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Gerald Salen, ... , E. H. Ahrens Jr., Scott M. Grundy

J Clin Invest. 1970;49(5):952-967. <https://doi.org/10.1172/JCI106315>.

Research Article

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Metabolism of β -Sitosterol in Man

GERALD SALEN, E. H. AHRENS, JR., and SCOTT M. GRUNDY

From The Rockefeller University, New York 10021

ABSTRACT The metabolism of β -sitosterol was compared to that of cholesterol in 12 patients. Sterol balance methods were supplemented by radiosterol studies, with the following results. (a) Plasma concentrations of β -sitosterol ranged from 0.30 to 1.02 mg/100 ml plasma in patients on intakes of β -sitosterol typical of the American diet. Plasma levels were raised little when intakes were increased greatly, and on fixed intakes they were constant from week to week. On diets devoid of plant sterols, the plasma and feces rapidly became free of β -sitosterol. (b) The percentage of esterified β -sitosterol in the plasma was the same as for cholesterol. However, the rate of esterification of β -sitosterol was slower than that for cholesterol. (c) Specific activity-time curves after simultaneous pulse labeling with β -sitosterol- ^3H and cholesterol- ^{14}C conformed to two-pool models. The two exponential half-lives of β -sitosterol were much shorter than for cholesterol, and pool sizes were much smaller. Values of turnover for β -sitosterol obtained by the sterol balance method agreed closely with those derived by use of the two-pool model. There was no endogenous synthesis of β -sitosterol in the patients studied; hence, daily turnover of β -sitosterol equaled its daily absorption. Absorption of β -sitosterol was 5% (or less) of daily intake, while cholesterol absorption ranged from 45 to 54% of intake. (d) About 20% of the absorbed β -sitosterol was converted to cholic and chenodeoxycholic acids. The remainder was excreted in bile as free sterol; this excretion was more rapid than that of cholesterol. (e) The employment of β -sitosterol as an internal standard to correct for losses of cholesterol in sterol balance studies is further validated by the results presented here.

INTRODUCTION

The fastidious work of Schönheimer (1, 2) in the 1930's demonstrated that little if any intestinal absorption of plant sterols occurs in mammals. In five animal species Schönheimer found no increase in the total sterol content of the liver after feeding large amounts of plant

sterols and concluded that plant sterols were not absorbed to any appreciable extent. These conclusions were sharpened in 1955 by Gould, Jones, Wissler, and Taylor (3, 4), who addressed the question of β -sitosterol absorption in moribund patients and in rats with the greater sensitivity inherent in the use of radioactive sterols; these authors estimated the absorption of radiosterol at less than 5% of the dose fed. In rats Borgström (5) in 1968 verified these low levels of absorption of β -sitosterol.

However, later investigators (6-9) reached different conclusions: they measured β -sitosterol absorption in rats by means of various sterol balance procedures and ascribed to absorption any difference between oral intake and fecal excretion. In this manner they ascertained that up to 50% of the intake was absorbable. However, these workers did not consider that losses of plant sterols during intestinal transit can be due to bacterial destruction of the sterol ring, a possibility that to us seems highly likely (10).

Since we have claimed (10) that β -sitosterol is an ideal internal standard to correct for degradative losses of cholesterol in sterol balance studies, we considered it necessary to reevaluate the question of β -sitosterol absorption in man. We measured the absorption of β -sitosterol by the isotopic balance method (11) and by analysis of specific activity-time curves after pulse labeling intravenously (12); we then compared these results with data for cholesterol absorption obtained simultaneously in the same patients. Our findings agree with the results of Gould and of Borgström, namely, that less than 5% of dietary β -sitosterol is absorbed. In the course of these studies we obtained other data concerning the metabolism of β -sitosterol in man, such as the question of its endogenous synthesis (13), its esterification and transport in the plasma, and its transformation into cholic and chenodeoxycholic acids.

METHODS

Patients

Studies were carried out on the metabolic ward at The Rockefeller University Hospital; the age, sex, body build, and clinical diagnosis of each patient are given in Table I. Eight patients had familial hypercholesterolemia with normal

Received for publication 17 October 1969 and in revised form 14 January 1970.

plasma triglyceride concentrations (type II by the lipoprotein phenotyping method of Fredrickson, Levy, and Lees [15]); three patients had elevations in both plasma cholesterol and triglyceride concentrations (one type IV and two type V); and one patient had normal concentrations of lipoproteins.

Diets

Food intakes consisted exclusively of orally administered liquid formula feedings in which dietary fats contributed 40%, protein 15%, and glucose 45% of total caloric intake, or were fat free (15% protein and 85% glucose); vitamin and mineral supplements were given as described previously (16). In each case the caloric intake was adjusted to maintain total body weight at a constant level throughout each study.

Table II lists the dietary fats used and the sterol contents of the various formulas. When the plant sterols and cholesterol were not inherent in the dietary fats, they were dissolved in them prior to their incorporation in the formulas during large scale homogenization (16). Aliquots of each formula were repeatedly analyzed for sterol content (17) as tests of formula homogeneity.

Experimental design

(a) *Patient 1a*. Patient 1 was studied on different diets in two successive years. In the earlier study (1a) a formula diet containing tracer amounts of β -sitosterol-22,23- ^3H was

fed five times daily for 83 days in order to attain the isotopic steady state (18) for β -sitosterol. During this steady state, measurements of the concentration of β -sitosterol in the plasma were found to be the same by two independent methods, gas-liquid chromatographic (GLC), and isotopic (see section Isotopes). After oral administration of labeled β -sitosterol was discontinued, the cumulative clearance of β -sitosterol from the body was determined as a measure of the amount of the sterol retained in the body.

(b) *Patients 1b, 2, and 3*. The rates of turnover of β -sitosterol and cholesterol in the plasma were determined from specific activity-time curves in patients 1b, 2, and 3 after simultaneous pulse labeling with β -sitosterol-22,23- ^3H and cholesterol-4- ^{14}C by the intravenous route; calculations of body pool sizes and intestinal absorption of cholesterol and β -sitosterol also were made. Conversion of β -sitosterol into the primary bile acids was demonstrated on samples of bile obtained by duodenal intubation.

(c) *Patients 4-8*. In these five patients the effects of moderate and high intakes of dietary β -sitosterol (designated as studies *a* and *b*, respectively) on plasma concentrations of cholesterol and β -sitosterol were assessed. Measurements of turnover and pool sizes of β -sitosterol were made in patient 4 on the two intake levels.

(d) *Patients 9-12*. Stools from these patients were analyzed for the presence of β -sitosterol at a time when this sterol had been absent from the diets for at least 4 wk. Measurements were made by gas-liquid chromatography (GLC), utilizing the maximum sensitivity that we can presently attain (see under Results).

TABLE I
Clinical Data

Patient No.	Initials	Age	Sex	Height	Weight	% of Ideal Weight*	Diagnosis†
		yr		cm	kg		
1	H. T.	57	M	162	46	84	Hypercholesterolemia (Type II)
2	M. R.	60	M	167	67	109	Hyperglyceridemia (Type V)
3	C. Z.	63	M	181	94	122	Normocholesterolemia, IHD,§ PVD§
4	M. S.	37	M	167	54	93	Hypercholesterolemia (Type II), IHD, xanthomatosis
5	J. R.	36	F	164	53	98	Hypercholesterolemia (Type II), IHD, PVD, xanthomatosis
6	R. G.	58	F	147	61	120	Hypercholesterolemia (Type II), IHD, xanthomatosis, essential hypertension
7	J. H.	39	M	172	74	104	Hypercholesterolemia (Type II)
8	N. A.	30	M	170	67	102	Hypercholesterolemia (Type II), IHD
9	A. M.	60	F	164	66	108	Hyperglyceridemia (Type IV), IHD, PVD, chronic lymphatic leukemia
10	E. K.	45	F	159	56	105	Hypercholesterolemia (Type II), IHD
11	R. W.	41	M	176	75	102	Hyperglyceridemia (Type V), PVD
12	R. F.	55	F	154	64	128	Hypercholesterolemia (Type II), IHD

* According to life insurance tables (14).

† Phenotyping of hyperlipoproteinemia by paper strip electrophoresis according to Fredrickson, Levy, and Lees (15).

§ IHD, ischemic heart disease; PVD, peripheral vascular disease.

TABLE II
Intakes of Dietary Fats, Sterols, and Total Calories

Patient	Cal/day*	Dietary fat† and duration of study (days)	Period I		Period II	
			Cholesterol	β -Sitosterol	Cholesterol	β -Sitosterol
			mg/day	mg/day	mg/day	mg/day
1a§	2000	Butter oil (I-40; III-36) Corn oil (II-83)	285	0	35	620
1b	1800	Lard (129)	402	242	—	—
2	2380	Lard (134)	565	342	—	—
3	2400	Lard (123)	685	415	—	—
4	2280	Cottonseed oil (170)	34	320	34	1909
5	1875	Butter oil (176)	285	125	315	7166
6	2062	Cottonseed oil (170)	33	293	33	6488
7	2688	Butter oil (184)	452	192	420	6211
8	2375	Butter oil (224)	378	166	380	6484
9	2100	None (42)	35	0	—	—
10	1900	Butter oil (40)	50	0	—	—
11	2800	None (55)	30	0	—	—
12	2000	Egg yolk fat (83)	2100	0	—	—

* To maintain constant body weight.

† In fat-containing formulas fat calories = 40% of total caloric intake.

§ Patient 1 was studied on two occasions in successive years. In the earlier study he was fed β -sitosterol-22,23-³H daily during period II; in periods I and III he was fed a butter oil formula free of β -sitosterol.

|| This butter oil was subjected to molecular distillation for removal of free cholesterol (Distillation Products Industries, Rochester, N. Y.).

Isotopes

β -sitosterol-22,23-³H was prepared according to the method of Steele and Mosettig (19). In this procedure the starting material, i-stigmasterol methyl ether, was prepared by Dr. M. J. Thompson, USDA, Beltsville, Md.; it was reduced with tritium gas by Dr. G. Gupta of The Rockefeller University, and the reduction product was converted into the acetate of β -sitosterol-22,23-³H by Dr. Thompson. We hydrolyzed the sterol ester and isolated the free labeled sterol by thin-layer chromatography (TLC) on Silica Gel G with heptane:diethyl ether, 2:1. The final product had a specific activity of 400 mCi/mmol. Cholesterol-4-¹⁴C was obtained from New England Nuclear Corp., Boston, Mass. Both labeled sterols were shown to be better than 99% pure by TLC on Florisil (Floridin Company, Tallahassee, Fla.), in the system diethyl ether:n-heptane, 55:45. Measurements of radioactivity were made in a Packard Tri-Carb Scintillation Counter, model 3003, (Packard Instrument Co., Inc., Downers Grove, Ill.) as previously described (17).

Patient 1a ingested β -sitosterol-22,23-³H (21.3 μ Ci, 620 mg) daily for a period of 83 days. At the time of homogenization the tracer sterol dissolved in 10 ml of ethanol was added to 40-kg batches of formula containing nonlabeled β -sitosterol in corn oil. A constant intake of isotopically labeled sterol was assured by feeding the same amount of formula each day (five divided doses).

Patients 1b, 2, and 3 were given a mixture of β -sitosterol-22,23-³H (136 μ Ci) and cholesterol-4-¹⁴C (90 μ Ci) as a single intravenous pulse at the beginning of their studies. Patient 4 was given a mixture of β -sitosterol-22,23-³H (50 μ Ci) and cholesterol-4-¹⁴C (27 μ Ci) during the first study, and β -sitosterol-22,23-³H (110 μ Ci) and cholesterol-4-¹⁴C (90 μ Ci) during the second study. In all cases the radiosterols

were dissolved in 1 ml of ethanol which was then dispersed in 150 ml of physiologic saline; the dispersion was immediately infused by vein.

Steroid analyses

Fecal steroids.¹ Complete stool collections were made throughout each study and combined into 4-day pools. Fecal neutral and acidic steroids were isolated separately, and their masses and specific activities were measured by methods developed in this laboratory (17, 20). These TLC and GLC procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit (5 β ,3 β -OH- and 5 β ,3-keto-compounds).

Plasma, erythrocyte, and bile steroids. For analysis of cholesterol and β -sitosterol in plasma, erythrocytes (RBC), and bile, it proved expedient to modify the fecal neutral steroid procedure in several ways. Because the accuracy of the results in this study depends upon the analysis of small amounts of β -sitosterol in the presence of large amounts of cholesterol, we present this procedure in some detail.

1 ml of plasma, packed RBC, or bile was refluxed for 1 hr with 20 ml of N NaOH in 90% ethanol; after refluxing, water (10 ml) was added to the alkaline saponification mixture, and nonsaponifiable components were extracted three times with 50-ml portions of petroleum ether, 60–80°C (PE). The combined PE extracts were evaporated to dryness in a round-bottom flask, and the residue was redissolved in

¹ The term *steroid* is used in preference to *sterol* because significant amounts of ketonic metabolites of cholesterol and of the plant sterols are usually present in the neutral and acidic fractions of the fecal lipids.

5.00 ml of ethyl acetate containing 350 μ g of 5 α -cholestane. 4 ml of this solution was utilized for radioactivity counting. The remaining 1 ml was taken for sterol analysis by GLC; solvent was evaporated and the trimethylsilyl (TMS) ethers of the sterols were formed by addition of 50 μ l of TMS reaction mixture (Sil Prep, Applied Science Laboratories, State College, Pa.). Generally, about 3 μ l of this mixture was analyzed by GLC; this was equivalent to about 1% ($1/5 \times 3/50$) of the neutral sterols in the original 1 ml sample of plasma, packed RBC, or bile.

Much greater sensitivity was required for analyses of materials obtained from patients fed diets free of β -sitosterol. In these cases the neutral sterol fraction was extracted as described above from 1 ml of plasma or fecal homogenate and dissolved in 1.00 ml ethyl acetate containing 70 μ g of 5 α -cholestane. This in turn was concentrated in the tip of a small conical tube during the evaporation of solvent; TMS ethers were formed by addition of 5 μ l of TMS reaction mixture. As much of this mixture as possible was then injected into the GLC column, and the analysis of the β -sitosterol derivative was accomplished at the maximum instrument sensitivity consistent with acceptable base line noise. Under our conditions a peak for TMS- β -sitosterol $2 \times$ base line noise was equivalent to no more than 65 ng per ml of plasma or per g of fecal homogenate.

Quantitative analysis of TMS ethers of cholesterol and β -sitosterol was carried out by GLC on 4-ft columns packed with 1% DC-560 (Applied Science Laboratories) at a column temperature of 240°C with a flame ionization detector, F and M Biomedical Gas Chromatograph, Model 400 (F and M Scientific Corp., Avondale, Pa.); these columns had 2000–3000 theoretical plates for TMS-cholesterol. Individual peak areas were measured by electronic integration, Model CRS-100, (Infotronics, Inc., Houston, Tex.). Because of the large excess of cholesterol relative to β -sitosterol in all samples, the sensitivity of the GLC detector was usually increased 32-fold after emergence of the cholesterol peak. The two sterols were individually quantified by relating their peak areas to that of the internal recovery standard, 5 α -cholestane; correction factors are not required (17). In tests with standard mixtures of these two sterols, we found we could reliably measure 65 ng of β -sitosterol per ml of plasma or per g of fecal homogenate, in the presence of large excesses of cholesterol.

In the analysis of bile, neutral sterols were removed by extraction with PE, and the unconjugated bile acids were isolated from the aqueous alcohol phase after alkaline hydrolysis of the conjugated acids according to our method for isolation of fecal acidic steroids (20). The methyl esters of the trihydroxy- and dihydroxycholic acids were isolated by TLC on Silica Gel H in the system chloroform:acetone:methanol, 70:25:5, and the specific activity of each bile acid class was measured. For quantification of bile acids by GLC, 6-ft columns containing 1% HiEff 8BP (Applied Science Laboratories) were used at 230°C; they offered about 3000 theoretical plates for TMS chenodeoxycholic methyl ester, with a separation factor of 1.10 between this derivative and that of deoxycholic acid.

Free and esterified sterols in plasma. The lipids in 2 ml of plasma were extracted into 38 ml of chloroform:methanol, 2:1. During evaporation of solvent small quantities of ethanol were added to facilitate the removal of water; the residue was redissolved in exactly 15.00 ml of ethyl acetate. 10.00 ml was taken for TLC on 0.5 mm Silica Gel H plates; the developing system contained petroleum ether:ethyl ether:acetic acid, 85:15:0.5, in which free sterols have an R_f of 0.30 and esterified sterols 0.90. The free and esterified sterols

were collected separately according to the method of Goldrick and Hirsch (21), and measurement of sterol content was made by GLC after saponification and TMS-ether formation, as described earlier in this paper.

Calculations

Corrections for neutral steroid losses. Corrections for losses of neutral steroids during intestinal transit and for variations in fecal flow rates were made with nonlabeled dietary plant sterols as internal standard (10).

Cholesterol absorption. The absorption of dietary cholesterol was measured as the difference between dietary intake and unabsorbed dietary neutral steroids in feces. The latter quantity was obtained by the procedure designated as Method I in this laboratory (22): unabsorbed dietary neutral steroids equal total fecal neutral sterols (determined by chromatographic methods) minus fecal neutral sterols of endogenous origin (determined by the isotopic balance technique after intravenous pulse labeling with radioactive cholesterol).

RESULTS

β -Sitosterol in feces of patients fed diets free of β -sitosterol. If β -sitosterol is synthesized by the body, it should be excreted in the feces continuously; the magnitude of this endogenous synthesis will be most clearly defined when the diet is free of plant sterols. With these considerations in mind we attempted to measure β -sitosterol in the feces of patients 9–12 who had been fed formula diets free of this sterol for periods longer than 4 wk. Diets and feces were analyzed, and no β -sitosterol or its bacterial transformation products could be detected.

Since 65 ng of β -sitosterol per ml of plasma or per g of fecal homogenate can be reliably measured by our GLC techniques, we can conclude that excretion of β -sitosterol in patients 9–12 was less than 10 μ g/day (the daily weight of feces of these patients ranged from 70 to 150 g). Thus, it appears improbable that these patients synthesized more than 10 μ g of β -sitosterol per day.

Measurement of cholesterol and β -sitosterol concentrations in plasma and RBC. Table III presents the results obtained for plasma and RBC levels of cholesterol and β -sitosterol in patients 1b, 2, and 3 who were fed diets in which the amounts of cholesterol and β -sitosterol approximated those of the average American diet. Measurements of plasma cholesterol and β -sitosterol concentrations were made twice weekly for 9 wk, as well as measurements of levels of RBC sterols. The average concentrations of plasma cholesterol ranged from 226 to 300 mg/100 ml, and of plasma β -sitosterol from 0.30 to 1.02; thus, the ratios of cholesterol to β -sitosterol in plasma ranged from about 300–800 to 1. The relatively small standard deviations of the means for plasma concentrations of cholesterol and β -sitosterol indicate a high degree of constancy of plasma levels of these two sterols throughout each study. RBC concentrations of cholesterol varied from 105 to 131 mg/100 ml of packed

TABLE III
Concentrations of β -Sitosterol and Cholesterol in Plasma and RBC as Measured by GLC

Patient	β -Sitosterol			Cholesterol			Cholesterol/ β -sitosterol	
	Diet	Plasma	RBC	Diet	Plasma	RBC	Plasma	RBC
	mg/day	mg/100 ml*	mg/100 ml packed cells*	mg/day	mg/100 ml*	mg/100 ml packed cells*		
1b	242	1.02 \pm 0.06 (18)	0.40 \pm 0.05 (9)	402	287 \pm 10 (18)	131 \pm 9 (9)	286	328
2	342	0.51 \pm 0.02 (18)	0.17 (2)	565	287 \pm 9 (18)	105 (2)	564	617
3	415	0.30 \pm 0.05 (18)	0.17 (2)	685	226 \pm 12 (18)	129 (2)	786	758
4a	320	0.90 \pm 0.07 (20)	0.29 (2)	34	300 \pm 10 (20)	120 (2)	350	413
4b	1909	1.73 \pm 0.15 (19)	0.88 (3)	34	254 \pm 6 (17)	129 (3)	150	147
5a	125	0.80		285	448		560	
5b	7166	1.46		315	354		242	
6a	293	0.73		33	267		366	
6b	6488	1.25		33	198		158	
7a	192	trace		452	196		—	
7b	6211	0.46		420	156		339	
8a	166	0.42		378	132		314	
8b	6484	0.60		380	102		170	

* Mean \pm SD. Figures in parentheses indicate number of determinations.

cells, and β -sitosterol concentrations from 0.17 to 0.40 mg/100 ml of packed cells. The cholesterol to β -sitosterol ratios in RBC were nearly the same as those in plasma, suggesting a free exchange of both sterols between plasma and RBC.

In five patients (patients 4–8) who received β -sitosterol at two levels of intake, concentrations of β -sitosterol in plasma were consistently higher during the period of the higher intake; but, despite eightfold or greater increases in intakes of β -sitosterol, plasma concentrations increased less than twofold. The increase in plasma β -sitosterol during the greater intake was associated with a similar increase in RBC β -sitosterol (patient 4b), and again the ratio of cholesterol to β -sitosterol in RBC was the same as that of plasma.

A comparison of the degree of esterification of β -sitosterol with that of cholesterol is presented in Table IV. Three patients (1b, 2, and 3) were pulse labeled simultaneously by the intravenous route with β -sitosterol- ^3H and cholesterol- ^{14}C , and the distribution of labeled sterols in the free and esterified sterol fractions of plasma was

analyzed 30–39 days after administration of isotopes; labeled β -sitosterol was found to be esterified to the same extent as labeled cholesterol (60–75% esterified). In patient 4, measurements of the free and ester ratios of the two radiosterols were begun earlier. Differences in esterification rate were most noticeable during the 1st few days; in this patient β -sitosterol appeared to be esterified at a slower rate than cholesterol.

The attainment of the isotopic steady state for β -sitosterol. In another experiment designed to examine whether β -sitosterol was synthesized endogenously, patient 1a received a diet free of β -sitosterol (40% butter oil) for 40 days in period I. After 1 wk and throughout the remaining 5 wk on the β -sitosterol-free formula, we found less than 65 ng of β -sitosterol per ml of plasma or per g of feces.

In period II (83 days) the patient was given a formula diet containing 40% of calories as corn oil in which β -sitosterol-22,23- ^3H had been incorporated during its manufacture; each day the patient ingested 620 mg of β -sitosterol of specific activity 74,245 dpm/mg. On the

2nd day of this regimen radioactivity was detected in the plasma; it rose to reach a constant level of approximately 1000 dpm/ml plasma in about 2 wk (Fig. 1). The plateau in plasma radioactivity persisted for the remainder of period II, suggesting the attainment of the isotopic steady state (18).

During this period when plasma levels of radioactivity were constant, the average specific activity of plasma β -sitosterol (calculated from plasma radioactivity and mass measurement of β -sitosterol by GLC) was 76,149 dpm/mg for the last four determinations (Table V). This agrees within 3% with that of the diet (74,245 dpm/mg). The near identity of specific activities of plasma and dietary β -sitosterol is added evidence that this patient did not synthesize β -sitosterol; if synthesis had occurred, the specific activity of plasma β -sitosterol would have been less than that of the diet, because of dilution of radioactive β -sitosterol in the plasma with that newly synthesized. Furthermore, during the same 16 day period, measurements of the specific activity of the fecal neutral steroids derived from β -sitosterol agreed with those of plasma and dietary β -sitosterol specific activities within $\pm 5\%$ (77,840 dpm/mg ± 1743 : mean \pm SD in five successive 4-day stool collections).

These findings, especially when coupled with the absence of β -sitosterol in plasma and feces during the β -sitosterol-free diet of periods I and III, are taken to

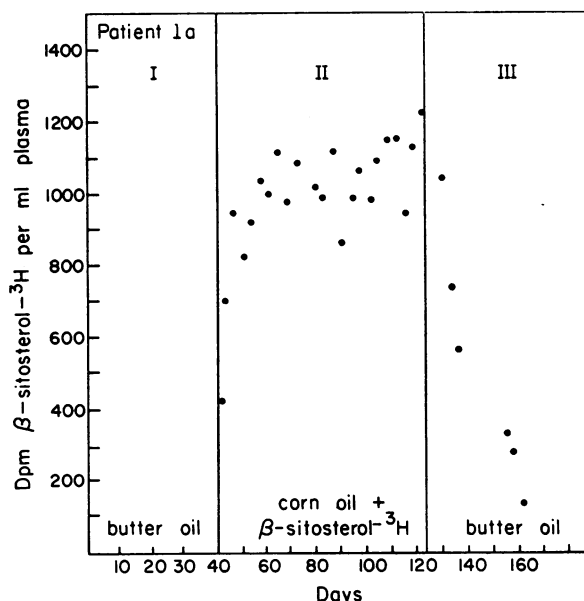


FIGURE 1 Isotopic steady state for β -sitosterol- ^3H (patient 1a). In period I a butter oil formula free of β -sitosterol was fed; the plant sterol was not detectable in plasma or feces after 3 wk. During period II a corn oil formula that furnished 620 mg/day of β -sitosterol- ^3H was fed; the isotopic steady state was attained in about 30 days. When the intake of β -sitosterol was discontinued (period III), the radioactivity in the plasma rapidly declined, and in 4 wk unlabeled β -sitosterol was no longer detectable in plasma and feces. In period III, 226 mg of β -sitosterol recovered in the feces was demonstrated to represent the total body pool of β -sitosterol in period II.

TABLE IV
Distribution of β -Sitosterol- ^3H and Cholesterol- ^{14}C
in Free and Ester Fractions of Plasma

Patient	Time after isotope days	β -Sitosterol		Cholesterol	
		Free	Ester	Free	Ester
	days	%		%	
1b	35	31	69	28	72
	39	33	67	27	73
2	30	40	60	36	64
	35	39	61	33	67
3	30	28	72	26	74
	35	30	70	28	72
4a	4	40	60	32	68
	5	35	65	29	71
	12	28	72	28	72
	15	29	71	29	71
4b	1	70	30	56	44
	2	53	47	43	57
	5	39	61	34	66
	6	31	69	34	66
	10	31	69	30	70
	14	29	71	30	70

indicate that endogenous synthesis of β -sitosterol did not occur.

In period III β -sitosterol feeding was abruptly discontinued by returning to the butter oil formula of period I. Plasma radioactivity declined sharply from 1000 to less than 150 cpm/ml of plasma after 30 days; by GLC the mass of β -sitosterol in plasma and feces fell to the base line limits noted in period I (Fig. 1). In the first 16 days of period III 369 mg of β -sitosterol was excreted in the feces; thereafter, our most sensitive assays failed to detect this sterol. We estimate that 143 mg of 369 mg represented β -sitosterol which lagged behind in the bowel after its feeding in period II had been discontinued.² The difference (369 - 143 = 226 mg) was

²Chromic oxide (300 mg/day) had been fed as an inert marker during period II (23). The ingestion of β -sitosterol and chromic oxide was discontinued simultaneously at the end of Period II; thereafter, 69 mg of chromic oxide was excreted in the feces in the first 4 days of period III and none thereafter. Since 69 mg of chromic oxide is equivalent to 23% of 1 day's intake, we have assumed that 23% of the last day's intake of β -sitosterol during period II was excreted in the feces during the 1st days of period III; 23% of 620 mg (1 day's intake) = 143 mg.

TABLE V
Plasma β -Sitosterol Concentrations Determined by GLC and Isotopic Methods in Patient 1a,
Fed β -Sitosterol-22,23- ^3H Daily for 83 Days (Period II)

Time after start of Period II	Plasma β -Sitosterol				Difference in concentration (GLC vs. isotopic calculation)
	Concentration measured by GLC	Radioactivity	Specific activity	Concentration* calculated isotopically	
days	mg/100 ml	dpm/100 ml	dpm/mg	mg/100 ml	%
8	0.91	79,100	86,923	1.06	-17
11	1.11	83,200	74,955	1.12	-1
15	1.37	93,500	68,248	1.26	+8
18	1.22	88,900	72,868	1.20	+2
29	1.38	104,100	75,434	1.40	-1
32	1.26	98,000	77,778	1.32	-5
36	1.28	100,500	78,516	1.35	-5
Average of last 4 values . . .	1.29 \pm 0.06	97,875 \pm 6,486	76,149 \pm 2,551	1.32 \pm 0.08	

* Plasma β -sitosterol concentration calculated by dividing plasma radioactivity (dpm/100 ml) by specific activity of dietary β -sitosterol (74,245 dpm/mg).

taken to represent the total body pool of β -sitosterol fluxing from the body after removal of β -sitosterol from the diet.

The fact that no further β -sitosterol was excreted in the feces or found in the plasma after 30 days on a β -sitosterol-free diet is offered as evidence that the synthesis of β -sitosterol in this man was not repressed by the β -sitosterol absorbed in period II.

TABLE VI
Measurement of β -Sitosterol Turnover by the Isotopic Balance
Method after Pulse Labeling with β -Sitosterol- ^3H

Patient	Neutral steroids*	Acidic steroids*	Turnover (neutral + acidic)
	mg/day	mg/day	mg/day
1b	7.82 (4)	2.25	10.07
2	7.15 (4)	1.45	8.60
3	6.34 (4)	2.31	8.65
4a	5.01 (6)	0.17	5.18
4b	13.91 (6)	0.08	13.98

* mg/day = dpm/day in each fraction of fecal steroids \div dpm/mg of plasma β -sitosterol 2 days earlier. This 2 day interval represents the average transit time for intestinal contents to pass through the intestine (23).

Analysis of β -sitosterol turnover by the isotopic balance method. The turnover of β -sitosterol in patients 1b, 2, 3, 4a, and 4b was measured by the isotopic balance method after pulse labeling with β -sitosterol-22,23- ^3H . This calculation was based on the assumption (proven true in section 3) that the specific activity of the fecal neutral steroids derived from β -sitosterol is the same as the specific activity of plasma β -sitosterol. Thus, the daily excretion (milligrams per day) of neutral and acidic steroids derived from β -sitosterol can be calculated by dividing the total number of disintegrations per minute of tritium excreted per day in each fecal steroid fraction by the specific activity (dpm/mg) of plasma β -sitosterol.

The values thus obtained are shown in Table VI: 5-14 mg/day were excreted as neutral steroids and 0.08-2.31 mg/day as acidic steroids. In the metabolic steady state the sum of the fecal neutral and acidic steroids reflects the daily turnover of β -sitosterol (since all other excretion routes are trivial in man except in biliary obstruction, in which the excretion of bile acids via the kidney may be sizable).

The isotopic balance method also provides a measure of daily bile acid synthesis from β -sitosterol, for in the steady state the daily excretion of fecal acidic steroids is equivalent to their daily synthesis. Patients 1b, 2, and 3 formed 2.25, 1.45, and 2.31 mg of bile acids from β -sitosterol each day, respectively. In contrast, this conversion was almost immeasurable in patient 4; as shown, this patient also converted very small amounts of cholesterol into bile acids.

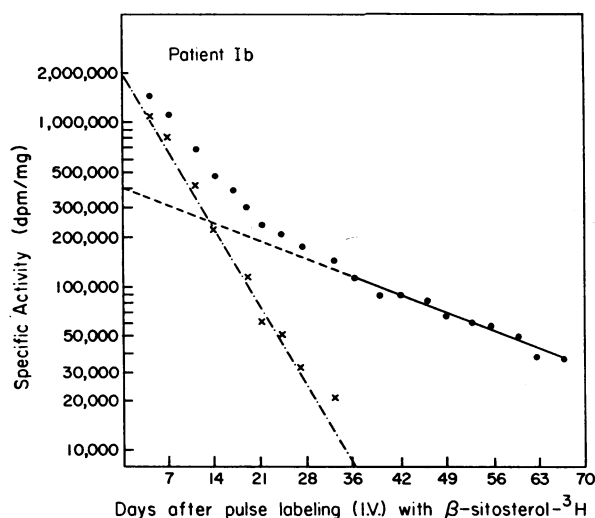


FIGURE 2 Specific activity-time curve for plasma β -sitosterol after pulse labeling intravenously with β -sitosterol- ^3H . A two pool model is suggested by the curve-peeling method: the difference values (small crosses) obtained by subtracting the points on the extrapolated line from the experimental points fit a straight line.

Analysis of sterol turnover and pool sizes by the two pool model. In the four patients pulse labeled by simultaneous intravenous administration of β -sitosterol-22,23- ^3H and cholesterol-4- ^{14}C , specific activities of each of the sterols in the plasma were measured twice weekly. Specific activity-time curves for each of the sterols in all four patients showed two components: a rapid non-linear decline followed by a period of more gradual, but log linear decay (Figs. 2, 3). When these four sets of curves were analyzed mathematically by the equations given by Goodman and Noble (12), each curve conformed to that described for a two pool model. Accordingly, we have calculated values for turnover and pool sizes for each of the sterols (Tables VII, and VIII).

The curves illustrated in Fig. 3 show that in each case the turnover of β -sitosterol was more rapid than that of cholesterol. This comparison is given numerically in Tables VII and VIII: the average value for the $t_{1/2}$ of the first exponential for cholesterol was 1.6 times longer than that of β -sitosterol; the $t_{1/2}$ for the second exponential was 3.5 times longer than that of β -sitosterol. The more rapid disappearance of labeled β -sitosterol previously demonstrated by Gould et al. (3) can be ascribed in part to the smaller body pool of β -sitosterol; however, in the next section we shall present evidence that β -sitosterol actually is excreted preferentially.

In these four patients the plasma β -sitosterol concentration varied from 0.3 to 1.73 mg/100 ml, a fivefold variation. Yet, the narrow range of variation of the two exponentials of radioactive β -sitosterol indicates

that the kinetics of β -sitosterol turnover were very much alike from patient to patient. Indeed, in patient 4 who was tested during periods of low and high dietary intakes of β -sitosterol, the two sets of half-lives of β -sitosterol were almost identical, even though the plasma concentration of β -sitosterol had doubled.

Estimates of β -sitosterol absorption. When the values for turnover of β -sitosterol derived by the two pool model (Table VII) were compared with the values obtained by the isotopic balance method (Table VI), close agreement was observed. Since all available evidence leads us to conclude that β -sitosterol was not synthesized by our patients, it becomes clear that in the metabolic steady state the daily turnover of β -sitosterol equals the amount absorbed each day from dietary sources (Fig. 4). When these four patients ingested 242 to 415 mg of β -sitosterol per day, the absorption of β -sitosterol varied from 6.5 to 12.5 mg/day according to the two pool model, and 5.2 to 14 mg/day according to the isotopic balance method; the differences are not significantly different (Student's t test). Patient 4 absorbed 5.2–7.0 mg/day out of a daily intake of 320 mg, and 13.5–14.0 mg when the diet contained 1909 mg/day, a doubling of absolute absorption with a sixfold increase in intake.

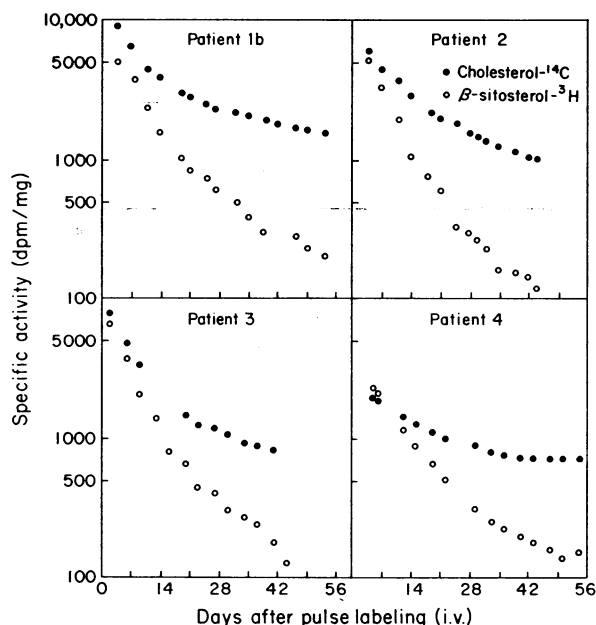


FIGURE 3 Comparative specific activity-time curves for plasma sterols after pulse labeling intravenously with β -sitosterol- ^3H and cholesterol- ^{14}C simultaneously in four patients. In each case the decay of β -sitosterol was more rapid than for cholesterol, indicating a more rapid turnover of β -sitosterol.

TABLE VII
Turnover of Tritiated β -Sitosterol

Patient	β -sitosterol intake	Plasma β -sitosterol \pm SD (n)	C_A^*	C_B^*	$t_{1/2}$ of first exponential	$t_{1/2}$ of second exponential	α^\ddagger	β^\ddagger
		mg/100 ml	dpm/mg	dpm/mg	days	days		days ⁻¹
1b	242	1.02 \pm 0.06 (18)	2,000,000	400,000	4.5	19.0	0.154	0.0864
2	342	0.51 \pm 0.02 (18)	5,000,000	800,000	3.8	13.0	0.187	0.0533
3	415	0.30 \pm 0.05 (18)	6,200,000	638,000	3.3	17.5	0.210	0.0396
4a	320	0.90 \pm 0.07 (20)	1,100,000	460,000	3.8	14.5	0.182	0.0479
4b	1909	1.73 \pm 0.15 (17)	1,480,000	300,000	3.7	15.2	0.182	0.0456
				Mean	3.8 \pm 0.2 (5)	15.8 \pm 2.4 (5)	0.183 \pm 0.017 (5)	0.0545 \pm 0.014 (5)

* Constants which represent the intercepts of straight line (Fig. 2) (12).

† Constants calculated from slope of straight lines (Fig. 2) (12).

§ M_A and M_B = size of pools A and B.

|| PR_A , amount of new sterol entering Pool A exclusive of recirculated sterol.

TABLE VIII
Turnover of Cholesterol-¹⁴C after

Patient	Cholesterol intake	Plasma cholesterol	C_A	C_B	$t_{1/2}$ of first exponential	$t_{1/2}$ of second exponential	α	β
	mg/day	mg/100 ml	dpm/mg	dpm/mg	days	days		days ⁻¹
1b	402	287 \pm 10 (18)	9000	3000	6	52	0.1194	0.0133
2	506	287 \pm 9 (18)	6300	1490	7	62	0.0990	0.0111
3	685	226 \pm 12 (18)	6500	1350	5.7	49.5	0.1215	0.0140
4a	34	300 \pm 10 (20)	1850	890	8	99	0.0866	0.0070
4b	34	254 \pm 6 (17)	5500	4050	5.6	46	0.1237	0.0150
				Average	6.2§	54.5	0.1133	0.0128

* Analysis of the two pool model for cholesterol cannot provide exact size of pool B (M_B), nor can precise values for rate constants k_{ba} , k_a or k_{ab} be determined (Fig. 4). However, we have estimated the lower limiting value of M_B (25) by assuming that S_B and k_B approach zero (no significant synthesis or degradation of cholesterol from pool B).

† Cholesterol intake was too low to permit valid measurement of absorption.

§ Averaged value obtained from patients 1b, 2, 3.

after Pulse Labeling Intravenously

M_A §	M_B §	Total body β -sitosterol [$M_A + M_B$]	Ratio M_A/M_B	PR_A	Daily turnover (isotope balance method)	β -sito- sterol absorbed	Recovery of dietary β -sitosterol	
mg	mg	mg		mg/day	mg/day	%	mg/day	% recovery
125.0	76.0	201.0	1.65	12.5	10.07	5.2	255 \pm 86 (11)	105
51.5	20.5	72.0	2.50	7.1	8.60	2.1	223 \pm 88 (10)	65
43.8	35.2	79.0	1.24	6.5	8.65	1.6	262 \pm 71 (8)	63
70.5	35.7	106.2	1.97	7.0	5.18	2.2	271 \pm 56 (12)	87
111.2	61.6	172.8	1.81	13.5	13.98	0.6	1398 \pm 62 (6)	73
80.4 \pm 36.1 (5)	45.8 \pm 22.5 (5)	126.2 \pm 57.7 (5)	1.83 \pm 0.46 (5)	9.3 \pm 3.4 (5)	9.3 \pm 3.2 (5)			

Simultaneous Pulse Labeling*

M_A	M_B	PR_A	By sterol balance method			Cholesterol absorption	
			Total endog. fecal steroids	= Endog. neutral steroids	+ Acidic steroids		
mg	mg	mg/day		mg/day		mg/day	% of intake
15,833	21,050	596	479	275 \pm 29 (11)	204 \pm 96 (11)	204 \pm 41 (11)	(50.7)
24,390	36,600	1001	742	513 \pm 90 (10)	229 \pm 111 (10)	311 \pm 64 (10)	(54.3)
24,203	37,800	1267	1421	1049 \pm 191 (8)	372 \pm 100 (8)	307 \pm 62 (8)	(44.8)
21,678	27,800	396	359	243 \pm 21 (12)	116 \pm 42 (12)		‡
21,884	17,200	644	679	569 \pm 39 (6)	110 \pm 27 (6)		‡
21,475 \pm 4,887	31,817 \pm 9,343	955 \pm 337	881 \pm 487				

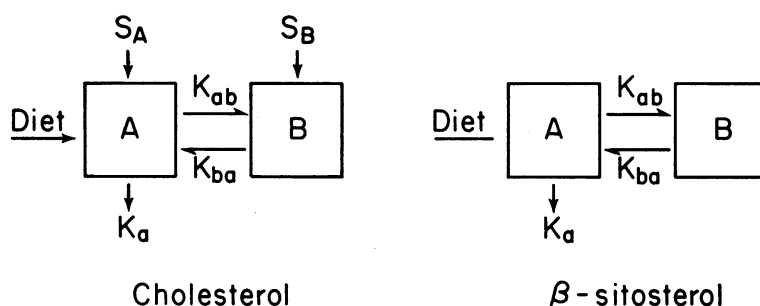


FIGURE 4 General two pool model for cholesterol and β -sitosterol in man. Rate constants are denoted by k values (after Goodman and Noble [12]). In the case of cholesterol, unlabeled sterol enters the body pools (A and B) either from new synthesis (S_A and S_B) or by absorption from the diet. For β -sitosterol, however, the only source of unlabeled sterol is from the diet, since there is no endogenous synthesis. Cholesterol and β -sitosterol are excreted only from pool A (k_a).

The recovery of nonlabeled dietary β -sitosterol in the feces by the sterol balance method varied from 63 to 105% of the daily intake; we have previously proposed that these losses must be due to degradation of the neutral sterol ring structure during intestinal transit (10). In view of the absorption data discussed in the previous paragraph, it is clearly erroneous to ascribe losses of β -sitosterol of this magnitude to absorption.

Turnover of cholesterol determined by the two pool model and by sterol balance methods. Measurements of cholesterol turnover by kinetic analysis of the two pool model were compared with turnover data determined by sterol balance methods (Table VIII) and found to be similar, as described previously in 10 other patients (24).

In patient 4a who was fed a diet virtually free of cholesterol and low in β -sitosterol, the half-lives of cholesterol in pools A and B were longer and the total turnover (359 mg/day) was less than in patients 1b, 2, and 3, whose diets contained much more cholesterol; thus, the increased rates of turnover in these three patients were the result of absorption of appreciable amounts of exogenous cholesterol as well as of a greater rate of daily synthesis. When large amounts of β -sitosterol were added to the diet (patient 4b), reabsorption of endogenous cholesterol was reduced; as a result, plasma concentrations of cholesterol were lowered, and the turnover of cholesterol in pools A and B was increased. Bile acid synthesis in patient 4a was considerably lower than in patients fed cholesterol in their diets; it was this patient in whom the conversion of β -sitosterol to bile acids (Table VI) was exceptionally low.

Comparison of cholesterol and β -sitosterol absorption during the steady state. Tables VII and VIII compare the percentage absorption of cholesterol and of β -sitosterol in patients 1b, 2, and 3; their diets con-

tained sterols in amounts typical of daily intakes in the United States. Cholesterol absorption varied from 204 to 307 mg/day (45–54% of daily intake). In contrast, the amount of β -sitosterol absorbed was only 6.5–12.5 mg/day (1.6–5.2% of daily intake). The absorption of β -sitosterol was apparently not affected by differing intakes of dietary cholesterol: no appreciable difference in β -sitosterol absorption was noted between patients 1b, 2, and 3 (fed cholesterol-containing diets) and patient 4a (fed a diet virtually free of cholesterol). The likelihood of this ought to be more rigorously tested by varying the cholesterol and β -sitosterol intake in the same patient.

Calculation of pool sizes of β -sitosterol. Table VII displays our calculations of the pool sizes of β -sitosterol, using the equations of Nestel, Whyte, and Goodman (25). The amount of β -sitosterol in pool A was found to vary in the four patients from 44 to 125 mg, while the quantity in pool B ranged from 21 to 76 mg; in general, the amount in pool B was about half that in pool A. Thus, the total amount of β -sitosterol in the body (pools A + B) varied from 72 to 201 mg.

Table VII shows that in patient 1b total body β -sitosterol was 201 mg when the dietary intake of β -sitosterol was 242 mg/day; this estimate agrees well with the calculation of total body β -sitosterol of 226 mg made 1 yr earlier in this patient by measuring the washout of β -sitosterol after shifting to a β -sitosterol-free diet from a diet containing 620 mg each day (see section 3). We consider that the agreement in results obtained in one patient in successive years by two different methods supports the premises on which both methods are based.

In patient 4, when the daily intake of β -sitosterol was increased from 320 to 1909 mg, the amount of β -sitosterol in pool A increased from 71 to 111 mg and that in pool

B rose from 36 to 62 mg. Thus, following a sixfold increase in dietary intake, the size of pool B was expanded to a greater extent than pool A.

Consequently, the amount of β -sitosterol retained in the body seems to be roughly proportional to the amount absorbed per day. Although our data are too few to permit rigorous analysis, a plot of these data can be extrapolated through the zero intercept; each milligram absorbed per day is equivalent to about 15 mg of total body β -sitosterol (Fig. 5). We take this to be additional evidence that endogenous synthesis of β -sitosterol did not occur in these patients.

Equilibration of labeled sterols between plasma, RBC, and bile after pulse labeling. At various intervals after simultaneous pulse labeling with β -sitosterol- ^3H and cholesterol- ^{14}C , samples of duodenal contents rich in bile were obtained in patients 1b, 2, and 3 (in some cases immediately following the injection of cholecystokinin intravenously); blood was drawn at the same time. Neutral sterols were isolated from plasma, RBC, and bile, and the specific activities of ^3H - and ^{14}C -labeled sterols were determined.

As shown in Table IX, the specific activities of the two neutral sterols were very similar in the plasma, RBC, and bile. While both sets of specific activities decayed with time (Fig. 3), the isotope ratio of β -sitosterol to cholesterol decreased significantly in all cases, indicating that the fractional rate of turnover of β -sitosterol was more rapid than that of cholesterol. Moreover, the isotope ratios of the two neutral sterols in bile were

consistently higher than those in the plasma. This clearly indicates that the liver can differentiate between β -sitosterol and cholesterol and that β -sitosterol is preferentially secreted into the bile from sterol pools.

The incorporation of β -sitosterol- ^3H and cholesterol- ^{14}C into bile acids. Table X compares the specific activity of the tri- and dihydroxycholic acids in bile with that of total neutral sterols (cholesterol + β -sitosterol) in plasma in three patients after simultaneous pulse labeling with β -sitosterol- ^3H and cholesterol- ^{14}C by the intravenous route. It is seen that the biliary bile acids were labeled both with ^3H and ^{14}C , indicating the simultaneous conversion of both neutral sterols into the primary bile acids. Moreover, the radioactivities per milligram of bile acids for both ^3H and ^{14}C were approximately the same as those in total plasma neutral sterols, suggesting that there was little if any enzymatic discrimination between the two sterols in this conversion. Comparing these results with those of section 5, it is apparent that, while β -sitosterol is somehow selected from cholesterol in the liver cell in the process of neutral sterol excretion, the enzymatic conversion of the two neutral sterols to the two primary bile acids is nonselective.

A separate communication will describe the formation of cholic- ^3H , chenodeoxycholic- ^3H , and deoxycholic- ^3H acids after pulse labeling with β -sitosterol-22,23- ^3H ; their isolation by countercurrent distribution and preparative TLC; and their identification with pure standards by TLC, GLC, melting points, and elemental analysis.

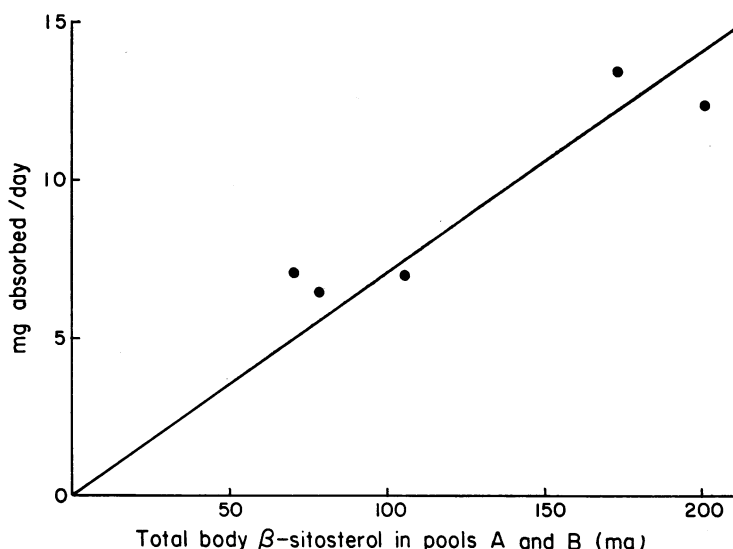


FIGURE 5 Relationship between total body β -sitosterol (pools A + B) and the amount of it absorbed each day. Extrapolation of the line through zero can occur only if β -sitosterol is not synthesized endogenously.

TABLE IX
Equilibration of Labeled Sterols Between Plasma, RBC, and Bile after Pulse Labeling Intravenously

Patient	Time after Pulse Labeling	β -sito.- ^3H SA*	$^3\text{H}/^{14}\text{C}$	β -sito.- ^3H SA	$^3\text{H}/^{14}\text{C}$	β -sito.- ^3H SA	$^3\text{H}/^{14}\text{C}$
		chol.- ^{14}C SA		chol.- ^{14}C SA		chol.- ^{14}C SA	
	days		dpm ratio per ml plasma		dpm ratio per ml packed cells		dpm ratio per ml bile
1b	27	313,732 3,023	0.40	—	—	235,143 2,829	1.00
	34	255,219 2,477	0.35	281,335 2,494	0.33	266,929 2,438	0.95
	41	140,707 2,097	0.27	153,457 2,056	0.32	130,576 1,877	0.82
	49	101,984 1,879	0.24	132,990 1,865	0.24	92,737 1,915	0.64
	56	90,156 1,602	0.20	116,909 1,540	0.20	86,617 1,525	0.56
	63	71,239 1,449	0.18	80,855 1,342	0.21	67,315 1,424	0.47
2	30	191,498 1,134	0.23	—	—	176,998 1,165	0.68
	42	136,200 820	0.15	—	—	132,000 695	0.44
3	25	437,694 1,190	0.36	—	—	410,140 1,100	0.81
	51	114,670 715	0.23	126,441 670	0.24	110,948 678	0.59
	58	74,187 658	0.20	83,333 785	0.18	72,452 587	0.42

* SA = dpm/mg.

TABLE X
Conversions of β -Sitosterol- ^3H and Cholesterol- ^{14}C into Bile Acids after Pulse Labeling Intravenously

Patient	Time after pulse labeling	Plasma		Cholic acid		Deoxy and chenodeoxycholic acids	
		^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
	days	dpm/mg of total neutral sterols		dpm/mg bile acids		dpm/mg bile acids	
1 b	27	1220	3022	1140	3316	900	3000
	34	871	2486	990	2680	865	2552
	41	565	2106	660	1960	745	1800
	49	464	1889	518	1610	502	1550
	56	333	1607	426	1500	478	1420
	63	265	1453	—	—	364	1120
2	42	127	820	212	645	127	550
3	25	429	1190	270	1016	426	1450
	51	171	715	250	730	178	634

DISCUSSION

Daily turnover of β -sitosterol by two methods. In the present studies the turnover of β -sitosterol was investigated in four patients by two independent methods. Analysis of the decay curve of plasma specific activity of β -sitosterol after pulse labeling intravenously indicated that the turnover of β -sitosterol conforms to a two pool model, just as in the case of cholesterol. According to the theory of the two pool model (12) the daily production rate of β -sitosterol (PR_Λ) should equal the amount of new β -sitosterol which enters the more rapidly exchangeable pool (pool A, Fig. 4) exclusive of recirculated sterol. In five studies of four patients the rate of turnover calculated in this manner varied from 6 to 14 mg/day. In the same patients turnover data were obtained independently by application of the isotopic balance method (11): here, the daily turnover of β -sitosterol is equal to the sum of the excretion of radioactivity

in the fecal neutral and acidic steroids derived from β -sitosterol- ^3H administered intravenously, divided by the specific activity of plasma β -sitosterol. Values for the total daily fecal excretion of neutral and acidic steroids derived by the latter method were almost identical with values for turnover calculated by kinetic analysis of the two pool model. This close agreement of data obtained by two independent methods appears to validate the assumptions on which both techniques are based.

Evidence for lack of endogenous synthesis. We have presented four lines of evidence that β -sitosterol was not synthesized in our patients. (a) In five patients fed diets free of β -sitosterol, the fecal neutral steroid fraction was free of β -sitosterol, 24-ethyl coprostanol, and 24-ethyl coprostanone. We use the term "free" operationally, in the sense that the sensitivity of the analytical methods used in the present work sets an upper concentration limit of 65 ng of β -sitosterol per ml of plasma or per g of homogenate. (b) When patient 1a was fed β -sitosterol labeled with β -sitosterol- ^3H of constant specific activity, plasma β -sitosterol specific activity reached the same level as that of the sterol fed. If β -sitosterol had been synthesized endogenously, the specific activity of plasma β -sitosterol would have been lower than that of the fed sterol, reflecting dilution by nonradioactive β -sitosterol produced endogenously. (c) In this patient the specific activity of the fecal neutral steroids derived from β -sitosterol was identical with that of the sterol fed. This correspondence would not have been obtained, had endogenous synthesis of β -sitosterol occurred in the intestinal mucosa. (d) In five studies a linear relationship was noted between the amount of β -sitosterol absorbed per day and that retained in the body; this line extrapolated to the zero intersect.

Turnover = absorption. Since all our evidence confirmed the widely held belief that β -sitosterol is not synthesized endogenously in man, the only entry into pool A must have occurred through absorption of exogenous β -sitosterol. Therefore, daily turnover of β -sitosterol in the steady state, whether calculated by analysis of the two pool model or through application of the isotopic balance method, must equal daily absorption. Table VI shows that 6–14 mg of β -sitosterol was absorbed each day, or 0.6–5.2% of the daily intake. These very low values for percentage absorption directly confirm the previous estimates of Gould (3, 4) and of Borgström (5).

β -Sitosterol as internal standard for sterol balance studies. Previous investigators (6–9) attempted to determine the absorption of β -sitosterol in rats by the method of sterol balance and reached very different conclusions from those presented here. They ascribed to absorption the differences between intake and fecal

output; these balance differences ranged from 22 to 53%. But it is our belief that losses of this magnitude were more likely due to degradation of the sterol ring structure during intestinal transit (10). In the present study in man, two patients (2 and 3) showed appreciable losses of nonradioactive dietary β -sitosterol by the sterol balance method (35 and 37%, respectively), while in a third patient (1b) the entire intake of dietary β -sitosterol was recovered in the feces. Despite these large differences in recovery that we have ascribed (10) to varying degrees of degradation of the sterol ring structure in different patients, the calculations of daily absorption from kinetic analysis of the two pool model were remarkably similar in all three patients. Therefore, we have concluded that the absorption of β -sitosterol cannot be accurately measured by subtracting fecal output from dietary intake. Indeed, all of the findings in the present study reinforce our reliance on the use of nonradioactive dietary β -sitosterol as an internal standard in sterol balance studies to correct for losses of cholesterol during intestinal transit: first, endogenous synthesis of β -sitosterol apparently does not occur in man, and second, its absorption in the human intestine is quantitatively so small (< 5% of intake) that it meets all the criteria of an ideal internal standard for balance studies on sterols (10).

Differences in metabolism of cholesterol and β -sitosterol. The present data emphasize certain differences in the metabolism of β -sitosterol (C_{27}) which is structurally similar to cholesterol (C_{28}) but contains an additional ethyl group at C-24. We have confirmed that this plant sterol is absorbed from the intestine only about one-tenth as effectively as cholesterol and that it is secreted into the bile more rapidly than cholesterol. It also appears to be esterified more slowly than cholesterol and it is also distributed differently between the two theoretical body pools. On the other hand, β -sitosterol is converted as rapidly as cholesterol into the same primary bile acids.

The mechanisms by which the two sterols are distinguished at the level of the intestinal mucosal cell remain unknown. In the rat, Sylvén and Borgström (26) have noted that the ratio of the two sterols in the intestinal lymphatics is the same as that in the mucosal cells of the intestine; hence, they concluded that the difference in absorption was not due to differences in any intracellular process nor in the transport of these sterols into the intestinal lymphatics. Rather, it seemed to them more likely that the distinction is made earlier in the absorptive process, either by a lesser micellar solubilization of plant sterol or by a slower transport through the outer surface of the mucosal cell. It seems to us, however, that the rate of esterification of β -sitosterol may be the factor limiting its absorption. Swell, Trout,

Field, and Treadwell (9) found that, while β -sitosterol, like cholesterol, is taken up by the intestinal wall, cholesterol is largely esterified before incorporation into chylomicrons. β -Sitosterol, on the other hand, is apparently not esterified in the intestinal wall: Kuksis and Huang (27), studying dogs with thoracic duct fistulas, observed that virtually all of the plant sterols in chyle are unesterified, whereas most of the cholesterol in chyle is esterified.

Two factors appear to contribute to the more rapid fractional turnover rate of β -sitosterol as compared with that of cholesterol. First, as discussed above, in passage through the small intestine at least 10 times more cholesterol than β -sitosterol is returned into body pools through reabsorption. Secondly, it seems clear from the results of the present study that mechanisms exist in the liver whereby β -sitosterol is preferentially selected for more rapid secretion into the bile as neutral sterol. The mechanisms by which the liver cell distinguishes between β -sitosterol and cholesterol are just as unclear as in the case of the intestinal cell. Again, we can visualize the possibility that a retardation in esterification of β -sitosterol, compared to that of cholesterol might make the free sterol more available for secretion into bile, but other possibilities should be considered. The intracellular routes of travel of the two sterols through the liver cell from plasma to biliary canaliculus are still not known; nor do we know whether the sterols in the endoplasmic reticulum are in equilibrium with those in the mitochondria or cell sap, nor how inconstant the sterol contents of the different membranous structures may be. At any rate, we have shown for the first time in any species that β -sitosterol is converted into the same primary bile acids as cholesterol, i.e., cholic acid and chenodeoxycholic acid. The similarities in isotope ratios in Table X suggest that the enzymes responsible for this conversion fail to differentiate one sterol from the other. At what stage the ethyl group at C-24 is removed remains to be determined. Several species of phytophagous insects are known to dealkylate C_{28} and C_{29} sterols to provide cholesterol during their larval growth (28). Therefore, it would be of considerable importance to ascertain whether the β -sitosterol incorporated into bile acids was initially dealkylated to cholesterol, or whether dealkylation occurred simultaneously with side chain oxidation during bile acid synthesis.

Striking differences in distribution of the two sterols in the two theoretical pools of readily exchangeable sterols (pools A and B) have been demonstrated in this study. According to theory (12, 24) the two pool model permits the calculation of the sizes of pools A and B. For cholesterol the size of pool A can be calculated from data obtained from specific activity-time curves of plasma cholesterol; but the size of pool B can be deter-

mined only if entry and removal of cholesterol from this pool are negligible (except for isotopic exchange with pool A). If one makes the latter assumption, the data of Goodman and associates indicate that in six adult normocholesterolemic men and women pool A varied from 15 to 27 g; pool B from 27 to 45 g. Our own data (similarly derived) in 21 adult patients with hyperlipoproteinemic states showed that pool A varied from 16 to 32 g, pool B from 21 to 49 g; the lack of difference between normo- and hypercholesterolemic patients is astonishing, if true. In contrast, the present study shows that, for β -sitosterol pool A was invariably the larger pool, ranging in hypercholesterolemic adults from 52 to 125 mg, with only 21 to 76 mg in pool B. Thus, in all adults tested, cholesterol is more richly distributed in pool B than in pool A; the converse holds for β -sitosterol. Thus, the total amount of readily exchangeable cholesterol is vastly greater than for β -sitosterol, and furthermore the distribution of the two sterols in the body is different. Why this difference exists remains to be shown, but it seems to rule out the possibility of using the distribution of the plant sterol as a guide to cholesterol distribution in specific tissue pools.

ACKNOWLEDGMENTS

We are grateful to Mrs. Eleanor Mathusek for cholesterol determinations, to Mr. Albert Angevin for the preparation of stool homogenates, to Miss Esperanza Piezas and Mr. John Crouse for GLC analyses, and especially to Miss Norma Glennon for excellent technical assistance.

This study was supported in part by U. S. Public Health Service Grant HE-06222 from the National Heart Institute, and by U. S. Public Health Service Grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources.

REFERENCES

1. Schönheimer, R. 1929. Über die Bedeutung der Pflanzensterine für den tierischen Organismus. *Hoppe-Seyler's Z. Physiol. Chem.* **180**: 1.
2. Schönheimer, R. 1931. New contributions in sterol metabolism. *Science*. **74**: 579.
3. Gould, R. G., R. J. Jones, G. V. LeRoy, R. W. Wissler, and C. B. Taylor. 1969. Absorbability of β -sitosterol in humans. *Metabolism*. **18**: 652.
4. Gould, R. G. 1955. Absorbability of beta-sitosterol. *Trans. N. Y. Acad. Sci.* **18**: 129.
5. Borgström, B. 1968. Quantitative aspects of the intestinal absorption and metabolism of cholesterol and β -sitosterol in the rat. *J. Lipid Res.* **9**: 473.
6. Ivy, A. C., T. Lin, and E. Karvinen. 1955. Absorption of dihydrocholesterol and soya sterols by the rat's intestine. *Amer. J. Physiol.* **183**: 79.
7. Roth, M. and P. Farvarger. 1955. La digestibilité des graisses en présence de certains sterols. *Helv. Physiol. Pharmacol. Acta*. **13**: 249.
8. Swell, L., T. A. Boiter, H. Field, Jr., and C. R. Treadwell. 1956. The absorption of plant sterols and their effect on serum and liver sterol levels. *J. Nutr.* **58**: 385.

9. Swell, L., E. C. Trout, H. Field, Jr., and C. R. Treadwell. 1959. Absorption of ^3H - β -sitosterol in the lymph fistula rat. *Proc. Soc. Exp. Biol. Med.* **100**: 140.
10. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary β -sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9**: 374.
11. Hellman, L., R. S. Rosenfeld, W. Insull, Jr., and E. H. Ahrens, Jr. 1957. Intestinal excretion of cholesterol: a mechanism for regulation of plasma levels. *J. Clin. Invest.* **36**: 898. (Abstr.)
12. Goodman, DeW. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. *J. Clin. Invest.* **47**: 231.
13. Gordan, G. S., M. E. Fitzpatrick, and W. P. Lubich. 1967. Identification of osteolytic sterols on human breast cancer. *Trans. Ass. Amer. Physicians (Philadelphia)*. **80**: 183.
14. Metropolitan Life Insurance Company Statistical Bulletin 40. 1959. November, December.
15. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276**: 34, 94, 148, 215, 273.
16. Ahrens, E. H. Jr., V. P. Dole, and D. H. Blankenhorn. 1954. The use of orally-fed liquid formulas in metabolic studies. *Amer. J. Clin. Nutr.* **2**: 236.
17. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral sterols. *J. Lipid Res.* **6**: 411.
18. Morris, M. D., I. L. Chaikoff, J. M. Felts, S. Abraham, and N. O. Fansah. 1957. The origin of serum cholesterol in the rat: diet versus synthesis. *J. Biol. Chem.* **224**: 1039.
19. Steele, J. A., and E. Mosettig. 1963. The solvolysis of stigmasteryl tosylate. *J. Org. Chem.* **28**: 571.
20. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6**: 397.
21. Goldrick, B., and J. Hirsch. 1963. A technique for quantitative recovery of lipids from chromatoplates. *J. Lipid Res.* **4**: 482.
22. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10**: 91.
23. Davignon, J., W. J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. *J. Clin. Invest.* **47**: 127.
24. Grundy, S. M., E. H. Ahrens, Jr., and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. *J. Lipid Res.* **10**: 304.
25. Nestel, P. J., H. M. Whyte, and DeW. S. Goodman. 1969. Distribution and turnover of cholesterol in humans. *J. Clin. Invest.* **48**: 982.
26. Sylvén, C., and B. Borgström. 1969. Absorption and lymphatic transport of cholesterol and sitosterol in the rat. *J. Lipid Res.* **10**: 179.
27. Kuksis, A., and T. C. Huang. 1962. Differential absorption of plant sterols in the dog. *Can. J. Biochem.* **40**: 1493.
28. Clayton, R. B. 1964. The utilization of sterols by insects. *J. Lipid Res.* **5**: 3.