Intestinal absorption and lymphatic transport of cholesterol and β-sitostanol in the rat

Aslam S. Hassan and Alfred J. Rampone

Department of Physiology, School of Medicine, University of Oregon Health Sciences Center, Portland, OR 97201

Abstract The intestinal absorption of cholesterol and β-sitostanol (the saturated analogue of β-sitosterol) were measured and their absorptions compared in the presence and absence of cholestyramine. After test meals containing [^{3}H]cholesterol and [^{14}C]β-sitostanol without added cholestyramine, 4-day fecal collections yielded an average of 51% of the fed cholesterol and 83% of the fed β-sitostanol. In separate lymph transport studies without cholestyramine, 36% of the fed cholesterol was recovered in lymph in 24 hours compared to only 2% of the fed β-sitostanol. Thus, while total recoveries of the two labeled compounds in feces plus lymph were nearly identical (51% + 36% = 87% for cholesterol and 83% + 2% = 85% for β-sitostanol) their distribution in the two compartments was markedly different, reflecting the relative nonabsorbability of β-sitostanol. Adding cholestyramine to the test meal caused fecal excretion of cholesterol to increase to 73%, independent of the dose of cholestyramine used. Cholestyramine had no effect on the fecal excretion of β-sitostanol (average excretion after cholestyramine, 85%). The relative nonabsorbability of β-sitostanol compared to cholesterol is clearly evident in this study and leads us to suggest its possible use as a lipid-soluble, nonabsorbable reference compound for measurement of the absorption of cholesterol and other lipids. Further data are presented to justify its use for this purpose.—**Hassan, A. S., and A. J. Rampone.** Intestinal absorption and lymphatic transport of cholesterol and β-sitostanol in the rat. *J. Lipid Res.* 1979. **20:** 646–653.

Supplementary key words plant sterols · cholestyramine · thoracic duct · fecal excretion of sterols

Plant sterols are structurally similar to cholesterol (**Fig. 1**). It is generally accepted that the physicochemical properties of some of the plant and animal sterols, specifically β-sitosterol and cholesterol, are also similar (1). However, the intestinal absorption of plant sterols has been shown to be limited (2) compared to the absorbability of cholesterol. In addition, it appears that modification of the sterol nucleus profoundly affects its absorbability. For example, cholestanol, a derivative of cholesterol formed by hydrogenation of the double bond in the B ring, is absorbed less than cholesterol (3).

In order to investigate the effect of hydrogenation of a plant sterol on its relative absorbability, the following experiments were designed to test the relative absorbabilities of cholesterol and β-sitostanol, a hydrogenated derivative of β-sitosterol. In one experiment rats were fed a test meal containing [^{14}C]β-sitostanol and [^{3}H]cholesterol with or without different amounts of cholestyramine so that absorption of the two substances could be tested under varying conditions. In a second experiment, rats were prepared with lymph fistulae and the lymphatic transport of the two compounds was studied in these rats after feeding them a test meal containing either [^{3}H]β-sitostanol or [^{14}C]cholesterol.

MATERIALS AND METHODS

Radioactive supplies

[1α,2α(n)-^{3}H]Cholesterol was obtained from New England Nuclear (Boston, MA); it was found to be 94% radiopure by thin-layer chromatography (TLC) and was used as such. β-[4-^{14}C]Sitostanol was prepared by Dr. T. D. Lee[1] by the catalytic hydrogenation of β-[4-^{14}C]sitosterol (98% radiopure by TLC) obtained from Amersham/Searle (Arlington Heights, IL). Nuclear magnetic resonance (NMR) spectral analysis of the product showed that the reduction was complete. β-[22,23(n)-^{3}H]Sitostanol was also prepared by Dr. T. D. Lee by the catalytic hydrogenation of β-[22,23(n)-^{3}H]sitosterol obtained from Amersham/Searle. Although NMR spectral analysis of the product showed that reduction was complete, the product was purified before use by thin-layer chromatography on 5% silver nitrate-impregnated silica gel plates developed in chloroform. This was

Abbreviations: TLC, thin-layer chromatography; NMR, nuclear magnetic resonance.

[1] Department of Chemistry, University of Oregon, Eugene, Oregon 97403.

necessary because of contamination of the product with silicone grease used in the hydrogenation apparatus. [4-^{14}C]Cholesterol was obtained from Amersham/Searle and found to be better than 97% radiopure by TLC.

Chemicals

Cholesterol (99% pure) was obtained from Sigma Chemical Co. (St. Louis, MO) and used as supplied. β-Sitostanol was prepared by Dr. Doyle Daves[2] by the catalytic hydrogenation of β-sitosterol obtained from Nutritional Biochemical Co. (Irving, CA); it was 91% β-sitosterol and 9% campesterol by gas–liquid chromatographic analysis. NMR spectral analysis of the product showed that the reduction was complete. Cholestyramine (Cuemid) was obtained from Merck, Sharpe and Dohme[3] (West Point, PA). All other chemicals were of reagent grade and used as supplied.

Lymph transport study

In order to evaluate the absorbability of β-sitostanol and cholesterol, lymph transport studies were carried out in male Sprague-Dawley rats in the following way. After anesthetizing the animals with sodium pentobarbital given intraperitoneally (35 mg/kg body wt.), the thoracic duct in the abdomen was cannulated with a P.E. 50 cannula as previously described (4). To avoid anesthetizing the animals again in order to feed the test meal, the stomach was also cannulated with a P.E. 50 cannula sutured in place. The stomach cannula was exteriorized through the original incision in the abdominal wall. The animals were kept in restraining cages and lymph was allowed to flow freely into a collection tube containing heparin. Only animals that had satisfactory overnight lymph flows (1–2 ml/hr) were considered for the study. Animals with satisfactory lymph flows were given either [4-^{14}C]cholesterol or [22,23-^{3}H]β-sitostanol in an ethanol solution through the gastric cannula. Injection of the test solution was followed by 1 ml of saline to flush out the cannula. Lymph was collected in hourly samples for 8 hr after the injection and as a single sample for the next 16 hr. At the end of 24 hr, the rats were killed with an overdose of sodium pentobarbital. The abdomen was then opened to check for the placement of the cannulae and for any overt signs of dilation of the mesenteric lymphatics.

The lymph samples were analyzed as follows. An aliquot of lymph was saponified for 90 min with 2 ml of 33% KOH in a hot water bath maintained at 90°C. After cooling, 2 ml of absolute ethanol was added to the saponified mixture and non-saponifiable neutral sterols were extracted into 20 ml of petroleum ether (boiling range 35–60°C). The solvent was washed with 5 ml of water. An aliquot of the petroleum ether extract was pipetted directly into a scintillation vial and the solvent was evaporated under nitrogen. To the dried lipid extract was added 10 ml of scintillation solution (4 g of 2,5-diphenyloxazole and 0.05 g of *p*-bis[2-(5-phenyloxazolyl)]-benzene in one liter of toluene) and the radioactivity was assayed in a Packard liquid scintillation spectrometer (Downers Grove, IL) with automatic external standard for quench correction. The radioactivity recovered was expressed as a percent of the amount injected.

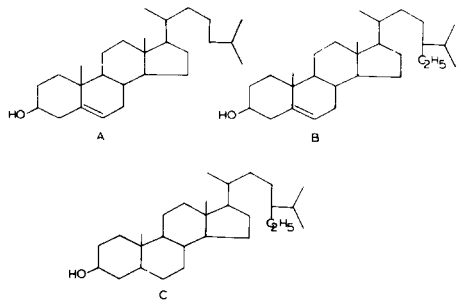

Fig. 1. Chemical structures. *A*, Cholesterol, *B*, β-sitosterol, and *C*, β-sitostanol. Note that the only difference between cholesterol and β-sitosterol is the ethyl group on the 24 position of the side chain. β-Sitosterol differs from β-sitostanol by the presence of a double bond in the B ring in β-sitosterol. Campesterol (not shown in this figure) is identical to cholesterol except for a methyl ($-CH_3$) group on the 24 position of the side chain.

Fecal excretion and blood analysis study

Twenty male Sprague-Dawley rats weighing between 240 and 270 g were divided into four groups with five rats in each group. Assignment of a rat to a group was based on the amount of cholestyramine (0, 100, 200, or 300 mg) the animal was to receive; this was determined in a random order. The rats were fasted overnight. On the day of the experiment, the animals were lightly anesthetized with ether; a gastric tube was introduced into the stomach and the following were injected in the order listed:

A. One ml of water as a check for placement of the tube.
B. Two ml of a fat-free milk solution (5) containing no cholestyramine or with added cholestyramine in one of three concentrations (25, 50, or 75 mg/ml).
C. Approximately 0.5 ml of an ethanolic solution

[2] Oregon Graduate Center for Study and Research, 19600 NW Walker Road, Beaverton, Oregon, 97005.

[3] From this point the concentration of Cuemid (90% cholestyramine resin) will be referred to as the concentration of cholestyramine for the sake of simplicity.

of cholesterol (2 mg/ml), β-sitostanol (1 mg/ml), [4-^{14}C]β-sitostanol (tracer), and [1,2-^{3}H]cholesterol (tracer) from a pre-weighed syringe which was weighed after injection to determine the exact volume injected.

D. Two ml of the same milk solution as in B above.

E. One ml of water to flush the tube.

After injection of the test meal the animals were returned to separate cages with wire mesh floors to allow for collection of feces. No food was available to the animals for 8 hr after receiving the test meal. They then had free access to rat chow for the duration of the experiment. Water was freely available to the animals at all times. Feces were collected daily for 4 days following the injection and frozen until analyzed. The experiment was terminated after the fourth fecal collection by killing the rats with an overdose of sodium pentobarbital.

Feces were analyzed according to Malinow et al. (6). The daily fecal samples were mixed with methanol, weighed, and homogenized in a blender (Osterizer, Oster Corp., Milwaukee, WI). An aliquot by weight was transferred to a centrifuge tube and 2 ml of 33% KOH was added to the homogenate in the tube. Minicondensers were fitted onto each tube and the feces were saponified under reflux for 90 min at 90°C. After cooling, 2 ml of absolute ethanol was added to the saponified mixture and the nonsaponifiable neutral sterols were extracted into 20 ml of petroleum ether (boiling range 35–60°C). The solvent was washed with 5 ml of water.

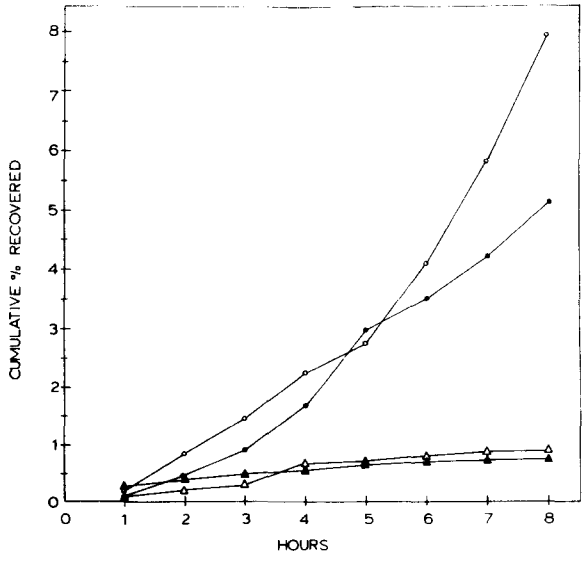

Fig. 2. Cumulative percent recovery in the thoracic duct lymph as a function of time. Circles, [^{14}C]cholesterol; triangles, [^{3}H]β-sitostanol. Each line represents an individual animal.

An aliquot of the petroleum ether extract was pipetted directly into a scintillation vial and the solvent was evaporated under nitrogen. The residue was dissolved in 2 ml of chloroform and the solution was ozonated to remove the color (5). The chloroform was evaporated under nitrogen and 12 ml of 10% Bio-Solv solubilizer (Beckman Instrument Co., Fullerton, CA) in toluene scintillation solution was added to the vial. Radioactivity was assayed in a Packard liquid scintillation spectrometer with automatic external standard for quench correction. This method recovered 95.2 ± 0.6% (mean ± SD, N = 6) of the cholesterol and 97.9 ± 1.6% (N = 6) of the β-sitostanol added to fecal homogenates. The recoveries were in fairly good agreement with those obtained by Malinow et al. (6) for cholesterol and β-sitosterol under similar conditions.

Forty-eight hours after injection of the test meal a blood sample, with heparin as the anticoagulant, was obtained from the tail of each rat under light ether anesthesia. The plasma was saponified and extracted with ethanol and petroleum ether and analyzed in a manner similar to the lymph.

Calculation of absorption

The absorption of labeled cholesterol from fecal excretion data was calculated in three different ways.

A. Isotope ratio method. Based on certain assumptions concerning the nonabsorbability of β-sitostanol to be described, the absorption of labeled cholesterol was calculated using the ratio of the two isotopes in the feces and test meal, according to the following formula:

$$\% \text{ Cholesterol absorbed} = [1 - (^{14}C/^{3}H \text{ in test meal}/ \; ^{14}C/^{3}H \text{ in first day fecal collection})] \times 100$$

B. Total fecal recovery. The percent labeled cholesterol absorbed was calculated by the following formula:

$$\% \text{ Cholesterol absorbed} = [1 - (\Sigma[^{3}H]\text{cholesterol recovered}/ \; [^{3}H]\text{cholesterol injected})] \times 100$$

C. Total fecal recovery corrected for nonabsorptive losses. This method corrects for incomplete recovery of cholesterol in feces due to nonabsorptive losses which may include possible bacterial degradation of cholesterol, incomplete fecal collection, variations in fecal flow, sequestration of cholesterol, etc. Based again on the assumption about β-sitostanol mentioned earlier, the fecal recovery of labeled β-sitostanol was used to make correction for nonabsorptive

loss of labeled cholesterol according to the following formula:

$$[1 - (\Sigma[^3H]\text{cholesterol recovered}/ [^3H]\text{cholesterol injected} \times [^{14}C]\beta\text{-sitostanol injected}/ \Sigma[^{14}C]\beta\text{-sitostanol recovered})] \times 100$$

RESULTS

Lymphatic recovery of [^{14}C]cholesterol and [22,23-^{3}H]β-sitostanol

The cumulative percent recoveries of [4-^{14}C]cholesterol and [22,23-^{3}H]β-sitostanol, over a period of 8 hr, in the thoracic duct lymph of rats are given in **Fig. 2,** showing an average recovery of 6.5% of the administered cholesterol and 0.8% of the administered β-sitostanol. The 24-hr recoveries averaged 36.7% and 2.2% for labeled cholesterol and β-sitostanol, respectively. The cholesterol recoveries are in agreement with the results of Sylven and Borgstrom (7). The lymphatic transport of β-sitostanol has not been studied previously.

Effect of cholestyramine

The effect of cholestyramine on the fecal excretion of labeled cholesterol and β-sitostanol is shown in **Fig. 3.** Rats receiving cholestyramine, as expected, excreted significantly greater amounts of labeled cholesterol than rats receiving no cholestyramine ($P < 0.001$ by analysis of variance). A near maximum effect of the drug was achieved at a dose of 100 mg. The fecal excretion of labeled β-sitostanol was unaffected by cholestyramine at any dose.

Absorption of labeled cholesterol and β-sitostanol

The fecal recoveries of labeled cholesterol and β-sitostanol in the presence and absence of cholestyramine are shown in **Table 1** together with computed values of cholesterol absorption using the three different methods. Note that the fecal excretion of β-sitostanol averaged 85%, independent of the presence or absence of cholestyramine. Because only 2% of the labeled β-sitostanol was recovered in the lymph, this means that 87% of the labeled β-sitostanol was accounted for. The remaining 13% must have been either degraded during passage through the intestine, sequestered in the gut, or absorbed through nonlymphatic channels (all of which were collectively referred to earlier as nonabsorptive losses). Because labeled β-sitostanol was not detected in the blood, we assume that none was absorbed through nonlymphatic channels.

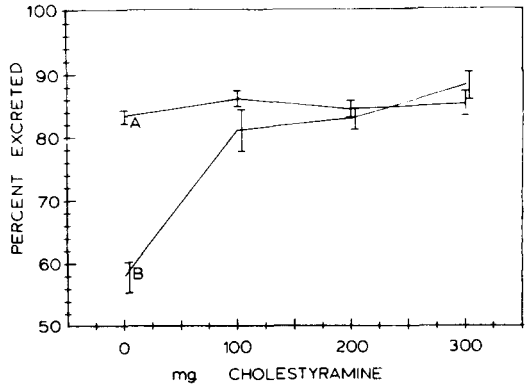

Fig. 3. Effects of cholestyramine on fecal excretion. *A*, [^{14}C]β-Sitostanol; *B*, [^{3}H]cholesterol. Values are percent of fed amounts as a function of cholestyramine dose. The fecal [^{3}H]cholesterol recoveries were corrected for nonabsorptive losses. Each point is the mean of five rats. The vertical bars represent standard errors offset in *B* to avoid overlapping.

The computation of cholesterol absorption by the three methods in the last three columns of Table 1 are based on two assumptions. First, labeled β-sitostanol was absorbed to the extent of only approximately 2% and this quantity was regarded as negligible. Second, the nonabsorptive losses of labeled β-sitostanol by bacterial or chemical degradation or by sequestration were matched by similar nonabsorptive losses of labeled cholesterol. There is no a priori reason to doubt the validity of this assumption since the two compounds are similar to one another in structure.

In **Fig. 4,** the values for absorption of labeled cholesterol from the isotope ratio method (method A) are plotted against each of the values from the two total fecal recovery methods—method B (uncorrected) and method C (corrected for nonabsorptive losses).

Fig. 4*A*, comparing methods A and B, shows a correlation coefficient of 0.978, but the values from these (uncorrected) fecal recoveries consistently exceeded those from the isotope ratios as evidenced by the positive *x*-axis intercept of the regression line. This would be expected if there were equivalent nonabsorptive losses of labeled cholesterol and β-sitostanol since equivalent nonabsorptive losses would yield reduced fecal recoveries of labeled cholesterol (and hence greater apparent absorption) while the ratio of the two compounds in the feces would be unaffected. That the nonabsorptive losses of both labeled cholesterol and β-sitostanol were similar was evidenced by the fact that when the fecal recoveries of labeled cholesterol and β-sitostanol, in the group not receiving cholestyramine, were summed with the recoveries of the two isotopes

TABLE 1. Fecal recoveries of [^{3}H]cholesterol and [^{14}C]β-sitostanol and percent cholesterol absorbed as calculated by the isotope ratio method (Method A) and two fecal recovery methods. (Method B, total fecal recovery and Method C, total fecal recovery corrected for nonabsorptive losses.)

Rat No.	Cholestyramine Fed	Cumulative % Recovery		Percent Cholesterol Absorbed		
				Isotope Ratio Method	Total Fecal Recovery Method	
		[^{3}H]Cholesterol	[^{14}C]β-Sitostanol	Method A	Method B	Method C
	mg					
3	0	45.11	83.38	52.14	54.89	45.90
4		55.06	83.40	38.75	44.94	33.98
10		55.23	84.87	39.32	44.77	34.92
12		49.53	85.22	47.22	50.47	41.88
17		48.90	79.80	44.32	51.10	38.72
Mean ± SEM		50.77 ± 1.94	83.33 ± 0.96	44.35 ± 2.51	49.23 ± 1.94	39.08 ± 2.21
5	100	71.70	85.70	16.91	28.30	16.34
11		75.64	82.23	10.97	24.36	8.01
13		64.40	86.27	27.19	35.60	25.35
16		73.24	86.20	16.33	26.76	15.03
19		69.58	90.19	24.73	30.42	22.85
Mean ± SEM		70.91 ± 1.90	86.12 ± 1.26	19.23 ± 2.96	29.09 ± 1.90	17.52 ± 3.06
1	200	73.99	84.97	14.17	26.01	12.92
6		74.18	87.64	15.63	25.82	15.36
8		75.25	86.28	14.19	24.75	12.78
9		68.98	82.63	19.71	31.02	16.52
15		63.27	80.83	23.21	36.73	21.72
Mean ± SEM		71.13 ± 2.25	84.47 ± 1.23	17.38 ± 1.77	28.87 ± 2.25	15.86 ± 1.63
2	300	73.75	79.50	7.93	26.25	7.23
7		74.11	82.68	11.22	25.89	10.37
14		79.12	85.19	7.32	20.88	7.13
18		73.75	89.38	18.39	26.25	17.49
20		77.75	89.36	14.21	22.25	12.99
Mean ± SEM		75.70 ± 1.14	85.22 ± 1.92	11.81 ± 2.06	24.30 ± 1.14	11.04 ± 1.94

in the thoracic duct lymph, the sums were nearly identical (51 + 36 = 87% for labeled cholesterol and 83 + 2 = 85% for labeled β-sitostanol). If 100% minus these values were taken to represent nonabsorptive losses, then the nonabsorptive losses of labeled cholesterol and β-sitostanol were similar.

In Fig. 4*B*, the isotope ratio method is compared in a similar fashion with method C (fecal recoveries after correcting for nonabsorptive losses). The correlation coefficient was increased to 0.998. In addition, the slope of the regression was closer (1.14) to the slope of the line of identity (1.0) and the intercept was not significantly different from the origin. Thus, one can see that the isotope ratio method for estimating cholesterol absorption compares favorably with estimates based on the total fecal recovery of labeled cholesterol, particularly when account is taken of a possible source of error

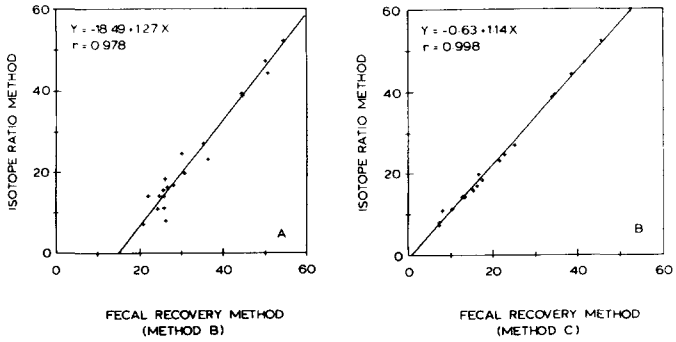

Fig. 4. Comparison of the isotope ratio method and two total fecal recovery methods. *A*, Total fecal recovery, uncorrected and *B*, total fecal recovery corrected for nonabsorptive cholesterol losses.

in the fecal recovery method, which is the nonabsorptive losses of labeled cholesterol during intestinal transit.

DISCUSSION

Fecal recovery and intestinal absorption of β-sitostanol

The mean recovery of labeled β-sitostanol in this study was 85%. This could mean that 15% of the compound was absorbed. However, we have presented three lines of evidence to suggest that the β-sitostanol was not absorbed or that it was absorbed in minimal amounts only.

1) In contrast to cholesterol, cholestyramine failed to cause an increase in the fecal excretion of labeled β-sitostanol. Cholestyramine binds bile salts (8) which have been shown to be essential for the absorption of cholesterol (9). The failure of cholestyramine to alter the fecal excretion of labeled β-sitostanol may mean either that β-sitostanol does not aggregate with bile salts to form mixed micelles as does cholesterol or that β-sitostanol does enter into the formation of mixed micelles with bile salts, but is still unable to be absorbed. In either case, cholestyramine would not be expected to have any effect on the fecal excretion of β-sitostanol.

It seems most unlikely that the lack of micellization of β-sitostanol was responsible for the lack of effect of cholestyramine on the fecal excretion of the compound, because micellar solutions of β-sitostanol have been prepared in our laboratory in connection with other studies. Therefore, the complete lack of effect of cholestyramine on the fecal excretion of β-sitostanol may be taken as indirect evidence that β-sitostanol was not absorbed to any appreciable extent, if at all, in this study.

2) No ^{14}C (β-sitostanol) activity was detectable in the nonsaponifiable fraction of the plasma taken from any of the animals 48 hr after feeding the test meal, although significant ^{3}H (cholesterol) activity was detectable in the same fraction of plasma of all but one rat (rat no. 14 in the group receiving 300 mg of cholestyramine). It is still possible, however, that absorption of β-sitostanol did not follow the same time course as cholesterol. Thus, labeled β-sitostanol absorbed after 48 hr may have escaped detection because later plasma samples were not taken. Considering the relatively short intestinal transit time of the rat (approximately 24 hr), it seems unlikely that any labeled β-sitostanol was absorbed after 48 hr. In any case, lack of ^{14}C activity in the plasma 48 hr after feeding the compound supports the conclusion that β-sitostanol was not absorbed to any appreciable extent.

3). An average of only 2.2% of the administered β-sitostanol was found in the thoracic duct lymph of two rats in 24 hr. Even though a small percentage of administered β-sitostanol was recovered in the lymph, it is interesting to note that the compound could not be detected in the plasma. This may have been due either to dilution of the small amount of the isotope in the circulation or to its possible rapid excretion or metabolism. Thus, it is evident that labeled β-sitostanol was absorbed only to a very limited extent, if at all. Further support for this conclusion has been provided by the studies of Sugano, Morioka, and Ikeda (10) and Ikeda and Sugano (11).

The apparent nonabsorbability of β-sitostanol provided the basis for using the isotope ratio method for calculating the absorption of cholesterol. In addition, because the nonabsorptive losses of β-sitostanol and cholesterol were shown to be similar, the fecal recoveries of labeled β-sitostanol were used as a correction factor to correct for nonabsorptive losses of labeled cholesterol.

β-sitostanol: a new marker for studying cholesterol absorption

An ideal marker or reference compound for studying the absorption of cholesterol should at least be *1*) nonabsorbable, *2*) similar to cholesterol in physicochemical properties, and *3*) subject to the same nonabsorptive losses as cholesterol during transit through the intestine. The last criterion has come to be recognized as important because of the possibility of degradation of neutral sterols in transit through the intestine (12, 13). The extent of degradation (or lack of it) has been shown to vary depending on species (14), intestinal transit rates (15), and diet (16). It is important, therefore, that an ideal marker for cholesterol absorption be treated in the same way as cholesterol by the intestinal bacterial flora.

Over the years, a number of substances such as yttrium chloride (17, 18), inulin (19, 20), dextran (19, 20) and β-sitosterol (12, 21–23) have been used as markers to study the absorption of cholesterol. Among these, only β-sitosterol is lipid-soluble and has been shown to have physicochemical properties similar to cholesterol (1). Evidence also indicates that it is treated by the intestinal bacteria in the same way as cholesterol (13). β-Sitosterol, however, is absorbed to varying degrees (2) and to the extent that it is absorbed, it is less than ideal as a marker.

Results from the present study indicate that β-sitostanol possesses properties required of a valid marker for studying cholesterol absorption. These include: *1*) The nonabsorptive losses (due to degradation by intestinal bacteria, etc.) of cholesterol and β-sitostanol were similar. The sum of the fecal and lymphatic recoveries of the two isotopes were nearly identical. *2*) The absorption of labeled cholesterol calculated by the isotope ratio method agreed closely with the corresponding values obtained by the total fecal recovery method, particularly when corrections for nonabsorptive losses of neutral sterols were made. As pointed out earlier, this correction should be made before comparing values of cholesterol absorption obtained by the two methods. *3*) The absorption of labeled β-sitostanol was minimal and may be regarded as insignificant.

Further, because β-sitostanol is prepared by the catalytic hydrogenation of β-sitosterol, it may be reasonably assumed that the physicochemical properties of β-sitostanol are similar to β-sitosterol and hence to cholesterol.

Thus, β-sitostanol may be considered to be a valid marker for studying cholesterol absorption in normal rats and in rats with impairment in cholesterol absorption induced by the administration of cholestyramine. The choice between the use of β-sitostanol and β-sitosterol as a marker for cholesterol absorption would seem to depend on the relative absorbability of the two substances in a given animal under given conditions.

It is important to note that measurement of the ratio of the two isotopes in feces was confined to the first day of fecal collection because the ratio of [^{14}C]β-sitostanol to [^{3}H]cholesterol in the feces was found to decrease following the first day's fecal collection. Borgstrom (5) and Sodhi et al. (24) have found similar changes in the fecal ratios of β-sitosterol and cholesterol and have attributed these changes to the secretion of absorbed labeled cholesterol into the lumen in the later stages of fecal collection (24).

Effect of cholestyramine on the excretion of labeled cholesterol

In the present study, various amounts of cholestyramine were fed to rats to achieve different degrees of malabsorption of cholesterol. Cholestyramine, by binding bile salts (8), reduces the availability of bile salts in the intestinal lumen and hence reduces the absorption of cholesterol. The binding of bile salts to cholestyramine, however, shows saturation (8). Binding of bile salts to cholestyramine is linear at low concentrations of cholestyramine. A further increase in the concentration of cholestyramine does not result in a directly proportional increase in the binding of bile salts. Since the presence of bile salts is essential for the absorption of cholesterol (9), the pattern of absorption of cholesterol in the presence of cholestyramine would be the reciprocal of the pattern of binding of bile salts to cholestyramine. In other words, the fecal excretory pattern of cholesterol when cholestyramine has been administered, would be expected to be similar to the pattern of binding of bile salts to cholestyramine. This expectation was realized in the present study in which the fecal excretion of cholesterol (Fig. 3) achieved a near maximum at a dose of 100 mg of cholestyramine. Further increase in the amount of cholestyramine administered led to only small additional increase in the fecal excretion of cholesterol.

Lymphatic recovery of [^{3}H]β-sitostanol

In order to directly evaluate the absorbability of β-sitostanol, the recovery of [^{3}H]β-sitostanol in the lymph was studied. Only 2.2% of the administered label was recovered in the thoracic duct lymph in 24 hr. Interestingly, both the rate of recovery and the total recovery of sitostanol in the lymph shown in the present study differed from that of sitosterol shown in a previous study (7). Why two substances so similar to one another should exhibit these differences is not clear. What is also not well understood is why these compounds, β-sitosterol and β-sitostanol, are absorbed so minimally when structurally they are quite similar to cholesterol. It is apparent that more work at a molecular level is needed to clarify the mechanisms involved in the intestinal absorption of sterols.

At this point it is interesting to compare the relative absorbabilities of the various stanols and sterols. It appears that there exists a pattern of decreasing absorbability and increasing potency as inhibitors of cholesterol absorption with substitution on the 24 position of the side chain and/or reduction of the double-bond in the B ring as shown below:

cholestanol < campesterol < β-sitosterol < β-sitostanol

According to the above, it appears that substitution on the 24 position of the side chain is more important with respect to the absorbability of the sterol (e.g., cholesterol and β-sitosterol). Reduction of the B ring further decreases the absorbability and increases the potency of inhibiting the absorption of cholesterol, e.g., β-sitosterol and β-sitostanol (10, 11).

Based on this, it may be hypothesized that, if there is a carrier molecule specific for cholesterol as Glover and Green (25, 26) proposed, the carrier is specific for the double bond in the B ring and the substituent group on the 24 position of the side chain. Such specificity of the carrier molecule could account for the pattern of absorbability of the various sterols and stanols and their potential for inhibiting cholesterol absorption. It would be interesting to see if other substitutions at the 24 position (other than CH_3 and C_2H_5) and/or around the double bond in the B ring lead to a substance that is less absorbable and even more potent than the plant sterols and stanols as an inhibitor of cholesterol absorption.

The authors gratefully acknowledge the help of Phyllis McLaughlin, Carolyn Stafford, and Muriel Schultz of the Oregon Regional Primate Research Center for technical assistance and Alice Fitzgerald and Lydia Welden for secretarial assistance. This work was supported in part by Grant 23.09 from The Medical Research Foundation of Oregon.

Manuscript received 18 September 1978; accepted 29 December 1978.

REFERENCES

1. Borgstrom, B. 1967. Partition of lipids between emulsified oil and micellar phases of glyceride–bile salt dispersions. *J. Lipid Res.* **8:** 598–608.
2. Subbiah, M. T. R. 1973. Dietary plant sterols: Current status in human and animal sterol metabolism. *Am. J. Clin. Nutr.* **26:** 219–225.
3. Ivy, A. C., T. M. Lin, and E. Karvinen. 1955. Absorption of dihydrocholesterol and soya sterols by the rat's intestine. *Am. J. Physiol.* **183:** 79–85.
4. Lambert, R. 1965. Surgery of the Digestive System in the Rat. Charles C Thomas, Springfield, Ill.
5. Borgstrom, B. 1967. Quantitative aspects of the intestinal absorption and metabolism of cholesterol and beta sitosterol in the rat. *J. Lipid Res.* **9:** 471–481.
6. Malinow, M. R., P. McLaughlin, L. Papworth, C. Stafford, G. O. Kohler, A. L. Livingston, and P. R. Cheeke. 1977. Effect of alfalfa saponins on intestinal cholesterol absorption in rats. *Am. J. Clin. Nutr.* **30** 2061–2067.
7. Sylven, C., and B. Borgstrom. 1969. Absorption and lymphatic transport of cholesterol and sitosterol in rats. *J. Lipid Res.* **10:** 179–182.
8. Kritchevsky, D., and J. A. Story. 1974. Binding of bile salts in vitro by non-nutritive fiber. *J. Nutr.* **104:** 458–462.
9. Siperstein, M. D., I. L. Chaikoff, and W. O. Reinhardt. 1952. ^{14}C Cholesterol: V. Obligatory function of bile in the intestinal absorption of cholesterol. *J. Biol. Chem.* **198:** 111–114.
10. Sugano, M., H. Morioka, and I. Ikeda. 1977. A comparison of hypochlesterolemic activity of β-sitosterol and β-sitostanol in rats. *J. Nutr.* **107:** 2011–2019.
11. Ikeda, I., and M. Sugano. 1978. Comparison of absorption and metabolism of β-sitosterol and β-sitostanol in rats. *Atherosclerosis.* **30:** 227–237.
12. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary β-sitosterol as an internal standard to correct for losses in sterol balance studies. *J. Lipid. Res.* **9:** 374–381.
13. Ahrens, E. H., Jr. 1970. A review of the evidence that dependable sterol balance studies require a correction for the losses of neutral sterols that occur during intestinal transit. *In* Atherosclerosis: Proceedings of the Second International Symposium on Atherosclerosis. R. J. Jones, editor. Section VIII, 248–252.
14. St. Clair, R. W., N. D. M. Lehner, and T. E. Hamm. 1975. Evaluation of chromic oxide, glycerol triether and β-sitosterol as fecal flow markers in two species of non-human primates. *Lipids.* **10:** 25–32.
15. Lofland, H. B., Jr., T. B. Clarkson, R. W. St. Clair, and N. D. M. Lehner. 1972. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. *J. Lipid Res.* **13:** 39–47.
16. Denbesten, L., W. E. Connor, T. H. Kent, and D. Lin. 1970. Effect of cellulose on the recovery of dietary plant sterols in the feces. *J. Lipid Res.* **11:** 341–345.
17. Sklan, D., P. Budowski, and S. Hurwitz. 1974. Effect of soy sterols on intestinal absorption and secretion of cholesterol and bile acids in the chick. *J. Nutr.* **104:** 1086–1090.
18. Sklan, D., M. Dahan, P. Budowski, and S. Hurwitz. 1977. Differential absorption of endogenous and exogenous cholesterol in the chick as effected by dietary oil level and phytosterols. *J. Nutr.* **107:** 1996–2001.
19. Sallee, V. L., F. A. Wilson, and J. M. Dietschy. 1972. Determination of unidirectional uptake rates for lipids across the intestinal brush border. *J. Lipid Res.* **13:** 184–192.
20. Lukie, B. E., H. Westergaard, and J. M. Dietschy. 1974. Validation of a chamber that allows measurement of tissue uptake and unstirred layer thickness in the intestine under conditions of controlled stirring. *Gastroenterology* **67:** 652–661.
21. Arnesjo, B., A. Nilsson, J. Barrowman, and B. Borgstrom. 1969. Intestinal digestion and absorption of cholesterol and lecithin in the human. *Scand. J. Gastroent.* **4:** 653–665.
22. Traber, M. G., and R. Ostwald. 1978. Cholesterol absorption and steroid excretion in cholesterol-fed guinea pigs. *J. Lipid Res.* **19:** 448–456.
23. Samuel, P., J. R. Crouse, and E. H. Ahrens, Jr. 1978. Evaluation of an isotope ratio method for measurement of cholesterol absorption in man. *J. Lipid Res.* **19:** 82–93.
24. Sodhi, H. S., B. J. Kudchodkar, P. Varughese, and D. Duncan. 1974. Validation of the ratio method for calculating absorption of dietary cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **145:** 107–111.
25. Glover, J., and C. Green. 1957. Sterol metabolism. 3. The distribution and transport of sterols across the intestinal mucosa of the guinea pig. *Biochem. J.* **67:** 308–316.
26. Glover, J., and C. Green. 1956. Studies on the absorption and metabolism of sterols. Mode of absorption. *In* Biochemical Problems of Lipids. G. Popjak and F. LeBreton, editors. Butterworths, London. 359–364.