Effects of dietary cholesterol on the regulation of total body cholesterol in man

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ABSTRACT Studies on the interaction of cholesterol absorption, synthesis, and excretion were carried out in eight patients using sterol balance techniques. Absorption of dietary cholesterol was found to increase with intake; up to 1 g of cholesterol was absorbed in patients fed as much as 3 g per day.

In most patients, increased absorption of cholesterol evoked two compensatory mechanisms: (*a*) increased reexcretion of cholesterol (but not of bile acids), and (*b*) decrease in total body synthesis. However, the amount of suppression in synthesis was extremely variable from one patient to another; one patient had no decrease in synthesis despite a large increment in absorption of dietary cholesterol, and two patients showed a complete suppression of synthesis.

In the majority of cases the accumulation of cholesterol in body pools was small because of adequate compensation by reexcretion plus reduced synthesis, but in a few patients large accumulations occurred on high cholesterol diets when absorption exceeded the compensatory mechanisms. These accumulations were not necessarily reflected in plasma cholesterol levels; these increased only slightly or not at all.

SUPPLEMENTARY KEY WORDS cholesterol homeostasis · compensatory mechanisms · sterol balance studies · absorption · excretion · storage · accumulation · pool sizes · feedback control · biliary cholesterol flux

TOTAL BODY cholesterol must be regulated by the interaction of three factors: absorption, synthesis, and excretion (or conversion into bile acids). In 1969 we reported (1) on the interaction of cholesterol absorption and synthesis and demonstrated that the total daily synthesis of cholesterol is related to the amount of cholesterol absorbed. In that report it was shown that interruption of the enterohepatic circulation of cholesterol through inhibition of absorption caused a reduction in plasma cholesterol, but this reduction was limited by a compensatory increase in cholesterol synthesis. Thus, feedback inhibition of cholesterol synthesis was released when the absorption of cholesterol was blocked. Although this study demonstrated that cholesterol synthesis in man is under feedback regulation by cholesterol itself, we did not determine how much dietary cholesterol can be absorbed or the extent to which this newly absorbed cholesterol can suppress synthesis. In the accompanying report (2) four methods for measuring cholesterol absorption are critically evaluated; these studies provide the basis for the investigations described in the present report.

In the present clinical studies we have extended our previous work by examining the following questions: What are the limits of cholesterol absorption in man? If the feeding of dietary cholesterol enhances the total absorption of cholesterol, how does the body compensate in order to reestablish a new steady state? Is synthesis inhibited by newly absorbed cholesterol? Is this excess cholesterol reexcreted as either neutral steroids or bile acids? Can it be shown that significant amounts of cholesterol accumulate in tissues when patients ingest high cholesterol diets?

The present study provides preliminary answers to some of these questions. Contrary to previous reports (3, 4), the absorption of dietary cholesterol is not always severely limited; indeed, up to 1 g per day can be absorbed when the intake is large. In most patients the

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TABLE 1 CLINICAL DATA

Patient	Initials	Age	Sex	Height	Weight		Calories	Diagnosis
		yr		*cm*	*kg*	*% of ideal**		
1	R.W.	54	F	155	79.5	132	2200†	Hypercholesterolemia, IHD‡
2	Y.R.	55	F	146	58.9	107	1800	Hypercholesterolemia and endogenous hyperglyceridemia, xanthomatosis, IHD
3	E.D.	59	F	145	43.4	77	1600	Hypercholesterolemia, xanthomatosis, IHD
4	A.R.	56	F	163	60.8	92	2150	Hypercholesterolemia and endogenous hyperglyceridemia, xanthomatosis, IHD
5	F.L.	67	F	156	65.0	105	2160	Hypercholesterolemia, xanthomatosis, IHD
6	W.P.	41	M	176	70.0	91	2840	Normolipidemia, IHD
7	J.C.	14	M	167	47.0	96	2115	Hypercholesterolemia (type II)§
8	S.C.	18	F	162	49.0	93	2000	Hypercholesterolemia (type II)

* According to life insurance table (5).
† Needed to maintain constant body weight.
‡ IHD, ischemic heart disease.
§ Typing of hyperlipoproteinemias according to Fredrickson, Levy, and Lees (6).

accumulation of cholesterol in tissue compartments is prevented through inhibition of endogenous synthesis and through reexcretion of excess cholesterol as neutral steroids. However, in occasional patients in whom synthesis is *not* inhibited, large amounts of cholesterol can be accumulated in the body. At present we are not able to distinguish one group from the other on clinical grounds, nor on the basis of the clinical laboratory tests in common use today, but clearly the ability to make this distinction deserves a high priority in future work.

METHODS

Patients

Studies were carried out on eight patients during hospitalization on a metabolic ward at The Rockefeller University Hospital, where balance studies lasting 6 to 19 wk were carried out. The age, sex, body build, caloric intake and clinical diagnosis of each patient are presented in Table 1. There were six females and two males, aged 14–67 yr; in only one case was body weight greater than 120% of normal. All but one patient had elevated plasma lipids on solid food diets; five had ischemic heart disease. Studies on absorption methodology in patients 1–5 are described in the accompanying report (2).

Diets

During all studies patients were maintained at constant body weight by orally administered liquid formula feedings as previously described (7, 8); dietary fat contributed 40%, protein 15%, and glucose 45% of the total caloric intake. The basic compositions of the two diets used are presented in Table 2. The dietary fat

TABLE 2 FORMULA DIETS USED AND THEIR STEROL CONTENTS

Diet	Dietary Fat	Cholesterol	Total Plant Sterols	β-Sitosterol	Source of Plant Sterols
		mg/1000 cal			
A	Cottonseed oil	12	160	142	Inherent
B	Corn oil (molecularly distilled)	12	124	82	Inherent

was cottonseed oil (diet A) for patients 1–6, and corn oil for patients 7 and 8. Plant sterols were inherent in both fats; the corn oil used was low in plant sterols because it had been submitted to molecular distillation (by Distillation Products Industries, Rochester, N.Y.) and steam deodorization (by E. F. Drew Co., Boonton, N.J.), processes which reduce the sterol content without impairing palatability. Crystalline cholesterol (Mann Research Laboratories Inc., New York) was added to the oil phase during formula preparation in order to obtain different levels of intake of cholesterol.

Isotopic Sterols

Cholesterol-1,2-^{3}H and cholesterol-4-^{14}C were obtained from New England Nuclear Corp., Boston, Mass. Radioactive sterols were purified by thin-layer chromatography on Florisil with ethyl ether–heptane 45:55 (v/v); about 4% of radioactivity remained at the origin in all cases, and only that material that chromatographed with the same R_F as the pure sterol standard was administered to patients. Patients 1–4 were given cholesterol-4-^{14}C daily in every formula feeding in order to attain an isotopic steady state; the labeled sterol in 5 ml of ethanol was dissolved in the oil phase during formula

preparation. Aliquots of formula sampled after storage at −15°C were found to be homogeneous in regard to sterol content and specific activity. For intravenous administration, radioactive material in 1 ml of ethanol was dissolved in 150 ml of saline and immediately infused intravenously.

Concentrations and specific activities of plasma cholesterol were determined twice weekly: concentrations by the method of Block, Jarrett, and Levine (9) on the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y.), and radioactivities on aliquots of the same plasma extracts in a Packard Tri-Carb scintillation counter (model 3003), as previously described (10).

Analysis of Fecal Steroids

Fecal neutral and acidic steroids were isolated separately from 4-day pools; their mass and radioactivity were measured by methods developed in this laboratory (10, 11). These isolation procedures permit the distinction to be made between plant sterols and cholesterol and their respective bacterial conversion products. β-Sitosterol was used as an internal standard to correct for losses of cholesterol during intestinal transit as well as for variations in fecal flow. Chromic oxide was employed as an internal standard to correct for fecal flow variations in bile acid excretion. The rationale for the use of these two markers in sterol balance studies has been presented previously (12–14).

Percentage recoveries in the feces of the two markers for each period of study are presented in Table 3. Patients 1 and 5 were nonideal excretors of chromic oxide according to criteria described in another publication from this laboratory (12). However, we justify the inclusion of these cases on the basis that losses of β-sitosterol and chromic oxide were very similar in all samples analyzed, as if the losses were entirely mechanical and not metabolic in origin. In this study the losses of β-sitosterol during intestinal transit were trivial in three of six patients; in the other three the losses relative to the recovery of chromic oxide ranged from 13% to 26%.

Measurement of Cholesterol Absorption

Daily absorption of exogenous cholesterol was measured by two methods previously described. Method I (equations 10 and 11, Ref. 15) makes use of data obtained after administration intravenously of a single dose of radioactive cholesterol, while Method II (equations 11, 15, and 16, Ref. 15) utilizes data obtained after continuous oral labeling. The similarity in results obtained by these two procedures was documented previously (15) and in the accompanying report (2).

Measurement of Biliary Flow of Cholesterol

The hourly flow of biliary cholesterol was determined on patient 6 on four occasions; this method has recently been described (16). The procedure required intubation with a double-lumen tube through which a liquid formula containing 40% of calories as cottonseed oil was infused at a constant rate into the second portion of the duodenum, along with cholesterol-1,2-^{3}H as an internal standard. The total amount of formula infused per 24 hr was the same as that needed to maintain constant body weight in the weeks preceding the test. A sample of the mixture of infusate plus intestinal contents was withdrawn at a constant rate from a site 10 cm distally; less than 5% of the total flow passing this point was removed from its enterohepatic circulation. The hourly flow of biliary cholesterol was determined according to the following equation: Biliary cholesterol (mg/hr) = rate of infusion of cholesterol-1,2-^{3}H (dpm/hr) ÷ specific activity

TABLE 3 INTAKES OF STEROLS AND INTERNAL STANDARDS

Patient		1	2	3	4	5	6	7	8
Sterols fed (cholesterol/β-sitosterol in mg/day)	Period I	46/324	38/275	30/217	43/306	44/310	57/404	55/271*	52/256*
	II	2936/306	2471/263	2119/235	542/296	241/284	2359/390	4058/289	4053/256
	III	43/306	—	33/235	—	909/270	—	—	—
Percentage recovery of internal standards (β-sitosterol/chromic oxide)	I	92/84	79/111	97/91	83/?	65/75	88/100	93*	87*
	II	70/78	85/111	93/91	76/93	70/59	77/88	96	112
	III	87/94	—	67/83	—	66/69	—	—	—
	Avg	83/85	82/111	86/88	76/93	64/68	82/94	—	—
Percentage losses of β-sitosterol relative to recovery of chromic oxide		2.3	26.1	2.2	18.2	5.8	12.7	—	—
Radioactive sterol administration									
Continuous oral dosage in period II (μCi/day)		0.94†	0.79†	0.68†	1.60†	—	—	—	—
Single intravenous dose at start of period I		—	—	—	—	108†	130‡	—	—

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

† Cholesterol-4-^{14}C.

‡ Cholesterol-1,2-^{3}H.

of withdrawn endogenous cholesterol (dpm/mg). When the diet was free of cholesterol, the cholesterol withdrawn was entirely of endogenous origin; its mass was measured by gas–liquid chromatography. When the diet contained cholesterol, the mass of endogenous cholesterol was measured isotopically: the patient was prelabeled with a single intravenous injection of cholesterol-4-^{14}C, and biliary flow studies were carried out several weeks later, at which time the specific activities of plasma cholesterol and biliary cholesterol were shown to be identical. The mass of endogenous biliary cholesterol was estimated as follows:

$$\text{Endogenous biliary cholesterol (mg)} = \frac{^{14}\text{C radioactivity withdrawn (dpm)}}{^{14}\text{C specific activity of plasma cholesterol (dpm/mg)}}$$

A more detailed description of these procedures will be published elsewhere.

Experimental Design

In this investigation, cholesterol balance studies were carried out during periods of low and high intakes of cholesterol (Table 4). During the periods of low intakes, base line values were obtained for plasma cholesterol and excretions of neutral and acidic steroids. We have assumed that patients were in a metabolic "steady state" during this period and that values for total steroid excretion were equivalent to daily synthesis of cholesterol. The validity of these assumptions is the subject of a previous report (15).

After this control period, cholesterol was incorporated into the diet of each patient; the amounts of cholesterol were 542–4058 mg/day. In those patients (nos. 1–6) who received isotopic cholesterol it was possible to make continuous estimations of cholesterol absorption throughout the entire period of cholesterol feeding. However, since estimations of cholesterol synthesis by the balance method are valid only in the metabolic "steady state" (15), we divided the periods of high cholesterol intake into subperiods, *A* ("nonsteady state") and *B* ("steady state"). This subdivision was not entirely arbitrary: by setting off at least three of the final 7- to 8-day stool collection periods as subperiod B, we were assured of sufficient numbers of sterol balance data with small enough variations to permit separate statistical treatment during the new metabolic "steady state." These subperiods B were 22–29 days in duration, whereas subperiods A were 12–54 days. In subperiod B, cholesterol synthesis was calculated as the difference between cholesterol intake and the excretion of all products of cholesterol. (Sterol balance data obtained in the first 7 or 8 days after introducing the high cholesterol diet were routinely omitted from the calculations in Table 4, since in no case did we administer a marker such as carmine in order to discriminate clearly the excreta formed thereafter from those of the preceding period.)

Patients 7 and 8 received no radioactive cholesterol; thus, in these patients cholesterol absorption could not be determined. Moreover, the period of high cholesterol intake was not sufficiently long to divide into subperiods A and B; therefore, in these two experiments it was possible to measure changes in total cholesterol balance, but not in synthesis.

RESULTS

Fig. 1 presents the individual sterol balance studies in patients 1–6 during varying intakes of cholesterol, with a summary of numerical data in Table 4. The duration of the balance period in each patient is shown, as well as plasma cholesterol level, intake and absorption of dietary cholesterol, excretion of endogenous neutral and acidic steroids, and total cholesterol turnover and balance.

Fig. 2 presents the balance data for patients 7 and 8, who were siblings. Since no labeled cholesterol was administered to these two patients, the fecal neutral steroids could not be subdivided into endogenous and exogenous fractions, and hence, cholesterol absorption could not be estimated; nevertheless, the total balance (intake minus excretion) for cholesterol is of considerable interest in these two patients, as discussed below.

What is most impressive in these data is the patient-to-patient variability in response to the imposition of a high cholesterol intake. Plasma cholesterol concentrations rose in five patients, and fell or remained essentially unchanged in three. Cholesterol absorption rates were from about 300 to 1000 mg/day. Total sterol balances became strongly positive in two patients, approached zero in two, decreased significantly in three, and remained unchanged in one. These first impressions indicate how highly individual the interactions between absorption, synthesis, and plasma levels can be. The following sections examine these relationships in greater detail.

A. Cholesterol Absorption

Since cholesterol enters the intestinal tract from both dietary and endogenous sources, the interaction of one input on the other must be considered in any quantitative evaluation of cholesterol absorption. In these paragraphs we will examine the possibility that different levels of exogenous cholesterol may have various effects on the reabsorption of endogenous cholesterol and hence affect the net absorption of cholesterol by the intestine.

In patients 1–6 the absorption of dietary cholesterol was measurable only in period II when the diet contained appreciable quantities of cholesterol (Table 4).

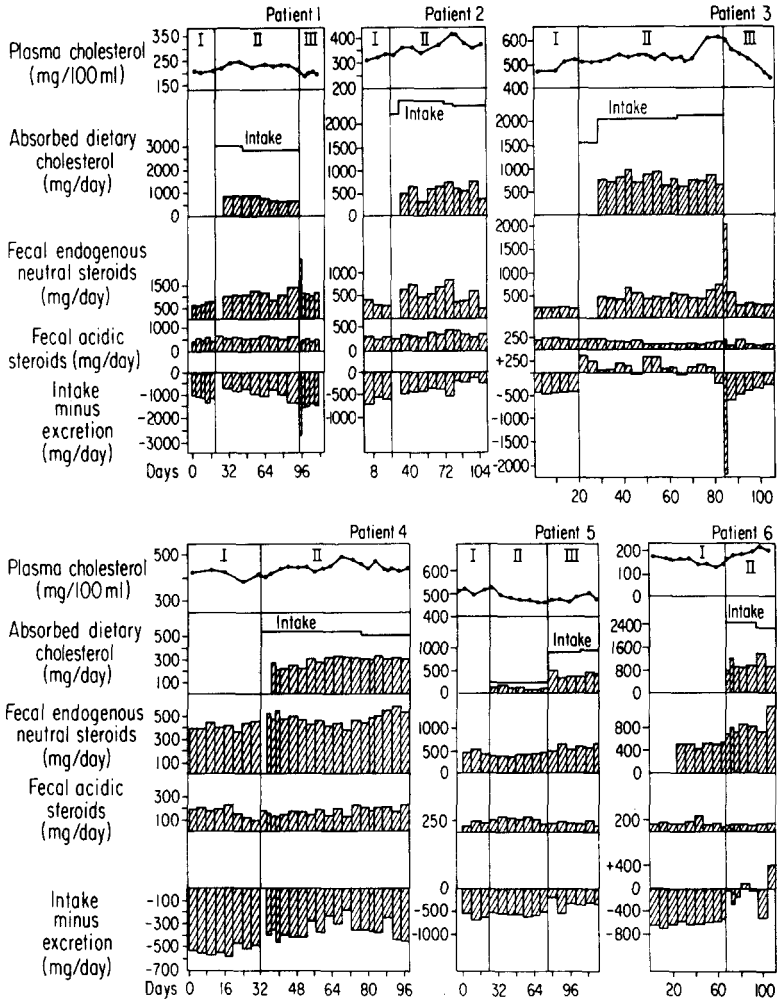

FIG. 1. Sterol balance data on different intakes of cholesterol in patients 1–6. In period I the diets were cholesterol-free; in period II the diets were rich in cholesterol. Total sterol balance (intake minus excretion) was unchanged in patient 1, signifying a complete lack of feedback control of cholesterol synthesis; balances were decreased significantly in patients 2, 4, and 5, and approached zero in patients 3 and 6. By contrast, plasma cholesterol levels rose in five patients, but fell in one. Absorption was measured by administration of radioactive cholesterol (Methods I and II, Ref. 2).

During this period of cholesterol feeding patient 4 received an amount that is typical of the average American diet (542 mg/day); she absorbed an average of 292 mg/day, or 53% of the intake. Patient 5 was studied at two levels of intake, 241 and 915 mg/day, from which she absorbed an average of 113 and 407 mg/day, respectively. In this patient, the absolute amount of cholesterol absorbed at the higher level of intake was clearly greater than at lower intakes. Patients 1, 2, 3, and 6 received much larger amounts of dietary cholesterol (2119–2936 mg/day), and values for absorption were 568–1028 mg/day. These studies in six different patients suggest that increased quantities of cholesterol in the diet are associated with increased absorption.

The relationship between daily intake and absorption can be seen more clearly in Fig. 3. Values presented here include the results obtained in the present study and also those in nine patients whose studies have been published previously from this laboratory (1, 2, 15). It is evident that at the lowest intakes at which cholesterol absorption can be measured, there was about 50% absorption; this decreased to 25% at the highest intakes. Up to 1 g of dietary cholesterol could be absorbed per day when the intake was in the range of 2–3 g/day.

The excretion of endogenous neutral steroids increased in every case when cholesterol was fed at more than one dose level (Fig. 1). This increment in endogenous neutral steroid excretion could have been due to (*a*) inhibition in reabsorption of endogenous cholesterol by exogenous cholesterol, or (*b*) reexcretion of absorbed dietary cholesterol. In explanation of the first possibility, the sites for absorption of exogenous cholesterol might be saturated by the endogenous cholesterol presented to the intestine through the biliary

TABLE 4A STEROL BALANCE AND ISOTOPE KINETIC DATA, PATIENTS 1–4

	Patient 1				Patient 2
Period	I	II A + B	II B	III	I
No. of days in each period	24	69	29	16	24
No. of successive stool collections analyzed	5	9	4	4	3
Cholesterol intake (mg/day)	46	2936	2890	43	38
Plasma cholesterol (mg/100 ml ± SD)	200 ± 26	232 ± 12	—	196 ± 7	316 ± 13
No. of determinations	9	18	—	5	7
Percentage change (vs. period I)	—	+16	—	—	—
Dietary cholesterol (dpm/mg)	—	700	700	—	—
Plasma cholesterol (dpm/mg)*	—	160	160	—	—
Fraction of plasma cholesterol derived from absorbed dietary radioactive cholesterol	—	0.228	0.228	—	—
Fecal steroids (mg/day ± SD)†					
Total neutral steroids (NS)	710 ± 164	3338 ± 227	3449 ± 325	1059 ± 108	376 ± 178
Endogenous NS	710 ± 164	1125 ± 187	1167 ± 284	1059 ± 108	376 ± 178
Unabsorbed dietary NS	—	2212 ± 91	2282 ± 52	—	—
Acidic steroids	502 ± 142	576 ± 41	563 ± 35	449 ± 98	283 ± 54
Total steroids	1213 ± 204	3914 ± 222	4013 ± 320	1509 ± 134	659 ± 178
Absorption					
Method I (mg/day ± SD)	—	—	—	—	—
Method II (mg/day ± SD)	—	724 ± 119	607 ± 51	—	—
Percentage absorption	—	25	21	—	—
Cholesterol turnover (mg/day ± SD)	1213 ± 204	1701 ± 188	1730 ± 281	—	659 ± 178
Cholesterol balance (intake − excretion; mg/day ± SD)	−1167	−978	−1123	−1466‡	−621

*Level of specific activity achieved by continuous labeling with radioactive cholesterol administered daily.
† Corrected according to the recovery of the markers, β-sitosterol and chromic oxide (12, 13, 14).
‡ Carmine red was given as a single dose when cholesterol feeding was stopped at the end of period II. Fecal steroids excreted up to the appearance of carmine red *and* as long as any remained visible in the feces were considered to belong to the last collection in period II; thereafter, all stools were ascribed to period III.

TABLE 4B STEROL BALANCE AND ISOTOPE KINETIC DATA, PATIENTS 5–8

	Patient 5				
Period	I	II A + B	II B	III A + B	III B
No. of days in each period	24	42	26	37	21
No. of successive stool collections analyzed	3	6	4	5	3
Cholesterol intake (mg/day)	44	241	234	915	930
Plasma cholesterol (mg/100 ml ± SD)	508 ± 10	477 ± 21	—	475 ± 19	—
No. of determinations	7	15	—	12	—
Percentage change (vs. period I)	—	−6	—	−7	—
Dietary cholesterol (dpm/mg)	—	—	—	—	—
Plasma cholesterol (dpm/mg)*	—	—	—	—	—
Fraction of plasma cholesterol derived from absorbed dietary radioactive cholesterol	—	—	—	—	—
Fecal steroids (mg/day ± SD)†					
Total neutral steroids (NS)	470 ± 102	533 ± 70	547 ± 23	1080 ± 206	1105 ± 87
Endogenous NS	470 ± 102	405 ± 44	416 ± 24	572 ± 116	600 ± 33
Unabsorbed dietary NS	—	128 ± 48	137 ± 12	508 ± 114	508 ± 31
Acidic steroids	157 ± 104	270 ± 106	253 ± 63	188 ± 72	175 ± 42
Total steroids	627 ± 104	803 ± 106	800 ± 63	1268 ± 206	1280 ± 88
Absorption					
Method I (mg/day ± SD)	—	113 ± 32	97 ± 12	407 ± 65	422 ± 52
Method II (mg/day ± SD)	—	—	—	—	—
Percentage absorption	—	47	41	44	45
Cholesterol turnover (mg/day ± SD)	627 ± 104	694 ± 53	670 ± 41	760 ± 69	775 ± 30
Cholesterol balance (intake − excretion, mg/day ± SD)	−583	−562	−566	−353	−350

* Level of specific activity achieved by continuous labeling with radioactive cholesterol administered daily.
† Corrected according to the recovery of the markers, β-sitosterol and chromic oxide (12, 13, 14).

tree: exogenous cholesterol would merely replace endogenous cholesterol at the mucosal surface, and there would be no net increase in absorption. In the second hypothesis, the feeding of cholesterol would result in a greater total absorption of cholesterol (endogenous + exogenous). Providing that this increment in absorption was not exactly counterbalanced by a decrease in endogenous synthesis of equal magnitude, the increment

TABLE 4A *Continued*

Patient 2			Patient 3			Patient 4		
II A + B	II B	I	II A + B	II B	III	I	II A + B	II B
78	24	20	56	28	21	24	59	22
10	3	5	16	8	7	6	15	6
2471	2429	30	2119	2187	33	43	542	529
360 ± 28	—	494 ± 19	536 ± 32	—	513 ± 56	412 ± 27	446 ± 22	—
22	—	6	17	—	4	5	23	—
+14	—	—	+8	—	—	—	+8	—
702	702	—	700	700	—	—	6672	6672
275	268	—	275	275	—	—	980	958
0.393	0.382	—	0.393	0.393	—	—	0.147	0.143
2452 ± 358	2286 ± 112	244 ± 6	1884 ± 390	2020 ± 155	332 ± 190	418 ± 56	738 ± 150	730 ± 39
549 ± 374	405 ± 171	244 ± 6	509 ± 192	521 ± 103	332 ± 190	418 ± 56	478 ± 112	523 ± 48
1903 ± 678	1880 ± 203	—	1374 ± 334	1500 ± 111	—	—	250 ± 96	205 ± 9
350 ± 82	333 ± 30	222 ± 38	150 ± 30	127 ± 24	114 ± 86	172 ± 88	178 ± 62	202 ± 21
2802 ± 380	2619 ± 107	467 ± 38	2033 ± 386	2147 ± 166	446 ± 312	590 ± 80	916 ± 242	932 ± 84
—	—	—	—	—	—	—	—	—
568 ± 147	549 ± 196	—	744 ± 116	688 ± 99	—	—	292 ± 41	314 ± 9
23	23	—	35	31	—	—	54	59
899 ± 239	738 ± 192	467 ± 38	659 ± 108	648 ± 118	—	590 ± 80	653 ± 70	738 ± 41
−331	−190	−437	+86	+40	−413‡	−547	−374	−403

TABLE 4B *Continued*

Patient 6			Patient 7		Patient 8	
I	II A + B	II B	I	II A + B	I	II A + B
44	36	24	23	16	23	16
6	5	3	6	4	6	4
57	2359	2331	55	4058	52	4053
153 ± 11	184 ± 19	—	259 ± 15	246 ± 12	175 ± 4	198 ± 8
17	10	—	5	6	5	7
—	+20	—	—	−4	—	+11
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
531 ± 100	2227 ± 792	2166 ± 460	329 ± 15	2686 ± 172	461 ± 50	2460 ± 135
531 ± 100	897 ± 394	906 ± 240	329 ± 15	—	461 ± 50	—
—	1331 ± 564	1259 ± 298	—	—	—	—
141 ± 96	105 ± 20	103 ± 11	225 ± 66	144 ± 50	124 ± 45	120 ± 27
672 ± 100	2332 ± 792	2269 ± 468	555 ± 77	2830 ± 130	586 ± 74	2580 ± 149
—	1028 ± 221	1072 ± 250	—	—	—	—
—	—	—	—	—	—	—
—	44	46	—	—	—	—
672 ± 100	1002 ± 205	1010 ± 251	555 ± 77	—	586 ± 74	—
−592	+27	+62	−500	+1228	−534	+1373

would be recognized by its reexcretion through the intestinal tract into feces.

Examination of the data for periods I and II in Table 4 suggests that when the diets were free of cholesterol the absorption capacities of these six patients were not saturated by endogenous cholesterol entering the intestine through the biliary tract, since in period II there was a measurable absorption of exogenous cho-

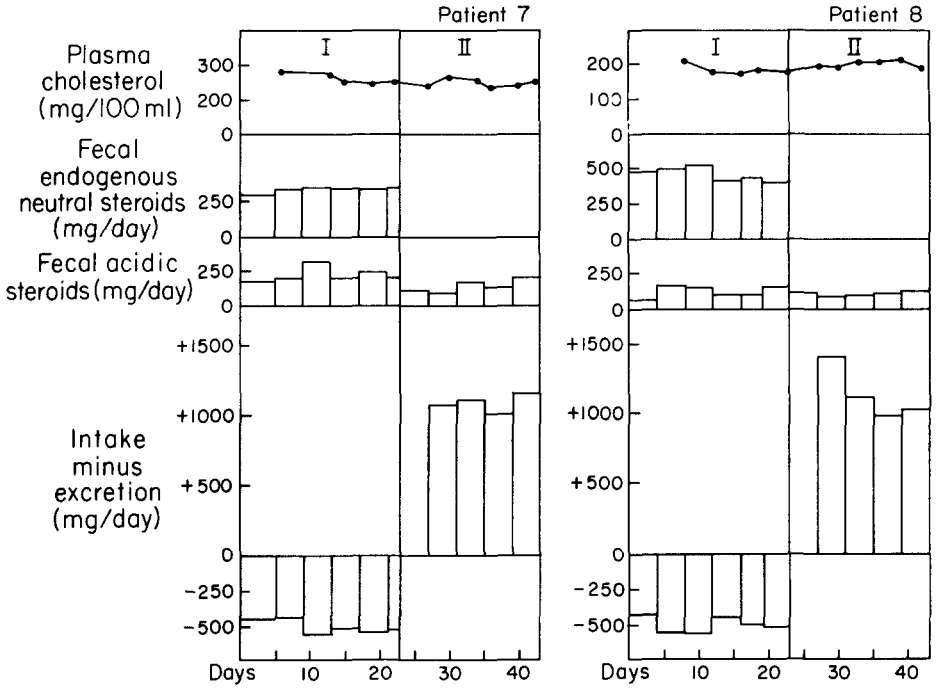

FIG. 2. Sterol balance data in patients 7 and 8, fed 4 g of cholesterol per day in period II after several weeks on a cholesterol-free diet (period I). Radioactive cholesterol was not administered to these patients; hence, in period II, neutral steroids of endogenous origin could not be distinguished from unabsorbed dietary cholesterol, nor could measurements of absorption be made. Nevertheless, the balance data showed a strongly positive balance for both patients throughout period II; plasma levels were essentially unaffected.

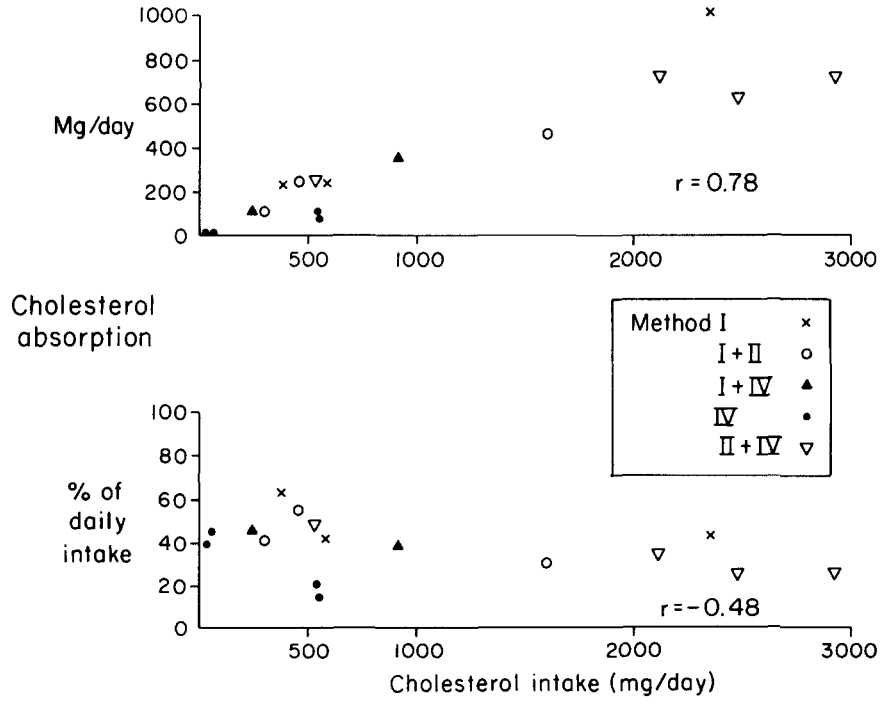

FIG. 3. 16 Determinations of cholesterol absorption by three methods in 15 patients. Upper graph shows absolute absorption; the lower shows percentage absorption. Each point represents an individual patient (means of many determinations are plotted for each patient). Data for Methods I, II, and IV represent all tests carried out in this laboratory up to the present time; those for Method III are not shown here because of failure to attain the isotopic steady state.

lesterol. However, this might have occurred at the expense of the absorption of endogenous cholesterol. What was needed, then, was a measure of the increment in total absorption (endogenous + exogenous).

We calculated these absorption increments (endogenous + exogenous) as follows (1). The increment in total cholesterol absorption for any given portion of period II (compared to period I) = absorbed dietary cholesterol during that time in period II − (fecal endogenous neutral steroids during that time in period II − mean excretion of fecal endogenous neutral steroids throughout period I), all in terms of mg/day. As Fig. 4 shows, there was in every case a definite increment in mean total cholesterol absorption throughout period II. (Figure 4 shows the results of the calculations made for each successive stool-collection period in all six patients.) The summation of these individual increments represents the total increments for total cholesterol absorption throughout period II; in the various patients these totals ranged from 7.3 to 30.8 g. Clearly, then, the absorption capacities of these six patients had not been saturated by reabsorption of endogenous cholesterol in period I. These results do not rule out the possibility that large amounts of exogenous cholesterol may partially inhibit the reabsorption of endogenous cholesterol, or vice versa; indeed, it seems highly probable that some of the increase in excretion of endogenous neutral steroids may be the result of competition between exogenous and endogenous cholesterol for absorption sites.

These results clearly demonstrate that more total cholesterol is absorbed when the diet contains cholesterol, and if some compensatory adjustment does not take place, cholesterol must accumulate in body pools. Either of two mechanisms might be invoked to cope with these increments in absorbed cholesterol: (*a*) reexcretion of cholesterol as neutral steroids or bile acids, or (*b*) reduction in total body synthesis of cholesterol.

B. Reexcretion of Absorbed Dietary Cholesterol

Cholesterol balance studies carried out in rats by Wilson (17) and in dogs by Pertsemlidis, Kirchman, and Ahrens[1] indicated that a significant amount of absorbed dietary cholesterol was excreted by these animals through enhanced formation of bile acids. However, in the present study in man the enhanced absorption of dietary cholesterol produced no statistically significant increases in excretion of acidic steroids (Table 4 and Figs. 1 and 2); similar findings in one patient have previously been reported (1).

By contrast, every patient showed an increased excretion of endogenous fecal neutral steroids, suggesting that absorbed cholesterol is reexcreted through the liver *before* being converted to bile acids. This was tested directly by comparing the rate of cholesterol flux through the biliary tree in patient 6, twice during period I and twice in period II. As shown in Fig. 5, the cumulative 24-hr secretions of cholesterol through the biliary tract were consistently greater when the diet contained cholesterol than when it was cholesterol-free; therefore, a portion of the absorbed cholesterol was reexcreted into the intestinal tract. This is clearly one mechanism by which a steady state in tissue cholesterol pools can be established on diets rich in cholesterol.

C. Cholesterol Synthesis

Although dietary cholesterol was partially reexcreted after institution of cholesterol feeding, the increments in total cholesterol absorption were nevertheless greater than the increments in excretion of fecal endogenous neutral and acidic steroids. Therefore, if synthesis had not been inhibited during period II, cholesterol would have accumulated to the extent shown in Fig. 4.

However, the cholesterol balance data indicate that in most patients the synthesis of cholesterol was indeed depressed by the feeding of dietary cholesterol. During the nonsteady state portion of the second period (IIA), reduction in synthesis could not be differentiated from accumulation of cholesterol; but when the new "steady state" was reached (Period IIB), daily cholesterol synthesis could be calculated as the difference between daily total fecal steroids and daily cholesterol intake (Eq. 9, Ref. 15). Table 4 shows these data. In patient 1 there was no change in net cholesterol balance between periods I and IIB, indicating that absorbed dietary cholesterol did not inhibit synthesis; patients 2, 4, and 5 had a distinct reduction in the negativity of balance in Period IIB, implying that cholesterol synthesis was partially reduced; and in patients 3 and 6 synthesis was apparently inhibited completely. Thus, in only one patient of this series (patient 1) did the feeding of large amounts of cholesterol fail to reduce total synthesis.

D. Accumulation of Body Cholesterol

Two compensatory mechanisms operate in man to prevent accumulation of stored cholesterol: repression of synthesis and reexcretion of absorbed dietary cholesterol in the form of neutral sterols. However, the data obtained from balance measurements also suggest that in some patients these two mechanisms fail to compensate completely for absorption, with the result that tissue pools of cholesterol expand.

Table 5 presents our estimate of the net accumulation of cholesterol during period II in patients 1–6, according to the following equation: Net accumulation of cholesterol in period II (g) = total absorption of dietary

[1] Pertsemlidis, D., E. Kirchman, and E. H. Ahrens, Jr. Unpublished data.

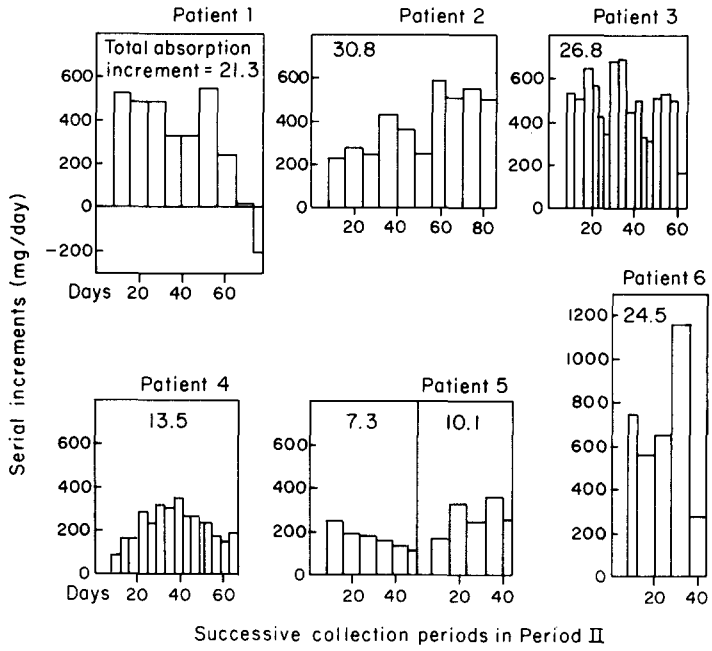

FIG. 4. Serial increments in total cholesterol absorption (exogenous + endogenous) are shown for successive stool collection periods in period II in patients 1–6, compared with the mean absorption of cholesterol in each patient in period I. The total absorption increment (in grams of cholesterol) is shown at the top of each diagram; it is the sum of all bars.

cholesterol in period II (g) − total increment in fecal endogenous steroids in period II (g) − total decrement in cholesterol synthesis in period II (g). In this formulation, total absorption of dietary cholesterol in period II = average daily absorption of dietary cholesterol in period II × days in period II; total increment in fecal endogenous steroids in period II = (average daily fecal endogenous steroids (period II) − that in period I) × days in period II; and total decrement in cholesterol synthesis in period II = (average daily cholesterol synthesis in period I − that in period IIB) × days in period II (A + B).

This calculation is only an approximation; it depends on the assumption that cholesterol synthesis throughout

TABLE 5 NET ACCUMULATION OF CHOLESTEROL IN TISSUE POOLS DURING PERIOD II (HIGH CHOLESTEROL INTAKE)

Patient	Diet	Cholesterol Intake	Days of Cholesterol Feeding	Total Cholesterol Intake	Total Absorption of Dietary Cholesterol	Increment in Total Fecal Steroids*	Total Decrement in Cholesterol Synthesis	Net Accumulation of Cholesterol	Accumulation	Difference in Sterol Balance† (Period III − I)	Increment in Plasma Cholesterol‡
		g/day		*g*	*g*	*g*	*g*	*g*	*mg/day*	*g in n days*	*g*
1	A	2.92	69	202	50	34	3	13	188	4.7 (16 days)	1.14
2	A	2.47	78	193	44	19	34	−9	−115	—	1.16
3	A	2.12	56	119	42	11	27	4	71	−0.7 (21 days)	0.82
4	A	0.54	59	32	17	3.9	8.5	4.6	78	—	0.93
5 (Period II)	A	0.24	42	10	4.7	2	0.7	2	47	—	−0.90
5 (Period III)	A	0.91	37	34	15	5	9	1	27	—	−0.96
6	A	2.34	36	84	37	12	24	1	28	—	0.97
7	B	4.06	16	65	not measured	not measured	not measured	20§	1228§	—	−0.27
8	B	4.06	16	65	not measured	not measured	not measured	22§	1373§	—	0.50

* Total fecal steroids = *endogenous* neutral + acidic steroids.

† In patient 1 the first 4 days' data in period III were excluded, and in patient 3, the first 3 days' data. This was done in order to exclude the cholesterol retained in the gut during the last days of period II (see footnote (‡) in Table 4A).

‡ Plasma volume × (increment in mean plasma cholesterol concentration in period II over that in period I). Plasma volume taken to be 4.5% of total body weight (18).

§ Positive balance of cholesterol on cholesterol feeding period (see Table 4).

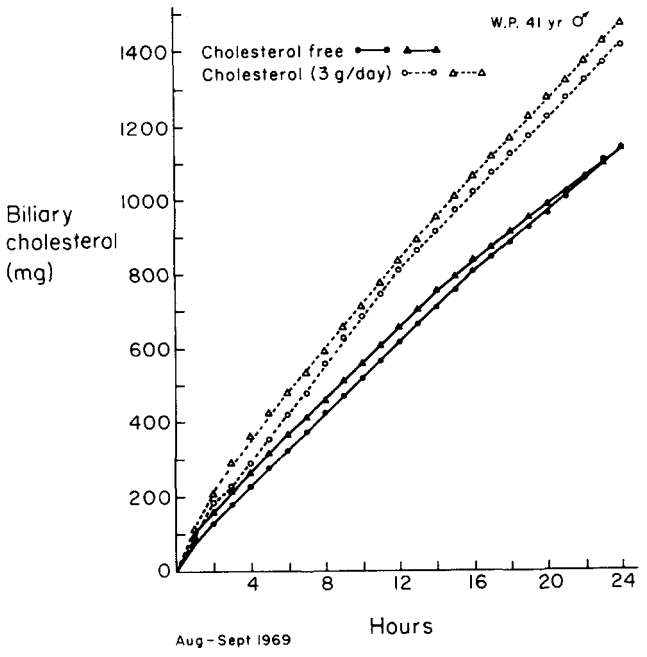

FIG. 5. Cumulative secretion of biliary cholesterol in four 24-hr tests in patient 6. Two tests on a cholesterol-free diet (solid lines) are contrasted with two on a diet containing 2.36 g of cholesterol per day (broken lines). The mean daily cholesterol flux through the biliary tree increased from 1.14 to 1.45 g, or 0.31 g per day, while at the same time the increment in total daily cholesterol absorption (exogenous + endogenous) was 0.68 g per day (see Fig. 4).

period II is identical to synthesis calculated by cholesterol balance studies in period IIB. If cholesterol synthesis in period IIA had been suppressed by dietary cholesterol to an extent greater than in period IIB, the estimation of accumulation in period IIA would be erroneously high; however, it seems unlikely that synthesis would be suppressed early in period II and later released in the face of a continuously high intake of cholesterol. If suppression of cholesterol synthesis in period IIA were not as great as in period IIB, the estimation of accumulation in period IIA would be erroneously low. It is not unreasonable to suppose that suppression of synthesis may not occur immediately upon feeding cholesterol, but rather that suppression develops as pool sizes swell; if so, the amount of cholesterol actually accumulated would have been more than that estimated by the equations described above. (The anomalous results for patient 2 (Table 5) showing a negative accumulation could be due to such an effect.)

In these patients the total intakes of dietary cholesterol in period II were 10–202 g, and total absorption was 4.7–50 g. In patient 1, the decrement in synthesis was very small; thus, despite a large increment in fecal endogenous steroid excretion, net accumulation was 13 g. In the remaining patients (nos. 2–6) considerable amounts of cholesterol were absorbed, but accumulations of cholesterol were prevented largely by sizable decreases in the amounts of cholesterol synthesized.

In the case of patient 1 additional evidence was obtained to support our contention that significant accumulations of cholesterol had occurred during period II (Table 5 and Fig. 1). In period III, when the feeding of cholesterol was discontinued, the excretion of endogenous neutral steroids continued at levels higher than the base line values measured in period I; this suggested that some of the excess dietary cholesterol accumulated in tissues in period II was reexcreted when cholesterol feeding was terminated. Indeed, in the 16 days of period III the excretion of total fecal steroids (endogenous) was 4.7 g larger than could have been expected from the base line excretion in period I (this amount might have been still larger if the balance study could have been continued longer). In contrast, in patient 3, in whom the net accumulation of cholesterol was small during period II, the excretion of total endogenous steroids in period II did not continue in period III.

An estimation of the amount of cholesterol accumulated in the plasma compartment during period II is also shown in Table 5. Any rise in plasma cholesterol associated with cholesterol feeding is a clear indication that compensation for excess absorption of cholesterol is incomplete. Nevertheless, the data in Table 5 indicate the lack of correlation between increments in tissues compared to those in plasma. The disparities are compellingly evident in patient 1, in whom the amount retained in tissues was more than 15 times greater than that in plasma.

Balance studies carried out during cholesterol feeding in patients 7 and 8 provide even more striking evidence that an accumulation of cholesterol can occur in body pools outside of the plasma compartment (Fig. 2). After a control period when the diet was free of cholesterol, these two teen-aged siblings were fed 4 g of cholesterol daily for 20 days; cholesterol balances in both patients were positive throughout the entire period of cholesterol feeding. Thus, even if synthesis was completely suppressed in these patients, they accumulated at least 20 and 21 g of cholesterol. These increases in body pools occurred despite the fact that plasma cholesterol levels were essentially unchanged. This finding suggests the operation of mechanisms (as yet undefined) that prevent plasma levels of cholesterol from rising even as tissue pools are rapidly expanded; conceivably this buffer consists of some interplay between the capacity of lipoproteins for cholesterol and the control of transfer of cholesterol from plasma into tissue storage sites (19).

DISCUSSION

The ultimate purpose of this study was to evaluate how

much cholesterol accumulates in body tissues during the ingestion of high cholesterol diets. However, with present methods the accumulation of cholesterol in tissues outside of the plasma compartment cannot be measured directly. Using the sterol balance procedure developed in this laboratory, we have studied the factors (absorption, excretion, and synthesis) that govern the levels of total body cholesterol, and then it was possible to make indirect calculations of how much cholesterol is added to body pools during the feeding of large amounts of cholesterol. We will now discuss the role played by each of these three factors in regulating the amount of exchangeable cholesterol in the body as a whole.

Cholesterol Absorption

One factor determining the quantity of dietary cholesterol that accumulates in body pools is intestinal absorption, yet the extent to which cholesterol can be absorbed by the human intestine has been the subject of conflicting claims. On the one hand, some workers (3, 4) have claimed that the absorptive capacity in man is severely limited, and that this prevents expansion of body cholesterol pools even when the diet is rich in cholesterol; but others (20–23) have presented evidence that large quantities of dietary cholesterol can be absorbed. In the present study we have attempted to resolve this disagreement by studying in the same patient the effects of increasing intakes upon absorption. In essence, we found that the amount of dietary cholesterol absorbed increases with the intake in a more or less linear manner. It is clear, then, that the capacity of the intestine to absorb cholesterol is not exceeded by the endogenous cholesterol entering the duodenum through the biliary tract; as much as 1 g of dietary cholesterol can be absorbed when intakes are as high as 3 g.

However, in one important sense we can say that absorption of cholesterol is limited in man (and this limitation reduces significantly the total load of cholesterol that can enter body pools): even when the diet is free of cholesterol, only about one-half of the endogenous cholesterol entering the intestinal tract is reabsorbed. Likewise, the absorption of exogenous cholesterol is limited to about 50% when intakes are small or moderate, and it decreases to about 25–30% when intakes are large.

Apparently, several factors restrict the amount of cholesterol that can be absorbed. The crucial roles played in cholesterol absorption by dietary fat and by bile acids have been examined previously (24, 25); the importance of each can be illustrated by two unpublished studies. In one, the requirement for exogenous fat to assist the absorption of dietary cholesterol was clearly shown. A patient was given 1 g of crystalline cholesterol in capsule form in three divided doses daily while receiving a fat-free diet; during one month of this regimen none of the dietary cholesterol was absorbed. In the second example, a patient with primary biliary cirrhosis who had complete obstruction to the outflow of bile was given 1 g of cholesterol daily for one month; however, in this case the cholesterol was dissolved in oil before its incorporation into a liquid formula diet. Again, none of this dietary cholesterol was absorbed, confirming previous reports that bile acids are obligatory for cholesterol absorption. These observations are in accord with the findings of Simmonds, Hofmann, and Theodor (26) that micellar solubilization of cholesterol may be rate-limiting in absorption of cholesterol. They found that, as fatty acids and monoglycerides are absorbed, cholesterol comes out of micellar solution and into the particulate, and presumably nonabsorbable, state.

Although intraluminal factors undoubtedly influence cholesterol absorption by imposing restrictions on the amount that can be made available for absorption in the proper physical state, the role of the intestinal mucosa cannot be overlooked. In the accompanying paper (2) we have shown that the proportion of a large single dose of cholesterol that is absorbed depends upon the previous intake of cholesterol. When cholesterol intake has been low, a larger fraction of any single dose is absorbed than when the intake has been high. This suggests that the degree of saturation of the mucosa is an important factor controlling cholesterol absorption.

Compared to such water-soluble substances as glucose, amino acids, fatty acids, and bile acids, the absorption of cholesterol is distinctly limited, but not so severely as has been suggested by some workers. With increasing amounts of cholesterol in the diet, more cholesterol is absorbed and can enter body pools.

Cholesterol Excretion

Since the absorption of cholesterol is greater when the diet is rich in cholesterol, compensatory mechanisms must come into play if progressive accumulation of cholesterol is to be prevented. One mechanism clearly demonstrated by the present study is enhanced reexcretion of cholesterol through the biliary tract; this is reflected by a greater fecal excretion of neutral steroids of endogenous origin.

However, it is of interest to note that, when cholesterol is absorbed in excessive amounts, reexcretion occurs mainly in the form of the neutral sterol; there is no enhanced conversion of cholesterol into bile acids. In this regard man differs from the dog[1] and the rat (17); these animals respond to the feeding of cholesterol with a distinct increase in excretion of bile acids. At the present time we have no explanation for this dif-

ference, but it is interesting to speculate about its possible significance. The high content of bile acids relative to cholesterol in the bile of dogs and rats and the low incidence of gallstones in these species may be related to a capacity to convert large amounts of cholesterol into bile acids. Since man apparently does not possess the same ability for transforming excess cholesterol into bile acids, he is forced to dispose of newly absorbed cholesterol in the form of neutral rather than acidic sterols. Thus, an apparent defect in the conversion of cholesterol into bile acids may render man more susceptible to the formation of cholesterol stones.

Cholesterol Synthesis

Another mechanism by which accumulation of cholesterol could be prevented is by feedback inhibition of cholesterol synthesis. Much interest has centered on the question of whether man is subject to feedback inhibition by dietary cholesterol. The possibility that this mechanism may not exist in man was first considered because of repeated observations that human beings respond to the daily feeding of radioactive cholesterol differently from dogs and rats. When large amounts of labeled cholesterol were fed continuously for many weeks to these animals, the specific activity of plasma cholesterol closely approached that of the dietary cholesterol, i. e., plasma cholesterol was derived almost entirely from labeled dietary cholesterol, and newly synthesized nonradioactive cholesterol contributed only a small fraction. These observations led earlier workers (17, 27) to the conclusion that in these animals the production of cholesterol is suppressed almost completely by dietary cholesterol.

In human beings a different response has been consistently found in several laboratories (3, 4): despite prolonged feeding of radioactive cholesterol, the specific activities of plasma cholesterol were always much lower than the specific activities of dietary cholesterol. This implied that in man the rate of synthesis of cholesterol was unchecked by the intake of large quantities of cholesterol, and it raised the question of whether absorbed dietary cholesterol ever suppresses cholesterol synthesis in man. However, as we have shown in the accompanying paper (2), the level of specific activity of plasma cholesterol attained in man is grossly misleading if the isotopic steady state has not been attained: absorption is underestimated, and synthesis is overestimated. A valid assessment of feedback control by dietary cholesterol in man demands that attainment of the isotopic steady state be proved, and the accompanying report (2) describes how this can be done.

Because of these difficulties we have approached the question of feedback control in man in a different way, namely, by blocking the absorption of cholesterol (and thus interrupting its enterohepatic circulation) by feeding large amounts of plant sterols (1). Subsequently, the production of cholesterol was measured by the sterol balance procedure. Synthesis increased markedly, indicating that previous inhibition of synthesis had been released. From such experiments we concluded that in the normal state, when the enterohepatic circulation is intact, synthesis of cholesterol is inhibited by continual reabsorption of endogenous cholesterol.

In this previous report (1), patient 1 (J.Sh.) failed to suppress cholesterol synthesis when fed large amounts of cholesterol, and patient 1 (R.W.) of the present report responded in the same manner. However, in three patients synthesis was reduced moderately when the intake was increased, and in two others suppression of synthesis was almost complete. What is new, then, is the demonstration of great variability from patient to patient in the degree to which cholesterol synthesis was suppressed by absorbed cholesterol. These variations may, in fact, determine the degree to which different patients compensate for cholesterol loading. The differences between patients who respond by feedback suppression and those who do not seems critically important, since it was the latter who showed the greater accumulations of cholesterol.

Cholesterol Accumulation

When the increment in cholesterol absorption on a high cholesterol diet exceeds the reduction in synthesis plus the enhancement in reexcretion, accumulation of cholesterol in body pools must occur. The interplay of these three parameters in regulation of body cholesterol has been measured in this laboratory as a function of several interventions: reduction of cholesterol absorption by oral administration of plant sterols (1); reduction of bile acid and cholesterol absorption through administration of the bile acid sequestrant, cholestyramine, and by surgical bypass of the terminal ileum;[2] increased efflux of cholesterol by dosage with Atromid-S (28); and redistribution of plasma cholesterol into tissue pools with exchange of unsaturated for saturated dietary fats (29). It is now urgent to corroborate the results obtained by sterol balance techniques with simultaneous measurements of pool sizes of readily exchangeable cholesterol, as carried out by isotope kinetic studies (30, 31); both approaches will be strengthened wherever agreement in results is obtained, just as previously demonstrated in comparative studies of daily synthesis rates (15).

In the present study two situations seemed to be associated with marked accumulation of cholesterol during the feeding of high cholesterol diets: failure of

[2] Grundy, S. M., and E. H. Ahrens, Jr. Unpublished data.

feedback inhibition of synthesis (patient 1), and excessive absorption that exceeds the body's capacity to compensate (patients 7 and 8). Other patients appeared to accumulate little because they were able to limit absorption, to reduce synthesis, and to reexcrete any increment in absorbed cholesterol.

Our balance data do not indicate which pools of cholesterol are expanded by increments in absorbed dietary cholesterol, and studies in animals also have failed to answer this question. When rabbits are fed large amounts of cholesterol, extensive accumulation occurs in many sites (32); in rats, cholesterol accumulation on high cholesterol diets seems to be restricted to the liver (32); and in dogs, cholesterol accumulation does not occur in any organ or tissue even when large amounts of dietary cholesterol are absorbed for many weeks.[1]

Even when large amounts of cholesterol accumulate in body pools, the effects on concentrations of plasma cholesterol are surprisingly small; increases rarely exceed 20%, regardless of intake and absorption. It appears that in man, unlike the rabbit, there are mechanisms that prevent a marked rise in plasma cholesterol when other pools are expanded. Indeed, plasma cholesterol concentrations reflect the total body content of cholesterol most imperfectly and may even misrepresent the effects of high cholesterol diets on tissue concentrations of cholesterol.

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