hr. Their results are not, however, strictly comparable as they fed cholesterol dispersed in oleic acid and bile salt.

Cholesterol absorption is complicated by the mixing of the dietary cholesterol with pools of endogenous cholesterol of various origins such as bile, intestinal secretions, and synthesis by the mucosal cells (10). It is possible that the thoracic duct lymph contains cholesterol derived from sources other than the absorbing cell, i.e. the liver and blood, which might be influenced by the dietary status (11).

Feeding triolein alone almost doubled the rate of transport of endogenous cholesterol into the thoracic duct lymph. This increase with fat feeding is related to an increase both in the lymph volume and in the concentration of total ester bonds; possibly the extra cholesterol is provided by the cell to make the composition of the chylomicron constant. It is also possible that the transport of cholesterol of endogenous origin to the chyle is dependent on the simultaneous transport of fat.

Addition of 25 and 50 μmoles of cholesterol to the test meal further increased the lymphatic transport of endogenous cholesterol, best documented in the cumulative transport for 24 hr. The decrease of the endogenous fraction of the lymph cholesterol with further increase in the amount of dietary cholesterol to 100 μmoles could be due to an inhibition of cholesterol synthesis and (or) a reflection of the exogenous cholesterol becoming an increasingly larger fraction of the cholesterol pool from which only a limited and constant fraction can be transported per unit time. The works of Dietschy and Siperstein (12) and Lindsey and Wilson (10) indicate that cholesterol synthesis in the intestinal mucosa is not influenced by dietary cholesterol. Their experimental conditions, however, were different in that cholesterol synthesis was studied in rats fed cholesterol-rich diets prior to the experiments, and not during the actual transport of dietary cholesterol. Our results indicate that at a high level of dietary cholesterol the cholesterol transported to the chyle is almost exclusively of dietary origin.

The amount of cholesterol transported in the lymph was only a fraction of that fed. This confirms earlier reports of a poor absorption of cholesterol (1, 7). Of more interest is the finding that a constant fraction of the dietary cholesterol is transported in the thoracic duct lymph irrespective of the size of the dose fed. These results correspond well with those recently obtained for cholesterol absorption in the rat calculated from fecal recoveries after a single feeding (1). The somewhat lower figures obtained for the lymphatic compared to the fecal recoveries—40 compared to 50%—are easily explained by the incomplete recovery in the lymph of absorbed fat (13) and the relatively short collection time used. The results of these investigations thus indicate that cholesterol absorption follows a specific pattern that in what follows is called a "fractional" type of absorption.

Fig. 8 gives a schematic presentation of the absorption process for cholesterol. The dietary cholesterol dissolves in the oil phase of intestinal content (mainly triglyceride). The normal transport of cholesterol from the oil phase to the cell membrane requires the presence of bile salt (14) and the polar split products of the oil phase formed by the action of lipase (15). V_1 and V_2 are the rates of transport of cholesterol from the oil phase to the micellar phase—composed of bile salts and monoglyceride and fatty acid—and from the latter to the cell membrane. The mechanism of transport in the intestinal cell is not

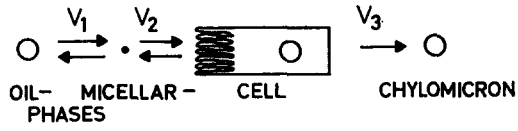

FIG. 8. Schematic diagram of the fat digestion and absorption processes. V_1, rate of transfer from oil phase to micellar phase; V_2, rate of transfer from micellar phase to cell membrane; V_3, rate of transfer from the intestinal cell to the lymph.

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considered; V_3 is the rate of transfer of cholesterol from the cell to the chyle.

As the results of the present investigation the characteristics of cholesterol absorption can be given as follows.

(*a*) A fraction, about 0.4, of the cholesterol fed is absorbed and transported to the chyle irrespective of the dose fed. What is not absorbed stays behind in the intestinal content and is transported to lower levels of the intestinal tract that are largely nonfunctional in absorption (16).

(*b*) The rate of transport of total cholesterol from the cell to the lymph, V_3, reaches a limiting value of close to 5 μmoles/hr when 50 μmoles of cholesterol is fed per 800 μmoles of triolein (Fig. 3). The transport to the chyle of exogenous cholesterol, however, continues to increase with further increase in dietary cholesterol, the transport of endogenous cholesterol correspondingly decreasing (Fig. 5). This finding indicates that V_3 is the rate-limiting step in the transport of cholesterol and not the transport of cholesterol from the micellar phase to the cell, V_2. Such an interpretation is also substantiated by the finding that dietary cholesterol accumulates in the intestinal wall with increase in amount of cholesterol fed (1).

The fact that the fractional absorption rate of cholesterol across the intestinal mucosa is constant is consistent with the idea that mucosal absorption of cholesterol is a passive diffusion process. The cholesterol available to the mucosal cell for absorption is most probably that of the micellar phase of intestinal content. The concentration of cholesterol in this phase is related to the concentration of cholesterol in the dietary fat and to the partition coefficient of cholesterol between the oil phase and micellar phase (16). The latter has been shown to be increased in favor of the micellar phase by the presence there of the polar end products of pancreatic lipolysis—monoglycerides and fatty acids (16). The incomplete intestinal absorption of cholesterol in the intact animal may be related to the fact that monoglyceride and fatty acid are absorbed more rapidly than cholesterol and leave behind a micellar phase deprived of these compounds when only a fraction of the cholesterol has been absorbed. In this situation V_2 of Fig. 8 will, because of the low concentration of cholesterol in the micellar phase, be rate-limiting for absorption, which will proceed at a much lower rate.

The maximal rate of transport of dietary cholesterol to the lymph must equal the maximal rate of absorption of exogenous cholesterol. The figure obtained in this investigation indicate this rate to be approximately 3–4 μmoles/hour or 72–96 μmoles/day at the most when large doses of cholesterol are fed. This figure is of interest in relation to the finding by Wilson (17) that cholesterol-fed rats will degrade cholesterol to bile acids at a rate of 17–20 mg/day (about 50 μmoles/day). Under these conditions the bile acids constitute around 70% of the total sterol excretion, which would thus be calculated as 70 μmoles/day, in good agreement with the above figure.

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