Cholesterol Balance and Fecal Neutral Steroid and Bile Acid Excretion in Normal Men Fed Dietary Fats of Different Fatty Acid Composition

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ABSTRACT Six normal men were fed formula diets containing either highly saturated fat (cocoa butter, iodine value 32) or polyunsaturated fat (corn oil, iodine value 125). The sterol balance technique was used to compare the changes in serum cholesterol concentration with the excretion of fecal steroids. The method used for the analysis of fecal steroids was chemical, with a final identification and quantification by gas-liquid chromatography. It was confirmed that the chemical method for fecal steroid analysis was accurate and reproducible.

The three dietary periods were each 3 wk in length. In sequence, cocoa butter (period I), corn oil, and cocoa butter (period III) were fed at 40% of the total calories. All diets were cholesterol free, contained similar amounts of plant sterols, and were identical in other nutrients.

Corn oil had a hypocholesterolemic effect. Mean serum cholesterol concentrations were 222 mg/100 ml (cocoa butter, period I), 177 during corn oil, and 225 after the return to cocoa butter. Individual fecal steroids were determined from stools pooled for 7 days. Both neutral steroids and bile acids were altered significantly by dietary polyunsaturated fat. The change in bile acid excretion was considerably greater than the change in neutral steroids. Corn oil caused a greater fecal excretion of both deoxycholic and lithocholic acids. The total mean excretion (milligrams per day) of fecal steroids was 709 for cocoa butter (period I), 915 for corn oil, and 629 for the second cocoa butter period.

The enhanced total fecal steroid excretion by the polyunsaturated fat of corn oil created a negative cholesterol balance vis-à-vis the saturated fat of cocoa butter. The hypocholesterolemic effect of polyunsaturated fat was associated with total fecal sterol excretion twice greater than the amount of cholesterol calculated to leave the plasma. This finding suggested possible loss of cholesterol from the tissues as well.

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INTRODUCTION

Since 1952 the serum cholesterol lowering effect of polyunsaturated fat in the diet of man has been demonstrated repeatedly by many investigators. Yet the precise mechanism of this effect has remained obscure. The disposition of the considerable quantity of cholesterol leaving the plasma compartment has not been identified. Earlier work suggested that polyunsaturated fat in the diet promoted the loss of cholesterol and its metabolites in the stool (2–5). Many of the recent experiments, however, have failed to document enhanced fecal excretion of neutral sterols and bile acids (6–9).

In addition to fecal excretion of steroids, there are at least four other possible effects that polyunsaturated fat might have upon lipid metabolism to account for the serum lipid lowering action. These are: (*a*) decreased intestinal absorption of cholesterol and bile acids, (*b*) reduced cholesterol biosynthesis, (*c*) greater bile acid formation in the liver, and (*d*) increased movement of plasma cholesterol into the tissues of the body.

In view of widespread recommendations that polyunsaturated fat be incorporated in the diet of man for the treatment and prevention of hypercholesterolemia and

TABLE I

Description of the Formulas for the Different Dietary Periods (3000 Cal Diets) and the Experimental Subjects*

Part A: Formulas

Period	Fat				Fatty acid composition‡			Protein		Carbohydrate	Sterols	
	Source of fat	Amount of fat	Per cent of calories	Iodine value	Saturated	Monounsaturated	Polyunsaturated	Casein	Cystine		Cholesterol	Plant sterol
		g				%		g		g	mg	
I & III	Cocoa butter	134	40	32	61	34	3	76	0.3	380	0	398
II	Corn oil	134	40	127	12	31	56	76	0.3	380	0	345

* All subjects received 3000 cal or an amount necessary to maintain body weight for each dietary period. Carbohydrates: cornstarch, 75 g; Dextrimaltose No. 1 (Mead Johnson & Co., Evansville, Ind.) 100 g; sucrose, 207 g. Vitamins and minerals were added to meet the daily recommended allowance of the National Research Council and then maintained constant throughout all periods.

‡ Fatty acid analyses for cocoa butter were performed by the Woodson-Tenent Laboratories, Memphis, Tenn. The fatty acid composition of corn oil was derived from reference 14. The saturated fatty acids were palmitic 24% and stearic 36% for cocoa butter and 10% palmitic and 2% stearic for corn oil. Oleic and linoleic acids were the monounsaturated and polyunsaturated fatty acids respectively.

Part B: Experimental Subjects

Subject	Age	Weight	Height	Calories per day
		lb.		
1	44	134	5 ft 7 in.	2600
2	40	141	5 ft 8 in.	2800
3	38	150	5 ft 6 in.	2600
4	36	157	5 ft 9 in.	2800
5	37	176	6 ft	3100
6	41	187	5 ft 7 in.	2800

coronary heart disease, it is especially important to know whether the cholesterol leaving the plasma compartment actually leaves the body or moves into the tissues. The studies of Gerson, Shorland, and Adams in the rat indicated that corn oil feeding increased liver and aortic cholesterol (10). Bieberdorf and Wilson found in the rabbit that muscle cholesterol became greater after safflower oil feeding (11). No information is available about the cholesterol content of human tissues after polyunsaturated fat feeding.

In the studies to be reported here we tested by the cholesterol balance technique the hypothesis that changes in fecal steroid [1] excretion might account for any changes in the serum cholesterol concentration which followed the substitution of polyunsaturated fat for saturated fat in the human diet. Several features of this investigation should be emphasized. The subjects tested were healthy men with average American serum cholesterol levels. Fecal steroids were determined directly by the chemical methods developed by Ahrens and colleagues. These methods measure all fecal neutral sterols and steroids, fecal bile acids, and their bacterial degradation products by gas-liquid chromatography (12, 13). Finally, the fat of the diets selected contained only long-chain triglycerides, in contrast to a number of other studies which have compared a "saturated" fat containing some short-chain fatty acids (i.e., coconut oil or butterfat) with a "polyunsaturated" fat (i.e. corn oil) containing only long-chain fatty acids.

In our study all six men had greatly enhanced neutral steroid and bile acid excretion when the polyunsaturated fat diet was ingested. The total fecal steroid excretion increased more than enough to account for the changes in the plasma cholesterol decrements and also for the presumed decrements in the primary pool of cholesterol (the plasma-liver pool).

METHODS

Six healthy men were selected from prison volunteers and were hospitalized on a metabolic ward at University Hospitals for the entire study. They ranged in age from 36 to 44 yr and in body weight from 140 to 190 lb. None was obese

[1] The term fecal steroids includes both neutral sterols and steroids (cholesterol, coprostanol, and coprostanone), and bile acids.

TABLE II

*Serum Cholesterol, Phospholipid, and Triglyceride Concentrations During the Three Dietary Periods (mg/100 ml)**

	Subject	General diet	Period I Cocoa butter			Period II Corn oil			Period III Cocoa butter		
					Mean			Mean			Mean
Cholesterol											
	1	231	178	188	183	137	158	147	189	178	184
	2	272	221	230	226	205	187	196	248	235	242
	3	307	214	237	226	161	162	162	205	215	210
	4	240	179	148	189	142	137	140	194	208	201
	5	329	246	262	254	221	225	223	260	272	266
	6	280	257	253	255	194	194	194	245	248	247
Mean ±SE		277 ±16	216 ±13	228 ±12	222 ±13	177 ±14	177 ±13	177 ±14	224 ±13	226 ±13	225 ±13
Per cent change					−20%			−20%			+27%
P value					<0.01			<0.001			<0.001
Phospholipid											
	1	149	143	149	146	113	129	121	157	152	155
	2	209	188	169	179	181	165	173	193	184	189
	3	201	168	182	175	128	138	133	155	174	165
	4	188	149	157	152	127	117	122	154	159	157
	5	229	188	197	193	148	159	154	208	210	209
	6	214	200	218	209	152	168	160	197	203	200
Means ±SE		198 ±11	173 ±9	179 ±11	176 ±10	142 ±10	146 ±9	144 ±9	177 ±10	180 ±9	179 ±9
Per cent change					−22%			−18%			+24%
P value					<0.02			<0.01			<0.001
Triglyceride											
	1	92	119	108	114	99	85	92	99	101	100
	2	123	159	138	149	127	89	158	144	154	149
	3	163	184	179	182	133	143	138	154	147	153
	4	100	113	129	121	96	86	91	111	112	112
	5	240	247	250	249	182	161	172	210	252	231
	6	120	136	133	135	97	131	114	116	137	127
Mean ±SE		140 ±22	160 ±21	156 ±21	158 ±21	122 ±14	116 ±14	128 ±14	140 ±17	151 ±22	145 ±19
Per cent change					+13%			−19%			+13%
P value					<0.001			<0.05			<0.2

* Two determinations of the serum lipids were made 3 days apart during the final week of periods I, II, and III. These are individually listed and averaged. SE represents the standard error of the mean.

or had metabolic diseases. The subjects received a general diet of mixed, natural foods for 1 wk, and then for 9 subsequent wk they were given liquid formula-type diets which differed only in fatty acid composition. Each of these dietary periods was 3 wk in duration. The composition of the formulas is listed in Table I. In period I, the fat was cocoa butter (the characteristic fat of chocolate). This predominantly saturated fat has an iodine number of 32. In period II, the highly polyunsaturated corn oil was substituted for cocoa butter as the source of fat. This corn oil had been redistilled[2] in order to remove enough of its high content of plant sterols, so that the plant sterol content of the corn oil and cocoa butter were approximately equivalent. Then, in period III, cocoa butter was substituted for corn oil. Periods I and III were thus identical. All formulas were cholesterol free. The plant sterol intake was 300–411 mg/day, dependent upon caloric intake and the type of dietary fat. The diets were constant in protein and carbohydrate. The fat constituted 40% of the total calories. The fatty acid composition of cocoa butter and corn oil are vastly different in per cent of saturated fatty acid (61 vs. 12) and polyunsaturated fatty acid (3 vs. 56). Monounsaturates (oleic acid) were closely similar. The diets were adjusted in caloric content, so that the men neither lost or gained weight during the period of study.

The serum cholesterol (15), phospholipid (16), and triglyceride (17) levels were determined twice weekly by methods previously reported. All stool specimens were individually collected and immediately frozen. At the end of each week of study, the stool specimens for each subject were thawed and pooled in 7-day lots. An equal weight of water was added and the stool was homogenized in a paint can shaker (18). Aliquots were then taken for analysis. The analytical methods involved the separation of the fecal steroids into their neutral steroid and bile acid fractions after saponification and extraction. These separate fractions were purified by thin-layer chromatography, and the individual components subsequently were measured by gas-liquid partition chromatography (12, 13). After such purification the individual steroids and bile acids were identified on the basis of retention times previously verified by the use of purified standards of the compound under question. The exceptions were that no standards were available for the stanol and stanone derivatives of the plant sterols. All neutral steroids and bile acids were converted to their

[2] Obtained from Distillation Products Industries, Rochester, N. Y.

TABLE III

Fecal Neutral Sterol and Bile Acid Excretion

Subject Nos.....		1			2			3		
Dietary periods	Wk	Neutral sterol	Bile acid	Total	Neutral sterol	Bile acid	Total	Neutral sterol	Bile acid	Total
I. Cocoa butter	1	522	569	1091	1218	852	2070	852	574	1426
	2	288	255	543	527	551	1078	525	346	871
	3	440	322	762	297	382	679	358	372	730
Mean for last 2 wk		364	289	653	412	466	878	442	359	801
II. Corn oil	4	366	313	679	312	284	596	233	196	429
	5	533	351	884	514	483	997	572	745	1317
	6	328	273	601	470	413	883	422	553	975
Mean for last 2 wk		431	312	743	492	448	940	497	649	1146
III. Cocoa butter	7	496	277	773	443	583	1026	517	367	884
	8	338	230	568	405	314	719	464	326	790
	9	376	206	582	303	187	490	438	198	636
Mean for last 2 wk		357	218	575	354	250	605	451	262	713

All data are expressed in mg/24 hr.

* Standard error of the mean.

trimethylsilyl ether derivatives before gas-liquid partition chromatography. Their identifications were based upon the assumptions provided in the work of Miettinen, Ahrens, and Grundy (12).

The gas-liquid partition chromatography was performed on an instrument equipped with a hydrogen flame ionization detector (F and M Biomedical Gas Chromatograph, model 400, Hewlett-Packard Co., Avondale, Pa.). The column was a 4-ft glass U-tube, 4 mm i.d. packed with Diatoport S (800/100 mesh) coated with 3.8% film of SE-30. Temperatures of column, detector, and flash heater were 230, 250, and 300°C, respectively, for neutral steroids and 240, 260, and 300°C, respectively, for bile acids. Helium was used as carrier gas at flow of 100 ml/min; the inlet pressure was 40 ψ.

Aliquots of the corn oil and cocoa butter formulas were analyzed for sterol content and composition according to the same procedure utilized for neutral steroids (12).

Statistical analyses were performed by standard methods (19). The accuracy and variance of the fecal steroid method was confirmed as similar to the reports of the originators (12, 13). The recovery of known amounts of cholesterol and deoxycholic acids added to eight stool samples was 99.3 ±2.7% (SD) for cholesterol and 102.4 ±9.4% (SD) for bile acids. The analysis of six aliquots of the same stool sample indicated the following standard deviations in terms of percentage variation from the mean: cholesterol: ±1.9%; plant sterols: ±1.4%; and bile acids: ±3.6%. In the original reports of this method, for comparison, the comparable standard deviation for bile acids was ±3%.

RESULTS

Serum lipid changes. The individual serum lipid concentrations in the six men fed the different dietary fats are listed in Table II. These men had a mean serum cholesterol concentration of 277 mg/100 ml when they consumed a general American diet. With the change to the cocoa butter formula, the serum cholesterol declined to 222 mg/100 ml or a change of − 55 mg/100 ml. This decline probably occured because dietary cholesterol had been eliminated from the general diet in the cocoa butter formula as had been observed previously in a study of similar design (20). This decrease is also of interest because the diet had actually become more saturated in terms of dietary fat; the iodine number decreased from about 60 (that of the usual American diet) to a value of 32 for the cocoa butter formula. When corn oil was the source of fat, as in period II, the serum cholesterol concentration decreased further to 177 mg/100 ml or a change of − 45 mg/100 ml. Finally, in period III, which repeated the cocoa butter diet of period I, the serum cholesterol increased to approximately the same level as in period I, an upward change of from 48 to 225 mg/100 ml. Serum phospholipid levels decreased and increased significantly in a parallel manner to the serum cholesterol changes during the different dietary periods. The serum triglycerides were decreased by the corn oil formula (period I–II) and increased less consistently by the subsequent cocoa butter feeding. These changes in serum lipids occurred from 7 to 14 days after the dietary change had been initiated. Thus, the analyses used for computation during the 3rd wk represented a relatively constant state.

Fecal steroid excretion data. The fecal steroid excretion of each subject was expressed in milligrams per day for each of the 3 wk of each dietary period (Table III) and was summarized in Table IV for statistical comparisons for the last 2 wk of each dietary period.

During the Different Periods

4			5			6			Means of all subjects by each week		
Neutral sterol	Bile acid	Total	Neutral sterol	Bile acid	Total	Neutral sterol	Bile acid	Total	Neutral sterol	Bile acid	Total
850	335	1185	1126	454	1580	994	298	1292	927 ±101*	514 ±83	1441 ±144
790	371	1161	373	187	560	288	88	376	465 ±78	300 ±66	765 ±130
321	168	489	371	332	703	336	210	546	354 ±20	298 ±36	652 ±45
556	270	825	372	260	632	312	149	461			
307	183	490	474	390	864	320	228	548	335 ±33	266 ±31	601 ±63
744	577	1321	577	510	1087	338	287	625	546 ±53	492 ±67	1039 ±109
383	241	624	492	292	784	488	387	875	431 ±27	360 ±47	790 ±62
564	409	973	535	401	936	413	337	750			
726	285	1011	771	570	1341	346	239	585	550 ±68	387 ±62	955 ±101
606	192	798	524	404	928	298	208	506	439 ±47	279 ±34	718 ±64
395	205	600	365	396	761	108	57	165	331 ±48	208 ±44	539 ±83
501	198	699	445	400	845	203	133	336			

Fig. 1 illustrates the reciprocal relationship between serum cholesterol changes and fecal steroid changes. The term fecal steroids, as used in this paper, included only cholesterol and its metabolites. The plant sterols were measured but were not included in the computation of fecal steroids since they do not enter the body's sterol pool to any appreciable extent by virtue of their inabsorbability. The neutral steroids consisted of cholesterol and its bacterially altered derivatives, coprostanol and coprostanone. The bile acids were largely deoxycholic acid and lithocholic acid, the bacterially altered products of the primary bile acids of man, cholic and chenodeoxycholic acids.

During the cocoa butter formula in period I, the mean neutral steroid excretion for the six men was 410 mg/day, the bile acid excretion 299 mg, and the total excretion 709 mg (Table IV). For period II, during the corn oil formula, the neutral sterol excretion increased to 489 mg/day, and bile acid output increased to 426 mg, a total of 915 mg. In period III, when cocoa butter replaced corn oil, the fecal steroid output returned to a pattern similar to that of period I. The neutral sterol excretion decreased to a mean of 385, bile acid to 244, and the total to 629 mg/day. All of these changes were statistically significant at the 0.01–0.05 level, both by paired data tests and by analysis of variance.

The fecal steroid data as analyzed in Table IV compared the excretion pattern for the last 14 days of each 21 day dietary period. When the data were broken down into fecal steroid excretion for the 2nd and 3rd wk of the three dietary periods, and 2nd and 3rd wk excretions of one dietary period were compared with the corresponding 2nd and 3rd wk of the subsequent diet, the fecal steroids during the corn oil diet (period II) again were significantly higher than during the cocoa butter diets (periods I and III) both by *t* test and by analysis of variance. This significant difference applied to both fecal neutral steroids and bile acids. The fecal steroid excretion for the 1st wk of each dietary period was not

TABLE IV

Summary of the Fecal Neutral Sterol and Bile Acid Excretion from Table III

	I	II	III
Dietary periods.....	Cocoa butter	Corn oil	Cocoa butter
Neutral sterol			
Mean	410 ±34	489 ±24	385 ±43
Change		+79	−104
Probability		$P < 0.02$	$P < 0.01$
Bile acid			
Mean	299 ±44	426 ±49	244 ±36
Change		+127	−182
Probability		$P < 0.05$	$P < 0.02$
Total excretion			
Mean	709 ±64	915 ±62	629 ±70
Change		+206	−286
Probability		$P < 0.01$	$P < 0.01$

Mean values for the last 2 wk (2 and 3) of each dietary period for all six subjects. All values are expressed in mg/24 hr.

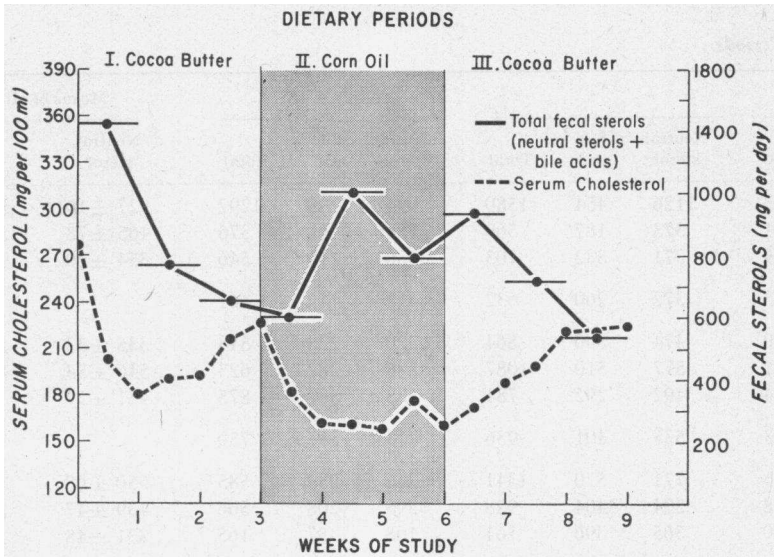

FIGURE 1 The changes in mean serum cholesterol levels and total fecal steroids for the six subjects during the three dietary periods. Each horizontal line represents the total fecal steroid daily output (neutral sterols and bile acids) taken from a 1 wk pool. The statistical comparisons are provided in Tables II–IV.

used in these calculations because it represented the metabolism and to some extent the diet of the preceding period (intestinal contents not yet excreted). For example, the neutral steroid excretion was 1089 mg/day for the 1st wk of period I and only 523 mg for the 2nd wk (Fig. 1). The 1st wk of the study reflected the preceding general diet which, to mention only one difference, contained considerable dietary cholesterol, much of which would not have been absorbed and thus would have contributed greatly to the fecal neutral steroids. The fecal steroid pattern during the 1st wk of the other dietary periods also tended to resemble that of the preceding diet; the new fecal excretion state had not yet been obtained. Similar considerations held for the calculations of the serum cholesterol changes. We did not use the 1st wk but the values of the 3rd and concluding wk of any dietary period.

The bile acid changes during these periods involved significant changes for both deoxycholic and lithocholic acid (Table V) from period II to III, and for deoxycho-

TABLE V

The Fecal Excretion of Individual Bile Acids During the Different Dietary Periods

	I. Cocoa butter				II. Corn oil				III. Cocoa butter			
Subject	Deoxycholic acid	Lithocholic acid	Others	Total	Deoxycholic acid	Lithocholic acid	Others	Total	Deoxycholic acid	Lithocholic acid	Others	Total
1	62	165	61	288	133	165	14	312	71	91	56	218
2	244	162	60	466	271	148	29	448	106	113	31	250
3	126	190	43	359	289	307	53	649	104	124	34	262
4	97	104	69	270	187	193	29	409	70	95	33	198
5	127	107	26	260	238	134	29	401	181	133	86	400
6	74	68	7	149	171	150	16	337	56	63	13	132
Mean & standard error	122 ±27	133 ±19	44 ±10	298 ±43	215 ±24*	183 ±26	28 ±6	426 ±44‡	98 ±18*	103 ±10‡	42 ±10	243 ±33‡

Values expressed in milligrams per day for the combined 2nd and 3rd wk of each dietary period.
Statistical comparisons:
* $P < 0.01$ vs. the preceding dietary period.
‡ $P < 0.05$ vs. the preceding dietary period.

TABLE VI

Changes in Fecal Neutral Sterol and Bile Acid Excretion and in Serum Cholesterol Level During the Feeding of Saturated and Polyunsaturated Fat

Subject	From cocoa butter (period I) to corn oil (period II)*				From corn oil (period II) to cocoa butter (period III)*			
		Fecal sterols				Fecal sterols		
	Serum cholesterol	Total	Neutral sterols	Bile acids	Serum cholesterol	Total	Neutral sterols	Bile acids
	mg/100 ml		*mg/day*		*mg/100 ml*		*mg/day*	
1	−34	+ 90	+ 67	+ 23	+35	−168	− 74	− 94
2	−30	+ 62	+ 80	− 18	+46	−336	−138	−198
3	−64	+345	+ 55	+290	+48	−433	− 46	−387
4	−49	+147	+ 8	+139	+61	−274	− 63	−211
5	−31	+304	+163	+141	+43	− 91	− 90	− 1
6	−59	+289	+101	+188	+53	−414	−210	−204
Mean	−45‡	+206§	+79‖	+127¶	+48‡	−286§	−104§	−182§

* The data were derived from the values for the combined 2nd and 3rd wk of each dietary period.
Statistical significances:
‡ $p < 0.001$.
§ $p < 0.01$.
‖ $p < 0.02$.
¶ $p < 0.05$.

lic acid from period I to II, with the increase in lithocholic acid in period II not attaining statistical significance ($P < 0.1$). The ratio of these two acids was not altered during the different dietary periods.

While the analysis of variance of the group results indicated significant differences for the fecal steroid excretions between the saturated and polyunsaturated diets, an occasional individual failed to follow the group trend for either neutral sterols or bile acids. From period I to II, subject No. 2 did not have enhanced bile acid excretion but did have the appropriate reduction in bile acid excretion with the change to a cocoa butter diet from period II to III (Table VI). Subject No. 5 had no bile acid change from period II to III but did from period I to II. Subject No. 4 had little neutral sterol change from period I to II but did from period II to III. For

TABLE VII

Fecal Excretion of Cholesterol and Its Bacterial Degradation Derivatives During Different Dietary Periods

Subject	I. Cocoa butter			II. Corn oil			III. Cocoa butter		
	Sterol	Stanol and stanone	Total	Sterol	Stanol and stanone	Total	Sterol	Stanol and stanone	Total
1	361	3	364	376	54	430	356	1	357
2	134	278	412	115	377	492	32	322	354
3	73	368	441	62	435	497	61	390	451
4	107	449	556	471	93	564	483	17	500
5	69	303	372	69	465	534	85	359	444
6	68	469	537	147	398	545	166	300	466
Mean & standard error	135 ±46	312 ±69	447 ±34	207 ±71	304 ±74	510 ±20*	197 ±74	232 ±71‡	429 ±24§

Values are expressed in milligrams per day for the combined 2nd and 3rd wk of each dietary period.
Statistical comparisons:
* $P < 0.05$ vs. the preceding dietary period.
‡ $P < 0.001$ vs. the preceding dietary period.
§ $P < 0.01$ vs. the preceding dietary period.

TABLE VIII

Fecal Neutral Sterol Excretion (mg/day) Corrected for Plant

Dietary period	Subject No.....	1		2		3	
	wks	Neutral sterol	Change	Neutral sterol	Change	Neutral sterol	Change
I. Cocoa butter	1	669	+147	1041	−177	1014	+162
	2	389	+101	462	−65	535	+10
	3	392	−48	396	+99	421	+63
Means of last 2 wk		390	+26	429	+17	478	+37
II. Corn oil	4	330	−36	339	+27	302	+69
	5	368	−165	381	−133	389	−183
	6	372	+44	373	−97	414	−8
Means of last 2 wk		370	−60	377	−115	402	−95
III. Cocoa butter	7	431	−65	503	+60	515	−2
	8	344	+6	465	+60	450	−14
	9	355	−21	404	+101	402	−36
Mean of last 2 wk		357	0	435	+81	426	−25

The change in milligrams per day represents the difference between the uncorrected values (Table III) and the corrected values.
* No statistical difference from the preceeding dietary period.

each subject the total fecal steroid change was appreciable and always in the right direction. If the bile acid did not change (i.e. for subject No. 2 as has been discussed), the neutral sterols did change. The same consideration applied to the other subjects. We would suggest that the fecal steroid excretion patterns of this study acquire added meaning because the subjects had the sequence of saturated fat, polyunsaturated fat, and saturated fat. The two saturated fat periods, differing of course in time, nonetheless produced statistically identical steroid and bile acid excretion values.

Bacterial transformation of fecal neutral sterols. The bacterially altered derivatives of cholesterol are coprostanol and coprostanone which result from reduction of the 5–6 double bond, isomerization, and, in the case of coprostanone, from the oxidation of the 3β-hydroxy to the 3-ketone. These substances as well as cholesterol were measured individually. The sum of cholesterol, coprostanone, and coprostanol provided the total neutral steroid excretion. The extent of the bacterial transformations is depicted in Table VII. In one subject (No. 1), virtually no bacterial transformation occurred, especially during the periods of cocoa butter feeding. Even in the corn oil period, very little coprostanol and coprostanone were found. Subject No. 4 also had fewer bacterial derivatives than the other subjects. The other four subjects converted most of their fecal neutral sterols to coprostanol and coprostanone consistently for all dietary periods. The conversion ranged up to 91% for the total fecal neutral steroids of cholesterol type.

Plant sterol recoveries in the stool. The plant sterols have been utilized as a marker for fecal flow and recovery (21). In our study, the dietary intake of plant sterols was measured and the amount in the stool was subsequently determined. For one subject (No. 6) there was a failure to recover considerable quantities of plant sterols for all dietary periods. The recovery was only 55% for period I, 75% for period II, and 42% for period III. This subject had a daily stool whose weight was similar to the stool weights of other subjects. With these considerations in mind and with our system for collection of stool, we did not consider it likely that any stool had been discarded each day. This man was studied again 1 yr later and still maintained this unique capacity to destroy or "lose" the steroid nucleus in the intestine. Because it has been shown that cholesterol and plant sterols are lost similarly when this phenomenon occurs in the gut (21), we provide information in Table VIII to indicate the extent to which the reported neutral sterol excretion of Table III might be changed when corrected by fecal plant sterol recoveries. The data of Table VIII lists the corrected neutral sterols and the actual amount of the change. It is apparent that this "correction" removes any significance from the neutral steroid excretions from the feeding of polyunsaturated fat vs. saturated fat. The mean values for the last 2 wk of each of the three dietary periods were 492, 471, and 438, all statistically similar. Note that the bile acid excretion still remained significantly different for polyunsaturated vs. saturated fat feeding, and that the bile acid changes constituted 60% or more of the total change in fecal steroids resulting from the polyunsaturated fat

Sterol Excretion During the Different Dietary Periods

4		5		6		Mean by week
Neutral sterol	Change	Neutral sterol	Change	Neutral sterol	Change	Neutral sterol
1026	+174	1235	+109	1968	+974	1159 ±178
608	−182	460	+87	637	+347	515 ±39
594	+273	553	+182	438	+102	466 ±35
601	+45	507	+135	538	+226	492 ±30
472	+165	515	+41	402	+82	393 ±35
440	−304	695	+118	514	+176	465 ±51
467	+84	656	+164	577	+89	477 ±48
454	−110	676	+142	546	+133	471 ±49*
519	−207	695	−76	594	+248	543 ±37
489	−117	557	+33	507	+209	469 ±29
434	+39	424	+59	425	+317	407 ±12
462	−38	491	+47	466	+263	438 ±20*

feeding. Until the end products of these neutral sterol losses can be identified, the significance of the plant sterol "losses" remains uncertain.

Weight changes. The caloric intake of each subject was carefully adjusted to avoid weight loss or weight gain, so that weight changes could be eliminated as having an effect upon the serum lipid levels. The mean weight change for the six men was + 1.6 lb. for the 9 wk study or only + 0.18 lb./wk. Each man had a virtually flat weight curve from the beginning to the end of the study.

DISCUSSION

Since the diets utilized in these studies were cholesterol free, the sum of the fecal neutral steroids and bile acids represented the excretion of endogenous cholesterol and its metabolites from the body. Under steady-state conditions, the total fecal steroid excretion equals the total cholesterol synthesis by the body, since all of the fecal steroids are derived ultimately from the cholesterol molecule. The direct sources of fecal steroids in any human are as follows: (*a*) the cholesterol and bile acids secreted into the bile by the liver and not reabsorbed via the enterohepatic circulation; and (*b*) cholesterol secreted by the intestinal mucosa or contained in sloughing mucosa cells and not reabsorbed. We recognize the great complexity of determining exactly how each of these heterogeneous sources fits into the final pattern of fecal steroid excretion.

The changes in fecal steroid excretion as occurred in this study from polyunsaturated fat feeding are interpretable in many ways, some more plausible than others. However, these results appear to substantiate the hypothesis that fecal steroid excretion can in fact quantitatively account for the changes in serum cholesterol concentration which follow the substitution of polyunsaturated fat for the saturated fat in the human diet. It is of interest that both neutral sterols and bile acids significantly increased or decreased depending upon the presence or absence of polyunsaturated fat in the diet. The bile acids represented over 60% of the total change in fecal steroid excretion and neutral sterols the remainder. Thus, the dietary polyunsaturated fat promoted more fecal bile acid excretion than it did the excretion of neutral steroids.

The simplistic explanation of these findings is that the

TABLE IX

Total Cholesterol Balance Induced by the Changes in Dietary Fatty Acid Composition

	From cocoa butter (period I) to corn oil (period II)	From corn oil (period II) to cocoa butter (period III)
Plasma cholesterol change	−1448 mg	+1629 mg
Change in fecal neutral steroids and bile acids	+2884 mg	−4004 mg
Difference	+1436 mg	−2375 mg
Per cent of total change in fecal steroids	50%	59%

Data from all six subjects have been combined.

polyunsaturated fat in the diet lowered the serum cholesterol, and that this cholesterol was excreted into the bile and then into the stool as neutral steroids and bile acids. Fecal steroid excretion increased 206 mg/day during the period of corn oil feeding and decreased 286 mg/day after saturated fat was substituted. If the total plasma cholesterol shifts occurring from one diet to another are calculated, then this figure can be compared with the changes in total fecal steroid excretion in order to compute the cholesterol balance (Table IX). The plasma volume was derived for each man from the formula provided by Edelman and Liebman (22). With this figure the total change of plasma cholesterol was computed. The total fecal steroid changes were obtained from the mean daily change multiplied by the 14 days in which the fecal steroid changes actually occurred. From period I to II (a change to the corn oil diet), the plasma compartment lost a total of 1448 mg of cholesterol. At the same time the fecal steroid excretion increased 2884 mg. From period II to III (a change back to cocoa butter) the total plasma cholesterol increased 1629 mg, while the fecal steroids decreased 4004 mg. Note the invariable reciprocal nature of these changes in plasma cholesterol and fecal steroids, and that the fecal changes were always much greater than the plasma changes.

We suggest that one possibility for these greater changes in fecal steroids might be a change in the cholesterol content of the tissues. For example, the plasma and liver cholesterol are usually considered as components of the same pool (23). In humans, the total liver cholesterol is roughly similar in amount to plasma cholesterol. Thus the "difference" figures of 1226 and 1867 mg might be explained by liver cholesterol shifts. We cannot exclude the possibility of the movement of cholesterol from the other tissues as well.

A second alternative explanation of this enhanced fecal steroid excretion might be that total cholesterol biosynthesis increased under the new conditions induced by polyunsaturated fat. This explanation seems unlikely to us as a primary factor in view of the decline in plasma cholesterol. Enhanced biosynthesis in the liver or intestinal mucosa might tend to increase the cholesterol in the primary cholesterol pool, and, assuming equilibrium between plasma and liver, the available evidence suggests that no enhanced biosynthesis or even the opposite effect might occur. Avigan and Steinberg have found that polyunsaturated fat increases cholesterol synthesis in the rat liver (24). Our study does not provide an answer to the very difficult problem of what happens to cholesterol biosynthesis in the whole animal.

A third explanation is that polyunsaturated fat, by inducing structural changes in plasma lipoproteins (25, 26), might make it possible for lipoprotein cholesterol to be reduced in quantity and at the same time provide adequate transport function as when saturated fat was fed. This alteration in lipid transport might then be followed by the fecal excretion of the now unneeded cholesterol. The fatty acid moiety of cholesterol esters, a structural component of lipoproteins, is influenced by the composition of dietary fat (27). Spritz and Mishkel recently found that polyunsaturated fat feeding reduced the lipid content but not the protein content of the low density lipoproteins (26). In particular, the fatty acid at the β-position of a plasma phospholipid (phosphatidylcholine) became largely polyunsaturated. They hypothesized that the feeding of polyunsaturated fatty acid altered the spatial configuration of the plasma lipids into which they were incorporated. Because a polyunsaturated fatty acid such as linoleic occupies a greater spatial area, the low density lipoproteins can carry less lipid, and their lipid content is thereby lowered.

If these physicochemical mechanisms do explain the cholesterol-lowering effects of dietary polyunsaturated fat, then the knowledge that the excess cholesterol is excreted into the stool seems most relevant to any recommendation that polyunsaturated fat be substituted for saturated fat in the diet of man. Should the "excess" cholesterol not be accounted for in steroid excretion, then its possible accumulation in the tissues (including arteries) would not support any such recommendation.

The results obtained in our study are at variance from those found by Spritz, Ahrens, and Grundy (8, 28). When the dietary fat was more polyunsaturated, these investigators did not find increased fecal steroid excretion with a change to polyunsaturated fat in the diet in 10 of 11 human subjects (28). One individual did have increased fecal steroid excretion in two separate studies conducted years apart (4, 28). Since in our study we employed similar chemical methods for the measurement of fecal steroid excretion for both neutral sterols and bile acids, methods which these scientists developed at the Rockefeller University, these different results are perhaps even more significant than if different methodologies had been used.

We would call attention to several possibilities in explanation of these different results. First, the subject material was drawn from two dissimilar population groups. Our subjects were men with characteristic serum cholesterol concentrations for the American population at their age and men without known metabolic disorders. In general, the Rockefeller group used hypercholesterolemic or hypertriglyceridemic subjects, individuals with xanthomatous disease in whom cholesterol storage in the skin, tendons, and perhaps other tissues was present. Second, we suggest that there may well be a considerable lag period before any change in the plasma-liver pool of cholesterol may be detected in en-

hanced fecal steroid excretion. Perhaps this lag period is even greater under conditions of metabolic abnormality (hypercholesterolemia and xanthomatosis) when the body pool of cholesterol may be greatly increased. In our subjects the lag period was at least 1 wk. In one of the Rockefeller subjects reported in detail in 1966, the lag period was also for some 6–7 days before the increased fecal steroid excretion became manifest (28). This was the single subject in whom polyunsaturated fat did enhance fecal steroid excretion. In their first study, they did not note any enhanced fecal steroid excretion in five subjects but looked especially for this possible change in fecal steroids during the transitional period, i.e., the time when the serum cholesterol was changing (8). Their collections for the subsequent period after the transition lasted for variable periods of 5–20 days, and stool collections were intermittent during this time rather than complete. In our study, stools were collected for a complete 21 day period after the change in dietary fat and analyzed in 7-day aliquots. Stools were analyzed for each entire dietary period.

Not only may there be a lag period, but the change in daily fecal steroid excretion which might occur when dietary fats are altered might be so small each day as to escape detection during the period of observation. Yet, if the examination is carried out over many days as was the case in our studies, perhaps the change might then be detectable. It must be appreciated that, as the changes in fecal steroid excretion approach the error of measurement of the method, then small changes of excretion might not be detected.

Another difference between the studies of various investigators relates to the kind of saturated fat actually tested in comparison with a certain polyunsaturated fat. In many studies, the saturated fat of the diets was either butter fat or coconut oil. Both of these fats contain short-chain fatty acids, especially coconut oil. We compared only long-chain fatty acids, those found in cocoa butter, which are largely palmitic and stearic acids. It may well be that the short-chain fatty acids in the diet do not affect fecal steroid excretion to the same extent as do long-chain saturated fatty acids. The metabolism of short- and long-chain fatty acids is different, especially the mode of absorption from the gastrointestinal tract, short-chain fatty acids being carried via the portal vein. In the single normal subject (study Nos. 3 and 5) tested by Spritz, Ahrens, and Grundy, corn oil and coconut oil were the two fats compared (8). In the one subject (study No. 1) in whom the action of long-chain fatty acids was compared (linoleic vs. palmitic and oleic), the fecal collection period was only 4 days. This collection period may well have been too short for the effect of polyunsaturated fat to be manifested. This subject was also hypercholesterolemic (8).

Avigan and Steinberg, using the isotopic balance technique for fecal steroid determination, compared the effects of coconut oil vs. corn or safflower oil (9). In this meticulously performed study, the dietary fats tested had no effect upon fecal steroid excretion. The same comments which were made for the Rockefeller studies, including the kinds of fats tested, apply here also. Five of the six subjects were hypercholesterolemic. In the one normocholesterolemic subject, the fecal collection period was for only 8 days, probably too short a time period for an effect on fecal steroid excretion to be manifested.

On the other hand, in an 80 yr old normocholesterolemic patient, the isotopic balance technique did indicate increased fecal steroid excretion after polyunsaturated fat feeding in a study reported by Sodhi, Wood, Schlierf, and Kinsell (29). The fecal collection periods were brief, 5–10 days. More recently again with the isotopic balance procedure, Moore, Anderson, Taylor, Keys, and Frantz compared the fecal steroid excretions in five men fed in sequence butter fat and safflower oil diets (30). The polyunsaturated fat increased fecal steroid excretion by 181 mg/day. The diets were not equivalent in cholesterol (347 and 197 mg) and plant sterol (420 and 720 mg) contents. Otherwise, the results of their study were similar to our findings.

In the two other studies which employed modern methodology for fecal steroid determination, either neutral sterols or bile acids were measured, but not both. The addition of corn oil to a standard cholesterol-containing diet did not enhance neutral steroid excretion (6). Bile acid output in the stool was not increased in three men given diets of corn oil and butter fat (7). However, the fecal collection period after the change to the new diet was only 4 days, much too short, as we have indicated, for the possible changes to be manifested.

As has been well demonstrated, probably the only two reliable methods for fecal steroid analysis are the isotopic balance technique (after cholesterol-4-^{14}C injection intravenously) and the quantification by gas-liquid partition chromatography (8). These methods do distinguish between plant sterols and cholesterol. They quantify accurately individual neutral steroids and bile acids. There are many possible methodological errors inherent in the techniques for measuring fecal steroids used in earlier studies (2, 3, 5). These points have also been discussed by Spritz, Ahrens, and Grundy (8). Neutral sterols were frequently quantified by digitonide precipitation or simply by Liebermann-Burchard color development. Such techniques did not distinguish the plant sterols of dietary origin which passed through the gut into the stool nor did they measure all of the fecal neutral steroids. Fecal bile acids were measured by titra-

tion of acidity in purified lipid extracts, a nonspecific method. Finally, the colorimetric determination of charred spots on thin-layer chromatographic plates offered some improvement but did not distinguish between plant sterol derivatives and cholesterol derivatives in the stool, nor was it specific in all instances for reproducible intensity of color development in steroidal spots they were sprayed with sulfuric acid.

Polyunsaturated fat and serum lipid levels. While there is apparently general agreement that dietary polyunsaturated fatty acids lower the serum lipid levels in contrast to saturated fatty acids (28), several investigations have failed to confirm this general view under certain experimental conditions (20, 31, 32). These divergent results have never been explained or refuted in the literature to date. In brief, increases in dietary polyunsaturated fat to the extent of 9% of the total calories (20), 7 and 11% of the total calories in two different studies (31), and 10% of total calories (32) did not increase serum cholesterol levels. Expressed in other terms, a change in the polyunsaturated to saturated ratio (P/S) from 0.1 to 1.6 (31, 32) or a change in iodine values from 64 to 100 (20) did not change the serum lipids. Ahrens had previously indicated in a point again seldom discussed that dietary fats with iodine values from 85 to 126 had similar lipid lowering effects (33).

It was because we have failed to obtain a change in serum lipids with a mild increase in dietary polyunsaturated fat (20) that the present study was conducted. After we increased the dietary polyunsaturated fat to 21% of the total calories and the iodine value of the fat from 32 to 127, a pronounced lowering of the serum cholesterol and phospholipid levels occurred. We suggest that, in some studies at any rate, there must be a certain threshhold exceeded as regards the amount of dietary polyunsaturated fat before serum lipid lowering effects are obtained.

Bacterial alterations in bile acids and neutral steroids during intestinal transit. The quantitatively significant fecal bile acids were the secondary bile acids, deoxycholic and lithocholic acids, which resulted from the bacterial dehydroxylation of the two primary bile acids in man, cholic and chenodeoxycholic. Deoxycholic and lithocholic acids were roughly similar in amount through all three dietary periods and made up about five-sixths of the total fecal bile acids. Bacteria apparently do not destroy the steroid ring structure of bile acids in contrast to the destruction of neutral sterols which may occur (21).

There was one known bacterial action upon neutral sterols: the conversion of the endogenously derived cholesterol and the three dietary plant sterols by hydrogenation and dehydrogenation to coprostanol and coprostanone compounds. These conversions were identical in a given subject for cholesterol and each of the three individual plant sterols. Subject No. 1 failed to have any alteration in the neutral sterols during the three different dietary periods, and subject No. 4 had a variable picture. Why these responses differed from the 60–90% bacterial conversion by the other subjects is conjectural but seems not related to diet.

The other alteration in neutral sterols was their loss in the intestinal tract on occasion. Any loss was monitored by a knowledge of the plant sterol intake and the output. This was consistent for subject No. 6 who ranged from only 42 to 75% recovery during the three dietary periods. Subject No. 6 has since been studied repetitively for this phenomenon and continues to manifest it. Grundy, Ahrens, and Salen first observed failure of recovery of dietary plant sterols in man (21). Their experiments have ruled out the possibility that the losses occurred because of intestinal absorption of plant sterols. However, they were unable to recover the breakdown products of labeled plant sterol or labeled cholesterol. We are currently attempting to study subject No. 6 again using plant sterol tolerance tests and incubating his stool bacterial with labeled sterols in a further effort to understand the basis for this interesting phenomenon.

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