Absorption and lymphatic transport of cholesterol and sitosterol in the rat

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ABSTRACT An attempt was made to determine the mechanism for the greater absorbability of cholesterol as compared to sitosterol.

Sitosterol-22,23-^{3}H in different combinations with cholesterol-4-^{14}C, dissolved in 0.8 ml of triolein, was fed to rats with lymph fistulae. Feeding 1.5, 50, or 100 μmoles of sitosterol resulted in a transfer to the lymph in 24 hr of 3–6% of the sitosterol, largely independent of the dose fed. The total amount of sitosterol transferred to the lymph was therefore almost linearly related to the dose fed. 30% of a tracer dose of cholesterol-4-^{14}C fed together with the sitosterol was transferred to the lymph in 24 hr.

When a total of 50 μmoles of sterol, containing cholesterol-^{14}C and sitosterol-^{3}H in the proportions 1:3, 1:1, and 3:1, was similarly fed, we found that sitosterol had no significant effect on the lymphatic transport of the simultaneously fed cholesterol.

The ratio of ^{3}H to ^{14}C in the lymph was between 0.1 and 0.2 (the ratio in each fed mixture being taken as 1.0). The ratio was constant during the absorption period and independent of the ratio of sterols in the fed sterol mixture. Thus the same percentage of each sterol was always absorbed, and the sterols exerted no mutual interference in each others' absorption.

We conclude that the mechanism for specificity in sterol absorption must be located early in the transport of the sterols within the intestinal mucosa cell.

SUPPLEMENTARY KEY WORDS intestinal absorption · competition

In a recent study (1) we found that dietary sitosterol was taken up by the small intestinal wall in the rat to a much lesser extent than cholesterol. This finding was in agreement with the absorption figures calculated from fecal excretion in the same study. We also found that addition of sitosterol to the diet had no influence on the uptake of cholesterol into the intestinal wall. These results do not necessarily refute the earlier conclusions, based on the work of Hernandez, Chaikoff, and coworkers (2, 3), that plant sterols inhibit the transfer of cholesterol to the lymph, as it is possible that the plant sterols taken up by the wall block the transport of cholesterol to the lymph.

The results obtained, however, prompted us to study the lymphatic transport of sitosterol and its relation to cholesterol in more detail.

METHODS

Rats with thoracic duct fistulae were produced and treated as described (4). Lymph was collected in hourly samples for the first 8 hr after feeding, and thereafter in one sample for the next 16 hr. Cholesterol-4-^{14}C (Radiochemical Centre, Amersham, Bucks., England) and sitosterol-22,23-^{3}H prepared as previously described (5) were fed in different combinations (see Table 1), dissolved in 0.8 ml of triolein. Nonradioactive sitosterol was a product obtained from Sigma Chemical Co. (St. Louis, Mo.). After recrystallization from acetone, gas–liquid chromatography revealed that it contained 30% campesterol.

The lymph was analyzed for ester bonds, sterols, and radioactivity as described earlier (4). The figures given for cholesterol absorption were taken from a previous investigation (4) except for the series of four rats fed 37.5 μmoles of cholesterol in 800 μmoles of triolein.

The significance of the difference between the means of two groups was assessed by Student's "t" test. We attached significance to those for which $P < 0.05$.

RESULTS

In the first series of experiments we fed the rats different amounts of sitosterol-^{3}H (1.5, 50, and 100 μmoles) and a tracer dose of cholesterol-4-^{14}C (0.03 μmole) dissolved in 0.8 ml (approximately 800 μmoles) of triolein. Fig. 1

TABLE 1 COMPOSITION OF TEST MEALS AND NUMBER OF RATS USED

Experiment 1				
Amount of sitosterol fed (μmoles) in 800 μmoles of triolein	1.5	50	100	
No. of rats	5	6	5	
Experiment 2				
Total amount of sterols fed (μmoles) in 800 μmoles of triolein	50	50	50	37.5
Per cent cholesterol	25	50	75	100
No. of rats	7	5	5	4

In experiment 1 a trace dose of cholesterol-4-^{14}C (0.03 μmole) was also included in each test meal. The balance of sterol in experiment 2 was made up by sitosterol.

TABLE 2 LACK OF EFFECT OF SITOSTEROL ON LYMPHATIC TRANSPORT OF CHOLESTEROL

	Amount Collected in Lymph*		
		μmoles	
A. Cholesterol fed	12.5	25.0	37.5
B. Cholesterol transported	5.7	9.7	14.0
C. Cholesterol/sitosterol fed (proportions)	12.5/37.5 (1:3)	25/25 (1:1)	27.5/12.5 (3:1)
D. Cholesterol transported	6.3	9.8	14.7
P for B minus D	0.92	0.26	0.68

Cholesterol-^{14}C and sitosterol-^{3}H were fed in 800 μmoles of triolein.

* During 24 hr after the feeding.

shows the cumulative percentage recovery in the thoracic duct lymph of labeled cholesterol and sitosterol for the first 8 hr after feeding. 1.5–2.7% of the sitosterol fed was transported to the lymph; the percentage recovered was not greatly influenced by the dose. The percentage transport of the tracer dose of cholesterol was about six to seven times as great; it too was not influenced much by the dose of sitosterol fed.

In the second series of experiments, test meals of 0.8 ml of triolein were fed with a total sterol content of 50 μmoles. Cholesterol and sitosterol were present in the proportions 1:3, 1:1, or 3:1. Table 2 shows the amounts of cholesterol from these diets transported to the lymph compared with the lymphatic transport of cholesterol when the same amounts of cholesterol were fed alone. No significant differences were found. The 24-hr recoveries for sitosterol in both series of experiments are summarized in Table 3 and show that the sitosterol transport is in the range of 3–6%, largely independent of the dose of sitosterol and also of cholesterol fed.

The ratio between labeled sitosterol and cholesterol in the hourly lymph specimens for the experiments in which a total of 50 μmoles of sterol was fed is given in Fig. 2. The ratio is between 0.1 and 0.2 of that of the diet sterols, constant for the whole absorption period, and independent of the composition of the sterol mixture fed.

DISCUSSION

The present experiments were undertaken in a study of the transport of sitosterol in the thoracic duct lymph of the rat and the possible interference of sitosterol with cholesterol transport. The design of the experiments was based on results obtained in an earlier study (4), in which the dose of triolein that dissolved the sterols in the present study (0.8 ml) was found to give the maximal rate of lymphatic transport of fat (ester bonds) and cholesterol for at least 8 hr after feeding.

The figures obtained for lymphatic transport of sitosterol in the present investigation, namely 3–6% in 24 hr, are in accord with the fecal excretion data previously reported (1). They are also in agreement with the absorption figures of 3–4% calculated by Gould (6) from activity retained in the rat 8 or 16 days after sitosterol-^{3}H had been continuously fed at a level of 0.1% of the diet. Our figures for sitosterol transport to the lymph are higher than those earlier reported (7–9). Swell, Trout, Field, and Treadwell (7) first reported

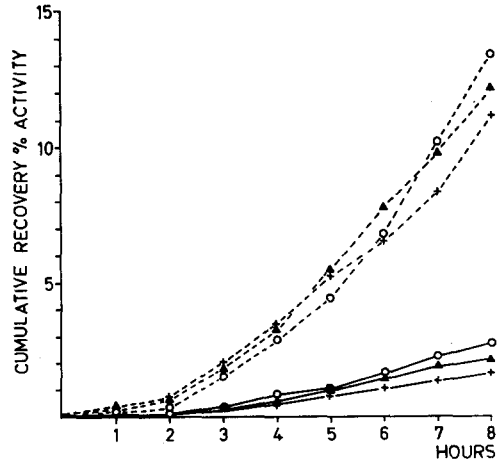

FIG. 1. Cumulative percentage recoveries in thoracic duct lymph of labeled cholesterol (broken line) and sitosterol (solid line) for the first 8 hr after feeding 0.03 μmole of cholesterol with 1.5 (○), 50 (▲), and 100 (+) μmoles of sitosterol in 800 μmoles of triolein.

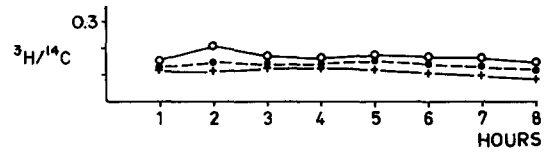

FIG. 2. Ratio between labeled sitosterol (^{3}H) and cholesterol (^{14}C) in the thoracic duct lymph of rats fed 50 μmoles of total sterol in 800 μmoles of triolein. The ratios of cholesterol to sitosterol were 1:3 (○), 1:1 (+), and 3:1 (●).

TABLE 3 LACK OF EFFECT OF CHOLESTEROL ON LYMPHATIC TRANSPORT OF SITOSTEROL

Fed Mixture		Recovery of Sitosterol in Lymph after 24 hr	
Sitosterol	Cholesterol		
μmoles		%	*μmoles*
1.5	0.03	5.3	0.08
12.5	37.5	4.9	0.61
25	25	3.4	0.85
37.5	12.5	6.4	2.4
50	0.03	3.4	1.7
100	0.03	3.2	3.2

Sterols were fed in 800 μmoles of triolein.

essentially no transfer of sitosterol-^{3}H to the lymph of rats, but later found a lymphatic transport of 2.1% when 48 mg of phytosterol-^{14}C dispersed in oleic acid and bile salt was fed (8). Dunham, Fortner, Moore, Culp, and Rice (9) reported a lymphatic transport of sitosterol of less than 1% in 24 hr when rats were fed 30 mg of sitosterol dispersed in 0.2 ml of corn oil. This amount fed was much greater than the ones used in the present investigation and corresponds to 290 μmoles per 0.8 ml of triglyceride, most of which is not in solution.

As with cholesterol (4), the percentage of sitosterol transferred to the lymph was largely independent of the dose of sitosterol fed. Thus 3% was recovered in the lymph in 24 hr whether 50 or 100 μmoles of sitosterol was fed. The actual mass of sitosterol was thus doubled when the dose fed was doubled and sitosterol absorption showed the same type of fractional absorption as cholesterol (4), the difference being that cholesterol is transported in quantities that are almost 10 times those of sitosterol.

In the present experiments no evidence was found for any competition between dietary cholesterol and sitosterol during the absorption and transfer of these sterols to the lymph. This is well demonstrated by the constant ratio of these sterols in the lymph independent of their ratio in the fed mixture. These results are not in accord with the commonly accepted view that cholesterol absorption (transport to the lymph) is decreased by the simultaneous feeding of soy sterols or sitosterol (2, 3). In the experiments of Hernandez, Peterson, Chaikoff, and Dauben (2), 4 mg (about 10 μmoles) of each sterol was fed in 0.25 ml of olive oil. In the first series of experiments the soy sterol decreased cholesterol transport to the lymph in 24 hr from 25–30% to less than 10 % (2). In the second series the effect was less pronounced but the simultaneous feeding of plant sterols decreased cholesterol absorption by 50%. On the other hand Dunham et al. (9) reported that the effect of sitosterol in reducing cholesterol absorption (in the rat) was striking only in the case where 30 mg of sitosterol was administered (in 0.2 ml of corn oil).

No significant difference between the nonradioactive sitosterol preparations used in these different investigations is apparent. In the present work we used a 2:1 mixture of sitosterol and campesterol (the 24-methyl analogue) derived from soy bean oil. In earlier investigations sitosterol has been obtained from the same source (7, 8) or from tall oil (9), or was reported to be pure sitosterol (6). The labeled sitosterol has been obtained by tritium exchange (6, 7, 9), by tritiation of stigmasterol (this investigation), or from tobacco plants grown in $^{14}CO_2$ (8). It is of interest that Daskalakis and Chaikoff (10) later reported that stigmasterol (Δ^{22}-24-ethyl-cholesterol) did not affect the transfer of cholesterol to the lymph in rats. Furthermore it has been reported that sitosterol and campesterol occur in the feces of humans in the same proportion as fed, which indicates no difference in intestinal handling (11).

We have no sound explanation for the differences in results obtained in studies of the effect of plant sterols on the lymphatic transport of cholesterol in the rat.

Earlier results from in vitro (5) and in vivo experiments (1) indicate that cholesterol and sitosterol are presented to the mucosa cell in micellar form in proportions directly related to those in the fed mixture. As sitosterol does not accumulate in the intestinal mucosa relative to cholesterol (1,12), the rate-limiting step in sterol absorption must be located in the uptake of sterols into the outer surface of the cell.

From the results previously discussed (4) it was concluded that cholesterol absorption had the characteristics of a passive diffusion process. The results of the present study show sitosterol absorption has similar characteristics and it is therefore tempting to suggest that the difference in rate of absorption of the two sterols depends on differences in diffusion rates into the intestinal cell evoked by the difference in the molecular structure of the side chain of the two sterols. With this type of mechanism, no competition between different sterols would be expected, an expectation borne out by the present investigation.

The difference in rate of absorption of closely related sterols has been ascribed to differences in affinity to the lipoproteins in the mucosal cell (13) or to lack of adequate esterification in the intestinal cell (14). The former mechanism might well be behind the different rates of passive diffusion into the outer surface of the cell. Inadequate esterification of the sterol is not, however, a probable mechanism, for several reasons. Sitosterol does not accumulate relative to cholesterol in the intestinal wall during absorption (1) and the activity ratio sitosterol:-cholesterol is almost identical in the intestinal wall and the lymph. Furthermore there is no relationship between rate of lymphatic transport of different dietary sterols and extent of esterification in the lymph (14–16).

Sitosterol has been used in animal (17) and human experiments (18) to lower blood cholesterol. In these studies large doses of sitosterol, up to 30 g or more in the human experiments, had to be fed to be effective. These amounts are relatively much higher than those used in the present study in which no more was fed than could be dissolved in the glyceride fat administered. It seems quite possible that sitosterol fed in such large quantities will interfere with cholesterol absorption, most probably in the intraluminal phase of absorption.

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