Absorption of H^3-β-Sitosterol in the Lymph Fistula Rat.* (24552)

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It has been established that plant sterols inhibit absorption of cholesterol and presumably, by this mechanism, they thereby lower blood and tissue levels of cholesterol(1-3). Furthermore, this inhibiting effect of plant sterols may be due to competition with cholesterol for the sterol absorptive system in the intestinal wall(4,5). After administration of H^3-β-sitosterol there was increased fecal excretion of cholesterol and related sterols(5). It was also observed that a considerable portion of H^3-β-sitosterol was not recovered in the feces and was presumably absorbed or converted to substances not precipitable with digitonin. Gould(6) fed tritium labeled sitostrol to humans and animals and from balance and blood data concluded that it was absorbed to a slight extent. Sitosterol balance studies in humans and rats have indicated considerable absorption of up to 40%(7-9). However, such experiments do not supply data on mechanism or route of absorption. Recent reports(10,11) on the mechanism of cholesterol absorption have indicated that there exists in the intestinal wall a pool with which cholesterol entering from the lumen is mixed prior to its esterification and transfer to the lymph. In light of these findings and the lack of definitive data on the mechanism of sitosterol absorption, it became of interest to determine the extent to which sitosterol enters the intestinal wall and is transferred to the lymph.

Methods and materials. Preparation and care of the thoracic duct fistula rats has been described(10). Twenty-four hours after operation each experimental animal received, by intubation, without anesthesia, 3 ml of aqueous emulsion containing 44 mg H^3-β-sitosterol† (6.8 μc), 50 mg blood albumin, 292 mg oleic acid, 279 mg sodium taurocholate, and 150 mg glucose; control rats received a comparable emulsion without sterol. There were 3 experimental groups of animals; A was killed at 6, B at 24, and C at 48 hours after the test meal. Lymph was collected in consecutive periods from 0-6, 6-24, 24-48 hours to time of sacrifice for each group. When animals were sacrificed, the small intestine was removed and saline washings from intestine were pooled with feces of the same animal. This gave combined samples of feces and intestinal contents for 0-6, 0-24, and 0-48 hours for groups A, B, and C, respectively. Lipid extracts of small intestine or segments of the intestine, and a non-saponifiable lipid extract of combined feces and intestinal contents were prepared according to procedures described earlier(10,11). Free and total cholesterol were determined colorimetrically on each extract of lymph and intestine; gravimetric sterol determinations were carried out on extracts of the combined feces and intestinal contents(10). Tritium activity of free, esterified, and total sterol fractions of all extracts were determined as previously described(5).

Results. At the end of 6-hour absorption period 8.7 mg or 19.9% of the fed H^3-β-sitosterol was not recovered in intestinal contents and feces (Table I). Analysis of feces and intestinal contents after 24 and 48 hour periods showed further disappearance of the fed sitosterol, 13.5 mg or 31.6% at 24 hours and 17.4 mg or 39.5% at 48 hours. Sterol absorption calculated from gravimetric sterol determination also indicated disappearance of the fed sitosterol, but such disappearance was less than indicated by activity data. These differences can be explained by the increase in fecal excretion of unlabeled cholesterol or related sterols when H^3-β-sitosterol is fed. Thus, for 24 hours excretion of unlabeled sterol increased from 6.0 to 15.9 mg. Also, specific activity of the sitosterol in the intestinal lumen indicated considerable dilution of the fed H^3-β-sitosterol with time.

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TABLE I. Recovery of Fed H^3-β-Sitosterol from Feces and Contents at Different Time Periods.

Time, hr	Feces and intestinal contents: Sterols total, mg	H^3-β-sitosterol, mg	Sterol other, mg	Specific activity† total sterol, μc/mg	H^3-β-sitosterol not recovered: mg	%
6	43.5 ± 2.2‡	35.7 ± 1.9	7.8 ± 1.4	.124 ± .011	8.7 ± 2.5	19.9 ± 5.9
24	46.4 ± .5	30.5 ± 3.5	15.9 ± 2.1	.100 ± .014	13.5 ± 2.6	31.6 ± 4.8
48	45.3 ± 2.5	26.6 ± 2.5	18.7 ± 1.3	.090 ± .010	17.4 ± 2.0	39.5 ± 3.8
24*	6.0 ± .4		6.0 ± .4			

* Represents 24 hr control excretion.
† Total H^3-β-sitosterol fed was 44 mg with specific activity of 0.154 μc/mg, 4 animals/group.
‡ Stand. error of mean.

At the end of 6 hours, 2 mg or 4.6% of the fed H^3-β-sitosterol was present in the intestinal wall (Table II). At 24 hours there was .8% and at 48 hours there was no H^3-β-sitosterol present. Virtually all of the H^3-β-sitosterol was present as free sterol; only a trace was present as esterified H^3-β-sitosterol. There was a small apparent increase in amount of free cholesterol in the intestinal wall at end of 6 hours. This increase could be accounted for by the uptake of H^3-β-sitosterol.

At various time intervals only traces of H^3-β-sitosterol could be detected in the lymph. These amounts were less than .3% of the fed dose. Also, there was no increase in the lymph total sterol level when compared to a group not receiving sitosterol in the test meal.

To locate area of maximum uptake of fed H^3-β-sitosterol by intestinal wall, the small intestine was sectioned into different length segments. The results were essentially the same as previously reported for cholesterol-4-C^{14}(11). About 70% of the H^3-β-sitosterol in the small intestine was present in the proximal half.

Discussion. Our results have confirmed and extended our earlier investigations(4,5,7). After feeding of H^3-β-sitosterol, there was increased fecal excretion of cholesterol and related sterols and from the balance data an apparent absorption of β-sitosterol. Based on these findings it was previously postulated that H^3-β-sitosterol was absorbed by the same mechanism as cholesterol and competed with cholesterol for the sterol absorptive system (5). The present results indicate that a considerable portion of ingested H^3-β-sitosterol is not accounted for by that in the lumen, intestinal wall, and lymph. The failure to account for all of the administered H^3-β-sitosterol in these 3 sites and the apparent absorption, from balance data, raises important questions regarding plant sterol absorption. These find-

TABLE II. Uptake of Fed H^3-β-Sitosterol by Intestinal Wall and Its Transfer to Lymph.

Time, hr*	No. rats†	Sterol free	H^3-sterol free	Sterol ester	H^3-sterol ester	Sterol total	H^3-sterol total	Admin. H^3-β-sitosterol recovered, %
				mg				
				Small intestine				
6	4	15.1 ± 1.0§	2.01 ±.50	1.3 ±.2	tr	16.4 ± .9	2.01 ±.50	4.6 ± 1.1
24	4	12.9 ± 1.1	.33 ±.10	1.0 ±.2	.0	13.9 ± .9	.33 ±.10	.8 ± .2
48	4	13.6 ± .5	.0	.6 ±.1	.0	14.2 ± .6	.0	.0
				Lymph				
0– 6	12	1.2 ± .4	tr‖	1.6 ±.3	tr	2.8 ± .3	tr	<.3
6–24	8	3.4 ± .3	tr	4.7 ±.5	tr	8.1 ± .8	tr	"
24–48	4	3.6 ± .4	.0	6.2 ±.6	.0	9.8 ±1.0	.0	.0
0–24‡	5	3.3 ± .3		6.1 ±.3		9.4 ±1.1		

* Represents collection time of lymph and time of sacrifice after test meal.
† Total H^3-β-sitosterol fed was 44 mg with a specific activity of .154 μc/mg.
‡ Represents 24 hr lymph collection period of control group receiving sodium taurocholate and oleic acid.
§ Gravimetrically determined.
‖ tr = trace.

ings suggest that part of the fed H^3-β-sitosterol may have undergone transformation in the intestinal wall or lumen so that it was no longer precipitated with digitonin and thus, would not have been detected in these experiments. Such changes occur with fed cholesterol-4-C^{14}(11). Another less probable alternative is that H^3-β-sitosterol was absorbed by some other route than through the lymphatic system. We are now using C^{14}-β-sitosterol to test these possibilities.

The present data also show that a considerable amount of H^3-β-sitosterol, like cholesterol(11), is taken up by the intestinal wall and is present almost entirely as free sterol. However, the amount taken up was about one-half that of cholesterol. Roth and Favarger (12) have also observed uptake of D-sitosterol by the intestinal wall. Thus, in this first step of the absorption process both sterols appear to be handled in a similar fashion, albeit, β-sitosterol is taken up to a lesser degree. After free cholesterol is transferred into the intestinal wall it is largely esterified and then transferred into the lymph as part of the chylomicron(11). It appears that β-sitosterol is not esterified in the intestinal wall or transferred to lymph in appreciable quantities. It should be emphasized that if the amount of H^3-β-sitosterol present in intestinal wall at end of 6 hours, had been transferred to the lymph during the next 18 hours, it could have been easily determined by the technics employed.

Summary. Administration of 44 mg of H^3-β-sitosterol to lymph fistula rats and analysis of feces, intestinal contents, intestine, and lymph gave the following results: (1) balance data indicated approximately 40% absorption in 48 hours, (2) there was increased fecal excretion of cholesterol and related sterols, (3) there was considerable uptake of H^3-β-sitosterol by the intestinal wall, but essentially no transfer into the lymph. Explanations for the apparent disappearance of H^3-β-sitosterol are discussed.

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Proteolytic Enzyme System of Skin. VI. Enzyme Patterns in Various Animal Species.*† (24553)

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Several enzymes which comprise, in part, the proteolytic enzyme system of rat skin have been described by Martin and Axelrod (1-5). In extracts prepared from rat skin acetone powder, 4 distinct enzymes, designated Proteinase A, Proteinase C, the A_1-esterase, and the A_2-esterase, have been characterized, partially purified, and in some cases separated from each other. With the exception of Proteinase C, synthetic sub-