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Research Article

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Since the majority of patients showed no change in cholesterol or bile acid excretions during the transitional period, we propose that when excretion changes did occur they were probably not the cause of the plasma cholesterol change. Furthermore, turnover data and specific activity curves suggested that cholesterol synthesis was not influenced by exchange of dietary fats. Thus, excluding changes in excretion and synthesis, we conclude that it is most likely that unsaturated fats cause plasma cholesterol to be redistributed into tissue pools.

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The Effects of Unsaturated Dietary Fats on Absorption, Excretion, Synthesis, and Distribution of Cholesterol in Man

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ABSTRACT Cholesterol balance studies were carried out in 11 patients with various types of hyperlipoproteinemia to determine the mechanism by which unsaturated fats lower plasma cholesterol. Unsaturated fats produced no increase in fecal endogenous neutral steroids in 10 of 11 patients and no decrease in absorption of exogenous cholesterol in 5 patients who received cholesterol in the diet. In 8 of 11 patients no changes occurred in excretion of bile acids during the period on unsaturated fat when plasma cholesterol was declining. However, in 3 of 11 patients small but significant increases in bile acid excretion were found during this transitional period; in 2 others increases also occurred after plasma cholesterol had become constant at lower levels on unsaturated fat.

Since the majority of patients showed no change in cholesterol or bile acid excretions during the transitional period, we propose that when excretion changes did occur they were probably not the cause of the plasma cholesterol change. Furthermore, turnover data and specific activity curves suggested that cholesterol synthesis was not influenced by exchange of dietary fats. Thus, excluding changes in excretion and synthesis, we conclude that it is most likely that unsaturated fats cause plasma cholesterol to be redistributed into tissue pools.

We have also examined the possibility that cholesterol which is redistributed into tissues could be secondarily excreted as neutral steroids or bile acids. In at least 5 of 11 patients excretion patterns were consistent with this explanation. However, we cannot rule out that excretion changes may have been due to alterations in transit time, to changes in bacterial flora, or to transitory changes in absorption or synthesis of cholesterol or bile acids.

Our conclusion that unsaturated fats cause a redistribution of cholesterol between plasma and tissue pools

points to the necessity in future to explore where cholesterol is stored, to what extent stored cholesterol can be mobilized, and to define the factors governing these fluxes.

INTRODUCTION

The mechanism by which unsaturated fats cause a lowering of plasma cholesterol concentrations remains in dispute despite years of work on this problem by several groups of investigators. Various sites of action have been proposed (Fig. 1), but to date no single mechanism has been shown to apply in all cases (1). Some workers (1-9) have suggested that unsaturated fats lower plasma cholesterol by causing an increased excretion of cholesterol or its metabolic products, the bile acids.

An increased excretion of cholesterol itself could occur either by decreasing absorption of cholesterol (Fig. 1, locus A) or by increasing secretion of cholesterol into the intestinal tract (locus C); an increase in bile acid excretion could be due to a decreased reabsorption of bile acids (locus B) or to an increased conversion of cholesterol into bile acids (locus E). The hypocholesterolemic effect of unsaturated fats might also be due to decreased synthesis of cholesterol (locus D) (10), or to a redistribution of cholesterol from plasma into tissues (loci F and G) (11-13).

In our view, the present confusion has several possible origins: results obtained from various animal species may not be applicable directly to man; patients with different kinds of lipid abnormalities may not respond by identical mechanisms to diets containing unsaturated fats; experimental designs may have been inappropriate to resolve the problem; or the methods used to describe the various aspects of cholesterol metabolism may not have been sufficiently critical or precise.

In an attempt to resolve some of the discrepancies in findings and in interpretation, we have reexamined this

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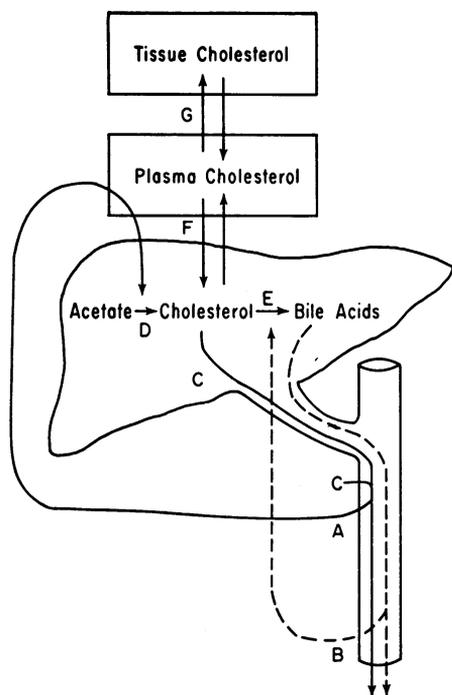


FIGURE 1 Schematic representation of the pathways of cholesterol metabolism in man. Possible sites of action of unsaturated fats in the lowering of plasma cholesterol are shown (loci A-G). A, interruption of the absorption of cholesterol. B, interruption of the reabsorption of bile acids. C, increase in cholesterol secretion into intestinal lumen. D, decrease in cholesterol synthesis. E, primary increase in oxidation of cholesterol into bile acids. F, redistribution of cholesterol from plasma into liver. G, redistribution of cholesterol from plasma into other tissues.

problem in 11 patients with various types of hyperlipoproteinemia; they were studied on the metabolic ward for periods ranging from 29 to 128 days. In order to overcome some of the technical deficiencies noted in previous studies, we have developed a method for the accurate measurement of all of the excretion products of cholesterol (14, 15); with its continued use over the last five years, it has become evident that the power of the method has been greatly enhanced by application of correction factors for variations in fecal flow and for losses of neutral steroids during intestinal transit (16). With these improvements, the sterol balance method supplemented by studies of isotope kinetics of cholesterol is a useful and dependable approach to the measurement of the absorption, synthesis, and turnover of cholesterol (17), and thus for describing the regulation of the movement of cholesterol and bile acids in the enterohepatic circulation (A-D in Fig. 1). With continued experience it has become clear that the distribution of cholesterol in and out of tissue pools (F and G in Fig. 1) can be assessed only indirectly.

In this investigation we carried out studies of cholesterol balance in 11 patients in whom unsaturated fats were substituted for saturated fats in the diet. Evidence will be presented to show that in almost all patients the excretion of neutral steroids of endogenous origin did not change when the type of dietary fat was altered. Although incremental changes in excretion of acidic steroids were noted in 5 of 11 patients, in only 3 of these did the change occur in the period when plasma levels were declining on unsaturated fat. In addition, we found no evidence to support the possibilities that unsaturated fat caused a decrease in either cholesterol absorption or synthesis. Thus, we have concluded that the primary action of unsaturated fat is to cause a redistribution of cholesterol between plasma and tissue compartments, (F or G in Fig. 1), and that in a few patients this effect can be reflected by secondary changes in the enterohepatic circulations of cholesterol or bile acids (A-E in Fig. 1).

METHODS

Patients. Studies were carried out on 11 hyperlipidemic patients during exchange of dietary fats on the metabolic ward at The Rockefeller University Hospital. The age, sex, stature, caloric requirement, and clinical diagnosis of each patient are given in Table I. In many of these same patients we have carried out cholesterol balance studies with other objectives, and certain data obtained in those experiments have been reported previously (20).

Diets. Food intakes consisted exclusively of orally administered liquid formula feedings as previously described (21), together with vitamin and mineral supplements. All formulas contained 15% of total calories as milk protein (R1-5, Ross Laboratories, Columbus, Ohio), 40% as fat, and 45% as glucose. In each case caloric intake was continually adjusted to maintain total body weight at a constant level.

Table II lists the dietary fat of each formula and its sterol content. Formulas A, B, and H contained corn oil or butter which had been subjected to molecular distillation (Distillation Products Industries, Rochester, N. Y.) and steam deodorization (Drew Chemical Corp., Boonton, N. J.), processes which reduced their sterol contents without impairing their palatability. Cholesterol and plant sterol contents of each formula were determined by gas-liquid chromatographic methods (15).

The patients of this study fell into three groups on the basis of paper-strip electrophoresis of lipoproteins (19) and intake of dietary cholesterol: patients 1-6 had familial hypercholesterolemia (type II lipoprotein pattern) and received diets low in cholesterol (46-83 mg/day); patients 7-9 also had familial hypercholesterolemia (type II) but ingested diets containing moderate amounts of cholesterol (457-679 mg/day); and the third group was composed of two patients (Nos. 10 and 11) with hypertriglyceridemia (types V and IV, respectively) who were maintained on formulas containing moderate amounts of cholesterol (586-679 mg/day).

Isotopes. A single dose (50-100 μ ci) of cholesterol-4- 14 C or cholesterol-1,2- 3 H was administered intravenously to several patients (Nos. 7 and 9-11) at the beginning of their studies. 1 ml of ethanol containing known amounts of the

TABLE I
Clinical Data

Patient No.	Initials	Age	Sex	Length of metabolic study	Weight	Height	% of ideal weight*	Clinical diagnosis and type of hyperlipoproteinemia
				days	kg	cm		
1	J. F.	39	M	51	78	177	105	IHD†, hypercholesterolemia (type II)§, xanthomatosis
2	E. Y.	49	F	100	46	163	93	Hypercholesterolemia (type II)
3	A. M.	19	M	44	83	169	131	Hypercholesterolemia (type II) xanthomatosis
4	J. C.	14	M	36	47	167	96	Hypercholesterolemia (type II)
5	R. M.	14	M	48	48	159	98	Hypercholesterolemia (type II)
6	S. C.	18	F	29	49	162	93	Hypercholesterolemia (type II)
7	B. K.	53	F	128	38	154	83	Xanthomatosis, hypercholesterolemia (type II)
8	J. Ca.	39	M	120	64	176	94	IHD, hypercholesterolemia (type II)
9	N. S.	21	F	79	52	166	90	IHD, xanthomatosis, hypercholesterolemia (type II)
10	H. Sa.	54	M	52	78	169	117	Hyperglyceridemia (type V)
11	N. R.	36	F	124	53	167	98	Hyperglyceridemia (type IV)

* According to life insurance tables of weight for height, age, and sex (18).

† IHD, ischemic heart disease.

§ According to Fredrickson, Levy, and Lees (19).

radioactive tracer was dispersed in 150 ml of physiologic saline; the mixture was immediately administered intravenously.

Patients 7 and 8 were fed a small amount of radioactive cholesterol daily in all formula feedings throughout their studies. Cholesterol-1,2-³H dissolved in 10 ml of ethanol was added to 40 kg batches of formula during homogenization in order to hold constant the isotope content of all feedings (homogeneity of labeled sterol content of all formulas was repeatedly verified by mass and specific activity measurements).

All isotopically labeled cholesterol was obtained from New England Nuclear Corp., Boston, Mass.; in every case it was shown that greater than 95% of radioactivity chromatographed with cholesterol on Florisil thin-layer plates in the system, ethyl ether-heptane (55:45). Total plasma cholesterol concentration and specific activity were determined biweekly: concentration by the spectrophotometric method of Abell, Levy, Brodie, and Kendall (22) and, on a portion of the same extract, radioactivity in a Packard Tri-Carb Scintillation Counter (Model 3003) as previously described (14).

Fecal steroid¹ analysis. Fecal neutral and acidic steroids were isolated separately, and their masses and specific activities were measured by methods presented previously (14,

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

15). These 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 α ,3 β -OH and 5 α ,3-keto compounds).

Excretions of neutral steroids were corrected for losses occurring during intestinal transit and for variations in fecal flow rates using dietary β -sitosterol as an internal standard. In a recent report (16) we showed that considerable amounts of neutral steroids (as much as 60%) may be lost during intestinal transit, presumably due to degradation of the sterol ring structure, but β -sitosterol is lost to the same extent; since less than 5% of this sterol is absorbed from the human intestine (23), ingested β -sitosterol can serve as an internal standard to correct for losses of cholesterol.

We have also demonstrated that acidic steroids are not lost during passage through the human intestine (16). However, in those patients (Nos. 7-11) who were given chromic oxide each day, corrections of acidic steroid excretions were made for day-to-day variations in fecal flow, as described previously (16, 24). Although a lag period in fecal excretion of steroids from one dietary period to another undoubtedly existed, the demarcation between periods was not made with a marker such as carmine; inclusion of this additional marker should constitute a desirable improvement in technique.

Measurement of cholesterol absorption. Daily absorption of exogenous cholesterol was measured in patients 7-11 by methods recently described. Method I (equations 10, 11

TABLE II
Formula Diets and Their Sterol Contents

Diet	Dietary fat	Cholesterol	β -Sitosterol
		mg/500 cal	mg/500 cal
A	Butter oil (distilled)	13	47*
B	Corn oil (distilled)	13	41‡
C	Butter oil	136	62§
D	Safflower oil	162	70‡
E	Butter oil	130	36§
F	Safflower oil	152	42‡
G	Butter oil (89%) Egg yolk (11%)	147	34§
H	Corn oil (distilled) (84%) Egg yolk (16%)	153	39‡
I	Butter oil	144	57§
J	Safflower oil	162	70‡

* β -sitosterol added as corn sterols (donated by Dr. S. S. Chang of the A. E. Staley Mfg. Co., Decatur, Ill.) contained 65% β -sitosterol, 30% campesterol, and 5% stigmasterol.

‡ 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 and Co., Indianapolis, Ind.; the final product contained 90% β -sitosterol and 10% campesterol.

of reference 17) for estimating absorption makes use of data obtained after pulse labeling with radiocholesterol by the intravenous route, while Method II (equations 11, 15, and 16 of reference 17) utilizes data obtained after continuous oral labeling.

Experimental design. In this investigation, saturated and unsaturated fats were fed in successive periods of balance study. Throughout the two feeding periods, intakes of cholesterol and β -sitosterol were held at similar levels by supplementing the unsaturated fat formulas with cholesterol and the saturated fat formulas with β -sitosterol, as shown in Table II.

We divided each patient's study into three metabolic periods during which complete stool collections were made for fecal steroid analysis. Period I was initiated after plasma cholesterol concentrations had stabilized on formula diets containing saturated fat, and it lasted until the institution of an unsaturated fat formula; during this first period stools were collected in 4- to 8-day pools. Period II represented the transitional period on unsaturated fat when concentrations of plasma cholesterol were decreasing; during this period stools were collected in 1- to 4-day pools. Period III represented those weeks on unsaturated fats after concentrations of cholesterol had stabilized at a new lower level; once again stool collections were made in 4- to 8-day pools. In two patients (Nos. 3 and 9) plasma levels of cholesterol continued to fall throughout the entire period of unsaturated fat feeding; in these two cases no clear demarcation could be made between periods II and III.

RESULTS

Plasma cholesterol changes

Concentrations of total plasma cholesterol for the 11 patients of this study are plotted in Figs. 2 and 3; nu-

TABLE III
Plasma Cholesterol Changes with Exchange of Unsaturated for Saturated Dietary Fat

Patient	Plasma cholesterol in "steady state"		Change	Plasma volume*	Change in plasma cholesterol content	Length of period II	Mean daily decrement of plasma cholesterol in period II
	Period I	Period III					
	mg/100 ml \pm SD (n)		absolute (%)	ml	mg	days	mg/day
1	485 \pm 11 (7)	381 \pm 12 (6)	104 (20)	3510	3650	10	368
2	421 \pm 21 (5)	293 \pm 16 (5)	128 (30)	2050	2630	32	82
3	450 \pm 10 (5)	340‡	110 (24)	3740	4114	28	147
4	321 \pm 11 (7)	256 \pm 10 (4)	65 (17)	2090	1358	17	80
5	242 \pm 11 (5)	176 \pm 9 (4)	66 (28)	2160	1425	8	178
6	291 \pm 14 (6)	176 (2)	115 (39)	2200	2530	12	211
7	320 \pm 26 (14)	255 \pm 11 (11)	65 (22)	1710	1111	16	69
8	289 \pm 14 (23)	184 \pm 12 (5)	105 (34)	2925	3071	32	96
9	549 \pm 11 (11)	468‡	81 (15)	2340	1895	49	39
10	460 \pm 48 (4)	450 \pm 15 (3)	10 (2)	3510	351	24	15
11	233 \pm 8 (9)	139 \pm 3 (3)	94 (40)	2925	2749	48	57

* 4.5% of total body weight (25).

‡ In patients 3 and 9 a new steady state in plasma cholesterol was never reached on unsaturated fat; in these cases the values shown represent the plasma cholesterol at the end of period II.

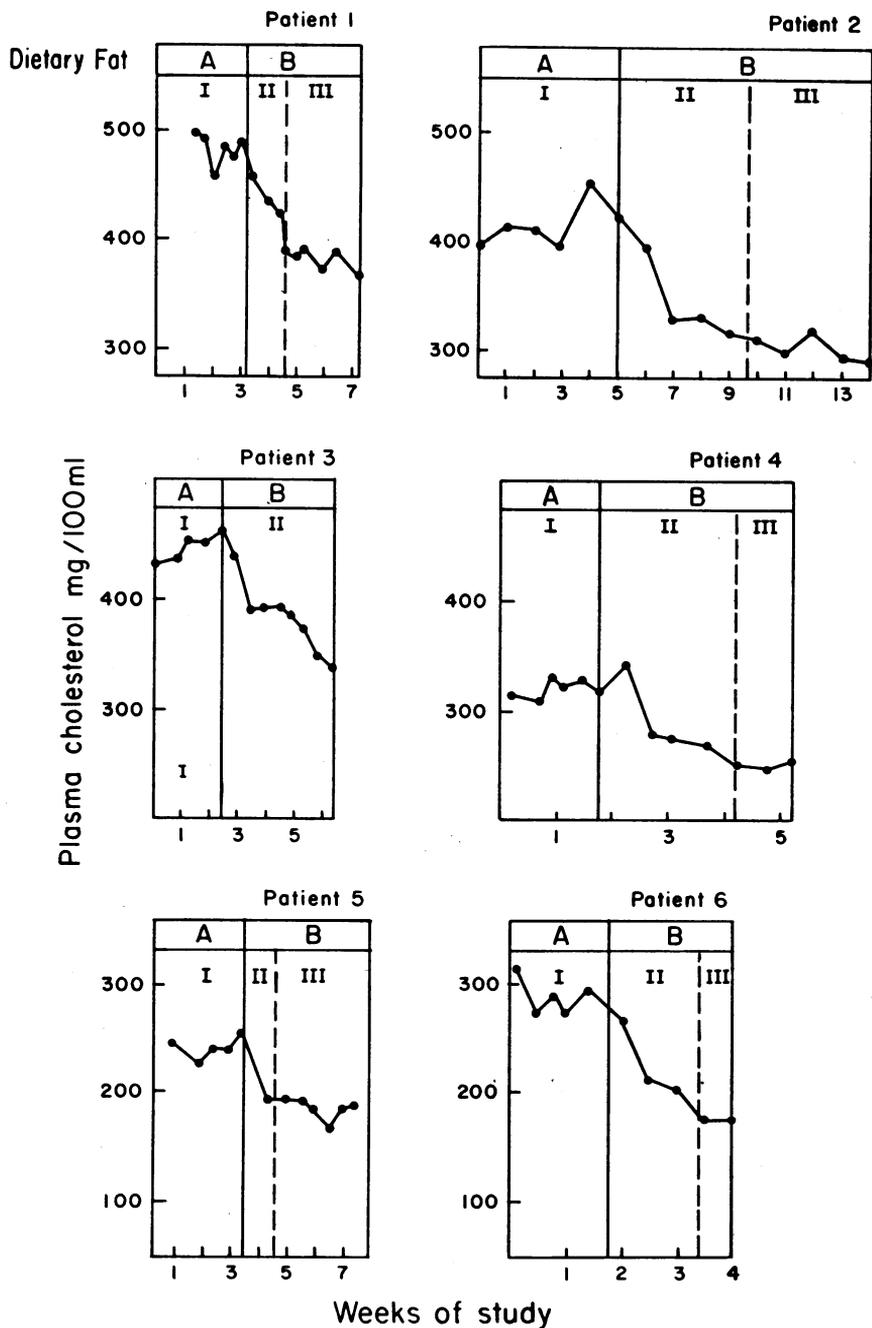


FIGURE 2 Decreases in plasma cholesterol after substitution of corn oil for butter oil in patients on low intakes of dietary cholesterol (patients 1-6). Dietary fats are lettered as in Table II. Period II is the transition period between steady states I and III.

merical data for all of the patients are given in Table III. Exchanges of unsaturated for saturated fats produced a significant lowering of plasma cholesterol in all but patient 10. Since plant sterol and cholesterol intakes were approximately the same throughout each patient's

study, the lowering of plasma cholesterol must be attributed entirely to differences in fatty acid compositions of the dietary fats.

Table III also displays the total and mean daily decrements in plasma cholesterol content that occurred as a

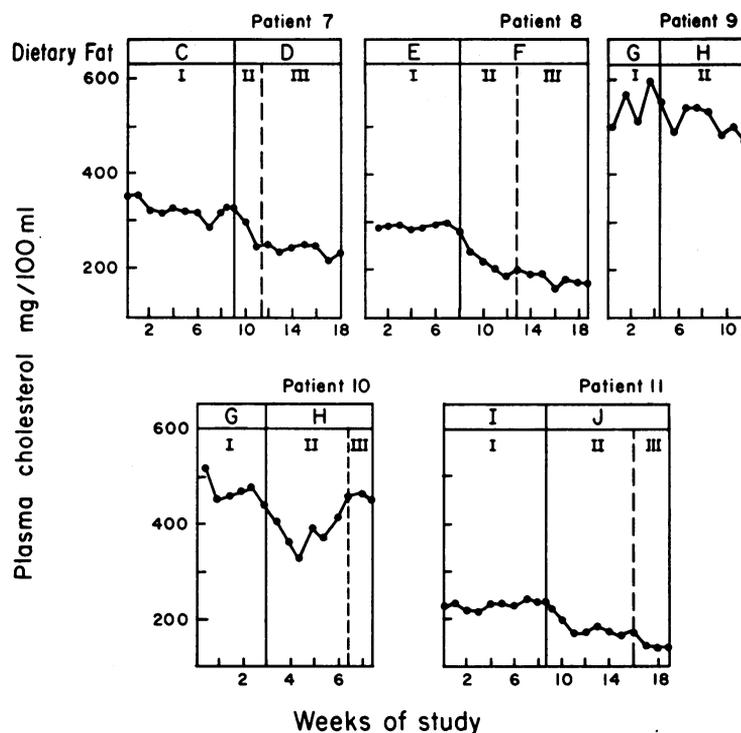


FIGURE 3 Decreases in plasma cholesterol after substitution of unsaturated fats for saturated fats in patients on moderate intakes of dietary cholesterol (patients 7-11). Dietary fats are lettered as in Table II. Period II = transition period between steady states I and III.

result of the dietary fat substitution. Plasma cholesterol levels declined for periods ranging from 8 to 49 days, and the rate of decline in cholesterol content varied from 39 to 368 mg/day in the 10 responsive patients.

Daily fecal steroid excretions

Endogenous neutral steroids. Table IV presents excretions of endogenous neutral steroids during periods I, II, III, and II + III. In 7 of 11 patients no significant change was found in excretion of this fraction when unsaturated fat was substituted for saturated fat. A highly significant *increase* was observed in only one patient (No. 11); this increased excretion of neutral steroids persisted throughout the entire period of unsaturated fat feeding. Three other patients (Nos. 7, 9, and 10) showed significant *decreases* in excretions of endogenous neutral steroids during the feeding of unsaturated fat.

Values for excretion of endogenous neutral steroids presented in Table IV have been corrected for losses and for variations in fecal flow with dietary plant sterols (or β -sitosterol) as an internal standard. We have previously demonstrated that cholesterol and plant sterols are lost to the same extent in their transit through the intestine; thus, the values for recoveries of plant sterols

(Table V) indicate the extent to which cholesterol also is lost. In patients 7-11 excretions of β -sitosterol have been corrected with chromic oxide as an internal standard for variations in fecal flow. A *t* test comparison of the plant sterol recoveries in the two periods showed that in 2 of 11 patients recoveries of plant sterols were greater on unsaturated than on saturated fat ($P < 0.05$); a sign test for the group as a whole also revealed significantly larger losses on saturated fats ($P < 0.05$).

We have postulated that losses of sterols are due to bacterial degradation of the sterol ring structure (16); if this suggestion proves to be true, the reduction in losses observed during unsaturated fat feeding could be due to a change in intestinal flora or to a more rapid intestinal transit time. We also have shown that the size of the loss of neutral sterols is inversely related to the rate of daily fecal turnover; i.e., the longer the colonic contents remain unexcreted, the greater the chance for neutral sterol losses. The recent report of Connor, Witiak, Stone, and Armstrong (9) showed the same trend toward greater recoveries of plant sterols on unsaturated fats. These findings illustrate the necessity for correction of excretion data for neutral sterol losses; in these workers' study, as well as in ours, failure to

make these corrections leads to falsely high values for neutral steroid excretion during the unsaturated fat feeding period.

Acidic steroids (Table IV). In only 3 (Nos. 3, 7, and 11) of 11 patients was a significant increase found in excretion of acidic steroids during the time in which plasma cholesterol levels were declining on unsaturated fat (period II); in one of these (No. 11) the increase persisted throughout period III without any further decrement in plasma cholesterol level. However, in two other patients (Nos. 4 and 8) who showed no changes in excretion of acidic steroids in period II, increases were noted during period III. In the remaining six patients no statistically significant increases were noted during either periods II, III, or II + III; in no patient was there a significant decrease in acidic steroid excretion on unsaturated fat.

Total endogenous steroids. In only one case (No. 11) was the excretion of total fecal endogenous steroids (endogenous neutral plus acidic steroids) significantly greater in period II than in period I. In three patients (Nos. 4, 8, and 11) total fecal steroid excretions were greater in periods II, III, or II + III than in period I.

Table IV presents the means and their standard deviations for each feeding period and for each patient. The mean coefficient of variation and the standard deviation for the 39 sets of data in each steroid class was $12.2 \pm 6.7\%$ for neutral steroids, $31.3 \pm 17.5\%$ for acidic steroids, and $13.5 \pm 6.2\%$ for total steroids; we consider this to be due more to biological rather than to methodologic variability. Because of the large number of individual values involved, we have not presented the excretion data for every stool collection in this study. However, we have carefully inspected the data for possible time trends in excretion of bile acids after period I; we concluded that the fluctuations in excretion were not systematically time related.

Comparison of plasma cholesterol decrement with fecal steroid changes

The foregoing results indicate clearly that unsaturated fats do not regularly induce a marked increase in excretion of either neutral or acidic steroids. Nevertheless, our results do not answer this question: if the cholesterol that was removed from the plasma compartment during unsaturated fat feeding had been excreted into the feces as neutral or acidic steroids (without altering the balance of cholesterol in any other way), could the incremental change in fecal steroids have been detected by our methods? Since daily variations in steroid excretion rates are sizable and the daily decline in plasma cholesterol on unsaturated fat is usually small compared to the daily excretion of total fecal steroids, the detection of an increase in fecal steroid excretion that matches the

decrement of plasma cholesterol might seem beyond the sensitivity of the balance method. Therefore, we have calculated how small an increase in excretion of fecal steroids we could have detected reliably (*a*) in the period when plasma cholesterol levels were falling (period II), or (*b*) in the entire period of unsaturated fat feeding (periods II + III).

First, the probability of detecting the decrement in plasma cholesterol during period II was calculated; the results are shown in Table VI. The daily decline in plasma cholesterol during the transition period is presented for each patient in the first column. Under the three steroid classes (neutral, acidic, and total) are given the minimal increases in steroid excretion that could have been detected in period II with 95% confidence at a probability of 90%. In other words the α -error was set at 0.05 and the β -error at 0.10, where the α -error is the probability of finding a significant difference between periods I and II when one does not exist, and the β -error is the probability of not detecting a difference between the two periods when they are truly different. A computer program (written by Dr. Bruce A. Barron of this University) compared successively increasing differences between means until a difference at the chosen levels of significance was reached. In carrying out this theoretical comparison between two means we have taken the conservative position of using the standard deviation of either period I or period II for both means, whichever was greater (see Table IV). (These calculations were made with an Olivetti-Underwood Programma 101; the program is available from the authors on personal request. The general concepts underlying this treatment are presented in standard statistical texts [26].) In Table VI, we present these threshold values: differences this great or greater would be required in order to attain statistical significance under the particular circumstances of each individual experiment.

Table VI also presents the actual differences between period II and period I. When in any one case the minimal detectable increase was less than the decrement in plasma cholesterol, we concluded that we could have detected this decrement with statistical validity, provided that it had occurred during period II. When in any one case the observed change was even less than the minimal detectable increase, we concluded that a false negative result was ruled out.

In the case of the neutral steroids we found that in 7 of 11 patients our methods were sufficiently precise to have detected an increase in these fecal steroids equal to the decrease in plasma cholesterol (yet, in none of these patients was the fecal increment as large as the plasma decrement). In two other patients (Nos. 9 and 10) we could not have found an increment in fecal

TABLE IV
Fecal Steroid Excretion during

Patient	Fecal endogenous neutral steroids				Fecal acidic steroids	
	Period I	Period II	Period III	Period II + III	Period I	Period II
	<i>mg/day</i> ±SD				<i>mg/day</i> ±SD	
1	453* ±83 (22:5) ‡ (A) §	423 ±30 (10:9) ‡ (B) §	401 ±77 (19:7) (B) §	418 ±71 (29:7) ‡ (B) §	121 ±20	185 ±98
2	416 ±108 (34:5) (A)	526 ±78 (32:5) (B)	497 ±54 (34:4) (B)	513 ±66 (66:9) (B)	253 ±56	260 ±53
3	751 ±34 (16:4) (A)	768 ±61 (28:7) (B)	—	—	81 ±20	186 ±101
4	322 ±12 (13:3) (A)	326 ±20 (17:4) (B)	335 ±4 (6:2) (B)	329 ±16 (23:6) (B)	107 ±19	170 ±85
5	475 ±59 (16:4) (A)	532 ±14 (8:2) (B)	538 ±55 (24:6) (B)	536 ±47 (32:8) (B)	187 ±102	164 ±129
6	467 ±14 (13:3) (A)	498 ±35 (12:3) (B)	330 (4:1) (B)	478 ±49 (16:4) (B)	133 ±94	155 ±44
7	495 ±64 (64:8) (C)	362 ±32 (16:4) (D)	281 ±88 (48:4) (D)	301 ±85 (64:8) (D)	180 ±53	292 ±42
8	503 ±79 (48:6) (E)	482 ±60 (32:4) (F)	496 ±54 (40:5)	490 ±56 (72:9) (F)	318 ±94	447 ±116
9	494 ±47 (30:6) (G)	318 ±23 (49:8) (H)	—	—	162 ±26	177 ±50
10	1138 ±144 (20:5) (G)	914 ±119 (24:6) (H)	958 ±138 (8:2) (H)	925 ±115 (32:8) (H)	899 ±86	820 ±213
11	705 ±192 (60:15) (I)	922 ±143 (48:12) (J)	930 ±116 (16:4) (J)	925 ±134 (64:16) (J)	269 ±56	367 ±71

* Patient No. 1 received dietary plant sterols only during the last 8 days of period I. In the entire study he had a total recovery of plant sterols of 87%; thus, on the assumption that losses were the same throughout the study, each value for neutral sterols obtained in period I was corrected upwards by 13%.

‡ Duration of balance study (days) for the period given and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this column gives the average stool collection period in days.

§ Diets administered in periods I, II, III, and II + III (see Table II).

|| Period II, III, or II + III significantly different from period I ($P < 0.05$) (Student's t test).

neutral steroids of the required magnitude, had it occurred; but in reality, significant *decreases* were observed. This finding eliminated the possibility that in these two patients the decrement of plasma cholesterol was matched by an increment in fecal steroids in period II. In the remaining two patients (Nos. 2 and 11) we would have failed to detect a change of the magnitude of the plasma cholesterol change. In one of these (No. 11) a significant increase in neutral steroid actually did occur; indeed, it was considerably greater than the plasma decrement.

Table VI also shows that we could have expected to detect a change in acidic steroids as great as the plasma

decrement in 6 of 11 patients. In actuality, two of these six patients (Nos. 7 and 11) showed increases in acidic sterols during period II that were greater than the plasma decrement, while a third patient (No. 3) had an increase that was statistically significant but considerably smaller than the plasma change. In three patients (Nos. 1, 2, and 6) acidic steroid excretion during period II clearly could not account for the change in plasma cholesterol. However, in four patients (Nos. 4, 5, 8, and 10) the results could have been false negatives.

For total fecal steroids the chance of detecting (with a β -error of 0.10) an increment equal to the fall of plasma cholesterol was present at the $P < 0.05$ level in

Exchange of Dietary Fats

Fecal acidic steroids		Total fecal endogenous steroids			
Period III	Period II + III	Period I	Period II	Period III	Period II + III
<i>mg/day</i> ±SD		<i>mg/day</i> ±SD			
100 ±36	98 ±30	574 ±86	608 ±91	501 ±86	516 ±76
303 ±65	279 ±59	670 ±96	795 ±59	800 ±68	792 ±59
—	—	832 ±50	894 ±139	—	—
224 ±26	205 ±97	429 ±19	496 ±95	559 ±29	524 ±105
134 ±68	139 ±98	662 ±102	696 ±143	672 ±134	675 ±126
70	104 ±43	600 ±105	614 ±78	500	582 ±90
226 ±30	243 ±44	683 ±53	653 ±12	507 ±109	544 ±144
580 ±95	521 ±121	821 ±68	929 ±83	1076 ±85	1012 ±112
—	—	656 ±55	495 ±72	—	—
1104 ±478	891 ±287	2037 ±220	1734 ±202	2062 ±617	1816 ±326
493 ±64	399 ±88	974 ±197	1290 ±177	1423 ±61	1321 ±172

only four patients. In two of these patients (Nos. 3 and 7) there were significant increases in acidic steroid excretion.

To recapitulate, we can conclude with reasonable certainty that the decrement in plasma cholesterol was not balanced by an increment in fecal neutral steroids in period II. On the other hand, a significant increase in acidic steroid actually did occur in three patients, but in three others we were able to eliminate the possibility that cholesterol removed from the plasma was not converted entirely into bile acids and excreted in the same period. Finally, in most patients we would not have been able to detect the fall in plasma cholesterol

as an increment in fecal steroids if that increment had been distributed between the neutral and acidic steroid fractions.

By the same statistical analysis the data from the entire period of unsaturated fat feeding (periods II + III) were compared with those in period I. Table IV showed that increases in fecal steroid excretion sufficient to account for decreases in plasma cholesterol occurred in 5 of 11 patients. In only two of the other six patients (Nos. 1 and 6) could we rule out with reasonable certainty (α -error 0.05 and β -error 0.10) that the plasma cholesterol decrement had not been excreted in the feces as neutral or acidic steroids. It is clear from these

TABLE V
Recoveries of Dietary Plant Sterols
 For each patient the saturated fat diet is listed first.

Patient	Diet	Days: No. of determinations	Plant sterol intake	Plant sterol excretion	Recovery	Difference
			mg/day	mg/day \pm SD	%	
1*	A	8:2	317	226 \pm 33	71.3	NS
	B	29:7	317	291 \pm 59	91.8	
2*	A	34:5	227	189 \pm 59	83.2	NS
	B	66:9	227	248 \pm 85	109.2	
3*	A	16:4	408 \ddagger	318 \pm 96	77.9	NS
	B	28:7	398	248 \pm 139	62.3	
4*	A	13:3	250 \ddagger	173 \pm 53	69.2	NS
	B	23:6	271	253 \pm 127	93.3	
5*	A	16:4	290 \ddagger	193 \pm 60	66.5	NS
	B	32:8	309	248 \pm 100	80.2	
6*	A	13:3	240 \ddagger	172 \pm 66	70.7	NS
	B	16:4	256	223 \pm 41	87.1	
7§	C	64:8	209 \ddagger	128 \pm 29	61.2	NS
	D	64:8	230	158 \pm 56	68.7	
8§	E	48:6	153 \ddagger	72 \pm 17	47.0	$P < 0.01$
	F	72:9	200	141 \pm 25	70.5	
9§	G	30:6	132	122 \pm 9	92.4	NS
	H	49:8	130	149 \pm 44	114.6	
10§	G	20:5	153 \ddagger	126 \pm 37	82.3	NS
	H	32:8	176	166 \pm 17	94.3	
11§	I	60:15	230 \ddagger	148 \pm 49	64.3	$P < 0.05$
	J	64:16	300	309 \pm 211	103.0	

*Patients 1-6: Intakes and excretions represent total plant sterols including campesterol, stigmasterol, and β -sitosterol. Excretions of plant sterols were not corrected for variations in fecal flow, since chromic oxide was not given to these patients.

\ddagger When intakes of plant sterols were different in the saturated and unsaturated fat periods, the excretion values were normalized before statistical treatment.

§ Patients 7-11: intakes and excretions represent β -sitosterol only; in these patients excretions were corrected for variations in fecal flow with chromic oxide as an internal standard.

analyses that the power of the sterol balance method to detect significant increases in fecal steroids becomes less and less as the period of time in which the incremental change is postulated to occur is lengthened.

Cholesterol absorption

It was technically feasible to measure changes in cholesterol absorption in those patients (Nos. 7-11) ingesting moderate amounts of cholesterol. Table VII shows no significant differences in three of five patients, while in two the absorption of exogenous cholesterol was *increased* significantly on the unsaturated fat regimen. One patient (No. 9) with increased absorption of ex-

ogenous cholesterol was shown to have a decreased excretion of endogenous neutral steroids on unsaturated fat (Table IV); presumably the absorption of endogenous cholesterol was also increased.

Patient 11 was exceptional in this series in showing a large increase in excretion of endogenous neutral steroids on the unsaturated fat regimen (Table IV). It is of interest, then, that in contrast to patients 8 and 9 she absorbed less exogenous cholesterol when ingesting unsaturated fat; although the change from 42 to 29% absorption was not statistically significant, it is noteworthy that this was the only patient out of seven who demonstrated decreased absorption of exogenous cholesterol of this magnitude.

TABLE VI
A Test for False Negatives
 Probability of detecting a fecal steroid excretion increment in period II equal to the plasma cholesterol decrement.

Patient	Decrement in plasma cholesterol in period II <i>mg/day</i>	Fecal endogenous neutral steroids		Fecal acidic steroids		Fecal total steroids	
		Minimal detectable increase*	Actual change (period II - I)	Minimal detectable increase*	Actual change (period II - I)	Minimal detectable increase*	Actual change (period II - I)
1	-368	+83‡	-30	+88‡	+64	+82‡	+34
2	-82	+155	+110	+58‡	+7	+138	+125
3	-147	+58‡	+17	+97‡	+106§	+134‡	+62
4	-80	+28‡	+4	+110	+63	+133	+67
5	-178	+83‡	+57	+250	-23	+333	+34
6	-211	+74‡	+31	+150‡	+22	+170‡	+14
7	-69	+60‡	-133**	+47	+112§	+49‡	-30
8	-96	+78‡	+31	+144	+129	+116	+108
9	-39	+40	-176**	+38‡	+15	+116	-161
10	-15	+143	-224**	+167	-79	+218	-303
11	-57	+141	+217§	+43‡	+98§	+145	+316§

* This value is the minimal increase in steroid excretion during period II as compared to period I which could be detected with an α -error of 0.05 and a β -error of 0.10.

‡ Symbol indicates that minimal detectable increase was less than the daily decrement in plasma cholesterol. In these cases an increase in fecal steroid excretion could have been detected if it was as great as the plasma change, and if it had occurred during period II.

§ These values represent average daily *increases* in fecal steroids during period II that were significantly different from the means of period I at $P < 0.05$ (Table IV).

** These values represent average daily *decreases* in fecal steroids during period II that were significantly different from the means of period I at $P < 0.05$ (Table IV).

Total cholesterol balance

Table VIII shows the balance of cholesterol during the two regimens: period I (saturated fats) and period II + III (unsaturated fats), where cholesterol balance equals the difference between daily intake of cholesterol and total daily excretion of steroids (total neutral + acidic steroids). Balances were negative at all times in all patients, due to continuing cholesterol synthesis; in six patients the balance was more negative during the unsaturated fat regimen, in five patients less negative. The mean difference between values for cholesterol balance (period II + III minus period I) on the two diets was 7 mg/day, with differences ranging from +293 to -403 mg/day. Thus, there was no consistent change in daily cholesterol balance when dietary fats were exchanged.

Specific activity-time curves of plasma cholesterol

Fig. 4 presents specific activity-time curves of plasma cholesterol in four patients following pulse labeling by intravenous administration of radiocholesterol. In three of these patients no change was observed in the slope of the curve which could be related to an exchange of

dietary fats. However, in patient 11 the transfer to unsaturated fat caused an increased slope of the curve; this patient also demonstrated an increased excretion of endogenous neutral and acidic steroids on unsaturated fat feeding and a decreased absorption of exogenous cholesterol. In this exceptional case the increased turnover of cholesterol suggested by the increased slope of the decay curve was consistent with an increase in cholesterol synthesis (as suggested also by Table VIII, especially since there was a decrease in absorption of exogenous cholesterol [Table VII]).

DISCUSSION

The purpose of this discussion is to examine the evidence we have obtained in 11 patients in an effort to determine which of the mechanisms proposed in Fig. 1 is the most likely to explain the cholesterol-lowering action of unsaturated fats.

Locus A. Change in absorption of cholesterol. If unsaturated fats cause a sustained inhibition in the absorption of cholesterol by the intestinal tract, a lowering of plasma cholesterol should result. Such a decrease in absorption of cholesterol would almost certainly be accom-

TABLE VII
Cholesterol Absorption Data on Saturated and Unsaturated Fat Feeding

Patient	Method	Period I				Period II + III			
		Days: No. of determinations	Cholesterol intake	Cholesterol absorption		Days: No. of determinations	Cholesterol intake	Cholesterol absorption	
				mg/day	%			mg/day	%
7	II	64:8	457	240 ±69	53	64:8	530	216 ±55 (NS)*	42
8	II	48:6	614	183 ±116	30	72:9	727	324 ±51 (P < 0.02)	45
9	I	30:6	577	231 ±59	40	49:8	568	363 ±43 (P < 0.001)	64
10	I	42:11	679	204 ±151	30	32:8	694	167 ±95 (NS)	24
11	I	60:15	586	248 ±88	42	64:18	671	192 ±83 (NS)	29

* Significance of differences: periods II + III vs. period I by Student's *t* test (NS, nonsignificant).

panied by an increased excretion of fecal endogenous neutral steroids as well as of exogenous neutral steroids if the diet contained cholesterol. However, as in several previous studies (12, 27, 28) in which no change in neutral steroid excretion was found during unsaturated fat feeding, in the present experiments no increase in fecal neutral steroids of endogenous origin was seen in 10 of 11 patients. Furthermore, in five patients in whom reliable measurements of absorption of exogenous cholesterol could be made, the substitution of unsaturated for saturated fat produced no significant decrease in the absorption of dietary cholesterol. These results seem to eliminate the possibility that unsaturated fats cause a lowering of plasma cholesterol concentrations by inhibit-

ing absorption of exogenous or endogenous cholesterol. In fact, two of the patients demonstrated an *increased* absorption of cholesterol when fed unsaturated fats.

The present findings contrast to other results observed in this laboratory in which absorption of cholesterol was clearly reduced. For example, we have recently confirmed by the sterol balance technique that absorption of exogenous and endogenous cholesterol is markedly inhibited by feeding large amounts of plant sterols (20). As a result of this continuous drain upon body sources of cholesterol, plasma cholesterol concentrations fall despite a marked compensatory increase in cholesterol synthesis. In five patients who ingested 5–10 g of plant sterols each day for long periods (48–120 days), the

TABLE VIII
Cholesterol Balance Data on Saturated and Unsaturated Fat Feeding

Patient	Period I			Period II + III			Difference Periods (II + III) - I
	Cholesterol intake	Total steroid excretion	Cholesterol balance	Cholesterol intake	Total steroid excretion	Cholesterol balance	
	1	60	574	-514	60	516	-456
2	46	670	-624	46	792	-746	-122
3	83	832	-749	83	894	-811	-62
4	55	429	-374	55	524	-469	-95
5	61	662	-601	61	675	-614	-13
6	51	600	-549	51	582	-531	+18
7	457	900	-443	530	858	-328	+115
8	614	1252	-638	727	1415	-688	-50
9	577	1002	-425	568	700	-132	+293
10	679	2037	-1833	694	2343	-1649	+184
11	586	1312	-726	671	1800	-1129	-403
	Mean		-679			-686	+7

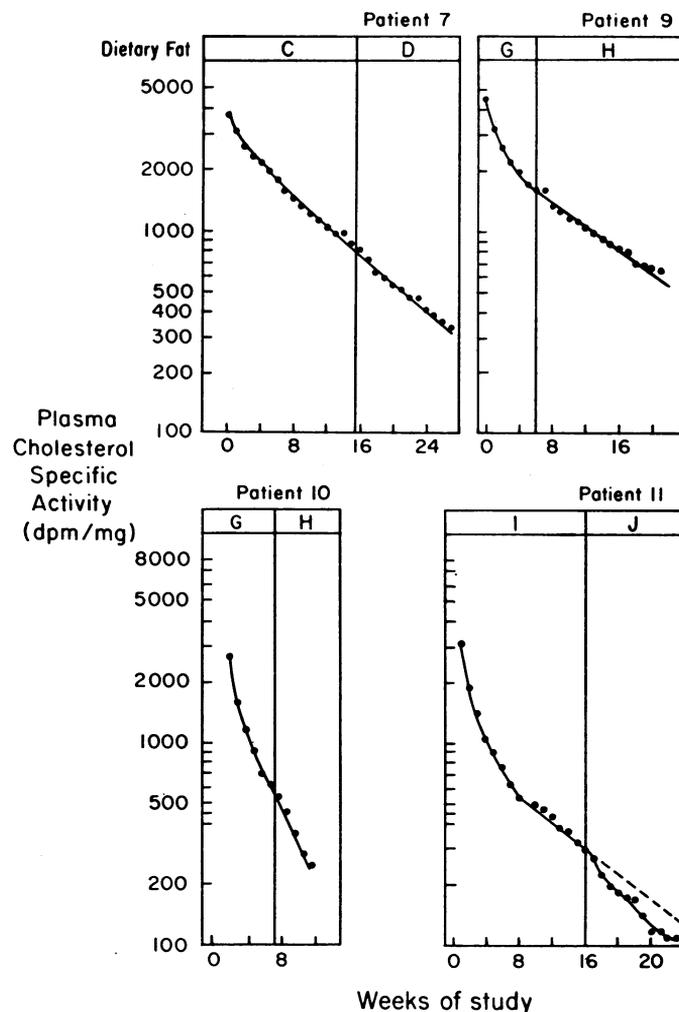


FIGURE 4 Specific activity-time curves of plasma cholesterol after pulse labeling during feeding of saturated and unsaturated fats (patients 7, 9-11). In patient 7, substitution of unsaturated fat for saturated fat produced no change in the slope of the decay curve. In patients 9 and 10, the change in regimen was made before 8 wk, and at this time the curves may not all have reached the phase of linear decline. However, no definite changes could be attributed to the unsaturated fat. Finally, in patient 11, introduction of unsaturated fat produced a distinct increase in the rate of decay in specific activity of plasma cholesterol. Cholesterol-1,2- ^3H was administered to patients 7 and 9, and Cholesterol-4- C^{14} was given to patients 10 and 11.

hypocholesterolemic effect persisted as long as the plant sterols were fed.

In addition, we have been able to detect a decrease in absorption of cholesterol in a variety of other conditions:³ (a) biliary obstruction, (b) ileal exclusion, (c) cholestyramine therapy, (d) neomycin therapy, and (e) abetalipoproteinemia. While the mechanisms by

³ Unpublished data.

which cholesterol absorption is reduced in these conditions differ, in all cases the reduction in absorption was readily revealed through use of the same methods employed in the present study. Therefore, we believe that our present findings justify the conclusion that the reduction of plasma cholesterol levels during the feeding of unsaturated fats is not due to an impairment of cholesterol absorption, as claimed by Wood, Shioda, and Kinsell (7).

Locus B. Change in absorption of bile acids. Another way in which unsaturated fats might act within the intestinal tract is through inhibition of reabsorption of bile acids. As examples of this mechanism, cholestyramine therapy and surgical exclusion of the terminal ileum both cause a reduction in bile acid absorption; this in turn appears to release feedback inhibition of the formation of new bile acids from cholesterol in the liver, and this enhanced removal of cholesterol is accompanied, in most cases, by a reduction in plasma cholesterol. Nevertheless, in order to compensate for the increased conversion of cholesterol into bile acids the synthesis of cholesterol increases strikingly, and a new metabolic steady state is rapidly established.⁸

When our patients were fed diets rich in unsaturated fats, we observed increases in fecal bile acids at some portion of the feeding period in 5 of 11 patients, but these changes were very much smaller than those we have observed with cholestyramine or ileal exclusion; yet, the extent of cholesterol lowering induced by unsaturated fats was as large, and in many cases larger, than those we have produced with cholestyramine or ileal exclusion. We suggest, therefore, that if small changes in bile acid excretion caused by exchange of dietary fats are due to a decreased reabsorption of bile acids, the changes probably have only a minor effect on plasma cholesterol levels. Nevertheless, in our view, alterations in the pattern of fecal acidic steroid excretion seen in some patients on unsaturated fats are entirely consistent with the possibility of small changes in reabsorption of bile acids. It has been estimated that 10–20 g of bile acids are reabsorbed daily by the intestinal tract; a change in reabsorption of only a few percentage points could easily account for the increases in excretion of acidic steroids we have sometimes seen during unsaturated fat feeding periods. This explanation is strengthened by the findings in Table V that suggest some decrease in intestinal transit time when unsaturated fats are fed.

Locus C. Increased cholesterol secretion into intestinal lumen. We recently reported (29) that the hypocholesterolemic action of chlorophenoxyisobutyrate (Clofibrate) may be explained in part by enhanced secretion of cholesterol into the intestinal tract, either through the biliary tract or intestinal wall. This evidence is based on the observation that this agent caused a marked increase in excretion of endogenous neutral steroids without affecting the reabsorption of cholesterol. Thus, a flux of cholesterol into the intestinal tract must have occurred; indeed, in most cases the accumulated increment in excretion of endogenous neutral steroids far exceeded the decrement in plasma cholesterol. Our inability to detect comparable changes in excretion of endogenous neutral

steroids when unsaturated fats are fed seems to eliminate the possibility that these two agents exert their effects through a common mechanism.

Locus D. Decreased cholesterol synthesis. Plasma cholesterol levels might be reduced if synthesis of cholesterol were decreased; yet, our balance data generally failed to indicate a decrease in cholesterol synthesis during feeding of unsaturated fats. If unsaturated fats had induced a sustained depression in synthesis, the difference between excretion of total steroids and the intake of cholesterol should have decreased, that is, the net negative balance should have been reduced. In fact, no statistically significant difference was found when mean balance data were compared during the feeding of the two types of fat. Furthermore, specific activity-time curves of plasma cholesterol after pulse labeling in four patients failed to show the decrease in rate of decay that would be expected if synthesis had been inhibited when unsaturated fat was introduced. Indeed, in one patient (No. 11) who showed the largest increments in fecal steroids on unsaturated fats, the decay curve steepened suggesting that an *increase* in cholesterol synthesis had occurred in this patient.

Locus E. Primary increase in oxidation of cholesterol into bile acids. Several investigators (1–3, 7, 9) have claimed that unsaturated fat promotes the excretion of acidic steroids. We can visualize three possible mechanisms which might give rise to such a change: (a) a decrease in reabsorption of bile acids (locus B), as with cholestyramine or ileal exclusion; (b) a primary increase in the oxidation of cholesterol into bile acids (locus E); or (c) redistribution of cholesterol from the plasma to liver compartments (locus F) with the subsequent removal of excess cholesterol through conversion into bile acids. Theoretically, it should be possible to distinguish the second mechanism (locus E) from the other two by combined use of the sterol balance method (14) and the isotopic method for estimating the pool size of bile acids (30, 31). A decrease in reabsorption of bile acids should produce a persistent increase in excretion of acidic steroids leading to a *smaller* pool of bile acids, while a primary increase in oxidation of cholesterol into bile acids should also cause a persistent increase in acidic steroid excretion, but with a *larger* pool size. Finally, if excess cholesterol redistributed from the plasma into the liver was removed by conversion into bile acids, the pool size would be expanded but changes in acidic steroid excretion would be only transitory (see next section).

Unfortunately, few data are available regarding pool sizes of bile acids on unsaturated and saturated fats, but in two such studies Lindstedt, Avigan, Goodman, Sjövall, and Steinberg (32) and Hellström and Lindstedt (33) found no consistent changes in the size of the

⁸ Unpublished experiments.

cholic acid pool when these two types of fats were exchanged. In other studies in which a persistent increase in acidic steroid excretion was reported, the authors did not determine whether the effect was due to a decrease in reabsorption or a primary increase in synthesis of bile acids, but the magnitude of the increases reported in fecal acidic steroids was relatively small compared to that induced by cholestyramine or ileal exclusion

In the present study no consistent increase in excretion of acidic steroids was found; in fact, 8 of 11 patients showed no change in output of acidic steroids throughout the period when concentrations of plasma cholesterol were falling. Nevertheless, in a significant number of cases (5 of 11), bile acid excretion was found to be increased at some portion of the unsaturated fat period. Therefore, if we seek a single explanation for the cholesterol-lowering effect of unsaturated fat, we interpret this inconsistency in excretion of bile acids to be (a) unrelated to the lowering effect, or (b) secondary to an altered partition of cholesterol between plasma and tissue compartments, as described below. In other words, changes in bile acid excretion *may* occur in association with decreasing plasma cholesterol on unsaturated fat diets, but such changes *need not* occur.

Loci F and G. Redistribution of cholesterol from plasma to tissues. The results of the present study offer little support for the possibilities that the cholesterol-lowering effect of unsaturated fats is due to a decrease in synthesis of cholesterol, to an increase in oxidation of cholesterol, or to an alteration in the enterohepatic circulation of either cholesterol or bile acids. Hence, only one mechanism remains to be considered, and it seems plausible: namely, that unsaturated fats cause plasma cholesterol to be transferred into tissue pools. In the present study the size of the decrement of total plasma cholesterol ranged from 0.351 to 4.114 g; we propose that these amounts could have been transferred into the liver (locus F) or into other tissues (locus G). In the case of locus F, four different secondary effects can be imagined.

(a) If cholesterol from the plasma is transferred primarily into the liver, it might be cleared rapidly from the liver into the intestinal tract and be excreted as fecal neutral steroids. However, in the present study, in only 1 of 11 cases did we actually find an increased excretion of neutral steroids, and in 9 we ruled out the possibility of a false negative result with reasonable confidence. While these findings lend no support to the concept that the decrement in plasma cholesterol is cleared rapidly from the body as neutral steroids, our data do not rule out the possibility that, in some patients at least, the excess cholesterol is rapidly converted into bile acids. As mentioned above, five of the patients showed increases in excretion of acidic steroids on unsaturated fats

which could have been derived from cholesterol transferred to the liver from plasma, but in six remaining patients no change in acidic steroid excretion was found; in three of these we ruled out the possibility that cholesterol could have been excreted as acidic steroids simultaneously with its decline in the plasma.

Our methods do not differentiate among three other mechanisms which might operate secondarily in the case of locus F: (b) cholesterol transferred into the liver could be excreted slowly over a period of many weeks either as neutral or acidic steroids and in amounts too small to be detected by present methods; (c) cholesterol transferred into the liver could cause a temporary depression of cholesterol synthesis in the liver with resultant decline in liver cholesterol concentrations to previous levels; or (d) concentrations of cholesterol in the liver could remain permanently at higher levels.

Information on the effects of unsaturated fats on cholesterol metabolism in various human tissues is meager, but several studies on this point have been carried out in animals. For example, with regard to the third possibility, (c) above, Wilson and Siperstein (34) found no evidence for decreased hepatic synthesis of cholesterol during unsaturated fat feeding in the rat. On the other hand, several workers (35-37) have found that ingestion of unsaturated fats leads to an increased concentration of cholesterol in the liver, while at the same time plasma levels are reduced. Avigan and Steinberg (35) have suggested that in the rat the increase in liver cholesterol results from enhanced synthesis, while Bloomfield (37) has obtained evidence that rats absorb more cholesterol on unsaturated fat diets; in view of this inverse relationship of the concentrations of cholesterol in liver and plasma compartments, these workers have proposed that unsaturated fats produce a shift in the partition of cholesterol between plasma and liver. However, in the only clinical study reported to date, Frantz and Carey (38) were not able to confirm the animal findings. They carried out serial punch biopsies on the livers of patients fed a saturated and then an unsaturated fat diet; they found a *decrease* in liver cholesterol concentration and concluded that plasma cholesterol is probably not transferred into the liver. In view of the differences between the findings in animals and those in man, it is clear that more definitive studies of this kind are required before the question can be answered with certainty.

If unsaturated fats caused plasma cholesterol to be distributed into tissues *other than* the liver and the intestinal tract, the decrement need not be cleared from the body (locus G): if synthesis of cholesterol within these tissues is not reduced temporarily, tissue concentrations would remain at slightly higher levels. This has not been tested in man: if the tissue pools thus involved

are the bulk tissues (muscle, adipose tissue, or connective tissue), the increase in cholesterol concentration would be too small to be detectable by present procedures. This point has been made by Bieberdorf and Wilson (13): they found no change in cholesterol synthesis or excretion during unsaturated fat feeding in rabbits studied by the isotopic steady-state methods and concluded that a redistribution of cholesterol must have occurred from plasma to the bulk tissues. Although they did not examine the amount of cholesterol in the livers in these animals, they showed that the bulk tissues contained up to 40% of the readily miscible pool of cholesterol, and concluded that the cholesterol lost from the plasma compartment could not have been detected in this large pool.

Although we lack *direct* evidence for or against loci F and G, we consider that loci A-E have been validly excluded, and thus we must seriously entertain the possibility that plasma cholesterol is transferred to tissue pools when the diet is rich in unsaturated fats. This conclusion has been supported by two lines of evidence developed by our colleagues Spritz and Mishkel. Spritz (39) has shown in rabbits that the fatty acid composition of the lipoproteins influences the rate of transfer of free cholesterol into tissues in a manner consistent with the concept that dietary unsaturated fats alter the equilibrium between tissue and lipoprotein pools of cholesterol. Later, Spritz and Mishkel (40) demonstrated in man that cholesterol-protein and phospholipid-to-protein ratios in low density lipoproteins fall during unsaturated fat feeding. They proposed that since unsaturated fatty acids have a greater space-occupying requirement than saturated acids, the apoprotein of the low density lipoprotein can accommodate fewer complex lipid molecules (phospholipids, cholesterol esters, and triglycerides) when these molecules are rich in unsaturated fatty acids. Thus, some of the cholesterol that had been previously carried in the plasma during saturated fat feeding would be excluded from the plasma when unsaturated fat is introduced in the diet.

The present study has been carried out on patients with various types of hyperlipoproteinemia; balance studies carried out elsewhere have employed normal people or patients with undefined types of hyperlipidemia. We recognize that the response to unsaturated fats may not be the same in everyone and, indeed, that the response could depend to some extent on the type of lipoprotein abnormality. Although the results of the present investigation reveal important differences in patterns of sterol excretion from one patient to another, we have attempted to develop a unitary hypothesis to explain the cholesterol lowering effects of unsaturated fats, even though we recognize that unsaturated fat may have more than one effect on lipid metabolism in man.

With regard to the relationship between cholesterol lowering and fecal steroid excretion, this investigation shows clearly that plasma levels of cholesterol *can* be reduced by diets rich in unsaturated fats without concomitant changes in excretion of either neutral or acidic steroids, and our unitary hypothesis attempts to explain how this may occur.

Since our study was restricted to patients with hyperlipoproteinemia, it is appropriate that we compare our findings with those of Connor, Witiak, Stone, and Armstrong (9) in six normal males. These workers used the formula-feeding technique and the same analytical procedures employed in the present study. Their experimental design included three successive periods in which dietary fats were cocoa butter, corn oil, and cocoa butter, respectively; results from all patients were pooled for statistical analysis. When excretions of neutral and acidic steroids were calculated without correction for cholesterol losses or variations in fecal flow, they found that excretions of both steroid fractions increased significantly when corn oil was substituted for cocoa butter and that both decreased significantly when cocoa butter was reinstated. However, as these workers indicated in their report the reported increments in excretions of neutral steroids disappeared when corrections were made for neutral sterol losses and for variations in fecal flow, using dietary β -sitosterol (plant sterols) as an internal standard.

Since chromic oxide had not been administered to the patients of this study, the authors did not correct excretions of acidic steroids for variations in fecal flow. Nevertheless, when the excretion of dietary plant sterols in these patients is examined in detail, an interesting pattern is noted that may be related to the excretion of acidic steroids as well as of neutral steroids: in five of six patients plant sterol recoveries were greater in the corn oil period than in the first cocoa butter period. Indeed, in four patients the recoveries in the second period were greater than 100%, suggesting that some of the dietary plant sterols had been retained in the intestine during the first period only to be excreted in the second period with the feeding of corn oil. If the excretion of acidic steroids followed a similar pattern, the higher values found in the corn oil period may have been due in part to retention from the first period and to a more rapid intestinal transit in the second and not to a specific effect of unsaturated fats on the formation of bile acids from cholesterol.

Summarizing, our studies illustrate how the method of sterol balance can be utilized in the study of any cholesterol-lowering regimen. This method is most useful in detecting the operation of mechanisms which exert their hypocholesterolemic effect by altering the enterohepatic circulation of cholesterol or of bile acids. The

method is less appropriate for studies of the possible redistribution of plasma cholesterol into tissue pools, for at present this can be recognized only by exclusion of other mechanisms; moreover, it has a very limited usefulness in determining the ultimate fate of redistributed cholesterol.

Since we have detected no consistent alteration in the enterohepatic circulation of cholesterol, we believe that the hypocholesterolemic effect is best explained by the mechanism of redistribution. Regardless of the fate of the cholesterol lost from the plasma, the sterol balance method demonstrates that the over-all economy of cholesterol is altered little by ingestion of unsaturated fats; we have obtained no evidence that they cause a depletion of body pools of cholesterol other than plasma. However, in an attempt to examine this possibility in more detail we have begun to study the cholesterol contents of various tissues. Our recent report on the sequestration of cholesterol in connective tissue as a function of age is a step in this direction (41).

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