

# $\beta$ -Sitosterolemia and Xanthomatosis

## A NEWLY DESCRIBED LIPID STORAGE DISEASE IN TWO SISTERS

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**ABSTRACT** Although the usual diet may contain 150–250 mg of plant sterols, chiefly  $\beta$ -sitosterol, only trace amounts of these sterols have heretofore been found in human or animal blood and tissues. We now report elevated plant sterol levels in the blood and tissues of two sisters with extensive tendon xanthomas but normal plasma cholesterol levels. Besides  $\beta$ -sitosterolemia and xanthomatosis, no other physical, mental, or biochemical abnormalities were detected.

Repeatedly, the plasmas of the two sisters have contained 27.1 and 17.7 mg/100 ml of  $\beta$ -sitosterol, 9.7 and 8.2 mg/100 ml of campesterol, and 0.5 and 0.5 mg/100 ml of stigmasterol, respectively. These plant sterols constituted 15.6 and 11.3% of the total plasma sterols. Some 60% of the plasma  $\beta$ -sitosterol and campesterol was esterified; the measurable stigmasterol was entirely unesterified. The transport of the plasma  $\beta$ -sitosterol and campesterol was largely in low density lipoproteins (76 and 83%, respectively). High density lipoproteins carried the remainder. Plant sterols were barely detectable in the very low density lipoprotein fraction. Only trace amounts of stigmasterol could be detected in the low density and high density lipoprotein fractions. The plant sterol content of the red blood cells averaged 12–13 mg/100 ml packed cells or about 13% of the total sterols. Two tendon xanthoma biopsies with the usual high concentration of cholesterol had 36.7 and 4.0 mg of plant sterols/g dry wt, of which 25.7 and 2.9 mg were  $\beta$ -sitosterol, entirely in the free form. Plant sterols were also found in adipose tissue

(0.2 mg/g wet wt) and in skin surface lipids (3.2 mg/g of lipid).

The intestinal absorption of  $\beta$ -sitosterol in both the patients, measured by two techniques, indicated greatly increased absorption of this sterol (about 24 and 28% in the patients L. H. and R. H., respectively, normal absorption being < 5%). We suggest that increased absorption of  $\beta$ -sitosterol must be considered as one cause of this disease.

The reason for the extensive xanthomatosis in these two patients remains unknown. Perhaps in some way plant sterols initiated the development of xanthomas with otherwise normal plasma cholesterol levels. Clinical atherosclerosis has not yet occurred. The occurrence of  $\beta$ -sitosterolemia in these two sisters with unaffected parents suggests an inherited recessive trait.

### INTRODUCTION

High concentrations of plant sterols in the blood and tissues of man and animals have not been previously reported. These sterols are usually found only in the lipids of plants and thus are particularly plentiful in vegetable oils, nuts, and other vegetable products. Structurally, the three plant sterols— $\beta$ -sitosterol, campesterol, and stigmasterol—resemble cholesterol except for minor differences in their side chains (Fig. 1).  $\beta$ -sitosterol and stigmasterol, both  $C_{28}$  sterols, have an ethyl group at the C-24 position; stigmasterol, in addition, has a double bond at the C-22 position. Campesterol, a  $C_{27}$  sterol, has a methyl group at the C-24 position.

Plant sterols are habitually consumed by man in the usual diet. A typical American diet may contain up to 250 mg of these sterols/day (1).  $\beta$ -Sitosterol is the predominant plant sterol, with campesterol the next

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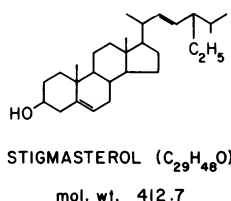
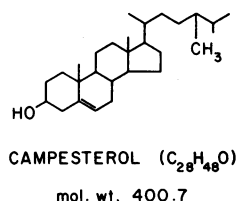
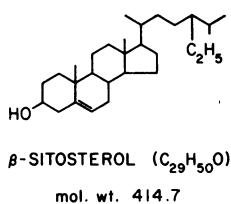
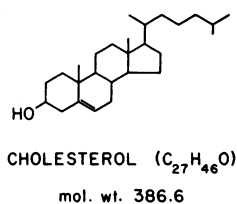


FIGURE 1 The chemical structures of cholesterol and plant sterols.

most abundant, and stigmasterol the least. These sterols pass through the intestinal tract almost completely unabsorbed and are usually recovered quantitatively in the feces. The intestinal absorption in man that does occur has been estimated at less than 5% of the amount in the diet, and only very small amounts of plant sterols are detectable in the blood (2, 3).

In this communication, we report for the first time high concentrations of  $\beta$ -sitosterol and the two other plant sterols in the blood and tissues of two sisters. The plant sterols were found to be widely distributed: in the plasma, lipoproteins, red blood cells, xanthoma, adipose tissue, and skin surface lipids. The intestinal

absorption of  $\beta$ -sitosterol was greatly increased, thus suggesting that one mechanism for the  $\beta$ -sitosterolemia was enhanced intestinal absorption of  $\beta$ -sitosterol from the diet. Furthermore, we postulate that in some way plant sterols initiated the development of the xanthomas in these two sisters, whose serum lipid levels were otherwise normal.

## METHODS

*The description of the patients.* The patients of this report were two Caucasian sisters, L. H. and R. H., who were university students (Table I). About 2 yr ago, patient L. H. attended the orthopedic clinic of the University Hospitals with a chief complaint of pains in both heels and knees. Tendon xanthomas were discovered, and she was referred to us for further investigations. Clinical history revealed that tendon xanthomas were first noted at the age of 8 yr in the extensor tendons of both hands. No treatment was given. Subsequently, over the years, xanthomas developed in the patellar, plantar, and Achilles tendon. Her younger sister, R. H., also had extensive tendon and tuberous xanthomas dating back to childhood. An excision biopsy of one lesion at that time indicated a "xanthoma." Patient R. H., when subsequently examined by us, was likewise found to have large xanthomas of the Achilles, plantar, and patellar tendons and of the extensor tendons of the hand. There were tuberous xanthomas of the elbows.

Aside from the xanthomas, their physical examinations and initial laboratory tests were normal. Dietary history revealed that both sisters were consuming the usual American diet. Their habitual diet provided an estimated plant sterol intake of 150–250 mg/day.

The family history indicated that the parents and the grandparents had no evidence of xanthomas. The parents were of German and Swiss-German origin. There was no history of consanguinity in the family. The two sisters were the only children in the family.

TABLE I  
*Clinical Description of the Two Sisters with  $\beta$ -Sitosterolemia and Xanthomatosis*

	Patient L. H.	Patient R. H.
Age, yr	22	20
Height, cm	165	165
Weight, kg	62	58
Xanthoma		
First appearance:		
Tuberous		At 7 yr, both elbows
Tendon	At 8 yr, extensor tendons of both hands At 14 yr, Achilles	At 7 yr, achilles
At present		
Tuberous	Both elbows	Both elbows, on both knees
Tendon	Extensor tendons of both hands, patellar, Achilles	Extensor tendons of both hands patellar, plantar, Achilles, both elbows
Plasma lipids, mg/100 ml		
Cholesterol	195	206
Triglycerides	81	63

*Determinations of sterols in plasma, erythrocytes, and tissues.* 0.5 ml of plasma or serum was saponified and extracted with hexane after the procedure of Abell, Levy, Brodie, and Kendall (4). The hexane extract was evaporated to dryness and analyzed by gas-liquid chromatography (GLC)<sup>1</sup> as trimethylsilyl ether (TMS) derivative (5), with 5 $\alpha$ -cholestane used as the internal standard. The erythrocytes were washed repeatedly with 0.9% sodium chloride solution, and the lipids were extracted with chloroform:isopropanol (7:11, vol/vol) (6). An aliquot of the extract was then subjected to GLC analysis as described above. Free and esterified sterols in plasma and erythrocyte were separated by thin-layer chromatography (TLC) using Silica-Gel G plates. The plates were developed in petroleum ether-diethyl ether-glacial acetic acid, 80:20:1. The free and ester sterol bands were scraped off and eluted with diethyl ether. The free sterol band eluate was subjected to GLC analysis directly, whereas the ester sterol band eluate was saponified, extracted with hexane, and then subjected to GLC analysis as described above.

Very low density lipoproteins (VLDL) were isolated by preparative ultracentrifugation from 6 ml of plasma (7). The infranate was made up to 5 ml and divided into two equal parts. From one, high density lipoprotein (HDL) fraction was obtained by precipitating low-density lipoprotein (LDL) with heparin and manganese chloride solution (8). The VLDL fraction, the fraction containing LDL and HDL, and the HDL fraction were separately extracted with chloroform:methanol (2:1, vol/vol) and made up to a suitable volume with the solvent. Aliquots of the extracts were taken for sterol analysis by GLC. The sterol value for LDL was obtained by subtracting the HDL sterol from that of the combined HDL + LDL fraction.

Patient R. H. underwent surgical removal of xanthomas from the patellar and the plantar tendons (through the cooperation of Dr. A. E. Flatt, Department of Orthopaedics). The tissue specimens were cut into small pieces and washed repeatedly with 0.9% sodium chloride solution to remove any blood. The pieces were blotted on a filter paper, weighed, and dried in a hot oven vacuum dessicator at 80°C to a constant weight. The dried tissues were repeatedly extracted with chloroform:methanol by boiling, and the lipid extracts were made up to 100 ml. Aliquots were taken for total, free, and esterified sterol determinations as described above. A sample of adipose tissue from patient R. H. was obtained by needle aspiration (9) for sterol analysis as described above.

*In vitro plasma esterification of sterols.* Blood samples in EDTA, 1 mg/ml blood were obtained from patient R. H. and two normal subjects (W. C. and A. B.), all in the fasting state. The plasma was added to a tube containing a known quantity of [4-<sup>14</sup>C] $\beta$ -sitosterol<sup>2</sup> (20,048 dpm/ml) and [1-2-<sup>3</sup>H]cholesterol<sup>3</sup> (20,208 dpm/ml) and mixed thoroughly. 0.5 ml of the plasma was immediately pipetted out

<sup>1</sup> *Abbreviations used in this paper:* GLC, gas-liquid chromatography; HDL, high density lipoprotein; LCAT, lecithin-cholesterol acyl transferase; LDL, low density lipoprotein; TLC, thin-layer chromatography; TMS, trimethylsilyl ether; VLDL, very low density lipoprotein.

<sup>2</sup> Obtained from Amersham/Searle Corp., Arlington Heights, Ill.

<sup>3</sup> Obtained from New England Nuclear, Boston, Mass. The radio-purity of both sterols was >98% as checked by TLC and GLC.

TABLE II  
*Test Breakfast for Sitosterol Absorption*

Substance	Amount
Calories	800
Carbohydrate	63 g
Protein	25 g
Fat	50 g
Mono-unsaturated	18.3 g
Saturated	16.6 g
Polyunsaturated	15.1 g
Cholesterol	0 mg
Plant sterols	3,500 mg
$\beta$ -Sitosterol	2,400 mg
Campesterol	1,000 mg
Stigmasterol	100 mg
Isotope: [4- <sup>14</sup> C] $\beta$ -sitosterol	2.0 $\mu$ Ci

and extracted as above. The rest were incubated at 37°C in a Dubnoff metabolic shaker, and at intervals of 24 and 48 h 0.5-ml aliquots were taken out and extracted with chloroform:methanol. Free and ester sterols were determined as described above, and the radioactivity was determined on another portion with 10 ml of scintillation solution (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis(2-(5-phenyloxazolyl) benzene)/liter of toluene), in a Packard Tri-Carb liquid scintillation counter (model 3380, Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with external standardization.

*Sterols of the skin surface lipids.* Two overnight collections of skin surface lipids were carried out on each patient after the procedure described previously (5). Sterol analyses were carried out by combination of TLC and GLC (5).

*$\beta$ -Sitosterol intestinal absorption.* The intestinal absorption of  $\beta$ -sitosterol was measured in both patients by feeding a single dose of  $\beta$ -sitosterol (about 2,500 mg and 2.0  $\mu$ Ci of [4-<sup>14</sup>C] $\beta$ -sitosterol) in a formula breakfast (Table II). The isotope was first dissolved in 5 g peanut oil with 150 mg crystalline  $\beta$ -sitosterol added as carrier and then mixed with the formula, which contained a total of 2,500 mg of  $\beta$ -sitosterol. An aliquot of the formula was analyzed for sterol mass and radioactivity to obtain the exact amounts fed (10). The feces were collected daily for the following 7-8 days and homogenized with 1:1 water as a single pool, and an aliquot was analyzed for sterol mass and radioactivity (10). The absorption of  $\beta$ -sitosterol was calculated as the difference between the amount fed and the amount excreted in the feces and expressed as percent of the intake (11). By this technique absorption was measured for both mass and radioactivity. The amount of  $\beta$ -sitosterol ingested daily in the background diet was considered in the calculations for mass. Fasting blood samples were also obtained subsequently for the determination of plant sterol radioactivity.

Patient R. H. received a second and larger test dose of about 60  $\mu$ Ci of [4-<sup>14</sup>C] $\beta$ -sitosterol in approximately 100 ml of skim milk with a breakfast that provided a total of 535 calories, 70 g carbohydrate, 21 g protein, 19 g fat, and 225 mg of cholesterol. This study was performed as a second test of absorption and to follow more accurately the appearance of [4-<sup>14</sup>C] $\beta$ -sitosterol in the blood.

TABLE III  
Plasma Total, Free, and Esterified Plant Sterols and Cholesterol in the Two Sisters

Patient	Date of sample	Total plant sterols	Plant sterols									Cholesterol			
			$\beta$ -Sitosterol			Campesterol			Stigmasterol			Total	Free	Ester	
			Total	Free	Ester	Total	Free	Ester	Total	Free	Ester				
<i>mg/100 ml</i>															
L. H.	5-18-72	32.2	24.5	9.4	15.1	7.5	2.6	5.0	0.2	0.2	Trace*	162.8	40.7	122.1	
	2-25-72	39.4	28.9	16.3	12.5	10.4	5.7	4.7	0.1	0.1	Trace	204.8	74.6	130.2	
	8-29-72	40.3	28.0	10.4	17.8	11.1	3.6	7.5	1.2	1.2	Trace	212.6	53.3	159.3	
	Mean $\pm$ SD	37.3 $\pm$ 4.4	27.1 $\pm$ 2.3	12.0 $\pm$ 3.7	15.1 $\pm$ 2.6	9.7 $\pm$ 1.9	4.0 $\pm$ 1.6	5.7 $\pm$ 1.5	0.5 $\pm$ 0.6	0.5 $\pm$ 0.6	Trace	193.4 $\pm$ 26.8	56.2 $\pm$ 17.1	137.1 $\pm$ 19.6	
R. H.	4-7-72	24.0	16.3	8.1	8.1	7.6	3.6	4.0	1.0	1.0	Trace	183.1	33.3	149.8	
	5-18-72	28.4	19.5	7.2	12.3	8.7	3.0	5.7	0.2	0.2	Trace	233.4	62.1	171.3	
	6-15-72	26.0	17.4	6.7	10.7	8.3	2.8	5.4	0.3	0.3	Trace	200.5	53.4	147.1	
	Mean $\pm$ SD	26.1 $\pm$ 2.2	17.7 $\pm$ 1.6	7.3 $\pm$ 0.7	10.4 $\pm$ 2.1	8.2 $\pm$ 0.5	3.1 $\pm$ 0.4	5.0 $\pm$ 0.9	0.5 $\pm$ 0.4	0.5 $\pm$ 0.4	Trace	205.7 $\pm$ 25.5	49.6 $\pm$ 14.8	156.1 $\pm$ 13.3	

\* Indicates <0.01 mg/100 ml.

## RESULTS

Plant sterols were found in relatively high concentrations in the plasmas of both sisters (Table III). On repeated determinations, the total plasma plant sterol concentration was 37.3 mg/100 ml in patient L. H. and constituted 16.2% of the total plasma sterols. These were distributed as 27.1 mg/100 ml  $\beta$ -sitosterol, 9.7 mg/100 ml campesterol, and 0.5 mg/100 ml stigmasterol. The plasma cholesterol in patient L. H. was 193 mg/100 ml or about 84% of the total plasma sterols. Similarly, in the sister, R. H., the total plasma plant sterols were 26.1 mg/100 ml or 11.3% of total plasma sterols. These were distributed as 17.7 mg/100 ml  $\beta$ -sitosterol, 8.2 mg/100 ml campesterol, and 0.5 mg/100 ml stigmasterol. The plasma cholesterol concentration in patient R. H. was 206 mg/100 ml, constituting about 89% of the total plasma sterols. The total plasma plant sterol concentration of their father was 0.19 mg/100 ml, and that of the mother was 0.27 mg/100 ml: barely detectable amounts. The total plasma plant sterol content in normal subjects, determined in our laboratory, averaged  $0.67 \pm 0.09$  mg/100 ml.

Both the plasma  $\beta$ -sitosterol and campesterol were largely esterified: 57 and 60% of their respective total

amounts (Table III). The amount of esterification was similar for both patients. The plasma stigmasterol, found in very small quantities, was measured only in the free form (0.5 mg/100 ml). In comparison, the plasma esterified cholesterol constituted 71.2 and 76.2% of the total cholesterol in L. H. and R. H., respectively.

The red blood cell content of plant sterols was 12-13 mg/100 ml of packed cells for both sisters, about 13% of the total erythrocyte sterols (Table IV). Red cell cholesterol concentration was, on the average, 83 mg/100 ml packed cells or 87% of the total sterols. All red cell sterols were entirely in the free form. The cholesterol-to- $\beta$ -sitosterol ratio in the red blood cells was nearly similar to the ratio in the plasma:  $933 \pm 22\%$  in the red blood cells and  $1,157 \pm 37$  in the plasma. This suggests free exchange of both sterols between the plasma and the red blood cells.

The plasma plant sterols were mainly transported in low density and high density lipoproteins. The very low density lipoproteins of both sisters carried only trace amounts of plant sterols (Table V). The low density lipoprotein  $\beta$ -sitosterol was 82 and 70% of total plasma  $\beta$ -sitosterol concentration in L. H. and R. H. respectively, very similar to the concentration of low density

TABLE IV  
Red Blood Cell Sterols in the Two Sisters

Patient	Plant sterols				Cholesterol
	Total	$\beta$ -Sitosterol	Campesterol	Stigmasterol	
<i>mg/100 ml packed cells</i>					
L. H.	12.1	7.0	3.1	1.0	82.2
R. H.	13.6 $\pm$ 2.0*	8.8 $\pm$ 1.2	4.0 $\pm$ 0.5	0.7 $\pm$ 0.4	84.3 $\pm$ 9.6

\* Mean  $\pm$ SD of three determinations.

TABLE V  
The Distribution of Plant Sterols and Cholesterol in the Plasma\* Lipoprotein Fractions of the Two Sisters

Patient	Very low density lipoprotein		Low density lipoprotein†				High density lipoprotein‡				
	Plant sterols, total	Cholesterol	Plant sterols			Cholesterol	Plant sterols			Cholesterol	
			Total	$\beta$ -Sito-sterol	Campe-sterol		Total	$\beta$ -Sito-sterol	Campe-sterol		
						<i>mg/100 ml</i>					
L. H.	Trace§	12.1	32.9	20.4	12.5	194.5	6.5	4.3	3.3	29.5	
	Trace	4.9	31.9	22.2	9.7	177.3	5.9	4.9	1.0	33.6	
R. H.	Trace	8.3	14.5	7.9	6.6	128.6	8.5	6.0	2.5	66.8	
	Trace	4.3	17.6	10.9	6.7	136.2	3.3	2.2	1.1	53.6	

\* Obtained on different days.

† Stigmasterol present in trace amounts (<0.01 mg/100 ml).

‡ Indicates <0.01 mg/100 ml.

lipoprotein cholesterol in relation to total cholesterol (Fig. 2). 18 and 30% of the total plasma  $\beta$ -sitosterol in L. H. and R. H. were transported in high density lipoprotein. The percent low density lipoprotein campesterol was 87.4 and 85.8 in L. H. and R. H., respectively—a little higher than that of cholesterol and  $\beta$ -sitosterol. About 13% of the total plasma campesterol was transported in the high density lipoprotein. Stigmasterol was only present in trace quantities in the lipoprotein fractions (Table V). The cholesterol distribution was normal: about 3% in very low density, 75% in low density, and 22% in high density lipoproteins.

The *in vitro* tests for esterification by the plasmas of R. H. and 2 normal control subjects indicated that  $\beta$ -sitosterol was esterified by the plasmas of both R. H. and the controls (Fig. 3). The esterification of [4-<sup>14</sup>C]- $\beta$ -sitosterol by R. H. was slower than the esterification of both  $\beta$ -sitosterol and cholesterol in the normal controls. However, the esterification of [1,2-<sup>3</sup>H]cholesterol was similar in R. H. as compared with normal controls.

After the *in vitro* incubation of R. H.'s plasma, the

amounts of esterified plant sterols (campesterol and  $\beta$ -sitosterol) as well as cholesterol increased simultaneously, as the concentrations of the unesterified sterols decreased (Table VI). By mass determination, the ester/free ratios of the various sterols were about twice their initial values after 24 h of incubation: from 2.7 to 5.2 for  $\beta$ -sitosterol, from 1.7 to 4.3 for campesterol, and from 2.4 to 6.0 for cholesterol. An additional 24 h of incubation provided much smaller increases in these ester/free ratios. Lecithin-cholesterol acyl transferase (LCAT), the enzyme known to be the cholesterol-esterifying enzyme of the plasma (12), was probably responsible for the esterification of the plasma plant sterols.

Plant sterols were found in the two tendon xanthoma biopsies and in the adipose tissue of R. H. (Table VII). The plant sterol content of the patellar xanthoma was 36.7 mg, and that of the plantar xanthoma was 4.0 mg/g dry wt or 17.5% and 12.3% of the total sterol content. As in the plasma,  $\beta$ -sitosterol was the most plentiful

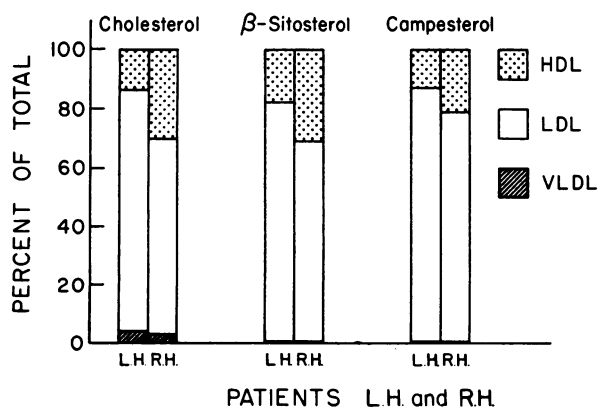


FIGURE 2 The distribution of sterols in the plasma lipoproteins of the two sisters.

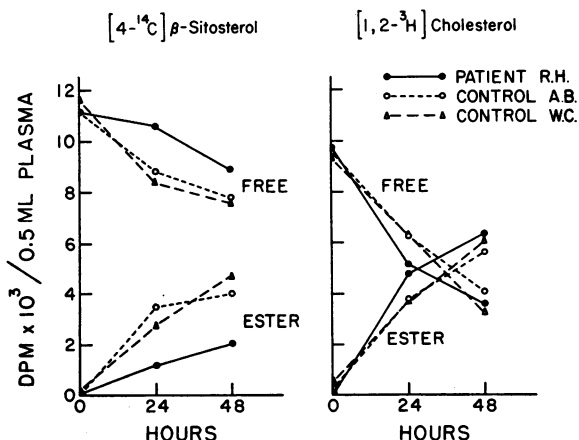


FIGURE 3 The *in vitro* esterification of [4-<sup>14</sup>C] $\beta$ -sitosterol and [1,2-<sup>3</sup>H]cholesterol by the plasmas of R. H. and two normal control subjects (W. C. and A. B.).

TABLE VI  
The Changes in the Plasma Free and Ester Sterol Concentrations After In Vitro Incubation

	Patient R. H.			Normal W. C.*			Normal A. B.*		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
	<i>mg/100 ml</i>								
<b>Cholesterol</b>									
Ester/free	2.4	6.0	6.7	3.1	6.6	8.0	2.5	6.5	7.6
Free	55.5	26.8	24.6	43.7	23.1	19.6	58.4	27.5	23.6
Ester	130.5	159.7	156.7	134.2	152.9	156.7	145.6	177.9	179.3
Total	186.0	186.5	188.6	177.9	176.0	176.3	204.0	205.4	202.9
<b><math>\beta</math>-Sitosterol</b>									
Ester/free	2.7	5.2	6.3						
Free	3.9	2.4	2.1						
Ester	10.4	12.2	12.9						
Total	14.3	14.6	15.0						
<b>Campesterol</b>									
Ester/free	1.7	4.3	4.6						
Free	2.6	1.2	1.2						
Ester	4.4	5.4	5.5						
Total	7.0	6.6	6.7						

\* W. C. and A. B. had no detectable plasma plant sterols.

plant sterol in the patellar xanthoma, 25.7 mg/g, followed by campesterol, 9.1 mg/g, and stigmasterol, 1.9 mg/g. The total sterol content was 210.1 mg, 32.3 mg/g dry wt. Cholesterol made up the remainder of the total sterols. Only trace amounts (< 0.01 mg/100 ml) of plant sterol ester were found in the xanthomas. The cholesterol ester content of the two xanthomas was 8.5 mg and 8.3 mg/g dry wt, or 4.9% and 29.4% of total cholesterol, respectively (Table VII). Adipose tissue contained 0.2 mg/g wet wt of  $\beta$ -sitosterol, 17.9% of the total sterol concentrations. Only trace amounts of the other plant

sterols could be detected. The percentage of  $\beta$ -sitosterol in adipose tissue sterols was slightly higher than that of the patient's plasma (11.3%). These results indicate that the tissue cholesterol esterase did not esterify the plant sterols and apparently is sterol-specific as regards its substrate. In contrast, the plasma cholesterol-esterifying enzyme, LCAT, as shown above, probably did not distinguish between its substrates, namely, cholesterol,  $\beta$ -sitosterol, and campesterol.

In both sisters, plant sterols were found in the skin surface lipids, about 19% of the total sterols.  $\beta$ -Sitos-

TABLE VII  
Tissue Sterols in Patient R. H.

Tissue	Plant sterols									
	Total	$\beta$ -Sitosterol		Campesterol		Stigmasterol		Cholesterol		
		Free	Ester	Free	Ester	Free	Ester	Total	Free	Ester
	<i>mg/g dry wt</i>									
<b>Tendon xanthoma</b>										
Patellar	36.7 (17.5)†	25.7	Trace*	9.1	Trace	1.9	Trace	173.4 (82.5)	164.9	8.5
Plantar	4.0 (12.3)	2.9	Trace	1.1	Trace	Trace	Trace	28.3 (87.7)	20.0	8.3
Adipose tissue§	0.2 (17.9)	0.2		Trace		Trace		0.7 (82.1)		

\* Indicates <0.010 mg/g dry wt.

† Figures in parentheses indicate percent of total sterols.

§ Values are milligrams per gram wet wt.

TABLE VIII  
The Plant Sterols and Other Sterols of the Skin Surface Lipids

Patient	Date of collection	Plant sterols				Cholesterol	Lathosterol
		Total	$\beta$ -Sitosterol	Campesterol	Stigmasterol		
					<i>% of total sterols</i>		
L. H.	8-28-72	17.4	15.2	1.27	0.98	81.0	1.52
	8-30-72	20.1	17.5	1.40	1.18	78.3	1.58
R. H.	9-13-72	21.3	19.6	1.50	0.17	76.1	2.61
	9-17-72	16.5	12.5	1.77	2.22	81.8	1.62
Normal subjects*	Mean $\pm$ SD	7.5 $\pm$ 2.6	6.8 $\pm$ 2.5	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	90.3 $\pm$ 2.9	2.2 $\pm$ 0.6

\* From reference 5.

terol was the primary plant sterol, constituting 16% of the total (Table VIII). More plant sterol was contained in the skin surface sterol than for normal persons; i.e., 19% of the total sterols versus the previously reported values of 7.5% in normal subjects (5).

The intestinal absorption of  $\beta$ -sitosterol in L. H. was 18% of the administered dose of  $\beta$ -sitosterol measured by mass. By radioactivity measurements, she absorbed a similar percentage, 29%, of the dose (Table IX). R. H. absorbed 27% of the dose of  $\beta$ -sitosterol by mass and 29% by radioactivity measurement. At the time of the second test, R. H. absorbed 35% of the oral [ $4\text{-}^{14}\text{C}$ ]- $\beta$ -sitosterol dose.

Another measure of intestinal absorption would be the amount of isotopic sitosterol that appeared in the blood after its oral administration in the test meal. In the second test R. H. received 60.3  $\mu\text{Ci}$  of [ $4\text{-}^{14}\text{C}$ ]- $\beta$ -sitosterol orally. At intervals of time from 2 to 9 days later, up to 7.1% of the total dose was present in her total

plasma volume<sup>4</sup> compared with less than 0.2% of the administered dose of [ $4\text{-}^{14}\text{C}$ ]-sitosterol in the plasma of a normal subject who received a comparable test meal (Table X). In other words, about 35 times more radioactivity after an oral dose of [ $4\text{-}^{14}\text{C}$ ]- $\beta$ -sitosterol appeared in the plasma of R. H., compared to the normal subject. This data provides additional evidence for the increased intestinal absorption of dietary  $\beta$ -sitosterol.

The  $\beta$ -sitosterol to campesterol ratios in the plasma, the erythrocytes, the tendon xanthoma and the feces of R. H. were 2.2, 2.2, 2.8, and 2.9, respectively. These ratios were very similar to the  $\beta$ -sitosterol/campesterol of the patient's diet, which was 2.9. This further suggested that the plant sterols of the blood and tissues of this patient were probably of dietary origin.

*The identification of plant sterols.* The plasma plant sterols of L. H. and R. H. were tentatively identified by comparing the retention times relative to 5 $\alpha$ -cholestane to those of pure sterols<sup>5</sup> on a SE-30 column in a

TABLE IX  
The Intestinal Absorption of  $\beta$ -Sitosterol in Patients L. H. and R. H. After a Single Oral Test Meal by Mass and by [ $4\text{-}^{14}\text{C}$ ]- $\beta$ -Sitosterol

Patient	$\beta$ -Sitosterol intake	$\beta$ -Sitosterol excretion in feces	% absorption
By mass, mg*			
L. H.	3509	2892	18
R. H.	3037	2219	27
By [ $4\text{-}^{14}\text{C}$ ]- $\beta$ -Sitosterol, $\mu\text{Ci}$			
L. H.	1.90	1.34	29
R. H.	1.93	1.37	29
	60.30	39.20	35

\* Total amount present in the test breakfast plus the daily intake contained in the usual diet.

TABLE X  
The  $\beta$ -Sitosterol Radioactivity in Total Plasma\* After an Oral Dose of Isotopic  $\beta$ -Sitosterol

Days after oral isotope	As Percent of dose fed	
	Patient R. H.	Normal subject
1	4.8	
2	6.7	0.16
4	7.1	
5	6.9	
6		0.17
9	6.6	0.18

\* Plasma volume calculated as 45 ml/kg body weight (13).

<sup>4</sup> Total plasma volume calculated as 45 ml/kg body wt (13).

<sup>5</sup> Obtained as a gift from the Upjohn Co., Kalamazoo, Mich. Purity of the sterols was > 95%.

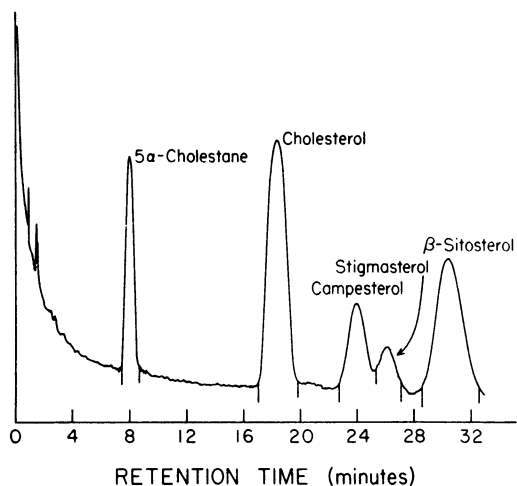


FIGURE 4 The GLC pattern of plasma sterols of patient L. H.  $5\alpha$ -Cholestane was used as the internal standard.

F and M high efficiency gas-liquid chromatograph (model 402) (F & M Scientific Corp., Hewlett-Packard Co., Avondale Div., Avondale, Pa.) The details of GLC conditions have been reported earlier (5). A typical gas chromatogram of the plasma sterols of patient L. H. is shown in Fig. 4. These sterols migrated together on the thin-layer plate and had the same  $R_f$  value as cholesterol. Addition of a known amount of pure  $\beta$ -sitosterol to the eluate of this band proportionately accentuated the peak, which we identified as  $\beta$ -sitosterol in the gas-chromatogram on the basis of retention time relative to  $5\alpha$ -cholestane. The recovery of the added amount of  $\beta$ -sitosterol was 99.0%. Comparison of the relative retention time (relative to  $5\alpha$ -cholestane) of the peak labeled as  $\beta$ -sitosterol (Fig. 4) to that of pure  $\beta$ -sitosterol either as free sterol or as the TMS derivative with SE-30 (3.8%) and QF-1 (2%) columns under similar conditions also supported the identification.

The identities of the plasma plant sterols of L. H. and R. H. were further confirmed by combined gas chromatography-mass spectrometry. The principal ions of fragmentation of the TMS derivatives of the plant sterols,  $\beta$ -sitosterol, campesterol, and stigmasterol and that of cholesterol were identical with those of authentic plant sterols<sup>5</sup> and cholesterol<sup>6</sup> and agreed with those reported by Brooks, Horning, and Young (14).

## DISCUSSION

Two basic questions must be asked about the problem of  $\beta$ -sitosterolemia and xanthomatosis in these two sisters. First, what was the origin of the high levels of plant sterols in their blood and tissues? Second, why did

<sup>5</sup> Obtained from the National Bureau of Standards, Washington, D. C., > 99% pure.

these two sisters develop extensive tendon and tuberous xanthomatosis when their serum cholesterol and triglyceride levels were normal?

There are three possibilities as to the origin of plant sterols in the body: (a) They may be derived from the diet because of increased intestinal absorption. (b) Their excretion into the bile by the liver either as neutral sterols or bile acids may somehow be blocked so that accumulation in the blood and tissues may occur. In fundamental metabolic studies in man, Salen, Ahrens, and Grundy found that about 20% of the absorbed  $\beta$ -sitosterol was ultimately converted to bile acids with the remainder excreted in the bile as the free sterol. This excretion has been shown to be more rapid than that of cholesterol (3). (c) Finally, plant sterols could conceivably be synthesized in the tissues of the body. There is no evidence to date that any animal or human tissues synthesize plant sterols (3); only plants have this capacity (15).

It is known that only small amounts (< 5%) of  $\beta$ -sitosterol are absorbed by man and that only trace amounts appear in the blood. There was greatly increased intestinal absorption of  $\beta$ -sitosterol in both of these sisters to provide experimental support for our hypothesis that increased absorption of  $\beta$ -sitosterol must be considered as a major cause of the  $\beta$ -sitosterolemia. Furthermore, the question may be raised as to whether the primary defect in this disease might well be an increased capacity to absorb all neutral sterols, including cholesterol. The intestinal absorption of cholesterol could be carried out in only one sister, R. H. It was 66% of a single oral dose. This value falls in the higher range of cholesterol absorption values obtained in this laboratory (> 60% cholesterol absorption occurred in only 14% of all humans studied, normals and type II hypercholesterolemic patients) (11). Probably, then, in this disease,  $\beta$ -sitosterolemia, one of the chief defects must lie in the intestinal mucosa. However, we cannot rule out that the excretion of  $\beta$ -sitosterol is impaired, a circumstance that would also favor the accumulation of plant sterols in the body. This possibility is under intensive investigation at present.

Why did these two patients have increased intestinal absorption of plant sterols? On the basis of the events involved in cholesterol absorption, the specificity determining the rate of  $\beta$ -sitosterol absorption could lie in any one of the following four steps: (a) partition of  $\beta$ -sitosterol between an oil and a micellar phase of the intestinal contents; (b) its uptake by the mucosal cell membranes; (c) its esterification in the mucosal cells; (d) its transport in chylomicrons.

All of the above four steps must have been carried out effectively for the increased absorption of plant sterols to have occurred in these two sisters. Borgström showed that the partition coefficient of  $\beta$ -sitosterol be-



tween micellar and oil phases of intestinal contents is similar to that of cholesterol (16). Thus, the state of the sterol in the intestinal lumen cannot explain the difference in the absorption of  $\beta$ -sitosterol and cholesterol in normal humans. The mode of transfer of sterols from micelles into mucosal cells is not known. There is evidence that mucosal cells can discriminate in their uptake of individual free sterols such as cholesterol and plant sterols (17, 18). Although the nature of this discriminatory process has not yet been elucidated, Glover and Green have proposed that the sterol absorption might be mediated via carrier lipoproteins and these lipoproteins might be specific for various sterols (19). However, there is evidence that  $\beta$ -sitosterol is taken up by mucosal cells (3). The carrier protein, of course, might be important in several of the steps in cholesterol absorption, including the formation of chylomicrons.

The third event in the absorption of sterols, namely the esterification of the sterols in the intestinal cells by cholesterol esterase, may well be the factor limiting  $\beta$ -sitosterol absorption (3).  $\beta$ -Sitosterol has been shown to be esterified to a lesser extent than cholesterol in the intestinal mucosal cells of rat (20, 21). Swell, Trout, Field, and Treadwell showed that while  $\beta$ -sitosterol, like cholesterol, is taken up by the intestinal wall, cholesterol is largely esterified before incorporation into chylomicrons.  $\beta$ -sitosterol, on the other hand, is apparently not esterified in the intestinal wall (22). Kuksis and Huang, studying dogs with thoracic duct fistulas, observed that virtually all of the plant sterols in chyle were unesterified, whereas most of the cholesterol in chyle was esterified (23). However, epicholesterol, a structural analogue of cholesterol, which is not esterified by the pancreatic cholesterol esterase, is absorbed in significant amounts (24), thus suggesting that esterification of a sterol in the intestinal mucosal cells may not always be an obligatory reaction for its absorption. Additional evidence that esterification may be the critical step in the usual failure of  $\beta$ -sitosterol absorption lies in the fact that this sterol prevents the absorption of cholesterol by preventing its esterification (25). The exact mechanism of this competition is not known. Reviewing all of the available evidence, we would suggest that the one reasonable hypothesis to account for the enhanced absorption of plant sterols in these two sisters involves their esterification and incorporation into chylomicrons.

It has been suggested that  $\beta$ -sitosterol is an "ideal" internal standard to correct for degradative losses of cholesterol in sterol balance studies (26). One of the primary reasons for the use of  $\beta$ -sitosterol as an internal standard in the sterol balance studies is that man absorbs hardly any plant sterols from the diet (2, 3). The present study indicates such was not the case in these two sisters, since both absorbed large amounts of plant sterols from the diet. In such patients  $\beta$ -sitosterol cannot be

used as marker to correct for sterol "degradation losses" in the gut. In these two sisters we cannot rule out the possibility of some sterol degradation which, if it occurred, would make  $\beta$ -sitosterol absorption appear greater than it actually was. However, we do not believe that degradation significantly could have affected the absorption test results. First, the blood levels of [4-<sup>14</sup>C]- $\beta$ -sitosterol give strong additional evidence of absorption, and second, both the sisters were consuming a mixed food diet at the time that the measurements of the intestinal absorption of  $\beta$ -sitosterol were carried out. Several previous studies have indicated that with mixed, solid food diets, no degradation of  $\beta$ -sitosterol or cholesterol occurs in the intestine of man (27-29). Degradation of cholesterol and  $\beta$ -sitosterol apparently occurs in men fed only formula diets (27, 30).

As regards the inheritance of this disease, the parents of these two sisters did not have  $\beta$ -sitosterolemia and xanthomas. The parents had total plasma plant sterol concentrations well within the normal range (see results) and similar to the values reported, 0.3-1.73 mg/100 ml of  $\beta$ -sitosterol, for eight patients ingesting the typical American diet (3). Thus, the occurrence of this disease in two sisters with unaffected parents suggests an inherited recessive trait.

The formation of tendon and tuberous xanthomas are poorly understood, even in hyperlipidemic states with which they are usually associated. The possible mechanisms include increased biosynthesis of cholesterol locally, decreased removal of cholesterol, or an increased affinity for cholesterol derived from the blood by the cells within the tissue. Current evidence suggests that xanthomas develop from imbibition of the plasma lipids by tissues in areas under physical stress and not from increased synthesis or decreased removal of cholesterol (31). The two sisters reported herein had extensive tendon and tuberous xanthomatosis but normal plasma cholesterol concentrations. The tendon xanthomas and adipose tissue contained plant sterols. Despite the high concentration of  $\beta$ -sitosterol in the xanthomas, the increase in xanthoma cholesterol was quantitatively more important, because cholesterol was the predominant sterol. Perhaps the accumulation of plant sterols in the tendons and skin may have initiated further cholesterol deposition in these tissues, either by attracting cholesterol from plasma by way of net transfer or by stimulating biosynthesis of cholesterol locally. Both processes could occur in the presence of normal plasma cholesterol levels. Perhaps the high concentration of plant sterols in the plasma may have affected the stability of both cholesterol and plant sterols in the circulating lipoprotein complex, thus favoring the deposition of these sterols into the tissues forming xanthomas. Tendon xanthomatosis with normal or low plasma cholesterol levels has also been described in cerebrotendinous xanthomatosis

(32-35). In this disease still another sterol, cholestanol, accumulates in the tissues in company with cholesterol. The two sisters reported herein did not have cholestanol in either the blood or the xanthomas analyzed.

In this context, the role of plant sterols in the development of atherosclerotic vascular disease should also be considered. It is possible in the light of the above discussion of xanthomas that these sterols may also favor deposition of cholesterol in the arteries producing atherosclerotic lesions at an early age. However, at the present time, there is no evidence to support this hypothesis, nor do we know the prognosis of this disease,  $\beta$ -sitosterolemia and xanthomatosis, as described in this report.

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