The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion

Don S. Lin and William E. Connor

The Divisions of Metabolism and Nutrition and Cardiology, the Department of Medicine and Clinical Research Center, University of Oregon Health Sciences Center, Portland, OR 97201 and the Clinical Research Center, University of Iowa College of Medicine, Iowa City, IA 55240

Abstract In order to study the metabolic responses of humans consuming a diet moderately high in cholesterol content, we carried out a long-term sterol balance study, up to 25 weeks in duration. Two subjects, one normocholesterolemic and one hypercholesterolemic, were given, in sequence, a very low cholesterol diet and then a diet containing 1000 mg cholesterol per day. The plasma lipids, lipoproteins, cholesterol absorption and synthesis, and fecal steroid excretion were then measured during the different dietary periods (10–14 weeks of a very low cholesterol diet and 11 weeks of a moderately high cholesterol diet). During the high cholestrol dietary period, the plasma cholesterol level increased from 280 to 427 mg/dl for Subject 1 and from 123 to 166 mg/dl for Subject 2. The low density lipoprotein (LDL) cholesterol increased from 215 to 318 mg/dl and from 76 to 112 mg/dl. The high density lipoprotein (HDL) cholesterol also increased. Of the possible compensatory mechanisms against cholesterol overloading from the diet, two mechanisms were partially effective: cholesterol biosynthesis decreased (feedback inhibition) and bile acid excretion increased. Cholesterol absorption remained unchanged after the high cholesterol diet and was not a compensatory mechanism despite earlier assumptions that it might be. In spite of these compensatory mechanisms, the cholesterol feeding led to a 44% increase in the plasma cholesterol levels of these subjects. The predominant component of the plasma cholesterol increase was in the cholesterol transported by LDL and with presumably greater atherogenicity as a result. In the hypercholesterolemic subject, the LDL/HDL ratio increased and there was a net storage of cholesterol in the body. Storage of cholesterol did not occur in the normal subject.—**Lin, D. S., and W. E. Connor.** The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. *J. Lipid Res.* 1980. **21:** 1042–1052.

Supplementary key words lipoproteins · cholesterol storage hypercholesterolemia

Cholesterol homeostasis in the body is governed by an interplay of cholesterol absorption, synthesis, storage, and excretion. When a given steady state is disturbed by cholesterol feeding, the responses to the changes in these parameters are different in different species and are correspondingly reflected in their serum cholesterol levels. In the rat, for example, there is only minor elevation of serum cholesterol after feeding large quantities of cholesterol. Control mechanisms prevent a disturbance in homeostasis by a reduction in the body's synthesis of cholesterol and by increased conversion of cholesterol to bile acids which promotes the excretion of steroids in the stool (1–3). On the other hand, the feeding of dietary cholesterol to rabbits results in an extraordinary hypercholesterolemia, atherosclerosis, and a debilitating cholesteryl ester storage disease (4). The rabbit has both high cholesterol absorption and a relative failure to synthesize and excrete bile acids in compensation.

In human beings, the plasma cholesterol level may be increased moderately by the addition of cholesterol to a baseline cholesterol-free diet; i.e., from 190 to 260 mg/dl (5). Similar elevations of serum cholesterol levels have been observed under wide-ranging

amounts of dietary cholesterol intake (up to 4500 mg/day) (5–11). Absorption data from this laboratory and others have pointed out the fact that, while there is always only a fraction of the dietary cholesterol absorbed in man, the net absorption has a roughly linear relationship with the amount of cholesterol in the diet (12–15). As much as 1 g of dietary cholesterol was absorbed when 3 g cholesterol was given daily (14). It is, therefore, conceivable that in order to prevent great elevations of serum cholesterol levels and expansion of the tissue cholesterol pool in the face of large cholesterol intakes, some compensatory mechanisms must be operative in man in response to the absorbed cholesterol. These could include feedback inhibition of cholesterol biosynthesis, enhancement of bile acid and/or neutral steroid excretion, and reduction of cholesterol absorption.

The reduction of cholesterol biosynthesis through the negative feedback mechanism has been demonstrated in different experimental animals after a high cholesterol diet and has been considered as an important protective mechanism for cholesterol homeostasis (16–20). In man, however, the experimental evidence has been somewhat contradictory. An in vitro study of human liver slices indicated that with prior cholesterol feeding there was marked reduction of cholesterol biosynthesis (21). However, studies by Wilson and Lindsey (22) and Taylor and his colleagues (23–25) in intact man using either the isotopic steady state technique or the deuterium-labeling technique did not confirm significant feedback control from the ingestion of dietary cholesterol. Other workers have obtained extremely variable results from subject to subject with feedback inhibition never attaining the significance that it has in animals (26–29).

With regard to increased fecal bile excretion after a high cholesterol diet, the results have been inconclusive. In fact, one review of the subject stated that increased bile acid excretion after cholesterol feeding has never been reported in man (30). The increased excretion of neutral steroids after cholesterol feeding was observed in some of the subjects in the previous studies (26, 27).

In the present experiment, long term sterol balance studies were carried out in two human subjects who were given, in sequence, a "very low cholesterol" diet and then a diet containing about 1000 mg cholesterol per day, an amount certainly consumed by some Americans. These subjects were hospitalized in the Clinical Research Center for the entire course of the study in order to have proper control of dietary intake and sample collection. These long-term feeding studies were carreid out for 21 to 25 weeks duration to ensure the establishment of a metabolic steady state. The baseline "very low cholesterol" diet was fed for 10 and 14 weeks to the two subjects, respectively, followed by 11 weeks of a high cholesterol diet. The plasma lipids, lipoproteins, cholesterol absorption cholesterol synthesis and fecal steroid excretion were measured during the first steady state period (baseline period), in the transition period, and during the second steady state period (the high cholesterol feeding period). The results indicated new and unequivocal findings about the effects of dietary cholesterol in man.

MATERIALS AND METHODS

Two subjects, one normal subject and one type II hypercholesterolemic patient, were hospitalized in the Clinical Research Center during the entire 22–25 week period of study. The protocol of study had been approved by the Human Ethics Committee and informed consent was obtained. Subject 1 was a 67-year-old woman who weighed 44.3 kg, and was 152 cm in height. She was a type IIa hypercholesterolemic patient and had xanthelasmas and extensor tendon xanthomas of the hands. Her thyroid, renal, and liver function tests were normal. She was not diabetic. All causes of secondary hyperlipidemia were ruled out. However, familial data could not be obtained; all family members were deceased. Subject 2 was a 31-year-old normocholesterolemic healthy man. His weight and height were 65.6 kg and 185 cm, respectively. Both subjects were slender. Their food intake for the entire experiment consisted exclusively of orally administered liquid formula feedings in which protein contributed 15%, fat 40%, and carbohydrate 45% of the total caloric intake (7) (**Table 1**). Vitamins and minerals were added to meet the daily recommended allowances of the National Research Council. The cholesterol content of the diets averaged 45 mg per day during the very low cholesterol period. The total caloric intake was adjusted to maintain constant body weight. The subjects were given this basic very low cholesterol formula diet for 10–14 weeks and then were fed, for another 10–11 weeks, a moderately high cholesterol diet which was composed of the basic formula diet with the daily addition of 1000 mg cholesterol. This cholesterol was supplied as egg yolk incorporated into the diet as in previous studies in a way to keep the total fat and fatty acid compositions of the two formulas very similar.

Venous blood samples, after an overnight fast of 14 hours, were collected twice weekly. Plasma lipo-

TABLE 1. The composition of the formulas for the two dietary periods[a]

Dietary Period	Cholesterol	Source of Fat: Whole Egg Yolk	Source of Fat: Vegetable Oil Mixture[b]	Iodine Value	Fatty Acids: Saturated	Fatty Acids: Monounsaturated	Fatty Acids: Polyunsaturated	P/S
	mg	*g*	*g*		*%*	*%*	*%*	
I Very low cholesterol diet	45	0	111	81	47.5	21.3	31.2	0.8
II Moderately high cholesterol diet	1126	28	83	83	48.0	22.2	29.8	0.8

[a] These representative formulas are calculated for a daily intake of 2500 calories. Vitamins and minerals were added to meet the recommended dietary allowances of the National Research Council and remained constant throughout the study.

[b] A combination of peanut oil and cocoa butter, typically 80% and 20%, respectively, for Period I, and 58% and 16% for Period II, to compensate for the incorporation of egg yolk fatty acids.

proteins were separated by ultracentrifugation and heparin-$MnCl_2$ precipitation (31). The cholesterol and triglyceride contents in the plasma and in lipoprotein fractions were determined by an Autoanalyzer-II (Technicon Instruments Corp., Tarrytown, NY). The intestinal absorption of cholesterol was measured by a single test meal technique (12). Four repeated absorption tests at 3-week intervals were performed during the cholesterol feeding period. [1,2-^{3}H]-Cholesterol used in the test meal was obtained from Amersham/Searle Corp., Arlington Heights, IL. A tracer dose of β-[4-^{14}C]sitosterol, obtained from the same company, was also incorporated into the meal to correct possible bacterial degradation (14). The purity of the isotopes was verified by thin-layer chromatography (32).

Daily stools of the last 5 weeks of the very low cholesterol feeding period and the entire moderately high cholesterol feeding period were collected and frozen immediately. At the end of the study, these fecal specimens were thawed and pooled in 7-day lots. The stools were homogenized with water in a paint can shaker (33). Aliquots were then taken for analyses. The analytical methods used for fecal neutral steroid and bile acid analysis were the same as we have reported previously (34). They are based on the original work of Miettinen, Ahrens, and Grundy (35) and Grundy, Ahrens, and Miettinen (36) involving the separation of the fecal steroids into neutral and acidic fractions. These separate fractions were purified by thin-layer chromatography, and the individual components subsequently were measured by gas–liquid chromatography. The gas–liquid chromatography was performed on an instrument equipped with a hydrogen flame ionization detector (Hewlett-Packard Model 7610A, Skokie, IL). The column was a 4-ft glass U-tube, 4 mm i.d., packed with Diatoport S (80/100) coated with 3.8% film of SE-30. Temperature of column, injection port, and flame detector were 230°C, 250°C and 280°C, respectively, for neutral steroids and the temperature of the column for bile acids was 240°C. Helium was used as carrier gas with a flow rate of 75 mm/min. The inlet pressure was 40 psi. An integrator (Hewlett-Packard Model 3370B) was used for quantitation of the peak area. Aliquots of the formula diets were analyzed for sterol content according to the same procedure utilized for neutral steroids. (35).

For the measurement of cholesterol absorption, the radioactivity in the fecal neutral steroid fraction was determined. The same procedure as described before for mass determination was used. Fecal steroids were first separated into neutral and acidic fractions after mild saponification. The neutral steroids extracted in hexane were dried and redissolved in 10 ml scintillation mixture (2, 5-diphenyloxazole (PPO) and 1,3-bis-(2(5-phenyloxazolyl)) benzene (POPOP) in toluene. The specimens were counted in a Packard Tri-Carb liquid scintillation counter (Model 3380, Packard Instrument Co., Downers Grove IL). The quench of the sample, if any, was corrected with an absolute activity analyzer (Model 544, Packard Instrument Co.).

Statistical analyses were performed by standard methods (37).

RESULTS

Changes in plasma lipids, lipoproteins and body weight

The weekly mean plasma cholesterol and triglyceride levels of these two subjects, consuming very low cholesterol and high cholesterol diets, are given in **Table 2**. In both subjects, the response to dietary cholesterol occurred quickly. Increased plasma cholesterol was observed in the first week of feeding for Subject 2 (the normal subject) and in the second week for Subject 1 (the hypercholesterolemic patient). The hypercholesterolemic patient not only responded

TABLE 2. The plasma lipids of two subjects fed very low cholesterol and high cholesterol diets

Dietary Period	Weeks		Subject 1 Plasma Lipids		Subject 2 Plasma Lipids	
	Subject 1	Subject 2	Cholesterol	Triglyceride	Cholesterol	Triglyceride
			mg/dl		*mg/dl*	
	1–4th	1–5th	333	84	134	99
	5–9th		263	77		
Low cholesterol	10th[a]	6th[a]	284	80	128	96
(<45 mg/day)	11th	7th	270	81	116	93
	12th	8th	286	85	123	96
	13th	9th	279	78	127	85
	14th	10th	290	88	120	76
Mean and S.D. (last 5 weeks)			280 ± 6.7	82 ± 4.0	123 ± 6.2	89 ± 8.6
Moderately high	1st		293	87	141	91
cholesterol	2nd		347	80	147	77
(1000 mg/day)	3rd		361	71	163	87
	4th		391	79	161	96
	5th		391	90	167	95
	6th		397	73	169	79
	7th		419	78	164	88
	8th		432	78	171	78
	9th		416	73	172	94
	10th		441	84	162	75
	11th		426	78	167	74
Mean and S.D. (last 5 weeks)			427 ± 14.5	78 ± 3.9	166 ± 7.0	82 ± 8.8

[a] Subject 1 received the very low cholesterol diet for a total of 14 weeks and Subject 2 received the same diet for 10 weeks. Each value represents two determinations per week.

more slowly than the normal subject, but her plasma cholesterol level also reached a plateau much later during the period of high cholesterol feeding: 6 weeks versus only 2 weeks for the normal subject. During the last 5 weeks of each dietary period, the plasma cholesterol level remained relatively constant: the variation being less than 5% from the mean value. The plasma cholesterol level of 280 ± 6.7 mg/dl during the very low cholesterol diet for Subject 1 increased to 427 ± 14.5 mg/dl during the high cholesterol dietary period, a rise of 147 mg/dl or +52.5%. For Subject 2, the plasma cholesterol level was 123 ± 6.2 mg/dl during the very low cholesterol dietary period and 166 ± 7.0 mg/dl during the moderately high cholesterol dietary period, an increase of 45 mg/dl or +35.8%. The plasma triglyceride levels of both subjects did not change appreciably during these two dietary periods (Table 2). The body weights of these two subjects remained relatively constant during the last 5 weeks of the low and moderately high cholesterol dietary periods: 44.0 ± 0.09 kg and 44.5 ± 0.16 kg during these two dietary periods for Subject 1, and 65.2 ± 0.16 kg and 66.5 ± 0.12 kg for Subject 2.

The cholesterol and triglyceride contents of the various lipoprotein fractions of these two subjects during both the low and moderately high cholesterol feeding periods are presented in **Table 3.** In both subjects during the low cholesterol feeding period, the bulk of the plasma cholesterol was carried in the LDL fraction (75% for the type II Subject 1, 62% for Subject 2).

When they were challenged with the moderately high cholesterol diet, there was an increase in cholesterol in both LDL and HDL fractions. The major increase in plasma cholesterol was carried in the LDL fraction. The cholesterol content of LDL increased from 215 ± 10.6 to 348 ± 13.4 mg/dl, a rise of 123 mg/dl or +62% ($P < 0.025$), for Subject 1, and from 76 ± 3.2 to 117 ± 14.8 mg/dl, a rise of 41 mg/dl or +54% ($P < 0.005$), for Subject 2. The cholesterol level of HDL increased from 67 ± 6.4 to 75 ± 4.8 mg/dl ($\Delta 12\%$ $P < 0.2$) for Subject 1 and from 38 ± 4.6 to 52 ± 9.9 mg/dl ($\Delta 37\%$ $P < 0.05$) for Subject 2. The increases in LDL were proportionately greater than the increases in HDL. Consequently, the LDL/HDL ratio for both subjects increased after the moderately high cholesterol diet (3.34 ± 0.36 to 4.69 ± 0.35, $P < 0.025$ for Subject 1 and 2.04 ± 0.24 to 2.32 ± 0.54, $P < 0.2$ for Subject 2).

Because the triglyceride level in the lipoprotein fractions of these two subjects changed little during the two dietary periods, we chose it as a marker of the composition of lipoprotein molecules and cal-

TABLE 3. Lipid content (mg/dl) of lipoprotein fractions of two subjects fed very low and high cholesterol diets

	Subject 1						Subject 2					
	Cholesterol			Triglycerides			Cholesterol			Triglycerides		
Diet	VLDL[a]	LDL	HDL	VLDL	LDL	HDL	VLDL	LDL	HDL	VLDL	LDL	HDL
Very low cholesterol	6 ± 2.1[b]	215 ± 10.6	67 ± 6.4	38 ± 4.2	31 ± 2.1	11 ± 2.1	8 ± 3.3	76 ± 3.2	38 ± 4.6	60 ± 12.5	16 ± 4.0	6 ± 2.7
Moderately high cholesterol	7 ± 3.7	348 ± 13.4	75 ± 4.8	30 ± 5.8	33 ± 2.7	12 ± 2.2	10 ± 2.5	117 ± 14.8	52 ± 9.9	58 ± 10.9	12 ± 2.6	12 ± 4.1
Change	+1	+123	+8	−8	+2	+1	+2	+41	+14	−2	−4	+6
P value (low vs high)[c]	NS	0.025	NS	NS	NS	NS	<0.05	<0.005	<0.05	NS	<0.05	NS

[a] VLDL, very low density lipoprotein fraction; LDL, low density lipoprotein fraction; HDL, high density lipoprotein fraction.

[b] Each value represents the mean and S.D. of the last 5 weeks of each dietary period except for those for Subject 1 in the very low cholesterol period which represent the mean of the last three determinations in that dietary period.

[c] Paired *t* test.

culated the cholesterol concentration in relation to it. The cholesterol/triglyceride ratio in LDL increased from 7.07 ± 0.84 during the cholesterol-free diet to 10.41 ± 0.54 ($P < 0.025$) during the moderately high cholesterol diet in Subject 1 and from 5.01 ± 1.42 to 10.46 ± 2.37 ($P < 0.005$) in Subject 2. The ratio of cholesterol/triglyceride in VLDL and HDL was not significantly different in the two dietary periods, except that there was a slight increase in the VLDL fraction of Subject 2 (from 0.13 ± 0.03 to 0.18 ± 0.04, $P < 0.05$).

Cholesterol absorption

Four absorption tests were carried out in each subject during the 11 weeks of the moderately high cholesterol feeding period. The average recoverage of β-[4-^{14}C]sitosterol in these tests was 93.7 ± 8.5% for Subject 1 and 98.3 ± 12% for Subject 2. The percentage of intestinal absorption of dietary cholesterol by both subjects was similar and remained constant throughout the feeding period (**Tables 4** and 5). The average absorption was 41.0 ± 2.4% for Subject 1 and 38.4 ± 1.4% for Subject 2. To avoid disturbing the steady state condition, the cholesterol absorption test was not performed in these subjects during the very low cholesterol dietary period. However, in another study, these same subjects had been given test meals containing 100 mg cholesterol under conditions of a cholesterol-free background diet. The cholesterol absorption was 40.4% for Subject 1 and 47.7% for Subject 2. These figures were used for the calculation of unabsorbed cholesterol and endogenous neutral steroid excretion in very low cholesterol period in this study (Table 4).

Fecal steroid excretion

The results of the fecal steroid excretion during the last 5 weeks of the very low cholesterol diet and the entire 11 weeks of the moderately high cholesterol diet are shown in Table 4. During the very low cholesterol dietary period, the fecal steroid excretion for Subject 1 was 539 ± 18.6 mg/day (unabsorbed dietary cholesterol 22 mg/day, endogenous neutral steroids 355 ± 15.1 mg/day, and bile acids 162 ± 25.4 mg/day) and 752 ± 27.6 mg/day (unabsorbed cholesterol 27 mg/day, endogenous neutral steroids 540 ± 23.7 mg/day, and bile acids 184 ± 14.2 mg/day) for Subject 2.

When the moderately high cholesterol diet was fed, there was a sudden decrease in endogenous neutral steroid excretion during the first 2 weeks for Subject 1. The fecal bile acid excretion began to increase during the second week. The total fecal steroid output increased gradually and became stabilized after 5 weeks of feeding. The average total fecal steroid excretion in the entire 11-week feeding period was 1201 ± 155.7 mg/day (unabsorbed dietary cholesterol 655 mg/day, endogenous neutral steroids 321 ± 139.8 mg/day, and bile acids 225 ± 52.8 mg/day). For Subject 2, there was also a decrease in endogenous neutral steroid excretion during the first week of the moderately high cholesterol diet. The trend toward the increase of fecal bile acids was noted after 2 weeks of feeding. The total fecal steroid output became steady after the second week. The average fecal steroid excretion for Subject 2 in these 11 weeks of feeding was 1489 ± 151.4 mg/day (unabsorbed dietary cholesterol 702 mg/day, endogenous neutral steroids 574 ± 154.5 mg/day and bile acids 214 ± 29.3 mg/day).

Sterol balance

The sterol balance data of these two subjects during the metabolic steady state (the last 5 weeks of the two dietary regimes) are tabulated in **Table 5**. The endogenous neutral steroid excretion was not different for the very low cholesterol and the moderately high

TABLE 4. Intestinal absorption, fecal steroid excretion, and balance of intake and excretion of Subjects 1 and 2 consuming low and high cholesterol diets

Dietary Period	Weeks	Cholesterol Intake	Cholesterol Absorption	Fecal Steroid Excretion					Balance (Intake less excretion)
				Neutral Steroids			Bile Acids	Total	
				Unabsorbed	Endogenous[b]	Total			
		mg/day	*%*	*mg/day*					*mg/day*
Subject 1									
Very low cholesterol	10th	37	40.4 ± 1.8[a]	22	345	367	165	532	−495
	11th	37	40.4 ± 1.8[a]	22	349	371	184	555	−518
	12th	37	40.4 ± 1.8[a]	22	377	399	153	552	−515
	13th	37	40.4 ± 1.8[a]	22	365	387	123	510	−473
	14th	37	40.4 ± 1.8[a]	22	341	363	184	547	−510
Moderately high cholesterol	1st	1109		652	46	698	143	841	+268
	2nd	1109	41.2	652	145	797	215	1012	+97
	3rd	1109		652	400	1052	284	1336	−227
	4th	1109		692	348	1040	155	1195	−86
	5th	1109	37.6	692	197	889	258	1147	−38
	6th	1109		692	308	1000	329	1329	−220
	7th	1109		635	388	1023	225	1248	−139
	8th	1109	42.7	635	363	998	223	1221	−112
	9th	1109		635	492	1127	197	1324	−215
	10th	1109		635	493	1128	220	1348	−239
	11th	1109	42.6	635	356	991	222	1213	−104
Subject 2									
Very low cholesterol	6th	52	47.7 ± 8.9[a]	27	552	579	191	770	−718
	7th	52	47.7 ± 8.9[a]	27	565	592	179	771	−719
	8th	52	47.7 ± 8.9[a]	27	531	558	206	764	−712
	9th	52	47.7 ± 8.9[a]	27	550	577	172	749	−697
	10th	52	47.7 ± 8.9[a]	27	504	531	174	705	−653
Moderately high cholesterol	1st	1142		689	460	1149	167	1316	−174
	2nd	1142	39.7	689	519	1208	199	1407	−265
	3rd	1142		689	688	1377	220	1597	−455
	4th	1142		694	715	1409	239	1648	−506
	5th	1142	39.2	694	314	1008	262	1270	−128
	6th	1142		694	887	1581	184	1765	−623
	7th	1142		707	524	1231	239	1470	−328
	8th	1142	38.1	707	482	1189	194	1383	−241
	9th	1142		707	638	1345	243	1588	−446
	10th	1142		725	608	1333	208	1541	−399
	11th	1142	36.6	725	484	1209	194	1403	−261

[a] This value was obtained at a different period with the same dietary background (see text).
[b] Endogenous neutral steroid excretion was calculated by subtracting unabsorbed dietary cholesterol from the total fecal neutral steroid excretion.

cholesterol feeding periods for Subject 1. Her fecal bile acids, however, were significantly increased by 34%, from 162 ± 25.4 mg/day to 217 ± 11.5 ($P < 0.01$). In response to dietary cholesterol, total cholesterol biosynthesis decreased (67.7%) from 502 ± 18.6 mg/day to 162 ± 61.5 mg/day ($P < 0.001$). The calculation of biosynthesis was based upon the prerequisite that the input of sterol was equal to the output during a metabolic steady state. Therefore, the balance (intake minus excretion) equals synthesis.

For Subject 2, the fecal endogenous neutral steroid excretion was also similar during these two dietary periods. An increase of fecal bile acids was observed from 184 ± 14.2 mg/day in the control period of 216 ± 23.9 mg/day during the moderately high cholesterol feeding period, an 18.7% increase ($P < 0.01$). Cholesterol synthesis decreased 52.1%, from 700 ± 27.6 to 335 ± 87.7 mg/day ($P < 0.001$).

The fecal neutral steroid excretion was corrected for β-sitosterol recovery (38). The recovery of β-sitosterol during the last 5 weeks of the control period was 95.2 ± 19.5% for Subject 1 and 99.7 ± 12.2% for Subject 2. For the last 5 weeks of the moderately high cholesterol dietary period the β-sitosterol recovery was 86.5 ± 8.5% and 91.3 ± 12.8% for Subjects 1 and 2, respectively.

Using the parameters measured in this present study (the plasma cholesterol, cholesterol absorption, cholesterol synthesis and cholesterol excretion), we have estimated the overall sterol economy in these two subjects consuming the moderately high cholesterol diet (**Table 6**). During these 11 weeks, Subject 1 ab-

TABLE 5. Sterol balance data of two subjects at metabolic steady states on two dietary regimens (last 5 weeks of each dietary period)

	Subject 1				Subject 2			
	Cholesterol-free Period (I)	1000 mg Cholesterol Period (II)	% Change	*P* Value[a] (I vs II)	Cholesterol-free Period (I)	1000 mg Cholesterol Period (II)	% Change	*P* Value[a] (I vs II)
Duration (weeks)	14	11			10	11		
Plasma cholesterol (mg/dl)	280 ± 6.7	427 ± 14.5	+52.5	<0.001	123 ± 6.2	166 ± 7.0	+35.0	<0.001
Cholesterol intake								
mg/day	37	1109			52	1142		
mg/kg/day	0.8	25.1			0.8	17.3		
Intestinal absorption (%)	40.4	42.7			47.7	37.4		
Fecal steroid excretion								
Neutral steroids (NS)								
Unabsorbed								
mg/day	22	635			27	714		
mg/kg/day	0.5	14.2			0.4	10.7		
Endogenous								
mg/day	355 ± 15.1	418 ± 68.7		NS	540 ± 23.7	547 ± 72.0		NS
mg/kg/day	8.1 ± 0.4	9.4 ± 1.6		NS	8.3 ± 0.4	8.2 ± 1.1		NS
Total								
mg/day	377 ± 15.1	1053 ± 68.7		<0.001	567 ± 23.7	1261 ± 72.0		<0.001
mg/kg/day	8.6 ± 0.4	23.7 ± 1.6		<0.001	8.7 ± 0.3	19.0 ± 1.1		<0.001
Bile acids (BA)								
mg/day	162 ± 25.4	217 ± 11.5	+34.0	<0.01	184 ± 14.2	216 ± 14.2	+17.4	<0.01
mg/kg/day	3.7 ± 0.6	4.9 ± 0.3	+32.4	<0.025	2.8 ± 0.2	3.2 ± 0.4	+14.3	<0.025
Total excretion (NS + BA)								
mg/day	539 ± 18.6	1271 ± 61.5		<0.001	752 ± 27.6	1477 ± 87.7		<0.001
mg/kg/day	12.3 ± 0.4	28.6 ± 1.5		<0.001	11.4 ± 0.5	22.2 ± 1.3		<0.001
Total body cholesterol synthesis measured by balance (intake less total excretion)								
mg/day	−502 ± 18.6	−162 ± 61.5	−67.7	<0.001	−700 ± 27.6	−335 ± 87.7	−52.1	<0.001
mg/kg/day	−11.4 ± 0.4	−3.6 ± 1.4	−68.4	<0.001	−10.7 ± 0.4	−5.0 ± 1.3	−53.3	<0.001

[a] Paired *t* test.

sorbed 35,011 mg of cholesterol and Subject 2 absorbed 33,765 mg cholesterol from the diet. The plasma cholesterol was elevated as the result of the intake of dietary cholesterol. Considering the plasma volume, we calculated the mass change of cholesterol in the plasma before and after cholesterol feeding (39). The increase of plasma cholesterol was 2,433 mg for Subject 1; this accounted for 6.9% of the absorbed cholesterol. For Subject 2, this comparable figure was 1292 mg or 3.8% of the absorbed dietary cholesterol.

In response to the absorbed exogenous cholesterol, the daily production of endogenous cholesterol decreased. If we assume that the rate of synthesis calculated during the last 5 weeks (the steady state period) of each dietary period (control and cholesterol feeding) is representative of each entire dietary period, the total decrease in cholesterol synthesis in the 11 weeks of the high cholesterol feeding was calculated to be 26,180 mg for Subject 1 and 28,108 mg for Subject 2. This reduction in synthesis amounted to 74.5% and 83.2% of absorbed dietary cholesterol for Subjects 1 and 2, respectively.

To calculate the changes in fecal steroid excretion (neutral steroids and bile acids) after feeding the high cholesterol diet, we used the mean excretion values during the last 5 weeks of the control period as baseline and compared it to the excretion during the 11 weeks of moderately high cholesterol diet period. For

TABLE 6. Sterol economy of the human body subjected to a daily intake of 1000 mg cholesterol for 11 weeks

	Cholesterol Balance	
	Subject 1	Subject 2
Absorbed dietary cholesterol	35,011 mg	33,765 mg
Increased plasma cholesterol	2,433 mg	1,292 mg
Decreased endogenous synthesis[a]	26,180 mg	28,105 mg
Changes in fecal neutral steroids[b]	2,583 mg (decreased)	2,653 mg (increased)
Increased fecal bile acids	4,823 mg	2,275 mg
Increased total fecal steroids excretion	2,240 mg	4,928 mg
Overall balance (or tissue storage)	+4,158 mg	−560 mg

[a] Assume that the synthetic rate of cholesterol measured at steady state (last 5 weeks) represents that of entire dietary period.

[b] Figure derived by comparison of actual total fecal steroid excretion (neutral steroids and bile acids) in entire 11 weeks of cholesterol feeding with that of baseline (mean of last 5 weeks of control period).

Subject 1, there was 4823 mg greater fecal bile acid excretion during the 11 weeks of consumption of high cholesterol diet compared with the baseline dietary period. For Subject 2, the increase in fecal bile acids excretion was 2275 mg. In regard to endogenous neutral steroid excretion, Subject 1 had 2583 mg less excretion than in the control period while Subject 2 had 2653 mg more. The total increase in excretion (the net sum of neutral steroids and bile acid excretion) therefore was 2240 mg (4823 mg − 2583 mg) for Subject 1 which equals 6.3% of the absorbed cholesterol and 4928 mg (2275 mg plus 2653 mg), or 14.5% of the absorbed cholesterol for Subject 2.

Therefore, the absorbed cholesterol for the 11 weeks of feeding was completely accounted for by the changes of the above metabolic parameters; namely, increased cholesterol in plasma compartment, decreased endogenous synthesis of cholesterol and an increase of fecal excretion in Subject 2. However, in the type II hypercholesterolemic Subject 1 with xanthomas, there was 4158 mg of the absorbed cholesterol or 14.5% of total absorbed cholesterol not accounted for. This may have been stored in the tissues of the body as will be discussed subsequently.

DISCUSSION

Sterol balance techniques require a metabolic steady state before the results are meaningful. A metabolic steady state exists when the body weight, plasma cholesterol levels, and fecal steroid excretion are all relatively constant (40). Only then will cholesterol synthesis equal the cholesterol balance (intake minus excretion). To ensure the establishment of steady state in experimental subjects during both control and experimental periods, we carried out a feeding study which totaled 21–25 weeks. This study provided data from what appears to be the longest sterol balance study ever conducted in humans.

The results demonstrate that, when cholesterol is incorporated into the diet of human beings, the body responds with multiple defense mechanisms to compensate for the ingested cholesterol. These mechanisms might include incomplete cholesterol absorption as has been stressed previously (22, 23), reduction in cholesterol synthesis, and increase in fecal steroid excretion (bile acids and/or neutral steroids). By these compensatory processes, the body can limit somewhat the expansion of its cholesterol pools. However, in spite of these compensatory mechanisms, to be discussed subsequently, the plasma cholesterol concentrations in this long term study increased 53% in the hypercholesterolemic subject and 35% in the normocholesterolemic subject. These results, therefore, further attest to the important role of dietary cholesterol in influencing plasma cholesterol levels in human beings.

The major increase in plasma cholesterol was in the LDL fraction of the plasma lipoproteins of these subjects fed a high-cholesterol diet. The HDL cholesterol level was also slightly increased in both subjects, although it was not statistically significant. Consequently, the LDL cholesterol/HDL cholesterol ratio increased from 3.34 ± 0.36 to 4.69 ± 0.35 ($P < 0.025$) for Subject 1 and 2.04 ± 0.24 to 2.32 ± 0.54 ($P < 0.2$) for Subject 2. Based on the data of the Framingham study, the ratio of LDL cholesterol to HDL cholesterol could be considered as an important index of the risk for coronary heart disease (41). Thus, the atherogenicity of the plasma lipoproteins is enhanced by the high cholesterol diet.

An important defense mechanism against the overloading effect of dietary cholesterol has been stated to be the incomplete absorption of dietary cholesterol by the human intestine. We did not find this to be the case at all in our study. In our subjects, cholesterol absorption in percentages was no different for Subject 1 and was slightly decreased for Subject 2 during the moderately high cholesterol diet than during the low cholesterol diet. In spite of a slight decrease in percentage of absorption, there were large increases in mass absorption. Therefore, we would suggest that cholesterol absorption proved not to be a defense against dietary cholesterol overloading at an intake of 1000 mg per day, which is at the upper limit of ingestion by most Americans. The amounts of cholesterol absorbed into the body were 455 mg/dl for Subject 1 and 439 mg/dl for Subject 2, not inconsequential amounts and hardly representing a barrier brought into play by the high cholesterol diet. As will be emphasized, the two defense mechanisms seen in our study were inhibitions of cholesterol biosynthesis and increased bile acid excretion.

In a short perfusion study (10 to 15 hr) with triple lumen tube, Grundy and Mok (42) found that a significant amount of isotope exchange occurred in the upper intestine. The effect of isotope exchange in our longer term (days) absorption test is not certain. However, in view of the quantitative recovery of β-[4-^{14}C]-sitosterol, which was administered simultaneously with [1,2-^{3}H]cholesterol, the problem of loss of isotopic cholesterol due to isotopic exchange in our test seems not great unless the intestinal cell selectively held isotopic cholesterol and not isotopic β-sitosterol.

The first protective mechanism observed was the reduction of cholesterol biosynthesis through the feedback inhibition by absorbed cholesterol. The rates

of cholesterol synthesis of our two subjects eating the very low cholesterol diet were 502 ± 18.6 and 700 ± 27.6 mg/day, respectively. These figures are in agreement with other reported values (43). During the moderately high cholesterol dietary period, our subjects only synthesized 162 and 335 mg cholesterol daily, 67.7% and 52.1% reductions in cholesterol production. Early studies in which isotopic techniques were used indicated that the specific activities of plasma cholesterol were always much lower than those of dietary cholesterol despite the prolonged feeding of a high isotopic cholesterol diet (22, 23). The investigators in those studies, therefore, could not confirm that significant feedback control was operative in man and suggested that limited absorption of dietary cholesterol was the important mechanism protecting human beings from hypercholesterolemia. In 1969, using the sterol balance technique, Grundy, Ahrens, and Davignon (44) found no feedback inhibition in one subject who was given 1.5 gm cholesterol daily. However, they did detect an increase in cholesterol synthesis in four subjects when their absorption of cholesterol was reduced by feeding large amounts of plant sterols, thus suggesting indirectly the existence of a negative feedback mechanism in man, but only operative under the unphysiological condition of plant sterol ingestion in very large quantities. Subsequently, investigators from this same group reported that there was great patient-to-patient variability in response to cholesterol feeding (26). Feeding these subjects 250–4000 mg cholesterol daily for 2 to 10 weeks after a very low cholesterol background diet, they found that feedback inhibition was demonstrable in some subjects. Nestel and Poyser (27) increased the cholesterol content of the diets of nine subjects from 100–300 mg per day to 550–890 mg per day for 4 to 6 weeks. A mean reduction of cholesterol synthesis of 22% (2%–53%) was also observed. From the evidence of these and from our studies, all performed with different experimental designs and for different periods of time, it seems clear that feedback inhibition of cholesterol biosynthesis after the feeding of dietary cholesterol is an important regulator of cholesterol metabolism in human beings. Even so, the amount of feedback control, 52 to 68% shutback in our studies, was less than that observed in many experimental animals in which over 90% inhibition after the feeding of dietary cholesterol has been observed (28, 29).

The second important metabolic response of our human subjects from the feeding of dietary cholesterol was an increased excretion of bile acid in the feces. Increased excretion of bile acids upon cholesterol feeding has also been observed in the rat and the dog (3, 45). Cholesterol feeding has been shown to increase bile acid synthesis in these animals (45–47). In the rat, both increased fecal bile acid and increased activity of cholesterol-7α-hydroxylase activity has been observed to occur simultaneously (47). It has been proposed that the increase of cholesterol-7α-hydroxylase activity from cholesterol feeding resulted from the increase in pool size of the substrate (48). However, increased bile acid excretion in man upon cholesterol feeding has not been stressed in previous reports. In the studies of Quintao, Grundy, and Ahrens (26) and Nestel and Poyser (27), increased bile acid excretion was not a consistent response in their human subjects. Instead, they observed the elevated excretion of endogenous neutral steroids, which we did not find in the two subjects of the present study. The reasons for these different results are not clear, but perhaps shorter time periods are responsible.

One of the major purposes of this study was to contrast the responses of normocholesterolemic and hypercholesterolemic subjects to a similar challenge of dietary cholesterol. The compensatory mechanisms were, if anything, more active in the hypercholesterolemic subject: greater inhibition of cholesterol synthesis and greater bile acid excretion, yet her plasma cholesterol increased 52.5% versus only 35.8%. Dietary cholesterol seemed to enhance further the basic metabolic error of this patient to increased LDL cholesterol concentrations. As has been alluded to previously, hypercholesterolemic patients are unusually sensitive to dietary cholesterol and respond dramatically to its restriction (49). It seems unlikely that these differences could be accounted for on the basis of sex and age differences.

Not only was the increase in plasma levels in response to cholesterol different, the time required for the increased plasma cholesterol level to reach its plateau was also dissimilar. The normocholesterolemic subject reached a steady state more rapidly than the type II hypercholesterolemic patient (2–3 weeks versus 6–7 weeks). The same phenomenon has been observed in hypocholesterolemic and hypercholesterolemic monkeys (50). This difference may result from a larger cholesterol pool of the hypercholesterolemic animal or human which requires a longer time for equilibration.

When overloaded with dietary cholesterol, the ability of these two subjects to minimize the expansion of their body cholesterol pool was also different. Taking into consideration the changes in plasma cholesterol level, cholesterol absorption, cholesterol

synthesis, and excretion upon cholesterol feeding, we estimated the overall sterol balance of the two subjects consuming 11 weeks of high cholesterol diet (Table 6). The normocholesterolemic Subject 2 was able to avoid the expansion of tissue cholesterol pool by compensatory measures. The accumulation of cholesterol in the body pools after cholesterol feeding was small. However, Subject 1, the hypercholesterolemic subject, had 4 g of absorbed cholesterol unaccounted for. This was presumably stored in the tissues, perhaps in the xanthelasmas and extensor tendor xanthomas of the hand, visible evidences of accumulation of cholesterol in the tissues, or elsewhere (arteries, connective tissue, etc.). Quintao, Brumer, and Stechharker (51) have also reported that an increase of tissue cholesterol occurred in some individuals after cholesterol feeding. The storage of cholesterol in the tissues of hypercholesterolemic patients defines also their major disease complication, atherosclerosis, an arterial wall reflection of tissue cholesterol storage.

Supported by U.S. Public Health Service Grants HL-19130 and HL-16336 from the National Heart and Lung Institute and by the General Clinical Research Centers Program (RR-334 and RR-59) of the Division of Research Resources of the National Institutes of Health.

Manuscript received 14 January 1980, in revised form 19 March 1980, and in re-revised form 27 May 1980.

REFERENCES

1. Chanutin, A., and S. Ludewig. 1933. The effect of cholesterol ingestion on tissue lipids of rats. *J. Biol. Chem.* **102:** 57–65.
2. Morris, M. D., I. L. Chaikoff, J. M. Felts, S. Abraham, and N. O. Fansch. 1957. The origin of serum cholesterol in the rat: diet versus synthesis. *J. Biol. Chem.* **224:** 1039–1045.
3. Wilson, M. D. 1964. The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: the influence of dietary cholesterol. *J. Lipid Res.* **5:** 409–417.
4. Wang, C. I., L. Strauss, and D. Aalersberg. 1957. Experimental xanthomatosis in the rabbit. *Arch. Pathol.* **63:** 416–422.
5. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. The serum lipids in men receiving high cholesterol and cholesterol-free diets. *J. Clin. Invest.* **40:** 894–901.
6. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. The effect of dietary cholesterol upon the serum lipids in man. *J. Lab. Clin. Med.* **57:** 331–342.
7. Connor, W. E., D. B. Stone, and R. E. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. *J. Clin. Invest.* **43:** 1691–1696.
8. Beveridge, J. M. R., W. F. Connell, A. Mayer, and H. L. Haust. 1960. The response of man to dietary cholesterol. *J. Nutr.* **71:** 61–65.
9. Steiner, A., E. Howard, and S. Akgun. 1962. Importance of dietary cholesterol in man. *J. Am. Med. Assoc.* **181:** 186–190.
10. Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. *Am. J. Clin. Nutr.* **25:** 589–594.
11. Erikson, B. A., R. H. Coots, F. H. Mattson, and A. M. Kligman. 1964. The effect of partial hydrogenation of dietary fat, of the ratio of polyunsaturated to saturated fatty acids, and dietary cholesterol upon plasma lipids in man. *J. Clin. Invest.* **43:** 2017–2025.
12. Connor, W. E., and D. S. Lin. 1974. The intestinal absorption of dietary cholesterol by hypercholesterolemic (type II) and normocholesterolemic humans. *J. Clin. Invest.* **53:** 1062–1070.
13. Kudchodkor, B. J., H. S. Sodhi, and L. Horick. 1973. Absorption of dietary cholesterol in man. *Metabolism.* **22:** 155–163.
14. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. An evaluation of four methods for measuring cholesterol absorption by the intestine in man. *J. Lipid Res.* **12:** 221–232.
15. Borgström, B. 1969. Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope-labeled meal. *J. Lipid Res.* **10:** 331–337.
16. Taylor, C. B., and R. G. Gould. 1950. Effect of dietary cholesterol on rate of cholesterol synthesis in the intact animal measured by means of radioactive carbon. *Circulation.* **2:** 467–468.
17. Gould, R. G. 1953. Cholesterol metabolism. I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue in vitro. *J. Biol. Chem.* **201:** 519–528.
18. Tomkins, G. M., H. Sheppard, and I. Chaikoff. 1953. Cholesterol synthesis by the liver. III. Its regulation by ingested cholesterol. *J. Biol. Chem.* **201:** 137–141.
19. Frantz, J. D., Jr., H. S. Schneider, and B. T. Hinkelman. 1954. Suppression of hepatic cholesterol synthesis in the rat by cholesterol feeding. *J. Biol. Chem.* **206:** 465–469.
20. Bricker, L. A., H. J. Weis, and M. D. Siperstein. 1972. In vivo demonstration of the cholesterol feedback system by means of a desmosterol suppression technique. *J. Clin. Invest.* **51:** 197–205.
21. Bhattathiry, E. P. M., and M. D. Siperstein. 1963. Feedback control of cholesterol synthesis in man. *J. Clin. Invest.* **42:** 1613–1618.
22. Wilson, M. D., and C. H. Lindsey. 1965. Studies on the influence of dietary cholesterol on cholesterol metabolism in isotopic steady state in man. *J. Clin. Invest.* **44:** 1805–1814.
23. Kaplan, J. A., G. E. Cox, and C. B. Taylor. 1963. Cholesterol metabolism in man. *Arch. Pathol.* **76:** 359–368.
24. Taylor, C. B., B. Mikkelson, J. A. Anderson, and D. T. Forman. 1966. Human serum cholesterol synthesis measured with the deuterium label. *Arch. Pathol.* **81:** 213–231.
25. Taylor, C. B., B. Mikkelson, J. A. Anderson, D. T. Forman and S. S. Choi. 1965. Human serum cholesterol synthesis. *Exp. Mol. Pathol.* **4:** 480–488.

26. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effect of dietary cholesterol on regulation of total body cholesterol in man. *J. Lipid Res.* **12:** 233–247.
27. Nestel, P. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. *Metabolism.* **25:** 1591–1599.
28. Taylor, C. B., D. Patton, N. Yogi, and G. E. Cox. 1960. Diet as source of serum cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **103:** 768–772.
29. Morris, M.D., I. L. Chaikoff, J. M. Felts, S. Abraham, and N. D. Fausah. 1957. The origin of serum cholesterol in rat: diet vs. synthesis. *J. Biol. Chem.* **224:** 1039–1045.
30. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. *New Engl. J. Med.* **282:** 1241–1249.
31. Manual of Laboratory Operations, Vol. 1. Lipid and Lipoprotein Analysis. Lipid Research Clinic Program.
32. Mangold, H. K. 1961. Thin-layer chromatography of lipids. *J. Am. Oil Chem. Soc.* **38:** 708–727.
33. Jover, A., and R. S. Gordon, Jr. 1962. Procedure for quantitative analysis of feces with special reference to fecal fatty acids. *J. Lab. Clin. Med.* **59:** 878–884.
34. Connor, W. E., D. T. Witiak, D. B. Stone, and M. L. Armstrong. 1969. The cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48:** 1363–1375.
35. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatography analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
36. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
37. Snedocor, G. W., and W. G. Cochran. 1967. *Statistical Methods.* Iowa State University Press, Ames, Iowa. 91–95.
38. Grundy, S. M., E. H. Ahrens, Jr., and F. Salen. 1968. Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9:** 374–387.
39. Edelman, I. S., and J. Liebman. 1959. Anatomy of body water and electrolytes. *Am. J. Med.* **27:** 256–277.
40. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis and absorption in man, carried out by isotopic kinetic and sterol balance method. *J. Lipid Res.* **10:** 91–107.
41. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am. J. Med.* **62:** 707–714.
42. Grundy, S. M., and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *J. Lipid Res.* **18:** 263–271.
43. Grundy, S. M., A. L. Metzger, and R. D. Adler. 1972. Mechanisms of lithogenic bile formation in American Indian women with cholesterol gallstones. *J. Clin. Invest.* **51:** 3026–3042.
44. Grundy, S. M., E. H. Ahrens, Jr., and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. *J. Lipid Res.* **10:** 304–315.
45. Pertsemlidis, D., E. H. Kirchman, and E. H. Ahrens, Jr. 1973. Regulation of cholesterol metabolism in the dog. I. Effects of complete bile diversion and of cholesterol feeding on absorption, synthesis, accumulation and excretion rates measured during life. *J. Clin. Invest.* **52:** 2352–2367.
46. Abell, L. L., E. H. Mosbach, and F. E. Kandall. 1956. Cholesterol metabolism in the dog. *J. Biol. Chem.* **220:** 527–536.
47. Raicht, R. F., B. I. Cohen, S. Shefer and E. H. Mosbach. 1975. Sterol balance studies in the rat. Effects of dietary cholesterol and β-sitosterol on sterol balance and rate-limiting enzymes of sterol metabolism. *Biochim. Biophys. Acta.* **388:** 374–384.
48. Mitropoulos, K. A., S. Balasubramaniam, and N. B. Myant. 1973. The effect of interruption of the enterophepatic circulation of bile acids and of cholesterol feeding on cholesterol 7-hydroxylase in relation to the diurnal rhythm in its activity. *Biochim. Biophys. Acta.* **326:** 428–438.
49. Carter, G. A., W. E. Connor, A. K. Bhattacharyya, and D. S. Lin. 1979. The cholesterol turnover synthesis, and absorption in two sisters with familial hypercholesterolemia (Type IIa). *J. Lipid Res.* **20:** 66–77.
50. Henderson, G. R., and R. W. St. Clair. 1977. Change in plasma cholesterol and bile specific activity associated with cholesterol feeding in hypo (HO) and hyper responsive (HP) African green monkeys. *Federation Proc.* **36:** 1105. (Abstract).
51. Quintao, E., S. Brumer, and K. Stechharker. 1977. Tissue storage and control of cholesterol metabolism in man on high cholesterol diets. *Atheroslerosis.* **26:** 297–310.