The interaction of cholesterol absorption and cholesterol synthesis in man

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ABSTRACT The total miscible pool of cholesterol in the body is determined largely by the interaction of cholesterol absorption and synthesis. In the present study we have examined the net effects of this interplay in one normal and five hypercholesteremic subjects when various amounts of cholesterol were made available for absorption.

Feeding large amounts of cholesterol to the normocholesteremic patient caused an expansion of body pools by as much as 20 g before the amount of cholesterol re-excreted as fecal neutral steroids each day came into balance with the cholesterol absorbed from the diet. There was no detectable decrease in total body synthesis of cholesterol nor any increase in conversion of cholesterol into bile acids. However, feedback control of cholesterol synthesis was demonstrable when large quantities of plant sterols were fed: in the hypercholesteremic patients thus studied, the absorption of both endogenous and exogenous cholesterol was then greatly reduced, and a compensatory increase in synthesis occurred.

Thus, the plant sterol experiments, but not the cholesterol feeding experiment, demonstrated that feedback control by dietary cholesterol does occur in man. That feedback control by dietary cholesterol is relatively unimportant in man seems to be due to the fact that in the metabolic "steady state" the absorption mechanism is essentially saturated by the large amounts of endogenous cholesterol available for reabsorption.

These findings demonstrate that there are important differences between man and various laboratory animals in regard to the interaction of absorption and synthesis as factors controlling the size of tissue pools of cholesterol.

SUPPLEMENTARY KEY WORDS   β-sitosterol · plant sterols · feedback regulation · cholesterol feeding · cholesterol balance

Constancy of total body cholesterol depends upon the rapidity and precision with which counterbalancing mechanisms (absorption, synthesis, and excretion) compensate for changes that expand or reduce the tissue pools of cholesterol. Different species of laboratory animals seem to vary considerably in the interplay of these compensatory mechanisms. For example, feeding cholesterol to rabbits causes a marked increase in the amount of cholesterol in blood and tissues (1): for a long time before a new steady state is established, cholesterol is absorbed faster than it is removed. In rats, on the other hand, cholesterol feeding produces only small increases in cholesterol concentrations in blood and in various tissues other than the liver (2): in this species any tendency to accumulate absorbed dietary cholesterol is compensated by a rapid inhibition of cholesterol synthesis (3) and by enhanced excretion of cholesterol in the form of its primary conversion products, the bile acids (4).

In man the quantitative interrelationships between absorption, synthesis, and excretion of cholesterol have not yet been fully defined, and information on the size of tissue pools of cholesterol is meager. Bhattathiry and Siperstein (5) concluded from in vitro studies of human liver fragments that cholesterol feeding in man causes a reduction in cholesterol synthesis in that organ by feedback control. On the other hand, Taylor, Patton, Yogi, and Cox (6) have been unable to confirm the existence of feedback control in man, and they suggested that limitation of cholesterol absorption is a more effective regulator of cholesterol metabolism than altered synthesis; Wilson and Lindsey (7) later reached the same conclusion through isotopic balance studies. However, as these workers have pointed out, appreciable quantities of dietary cholesterol continue to be absorbed in the steady state, and progressive accumulation of cholesterol into body pools would necessarily occur if not compensated by a decrease in synthesis or an increase in excretion of cholesterol or bile acids.

In the present study in man we have attempted to

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TABLE 1   Clinical Data

Patient No.	Initials	Age	Sex	Height	Wt. and % of Ideal Wt.*		Diagnosis
		yr		cm	kg	%	
1	J.Sh.	68	F	165	63	117	IHD,† normocholesteremia
2	N.A.	30	M	170	67	102	IHD, hypercholesteremia (Type II)‡
3	J.H.	38	M	172	74	104	Hypercholesteremia (Type II)
4	J.R.	36	F	164	53	98	IHD, PVD†, xanthomatosis, hypercholesteremia (Type II)
5	R.G.	58	F	147	61	120	IHD, xanthomatosis, hypercholesteremia (Type II), essential hypertension
6	J.St.	35	M	175	70	104	IHD, hypercholesteremia (Type II)

* According to life insurance tables (24).
† IHD = ischemic heart disease; PVD = peripheral vascular disease.
‡ Typing of hyperlipidemias according to Fredrickson, Levy, and Lees (12).

TABLE 2   Dietary Constituents

Patient	Caloric Intake	Fat	Cholesterol-³H Intake		Period I		Period II	
			Position of Label	Amount	Cholesterol	β-Sitosterol	Cholesterol	Plant Sterols
	cal/day			dpm/day × 10⁻⁶	mg/day		mg/day	
1	2188	Corn oil	7	7.15	34	656*	1587	875*
2	2375	Butter oil	1,2	10.93	378	166†	380	9975‡
3	2688	Butter oil	1,2	6.60	452	192†	425	9555§
4	1875	Butter oil	1,2	3.74	285	125†	310	11025‡
5	2062	Cottonseed oil	None		33	293*	33	9982‡
6	2562	Butter oil	None		304	None	418	5530‡

* β-Sitosterol inherent in oil used for formula.
† β-Sitosterol (Mann Research Laboratories, New York) purified and prepared in microcrystalline form by Dr. Erold R. Diller, Eli Lilly Co., Indianapolis, Ind.; the final product contained 90% β-sitosterol and 10% campesterol.
‡ Mixed plant sterols also furnished by Dr. Diller contained 65% β-sitosterol, 30% campesterol, and 5% stigmasterol.
§ A special preparation of β-sitosterol prepared by Dr. Diller containing 95% β-sitosterol and 5% campesterol.

assess the quantitative changes in synthesis and excretion that serve to compensate when absorption is abruptly altered. We have examined these parameters by disturbing the steady state in two ways: (a) by markedly expanding the intestinal load of cholesterol through feeding large amounts daily, and (b) by interrupting the reabsorption of endogenous cholesterol by feeding large amounts of plant sterols. The sterol balance methods developed in this laboratory (8–11) have been supplemented by pulse- and continuous labeling with isotopic cholesterol in these studies in six patients.

METHODS

Patients

Cholesterol balance studies were carried out on six patients in the metabolic ward at The Rockefeller University Hospital; all patients were hospitalized for approximately 6 months. The age, sex, body build, and clinical diagnosis of each patient are given in Table 1. Five patients had familial hypercholesterolemia with normal plasma triglyceride concentrations (Type II of Fredrickson, Levy, and Lees [12]); one patient had a normal concentration 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, as previously described (13), together with vitamin and mineral supplements. In each case, caloric intake was adjusted so as to maintain total body weight at a constant level for the many weeks of each study.

Table 2 lists the dietary fats used, and the sterol contents of the various diets. Dietary cholesterol was an inherent component of the formula ingredients in all cases, a minor amount being furnished in the "fat-free" milk protein mixture used for all formulas, a larger amount as a component of the butter oil in the formulas of Patients 2, 3, 4, and 6. Plant sterols were inherent in the corn and cottonseed oils used in Patients 1 and 5; in other cases the plant sterols described in Table 2 were dissolved in the dietary fats prior to mixing these fats with other constituents of the formulas at the time of large-scale homogenization (13).

The formula of Patient 1 in Period I contained corn oil (40% of calories) without added cholesterol, and in Period II the same amount of corn oil with cholesterol added to furnish a daily intake of 1.587 g/day. To

assure a homogeneous solution of cholesterol in the finished formula, crystalline cholesterol was completely dissolved in corn oil before the addition of the oil to other dietary constituents. Patients 2–6 were studied during successive periods of low and high intakes of plant sterols. In two patients (Nos. 3 and 6) plant sterol feedings were continued into a third period at the same level of sterol intakes as in Period II but with the following changes: Patient 3 was given mixed plant sterols instead of purified β-sitosterol in amounts such that the total intake of plant sterols was not altered; and Patient 6 received corn oil instead of butter as the source of dietary fat (the amount of added plant sterols being reduced in the third period to compensate for the amount of these sterols inherent in the corn oil).

Isotopes

Patients 2, 3, and 4 ingested a small amount of radioactive cholesterol daily in all formula feedings throughout both study periods (Table 2). Cholesterol-1,2-^{3}H, dissolved in 10 ml of ethanol, was added to 40-kg batches of formula during homogenization in order to hold the isotope content of all feedings constant (this constancy was repeatedly verified by specific activity measurements). Patient 1 was given isotopic cholesterol in this manner only during Period II.

All six patients received a single dose of either cholesterol-4-^{14}C or cholesterol-26-^{14}C intravenously at the beginning of the study. 1 ml of ethanol containing known amounts of the radioactive tracer (approximately 100 μc) was dispersed in 150 ml of physiologic saline; the mixture was immediately administered intravenously.

All isotopically labeled compounds were obtained from New England Nuclear Corp., Boston, Mass.; before use their radiopurity was confirmed by thin-layer chromatography. Plasma total cholesterol levels and specific activities were determined biweekly. Plasma cholesterol concentration was measured by the spectrophotometric method of Abell, Levy, Brodie, and Kendall (14); on a portion of the same extract, radioactivity was measured in a Packard Tri-Carb scintillation counter (model 3003) as previously described (8).

Fecal Steroid Analysis

Complete collections of each patient's stools were made throughout the study; stools were usually combined into 4-day pools, and after homogenization aliquots were taken for analysis. Fecal neutral and acidic steroids were isolated separately, and their mass and specific activities were measured by methods developed in this laboratory (8, 9). These procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products (5β,3β-OH and 5β,3-keto compounds) derived from plant sterols and cholesterol during intestinal transit.

Values obtained for excretion of neutral steroids were corrected for losses during intestinal transit and for variation in fecal flow rates with dietary plant sterols as an internal standard (10) during periods of both low and high intakes of plant sterols. We have previously reported that percentage losses of plant sterols in these same patients were similar whether the patients' intake of plant sterols was low or high (Table 4, and Ref. 10); losses of plant sterols in Patients 2–5 ranged from 21 to 41%. Patient 6 received no plant sterols in his formula feedings during Period I, so that it was impossible to correct for any losses of neutral steroids in this period. Nevertheless, we have justified the inclusion of Patient 6 in this study on the basis that the recovery of plant sterols was 94% in Period II; in Period I neutral steroid excretion was corrected for variation in fecal flow with an inert marker, chromic oxide, as described earlier (15). On the basis of our previous observation that acidic steroids are not degraded significantly in their passage through the intestine (10), we have corrected excretions of acidic steroids only for variations in fecal flow with chromic oxide. The rationale and justification for the use of these internal standards have recently been presented in detail (10).

Measurement of Cholesterol Absorption

In a recent report (11) we have discussed three methods for measurement of cholesterol absorption using sterol balance data and isotopic techniques. In the present report, absorption of dietary cholesterol was measured as the difference between dietary intake and unabsorbed dietary neutral steroids in feces by techniques that we have designated Methods I and II (11). Values for unabsorbed dietary neutral steroids were obtained as the difference between total fecal neutral steroids (determined by chromatographic methods) and fecal endogenous neutral steroids (determined by isotopic techniques). Calculations of fecal endogenous neutral steroids were made with the isotopic data derived from a single intravenous injection of radioactive cholesterol (Ref. 11, Equation 4) or from continuous oral administration of the isotope (Ref. 11, Equations 15 and 16). Method I (Ref. 11, Equations 10 and 11) for estimating absorption makes use of data obtained after pulse labeling, while Method II (Ref. 11, Equations 11, 15, and 16) utilizes data obtained after continuous labeling. Thus, when simultaneous pulse- and continuous labeling with two isotopes of cholesterol was carried out, values for cholesterol absorption were obtained in the same patient by both Methods I and II.
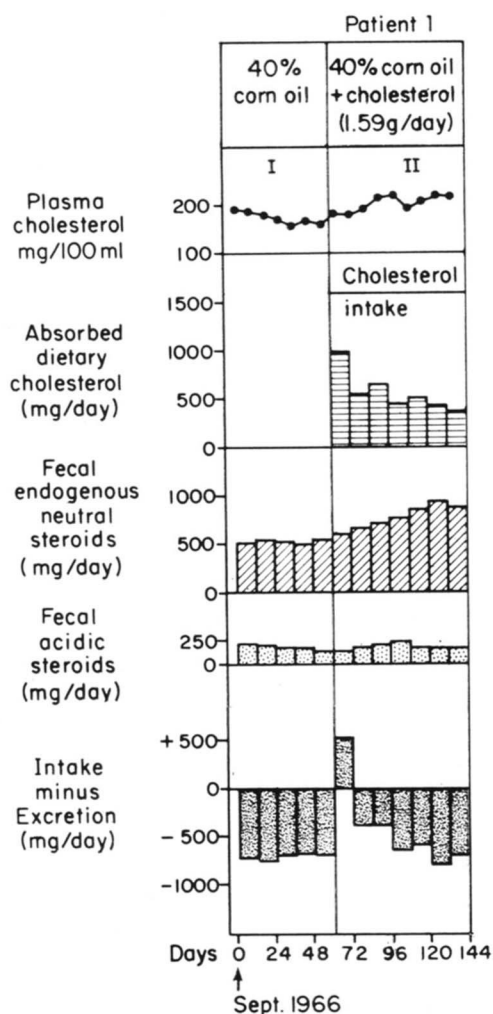
Patient 1

FIG. 1. Cholesterol balance data on low and high intakes of cholesterol in Patient 1. Total cholesterol balance (intake − excretion) = intake of dietary cholesterol minus [total fecal excretion of neutral steroids (unabsorbed dietary and endogenous) plus acidic steroids]. In the "steady state" at the ends of Periods I and II, when daily balance = daily synthesis, note that synthesis was unaffected by a high cholesterol intake, even though plasma levels increased slightly.

Measurement of Cholesterol Synthesis in the "Steady State"

We have also discussed the use of the method of sterol balance in the estimation of cholesterol synthesis in man (11). The sterol balance method is strictly valid only during the true metabolic steady state, defined as the time during which the total body content of cholesterol is constant, and when the synthesis of cholesterol equals the difference between the intake of cholesterol and the excretion of cholesterol and its metabolic products (total fecal steroids exclusive of plant sterols). Unfortunately, present methods do not permit the direct assessment of the constancy of the total body pool of cholesterol. Since this constancy is critical to the definition of the metabolic steady state, we will subsequently

TABLE 3   STEROL BALANCE DATA IN PATIENT 1 DURING "STEADY STATE" ON LOW AND HIGH INTAKES OF CHOLESTEROL

	Period	
	I	II
Total days in period	60	84
Terminal "steady state" period (Days:No. of determinations) *	24:6	24:6
Cholesterol intake (mg/day)	34	1587
Plasma cholesterol [mg/100 ml ± SD(n)] in "steady state"	180 ± 12(11)	199 ± 16(13)
Fecal steroids (mg/day ± SD)		
Neutral steroids		
Total neutral steroids	529 ± 41	2101 ± 276
Endogenous neutral steroids		881 ± 131
Unabsorbed dietary neutral steroids		1220 ± 153
Absorbed dietary cholesterol		367 ± 143†
Acidic steroids	152 ± 25	183 ± 31
Total steroids (total neutral + acidic)	681	2284
Cholesterol balance		
(Cholesterol intake − total fecal steroids, mg/day)	−647	−697

* Duration of "steady state" (days) and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this row gives the average stool collection period in days.

† Calculated by equations 10 and 11, ref. 10, absorption being determined by Method I.

enclose the term steady state in quotation marks as a reminder of the arbitrariness of its present-day use. We assume the "steady state" to be present when the following conditions are met: constant plasma cholesterol concentrations, unchanging fecal excretion of steroids, and constant body weight must all co-exist during long periods of study of patients who are clinically stable and free from complicating metabolic abnormalities. It must be stressed that, when plasma and (or) tissue pools of cholesterol are changing in size, any measurement of synthesis rates by current methods may yield erroneous results.

RESULTS

Effects of Dietary Cholesterol on Absorption and Synthesis of Cholesterol

Fig. 1 presents data for measurements of cholesterol balance in Patient 1 throughout Periods I ("cholesterol-free" diet, 60 days) and II (high-cholesterol diet, 84 days). Values for different parameters of cholesterol metabolism during the last 24 days of each period ("steady state" portion) are given in Table 3. During the feeding of large amounts of cholesterol, plasma cholesterol levels were found to increase slightly but significantly.

Cholesterol Absorption. During Period I, when the patient was receiving a cholesterol-free diet, all cholesterol

absorbed by the intestine was of endogenous origin. In Period II she received a large intake of dietary cholesterol (1587 mg/day) of which 367 mg or 23% was absorbed daily during the "steady state" portion of the period (Table 3). Absorption of dietary cholesterol was somewhat higher during the early portion of Period II, as shown in Fig. 1.

Cholesterol Synthesis. The cholesterol balance in successive 12-day periods is shown in Fig. 1 (bottom section) as the difference between intake and excretion of cholesterol (and its metabolic products). Throughout Period I the balance of cholesterol was negative, and during the last 24 days of this period excretion exceeded intake by an average of 647 mg/day; we consider this to represent the amount of cholesterol synthesized daily in the first "steady state." During the first 12 days of Period II the patient was in positive cholesterol balance, indicating that cholesterol was accumulating in the body during those days. In the next two 12-day periods the patient gradually returned to a state of negative balance. However, the extent of negativity was less than in the control period, and in the next 24 days (designated 72–96 in Fig. 1) the patient was either retaining cholesterol or was producing less new cholesterol each day in response to the absorption of dietary cholesterol. Thereafter (last four 12-day periods of Period II) the patient was in a new "steady state," in which, despite the absorption of significant amounts of exogenous cholesterol, cholesterol synthesis was approximately equal to that of the control period.

Values for cholesterol balance during the "steady state" portion of each period are presented in numerical

form in Table 3. During the last 24 days of Period II the absorption of exogenous cholesterol (367 mg/day) was found to be balanced by an equal increment in excretion of endogenous neutral steroids (881 − 529 = 352 mg/day). Hence, in the two "steady states" the average negative balance of cholesterol in Period II (697 mg/day) was not significantly different from that in Period I (647 mg/day), indicating equal rates of daily synthesis in the two "steady states." Changes in excretion of fecal steroids were confined to the neutral fraction; feeding of dietary cholesterol did not increase excretion of bile acids significantly. Thus, despite the incorporation of large amounts of cholesterol in the diet, synthesis of cholesterol was apparently not significantly changed after attainment of a new "steady state."

The ingestion of large amounts of dietary cholesterol was associated with a gradual increase in the excretion of endogenous neutral steroids (Fig. 1). This increment could have been due to an inhibition in reabsorption of endogenous cholesterol by exogenous cholesterol; or alternatively, it could have been derived from the re-excretion of absorbed dietary cholesterol. During the early portion of Period II more cholesterol from combined endogenous and exogenous sources must have been absorbed. This increment in total cholesterol absorption (endogenous plus exogenous) can be calcu-
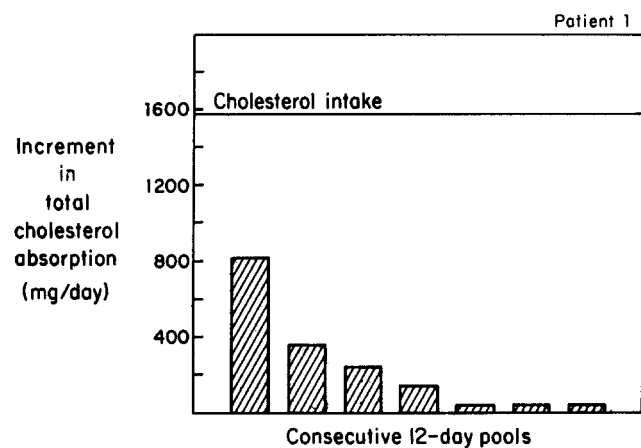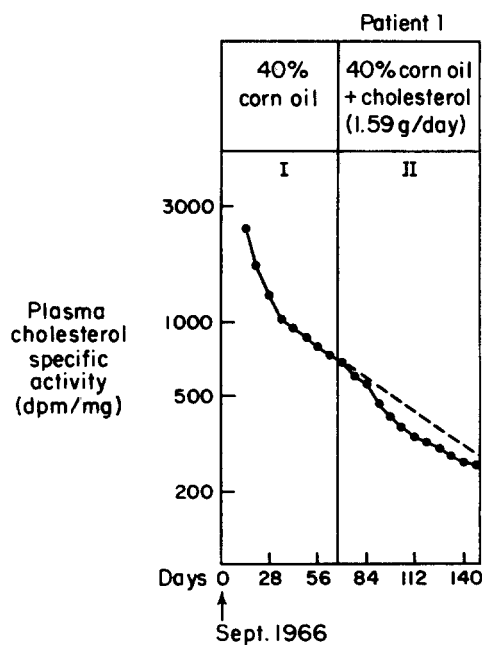

FIG. 2. Increment in absorption of total cholesterol (endogenous plus exogenous) in Patient 1 on a high cholesterol intake. (Increment = change in total cholesterol absorption, Period II vs. Period I, in mg/day). Immediately after institution of cholesterol feeding, net absorption was large but progressively decreased until the last three 12-day collection periods; at that time the amount of dietary cholesterol absorbed was balanced by increased fecal excretion of endogenous neutral steroids.

FIG. 3. Specific activity–time curve of plasma cholesterol after pulse labeling in Patient 1 during low and high intakes of cholesterol. The patient received 85 μc of cholesterol-4-^{14}C intravenously at zero time. With addition of cholesterol to the diet, the slope increased significantly. This suggests that, even during the first 48 days of cholesterol feeding, the daily synthesis of cholesterol was not inhibited to an extent equal to the increment in cholesterol absorption.

TABLE 4   STEROL BALANCE DATA IN PATIENTS 2–6 DURING "STEADY STATE" ON LOW AND HIGH INTAKES OF PLANT STEROLS

	Patient									
	2		3		4		5		6	
Period..................	I	II	I	II	I	II	I	II	I	II
Total days in period	80	112	60	48	72	48	48	84	24	48
Terminal "steady state" period (Days:No. of Determinations)*	20:5	20:5	20:5	20:5	21:5	20:5	20:5	20:5	20:5	20:5
Cholesterol intake (mg/day)	378	380	452	425	285	310	33	33	304	418
Plasma cholesterol in "steady state" [mg/100 ml ± SD(n)]	257±7 (5)	205±15 (14)	447±13 (7)	372±25 (6)	464±24 (12)	393±12 (15)	407±13 (9)	323±14 (5)	422±11 (5)	301±12 (5)
Per cent decrease		20		17		15		20		29
Fecal steroids (mg/day ± SD)										
Neutral steroids (corrected with β-sitosterol)										
Total neutral steroids	626±49	1074±49	1044±83	1685±186	679±56	1145±71	681±33	797±43	405±74	1310±77
Endogenous neutral steroids	477±53	833±95	840±66	1414±135	502±38	896±95			347±74	1000±54
Unabsorbed dietary neutral steroids	149±8	241±40	204±18	271±93	177±23	249±26			58±30	310±43
Absorbed dietary cholesterol†	229±8	139±40	248±18	154±93	108±23	62±28			247±30	108±43
Per cent absorption	61	37	52	36	38	20			81	24
Acidic steroids (corrected with chromic oxide)	206±31	238±63	108±14	154±58	96±19	72±19	360±38	355±139	258±50	321±88
Total steroids (total neutral + acidic)	832	1312	1152	1839	775	1217	1041	1152	663	1631
Cholesterol balance										
(Cholesterol intake − total fecal steroids, mg/day)	−454	−932	−700	−1414	−490	−907	−1007	−1118	−359	−1213

* See footnote*, Table 3.
† Calculated by equations 10 and 11, ref. 10, absorption being determined by Method I.

lated: increment in total cholesterol absorption in each successive 12 days of Period II, compared to "steady state" portion of Period I, in mg/day = absorbed dietary cholesterol in Period II (mg/day) − [fecal endogenous neutral steroids in Period II (mg/day) − average fecal endogenous neutral steroids throughout "steady state" portion of Period I (mg/day)]. The data thus obtained are shown in Fig. 2. The net change in total absorbed cholesterol was maximal in the first 12-day period and then decreased progressively through Period II, approaching zero at the end of the period (the metabolic "steady state") when the increase in excretion of endogenous neutral steroids nearly balanced the amount of dietary cholesterol absorbed.

Throughout Period II a total of 20.5 g of dietary cholesterol could not be accounted for by the method of sterol balance (the summation of all bars shown in Fig. 2); this value represents the net increment in cholesterol absorption in Period II. If no decrease in synthesis occurred during the first part of Period II, plasma and tissue pools increased by 20.5 g. On the other hand, if cholesterol synthesis was completely inhibited immediately after the introduction of dietary cholesterol, very little additional cholesterol was retained in the body. To assess whether cholesterol synthesis had in fact decreased in response to this increment in absorption, we determined a specific activity–time curve of plasma cholesterol after pulse labeling (Fig. 3). 10 days after cholesterol was added to the diet in Period II the curve

became steeper, and for the next 28 days it appeared to decline faster than in the control period; during this time synthesis could not have been inhibited to an extent equal to the increment in cholesterol absorption (which would have led to an unchanged slope). Hence, in Period II from day 84 to day 112 more nonlabeled cholesterol (from the diet and from new synthesis) must have entered the body pools per day than during Period I. On the other hand, for the first 10 days of Period II we cannot rule out the possibility that synthesis was depressed almost completely; thereafter, cholesterol entered body pools at a greater rate, but the total amount of cholesterol accumulated in the body cannot be measured accurately.

Effects of Dietary Plant Sterols Upon Cholesterol Absorption and Synthesis

Graphical data in Patients 2–6, who were fed large amounts of dietary plant sterols, are shown in Fig. 4; values obtained during the final 20-day "steady state" portion of Periods I and II are presented in Table 4.

Treatment with plant sterols produced a prompt lowering of plasma cholesterol levels in three patients in whom the initial hypocholesteremic response was maintained as long as these sterols were fed (from 48 to 112 days); in Patients 5 and 6 the decrease in plasma cholesterol levels was effected slowly. The mean plasma cholesterol decrease in all five patients was 20% (range 15–29%).
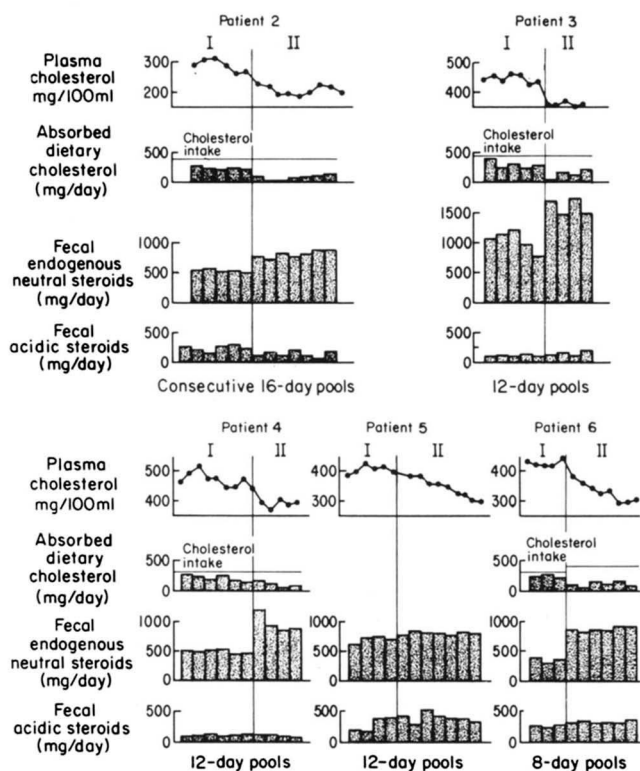
FIG. 4. Cholesterol balance data during low and high intakes of plant sterols (Patients 2–6). In Period I, intakes of β-sitosterol ranged from zero to 293 mg/day; during Period II, intakes of total plant sterols ranged from 5530 to 11,025 mg/day (Table 2). In Period II all patients demonstrated decreased plasma cholesterol levels and increased fecal excretion of endogenous neutral steroids, but essentially no change in fecal excretion of acidic steroids. High intakes of plant sterols also caused decreased absorption of exogenous cholesterol in the four patients whose diets contained significant amounts of cholesterol.

Cholesterol Absorption. Administration of plant sterols decreased the absorption of dietary cholesterol in Patients 2, 3, 4, and 6; this decreased absorption persisted throughout the period of plant sterol feeding (Fig. 4). In addition, the excretion of endogenous neutral steroids was increased. Thus, plant sterols inhibited not only the absorption of exogenous cholesterol, but also the reabsorption of cholesterol entering the intestinal tract from the liver and intestinal mucosa. In Period II there was a sudden decrease in the specific radioactivity of plasma cholesterol derived from labeled dietary cholesterol, which is indicative of reduced absorption (Fig. 5).

The percentage absorption of exogenous cholesterol (Table 4) dropped from 38–81% (control) to 20–36% (plant sterol feeding). Absorption data calculated by Method I (pulse labeling) checked well with those obtained by Method II (continuous labeling) in the three patients simultaneously studied by both methods (Fig. 6). [We have recently presented a more detailed com-

parison of Methods I and II in these same patients (Ref. 11, Fig. 6), but that comparison was for the purpose of evaluating methods for measuring absorption and it included results from the control period only.]

Preliminary evidence has been obtained by Dr. E. Quintao in this laboratory (unpublished data) that exogenous and endogenous cholesterol are absorbed at similar rates; these comparisons were made in man and in rats. If this proves to be true in more extensive trials, then it is reasonable to calculate the amount of endogenous cholesterol available for absorption if two data are at hand: the percentage absorption of exogenous cholesterol and the total quantity of endogenous cholesterol in the feces. Endogenous contribution to cholesterol in the intestinal lumen (mg/day) = fecal endogenous neutral steroids (mg/day) ÷ (1 – fraction of exogenous cholesterol absorbed). Fig. 7 shows calculations for the total load of endogenous cholesterol presented for absorption in Patients 2, 3, and 4 using Method II for estimation of absorption of exogenous cholesterol (Fig. 6). This calculation suggests that, despite the decrease in absorption of cholesterol produced by the feeding of plant sterols, these sterols have almost no effect upon secretion of cholesterol into the intestinal tract from whatever source.

Cholesterol Synthesis. Values for the balance between dietary intake and excretion of cholesterol and its products during the "steady state" portions of Periods I and II are presented in Table 4 and Fig. 8. If it is true that steady states had actually been attained in the two periods, the results indicate that the administration of plant sterols caused a marked increase in the rate of synthesis of new cholesterol in four of five patients.

This conclusion is also supported by the data in Table 5, which shows the magnitude of the increments in excretion of endogenous cholesterol during extended periods of plant sterol feeding. In Patients 4 and 5 the increases were 20 and 18 g, respectively, but in the other three the accumulated increments in excretion of cholesterol ranged from 49 to 70 g. These increments could have been derived from tissue stores of cholesterol or, alternatively, from new synthesis. The first explanation seems unlikely in view of Cook's estimate (16) that the total body content of cholesterol of a 70 kg man is approximately 108 g (exclusive of the nervous system): treatment with plant sterols for 84–120 days could hardly have caused a depletion in total body stores of cholesterol of 40–60%, especially since the largest excretion increment (70 g) was observed in Patient 2 who was free of xanthomata. Moreover, the increase in excretion of endogenous neutral steroids persisted throughout the feeding of plant sterols and showed no lessening at the end of the study period, as would be expected if tissue stores were being depleted. Our interpretation of the
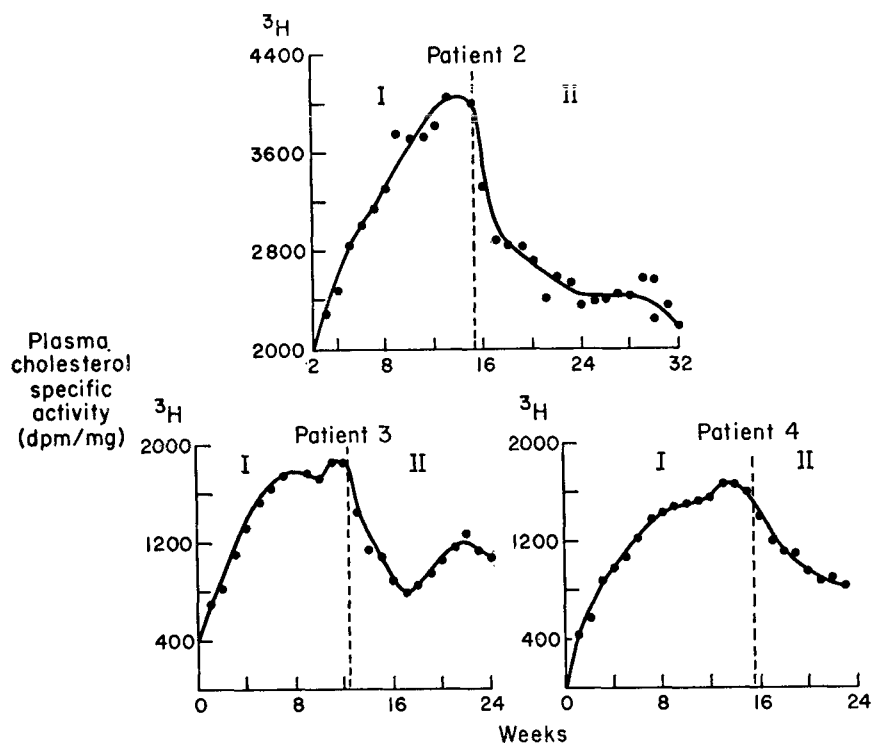
FIG. 5. Specific activity–time curves of plasma cholesterol during daily oral administration of cholesterol-³H in patients fed low and high intakes of plant sterols. In no patient was the isotopic steady state (17) attained in Period I, but after introduction of large amounts of plant sterols in Period II a marked decline in specific activity was noted in each patient, indicating a striking reduction in absorption of exogenous cholesterol.
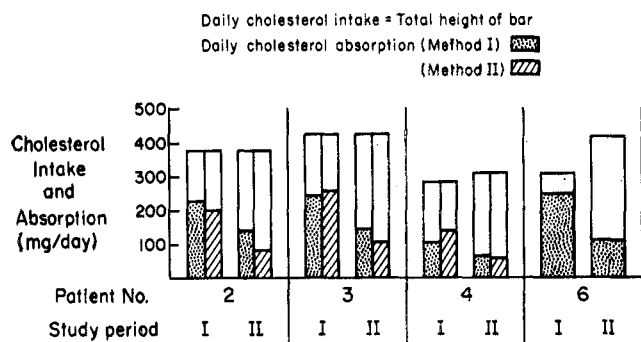

FIG. 6. Effects of large intakes of plant sterols on absorption of dietary cholesterol. In Patients 2, 3, and 4, values for absorption calculated by Method I (pulse labeling) are compared with those obtained by Method II (continuous labeling orally); Patient 6 was studied by Method I only. All data were derived from five 4-day stool collection periods in the last 20 days of low (Period I) and high (Period II) intakes of plant sterols ("steady states"). Absorption of dietary cholesterol was consistently lower in Period II. Similar results were obtained by the two methods for calculating absorption (11).

data is that the major portion of the increase in fecal excretion of cholesterol was due to increased synthesis.

This conclusion is neither supported nor weakened by study of the specific activity–time curves after pulse
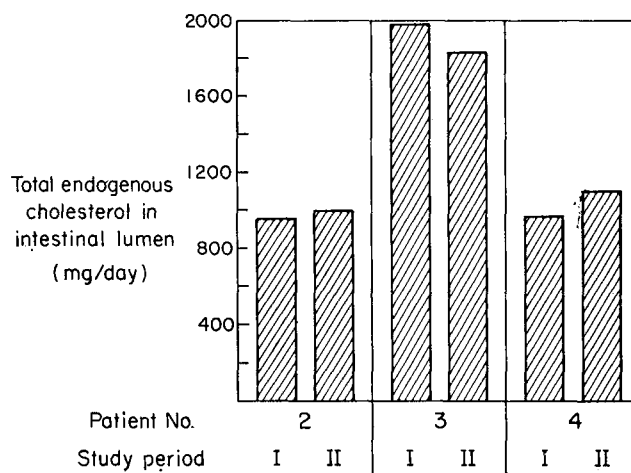

FIG. 7. Total endogenous cholesterol presented for intestinal absorption during low and high intakes of plant sterols. If cholesterol of exogenous and endogenous origin is absorbed at similar rates, the amount of endogenous cholesterol presented to the intestinal lumen = fecal endogenous neutral steroids ÷ (1 − the fraction of exogenous cholesterol absorbed). The bars represent the average of five 4-day collection periods in the last 20 days ("steady state") of Periods I and II; absorption of dietary cholesterol was calculated by Method II (10). In each patient the amount of endogenous cholesterol presented for absorption was the same in Periods I and II.
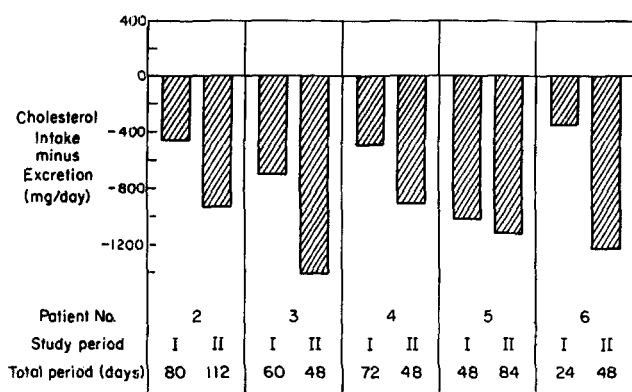
Fig. 8.   Cholesterol balance in "steady state" on low and high intakes of plant sterols. Cholesterol balance = intake of dietary cholesterol minus [total fecal excretion of neutral steroids (unabsorbed dietary and endogenous) plus acidic steroids]. In the "steady state," daily cholesterol balance equals daily cholesterol synthesis. In Period II all patients except No. 5 showed a marked change in cholesterol balance, indicative of increased synthesis.

labeling with isotopic cholesterol (Fig. 9). (In Patient 6, log-linearity in the decay curve was not obtained prior to the shift in regimens from Periods I to II, and since the conformation of the curve has no meaning in this context, it is not included in Fig. 9.) After introduction of plant sterols into the diet the "decay" curves for Patients 2, 3, and 4 showed an increased slope. There are four possible explanations for such an increase (15): (a) a decrease in pool size of readily miscible cholesterol in the tissues; (b) an increase in absorption of nonlabeled dietary cholesterol; (c) an increase in cholesterol synthesis; or (d) combinations of these factors. Increased cholesterol absorption can be ruled out on the basis of the evidence already presented, but we find it impossible to choose among the other possibilities. However, it is of interest to note that, in contrast to these three patients, Patient 5 exhibited no appreciable change in the slope of the specific activity–time curve; in this patient the excretion of neutral steroids showed comparatively little change after introduction of plant sterols (Table 4), and total cholesterol balance changed less than in any other patient studied (Fig. 8).

DISCUSSION

Cholesterol Absorption

A number of workers have come to the conclusion that the human being can absorb only limited amounts of dietary cholesterol. For example, Kaplan, Cox, and Taylor (17) determined cholesterol absorption in 24 healthy volunteers by daily feeding of labeled cholesterol diluted with various amounts of unlabeled cholesterol; they measured the specific activity of plasma cholesterol over many weeks and calculated that the absorption

TABLE 5   INCREMENTS IN EXCRETION OF CHOLESTEROL AND ITS METABOLIC PRODUCTS DURING PROLONGED PERIODS ON HIGH INTAKES OF PLANT STEROLS

		Control Period		Plant Sterol Feeding Period		
Patient	Body Wt	Daily Cholesterol Balance*	Days†	Average Cholesterol Balance‡	Increment in Average Daily Excretion§	Increment in Total Excretion ‖
	kg	mg/day		mg/day	mg/day	g
2	67	−454	120	−861	407	48.8
3	74	−700	88	−1496	796	70.1
4	53	−490	48	−952	462	20.3
5	61	−1007	72	−1182	175	17.7
6	70	−359	84	−1244	885	63.8

* Cholesterol balance in the control period was calculated as the difference between total fecal steroids (neutral + acidic) and dietary cholesterol during the last 20 days of Period I (Table 4); in the true metabolic steady state, cholesterol balance equals cholesterol synthesis.

† This column shows the total duration of plant sterol feeding. In Patients 2, 4, and 5, plant sterols were fed throughout Period II only (Fig 1), but in Patients 3 and 6, an additional period of plant sterol feeding is included beyond Period II as described in Methods.

‡ The average cholesterol balance in this period was calculated as the average difference between daily total fecal steroids derived from cholesterol and daily intake of cholesterol throughout the entire period of plant sterol feeding.

§ The average increment in daily excretion of fecal steroids is the difference in cholesterol balance between control and plant sterol treatment periods.

‖ The total excretion increment is the product of the average daily excretion increment and the number of days of plant sterol feeding.

maximum might be as low as 150–300 mg/day. In similar studies of two healthy men fed high and low amounts of cholesterol during the isotopic steady state, Wilson and Lindsey (7) found that cholesterol absorption was small even when the intake was very large: 300 mg absorbed daily from an intake of 3 g. In our present study of a 68-yr-old normocholesteremic woman with ischemic heart disease (Patient 1) we observed (Table 3) that in the metabolic "steady state" the amount of cholesterol absorbed (367 mg/day) was considerably lower than the intake (1587 mg/day), and, in another patient fed 2794 mg/day cholesterol in the form of egg yolk for 42 days, absorption of cholesterol in the "steady state" was only 352 mg/day (unpublished data). Thus, all results obtained in three different laboratories have led to the conclusion that the amounts of dietary cholesterol absorbed by man remain relatively low even in the face of large intakes. However, the amounts absorbed are not insignificant: if other mechanisms fail to compensate for this absorption, progressive accumulation of cholesterol in body pools must result.

The results of the present study also seem to indicate that less endogenous cholesterol is reabsorbed during the feeding of plant sterols. However, little is known about the comparative absorption rates of dietary cho-
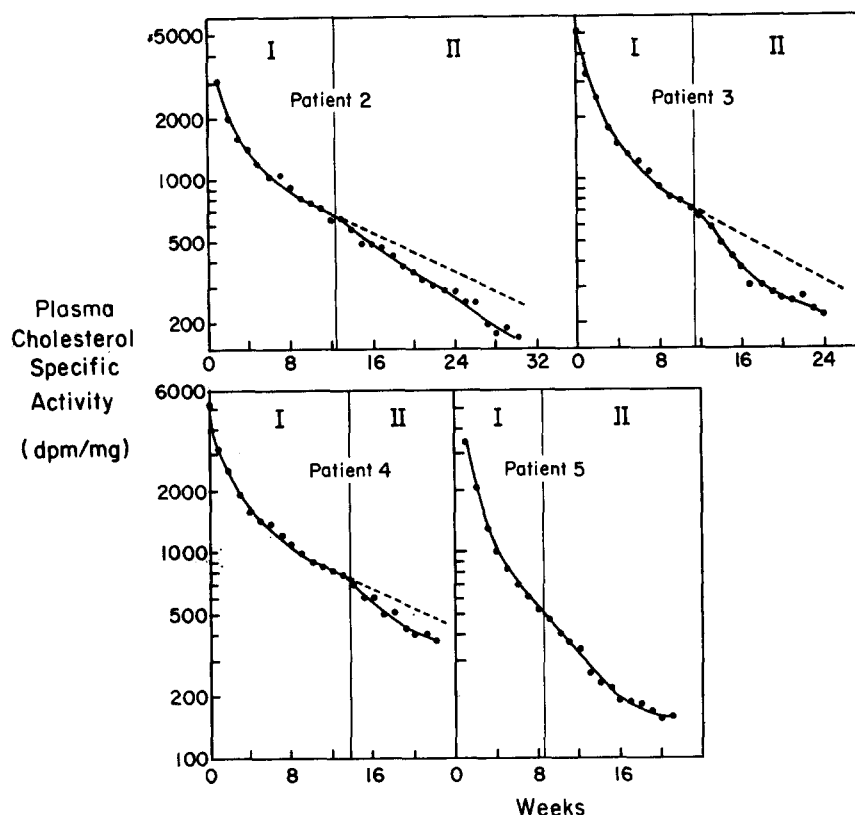
FIG. 9. Specific activity–time curves of plasma cholesterol after pulse labeling during low and high intakes of plant sterols. The first three patients showed an accelerated rate of decrease in specific activity in Period II; the curve for Patient 5 showed no change in slope. An increased negative slope could have been due to decreased pool size of tissue cholesterol or to increased cholesterol synthesis; increased absorption of cholesterol was ruled out by other data (Fig. 6). Cholesterol-26-^{14}C was administered to Patients 2, 3, and 4; cholesterol-4-^{14}C was given to Patient 5.

lesterol and of endogenous cholesterol secreted into the intestinal tract in bile or through the intestinal wall. While the amount of endogenous cholesterol presented to the intestine for reabsorption may range from 1 to 3 g daily (18), there are at present no direct methods for determining the size of this load or the fraction of it that is reabsorbed. If the assumption is made that exogenous and endogenous cholesterol are absorbed to a similar extent, as indicated by unpublished data obtained here by Quintao, it is possible to estimate the total amount of endogenous cholesterol presented to the intestine: in three patients we found, using this assumption, that feeding plant sterols had no effect upon the size of this load.

The feeding of plant sterols decreases the total quantity of cholesterol that is absorbed from the intestinal tract. The mechanisms by which plant sterols inhibit cholesterol absorption have not been elucidated at the cellular or molecular level, but at least three possibilities have been considered: (a) plant sterols may competitively block sites for cholesterol absorption in the intestinal mucosa (19, 20), or (b) esterification of

cholesterol and transport out of the mucosal cell and into the lymph may be hindered (21), or (c) plant sterols may displace cholesterol from bile salt micelles in the intestinal lumen or render it insoluble by the formation of mixed crystals of cholesterol and β-sitosterol (22, 23). Whatever the mechanism involved, inhibition in reabsorption of cholesterol from endogenous sources is associated with a simultaneous increase in its excretion into feces in the form of neutral steroids. Thus, prolonged feeding of plant sterols will necessarily produce a drain on body pools of cholesterol if synthesis fails to increase to replace that lost from the body. Presumptive evidence for increased synthesis is discussed below.

Cholesterol Synthesis

The synthesis of cholesterol in Patient 1 was essentially the same during "steady state" portions of the two periods when the diet was low and high in cholesterol. This observation would be predicted on the basis of the isotope studies of Taylor and coworkers (6), who indeed questioned the extent to which cholesterol synthesis in the human being is under feedback control by choles-

terol itself. On the other hand, evidence for the existence of a partial feedback control of cholesterol synthesis in man was obtained by Bhattathiry and Siperstein (5), who demonstrated that cholesterol synthesis in liver is inhibited shortly after addition of large amounts of cholesterol to diets of human beings. Nevertheless, results obtained in the present study have led us to the conclusion that the extent of feedback control by cholesterol cannot be adequately evaluated through experiments with high cholesterol diets: once the "steady state" has been attained, the increment in absorption of dietary cholesterol is balanced almost precisely by an increased excretion of endogenous neutral steroids into feces. Therefore, we have taken another approach to the problem: we have studied the effects of interrupting the reabsorption of cholesterol by feeding large amounts of plant sterols.

Plant sterol feeding resulted in an increase in excretion of endogenous neutral steroids that persisted as long as plant sterols were fed. In periods lasting as long as 112 days there was no diminution in this increment of neutral steroid excretion in the feces; any such diminution might have suggested an emptying of pools of stored cholesterol. Furthermore, the magnitude of the increments (as much as 70 g in Patient 2, who was free of xanthomatosis) makes it likely that these increments could not have been derived from tissue stores. By exclusion, the major portion of the increments in endogenous neutral steroids in the feces must have originated from new synthesis.

We conclude that an interruption of the enterohepatic circulation of cholesterol causes a release of feedback inhibition of cholesterol synthesis (since the excretion of bile acids is not affected by plant sterol feeding). Therefore, it is clear that in man the amount of cholesterol absorbed is an important factor regulating the amount of cholesterol synthesized each day. Even when the diet is cholesterol-free, endogenous cholesterol is reabsorbed in amounts sufficient to limit substantially the synthetic process, and the incorporation of cholesterol into the diet appears to produce little if any additional effect upon total body synthesis of cholesterol. However, when reabsorption of cholesterol is diminished by plant sterol feeding, feedback inhibition of cholesterol synthesis is released, and the daily production of cholesterol is increased significantly.

Cholesterol Excretion

The cholesterol balance study in Patient 1 indicated that continuous accumulation of absorbed dietary cholesterol into body pools was prevented by an increase in the fecal excretion of cholesterol from endogenous sources. This compensatory mechanism could have been activated in any of the following ways: (*a*) through a decrease in reabsorption of endogenous cholesterol; (*b*) through an increase in the rate of its secretion into the intestinal tract; or (*c*) by both. Conceivably, the reabsorption of endogenous cholesterol may be partially inhibited by exogenous cholesterol simply because the absorption capacity is already exceeded by the load of endogenous cholesterol presented for reabsorption. On the other hand, an increase in secretion of cholesterol into the intestinal tract may result from an expansion of the enterohepatic pool caused by an increase in total absorption of cholesterol. The sequence of events shown in Figs. 1 and 2 leads us to favor this second explanation: a definite lag period was noted in the first 36 days of high cholesterol feeding, during which time the absorption of exogenous cholesterol was maximal, then tapered off as a new "steady state" was approached. Over the entire period the amount of dietary cholesterol absorbed exceeded the increase in excretion of endogenous neutral steroids by approximately 20 g, and only after 48 days was the absorption of dietary cholesterol balanced by an increase in excretion of endogenous cholesterol. The existence of this lag period suggests a gradual increase in the size of the enterohepatic pool of cholesterol; with the attainment of a new "steady state," increased absorption was balanced by increased secretion, and synthesis was the same as in Period I. The size of the increment (20 g) suggests that newly absorbed cholesterol found its way into tissues other than plasma and liver. How much remained in the intestinal mucosa and how much in other tissues was not assessed, but the different degrees of responsiveness in plasma cholesterol concentrations of different patients to cholesterol-containing diets that have been noted by many investigators suggests that some patients may compensate more slowly for newly absorbed cholesterol, as rabbits do, whereas others either absorb less or re-excrete it more efficiently. The rat, in contrast to man, compensates for increased absorption primarily by increasing the catabolism of cholesterol to bile acids (4), and in this manner the rat keeps its tissue stores low.

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