The Intestinal Absorption of Dietary Cholesterol by Hypercholesterolemic (Type II) and Normocholesterolemic Humans

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ABSTRACT The incomplete absorption of dietary cholesterol may represent an adaptive intestinal barrier that prevents hypercholesterolemia. To explore this mechanism, we compared cholesterol absorption in 15 normocholesterolemic and 6 hypercholesterolemic (type II) subjects fed background cholesterol-free formula diets with 40% of calories as fat. Each test meal consisted of a breakfast into which was incorporated scrambled egg yolk containing 300–500 mg of cholesterol and [4-^{14}C]cholesterol (3–22 μCi), either naturally incorporated into the yolk cholesterol by previous isotope injection into the laying hen or added in peanut oil to the yolk of the test breakfast. In some instances [1α-^{3}H]cholesterol was the radioactive marker.

The radioactivity of the fecal neutral sterol fraction was determined in daily stool samples for the next 7 days to provide an estimate of unabsorbed dietary cholesterol. The amount of absorbed and reexcreted labeled cholesterol proved negligible. Most unabsorbed dietary cholesterol appeared in the stool on the second or third day after the meal, and 95% or more was recovered in the stool by 6 days. Plasma specific activity curves were usually maximal at 48 h. Normal subjects absorbed 44.5±9.3 (SD) of the administered cholesterol (range 25.9–60.3). Hypercholesterolemics absorbed the same percentage of cholesterol as normals: 47.6±12.6% (range 29.3–67.3). Absorption was similar whether the radiolabeled cholesterol was added to egg yolk or naturally incorporated in it (42.1±9.3 vs. 48.9±9.8%).

Six normal subjects were fed a cholesterol-free formula for 4 wk, and then different amounts of cholesterol (110–610 mg/day) were added for another 4 wk. At the end of each period, single test meals containing either 110, 310, or 610 mg of cholesterol and [1α-^{3}H]cholesterol were administered. Cholesterol absorption was 42.3±6.0% and 45.4±8.3% for the two dietary periods, respectively. The absolute cholesterol absorption was linearly related to the amount of cholesterol in the test meal, and absorption was not affected by background diets high or low in cholesterol content.

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INTRODUCTION

At any moment the plasma cholesterol is composed of components derived from two different sources (1). There is a biosynthetic component, and there is a second component derived from cholesterol absorbed from the diet. Ultimately, the cholesterol from these two sources intermixes and is indistinguishable one from the other. Past isotopic studies in man under steady-state conditions have shown, in general, that the input of cholesterol into the plasma from biosynthesis in the liver and intestinal mucosa remains rather constant, and that cholesterol biosynthesis is not usually inhibited by various quantities of dietary cholesterol (2–6). The plasma cholesterol level is thus directly related to the amount of cholesterol secreted into the plasma from biosynthesis. Furthermore, the input of cholesterol from the diet is additive to the amount synthesized. With a constant synthetic rate, the plasma cholesterol level may be altered considerably by the amount of cholesterol in the diet (7–9).

Unlike other animals, such as the monkey and the rabbit, which may develop serum cholesterol levels of 600–2,000 mg/100 ml after cholesterol feeding, man ordinarily has a much more limited serum cholesterol increase from even very large quantities of cholesterol in the diet (7–9). Increases of only 50–100 mg/100 ml from base-line levels of 200–250 mg/100 ml after cholesterol feeding indicate the limited extent of this

TABLE I

Clinical Data of Experimental Subjects

Subject no.	Initials	Age	Sex	Height	Weight	Serum lipids: Cholesterol	Serum lipids: Triglyceride	Medical diagnosis
		yr		*cm*	*kg*	*mg/100 ml*		
Group I								
1	A. M.	36	M	178	84	169	139	Healthy
2	K. W.	33	M	171	63	173	—	Healthy
3	S. D.	40	M	173	74	160	75	Healthy
4	J. F.	44	M	170	62	196	100	Healthy
5	C. L.	40	M	173	65	230	149	Healthy
6	D. M.	38	M	165	67	207	153	Healthy
7	P. M.	36	M	175	73	208	112	Healthy
8	R. R.	37	M	183	80	225	231	Healthy
9	S. J.	40	M	168	84	215	127	Healthy
10	M. T.	49	F	165	65	295	88	Hypercholesterolemia (type II), xanthelasma
11	A. A.	56	F	155	39	400	103	Hypercholesterolemia (type II), xanthelasma
12	R. N.	66	F	170	65	333	292	Hypercholesterolemia, xanthelasma, tendon xanthoma
13	G. K.	73	M	173	78	474	265	Hypercholesterolemia, xanthelasma, tendon xanthoma, cerebral atherosclerosis
14	E. M.	61	F	155	48	618	126	Hypercholesterolemia, tendon xanthoma
15	B. J.	41	F	155	57	862	177	Hypercholesterolemia, xanthelasma, tendon xanthoma, coronary heart disease
Group II								
16	E. G.	48	M	170	70	193	—	Healthy
17	L. H.	32	M	173	78	158	—	Healthy
18	O. K.	43	M	183	75	173	—	Healthy
19	W. M.	32	M	180	79	149	—	Healthy
20	R. P.	30	M	185	77	113	—	Healthy
21	R. U.	38	M	178	88	103	—	Healthy

response. One reason for the different response in humans compared with the other animals may be the incomplete absorption of dietary cholesterol in man (2, 3, 5). It may even be suggested that the intestinal mucosa represents an evolutionary adaptive barrier for the relative prevention of hypercholesterolemia.

Yet the fact that human beings of similar age and sex consuming similar diets have a wide range of serum cholesterol levels (120–320%) suggests the existence of certain metabolic differences responsible for these wide variations. A consideration of the body's input and output of the cholesterol or the "sterol balance," the factors important in the maintenance of any given serum cholesterol concentration, suggests that one possible metabolic difference might lie in the area of cholesterol absorption. Absorption controls the input derived from the diet and also from endogenous cholesterol excreted into the gut and available for reabsorption. Other important factors for consideration of these metabolic differences would be cholesterol synthesis, cholesterol conversion to bile acids, and cholesterol and bile acid excretion into the stool.

In the experiments to be reported we posed the following questions: (*a*) What is the absorption of cholesterol by hypercholesterolemic (type II) and normal subjects? (*b*) Is the absorption of isotopic cholesterol in a natural food (egg yolk) similar to the absorption of crystalline labeled cholesterol dissolved in oil? (*c*) Is cholesterol absorption affected by the amount of cholesterol in the background diet and in the test meal itself?

METHODS

Experimental subjects and protocols

Group I (subjects 1–15). Nine normal male subjects and six hypercholesterolemic patients were hospitalized in the Clinical Research Center for 2–3-mo periods of time. The clinical data of the experimental subjects are provided in Table I. All subjects had normal gastrointestinal

TABLE II

Composition of the Test Meal Breakfast

Calories	800
Fat	51 g
Protein	25 g
Carbohydrate	63 g
Cholesterol (egg yolk)	500 mg
[4-^{14}C]cholesterol	3–22 μCi
or	
[1-^{3}H]cholesterol	50 μCi

TABLE III

Intestinal Absorption of Cholesterol in Normal Subjects and Hypercholesterolemic Patients Fed the Isotopic Test Breakfast

Subjects	Test meal contents: Cholesterol	Labeled cholesterol: Isotope	Form	Amount	Excretion of radioactivity, days after meal: 1	2	3	4	5	6	Total	Absorption		Recoverage of β-sitosterol	Corrected absorption
	mg			*μCi*	*μCi*						*μCi*	%	*mg*	%	%
Normal															
1A	398	[4-^{14}C]cholesterol	Egg yolk	11.4	0.54	6.26	1.21	0.30	0.10	0.05	8.5	25.9	103	—	—
1B	308	[4-^{14}C]cholesterol	Egg yolk	8.4	0.63	3.98	0.65	0.07	0.03	—	5.4	35.7	110	—	—
1C	2,799	[4-^{14}C]cholesterol	Egg yolk	13.1	0.05	4.84	4.47	0.62	0.01	—	10.0	23.9*	669	—	—
2A	469	[4-^{14}C]cholesterol	Egg yolk	14.7	—	3.38	3.78	1.23	—	0.80	9.2	37.4	175	—	—
2B	396	[4-^{14}C]cholesterol	Egg yolk	10.1	0.54	4.00	0.50	0.27	0.12	—	5.4	46.4	184	—	—
3	412	[1α-^{3}H]cholesterol	Crystalline	46.4	1.35	9.43	3.36	2.55	1.06	0.67	18.4	60.3	248	70	43.3
4	560	[4-^{14}C]cholesterol	Egg yolk	15.0	—	—	—	—	—	—	7.1	52.7	295	100	52.7
5	540	[4-^{14}C]cholesterol	Egg yolk	13.8	—	—	—	—	—	—	7.8	43.5	235	100	43.5
6	540	[4-^{14}C]cholesterol	Egg yolk	19.6	—	—	—	—	—	—	9.9	49.5	267	100	49.5
7	544	[4-^{14}C]cholesterol	Egg yolk	20.8	—	—	—	—	—	—	11.5	44.7	243	100	44.7
8	452	[4-^{14}C]cholesterol	Egg yolk	16.7	—	—	—	—	—	—	9.7	41.9	189	81	28.3
9	560	[4-^{14}C]cholesterol	Egg yolk	22.1	—	—	—	—	—	—	10.8	51.1	286	—	—
Mean and SD												44.5±9.3			43.7±8.4
Hypercholesterolemic patients															
10	482	[4-^{14}C]cholesterol	Egg yolk	4.1	0.01	0.83	0.64	0.82	0.47	—	2.8	32.0	154	100	32.0
11A	353	[4-^{14}C]cholesterol	Egg yolk	3.6	0	0	0.11	0.38	1.60	—	2.1	41.7	147	96	39.3
11B	366	[4-^{14}C]cholesterol	Egg yolk	3.1	0	1.80	0.05	0.14	0.19	—	2.2	29.3	107	97	26.4
11C	410	[4-^{14}C]cholesterol	Crystalline	34.0	0	11.23	0.96	1.18	0.20	0.14	13.7	59.7	245	96	58.0
12A	370	[4-^{14}C]cholesterol	Egg yolk	3.8	0	0.50	1.57	0.25	0.01	—	2.3	38.8	144	—	—
12B	389	[4-^{14}C]cholesterol	Egg yolk	4.0	0	0	0.12	0.72	0.77	—	1.6	60.3	234	—	—
13	468	[1α-^{3}H]cholesterol	Crystalline	53.1	2.85	16.11	8.40	0.71	0.29	0.14	28.5	46.3	217	81	33.7
14	434	[1α-^{3}H]cholesterol	Crystalline	53.4	0	7.72	15.90	3.41	0.53	0.33	27.9	47.8	207	—	—
15A	412	[1α-^{3}H]cholesterol	Crystalline	45.9	0.25	0.66	4.06	4.06	5.25	0.60	14.9	67.5	278	85	61.8
15B	472	[4-^{14}C]cholesterol	Crystalline	53.7	0.01	6.80	10.06	7.11	0.64	0.68	25.3	52.9	250	86	44.6
Mean and SD												47.6±12.6			42.3±13.4

* This figure has been excluded from the mean because of the unusually high cholesterol content of the meal.

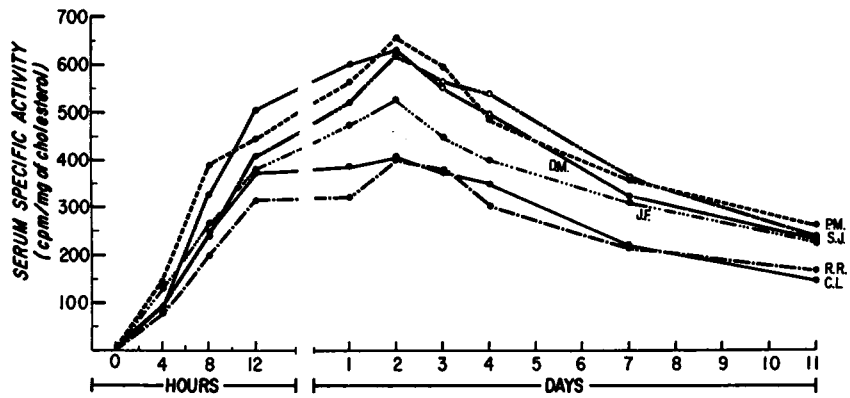

FIGURE 1 The serum cholesterol specific activities of six normal men after the ingestion of the test breakfast containing [4-^{14}C]cholesterol.

tract function and had regular daily bowel movements. Their food intake consisted exclusively of orally administered liquid formula feedings in which protein contributed 15%, fat 40%, and carbohydrate 45% of the total calorie intake (8). Vitamins and minerals were added to meet the daily recommended allowances of the National Research Council. The cholesterol content of the diets was less than 10 mg/day except as otherwise stated. The total calorie intake was adjusted to maintain constant body weight.

All subjects received a single dose of either egg yolk [4-^{14}C]cholesterol or labeled crystalline cholesterol ([4-^{14}C]-cholesterol or [1α-^{3}H]cholesterol) in a breakfast meal. Some subjects had more than one test. Both of the cholesterol isotopes were purified before use by thin-layer chromatography (10). The radioactive egg yolk, obtained by injecting [4-^{14}C]cholesterol into laying hens (11), was mixed thoroughly with water in a Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.). The total weight of the mixture was measured, and aliquots were taken for cholesterol and radioactivity determination. The mixture was cooked as "scrambled eggs" and incorporated into the usual formula breakfast of subjects 1–2, 4–12. For calculation of the exact intake of radioactive cholesterol, the residue cholesterol and the radioactivity left in the eating utensils of each subject were measured. The test meal usually contained 300–560 mg cholesterol with 3–22 μCi of radioactivity. The detailed composition of the basic test meal is listed in Table II. Study 1C was an exception in that the subject was fed a test meal of 10 egg yolks, containing 2,800 mg of cholesterol.

For subjects 3 and 13–15, crystalline [1α-^{3}H]cholesterol or [4-^{14}C]cholesterol dissolved in 5 g peanut oil was cooked with the egg yolk and incorporated into the usual test breakfast. This meal contained the usual amount of cholesterol and 34.0–53.7 μCi of radioactive cholesterol.

Group II (subjects 16–21). Six additional normal men, aged 30–48, were hospitalized in the Clinical Research Center. They received low cholesterol diets (the same as for group I) for 4 wk. For the next 4 wk, they were given the same diet plus the daily addition of 110, 310, or 610 mg of cholesterol to two each of the six subjects, respectively, for another 4-wk period. This cholesterol was supplied as egg yolk which was incorporated into the diet in such a way as to maintain constancy of all nutrients (calories, protein, fat, etc.) as carried out in previous studies (8). Single meals containing 110–610 mg of cholesterol from egg yolk and 18.2–18.8 μCi of crystalline [1α-^{3}H]cholesterol were given at the conclusion of this dietary period to determine cholesterol absorption as indicated for group I subjects.

Analytical procedures

Venous blood samples of the subjects were collected at 4, 8, and 12 h and daily for 4 days after giving the isotope meal. Periodic blood samples were also taken subsequently. The cholesterol concentration of the serum was determined by the method of Abell, Levy, Brodie, and Kendall (12). For determination of radioactivity, the serum samples were saponified with alcoholic KOH. The nonsaponifiable residue was extracted with hexane, dried, and then dissolved in 10 ml scintillation mixture (4 g of 2,5-diphenyloxazole) and 0.1 g of 1.4 *bis*{2-(5-phenyloxazolyl)} benzene in 1 liter of toluene. The samples were then counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) with an efficiency of 87% for ^{14}C and 41% for ^{3}H. The results were expressed as specific activity in counts per minute per milligram of cholesterol. Aliquots of the egg yolk meal (approximated 0.5 g) were weighed accurately and extracted with chloroform-methanol (2:1) (13). Its cholesterol content and radioactivity of the lipid extract were determined as described above.

Stool samples were collected daily and frozen. For analysis, stools were thawed and homogenized with an equal amount of water. The radioactivity of the fecal neutral steroids was determined by the method described by Mietinen, Ahrens, and Grundy (14). Approximately 0.5 g of homogenized stool sample was weighed out accurately and saponified with alcoholic NaOH. Neutral steroids were extracted with hexane. The extracts were concentrated by Rinco evaporator (Rinco Instruments Co., Greenville, Ill.) under vacuum to a small volume and quantitatively transferred into counting vials. Radioactivity was measured by Packard Tri-Carb Scintillation Counters. Any color quenching by the sample was corrected with the automatic external standardization system. Fecal bile acid radioactivity was determined as previously described (15).

Cholesterol absorption was computed as the difference obtained by subtracting the amount of unabsorbed radiolabeled cholesterol contained in the stool from the total

TABLE IV

Comparison of Cholesterol Absorption of Normal Men with Dietary Backgrounds of Different Cholesterol Intake

Dietary period	Subjects	Daily cholesterol intake	Intake in single test meal: Cholesterol	Intake in single test meal: [1-³H] cholesterol*	Fecal excretion of [1α-³H] cholesterol, days after meal: 1	2	3	4	5	6	Total (1–6 Days)	Absorption	
		mg	*mg*	*μCi*	*μCi*							%	*mg*
I	16	10	110	18.8	0	2.48	—	6.48	—	0.58	9.5	49.5	54
	17	10	110	18.8	0.01	—	7.15	—	1.68	0.97	9.8	47.9	53
	18	10	310	18.7	3.53	7.12	—	0.87	0.21	—	11.7	37.4	116
	19	10	310	18.8	0.51	2.22	6.29	0.58	0.40	0.23	10.2	45.7	142
	20	10	610	18.8	2.60	7.21	1.46	0.52	—	0.18	12.0	36.2	221
	21	10	610	18.7	0.38	—	—	0.59	7.67	3.12	11.8	36.9	225
Mean and SD												42.3±6.0‡	
II	16	110	310	18.5	0.01	0.14	2.73	3.69	1.24	0.38	8.2	55.7	173
	17	110	610	18.2	0.02	—	8.41	—	—	0.94	9.4	48.4	295
	18	310	610	18.8	4.74	6.96	0.68	0.43	0.20	0.12	13.1	30.3	185
	19	310	110	18.5	0.87	7.34	—	0.83	0.47	0.41	9.9	46.5	51
	20	610	110	18.7	0.91	5.76	3.43	0.15	0.18	—	10.4	44.4	49
	21	610	310	18.6	0	5.32	1.53	2.71	0.20	0.18	9.9	46.8	145
Mean and SD												45.4±8.3	

* Crystalline [1α-³H] cholesterol, dissolved in peanut oil, was fed.

‡ Average percent of cholesterol absorption of dietary period I vs. that of period II ($P < 0.5$).

amount fed in the test breakfast. The peak fecal excretion of the dietary unabsorbed cholesterol usually appeared on the second or third day after the test meal provided there were daily or almost daily bowel movements. In all instances, over 95% of the unabsorbed cholesterol had appeared by the sixth day (Table III). Accordingly, 6- or 7-day pools of stool after the test breakfast could be conveniently pooled for the purpose of determining the amount of unabsorbed cholesterol. In order to correct for possible sterol degradation in the gut that might then have affected the calculations for cholesterol absorption, we corrected for sterol losses in our subjects in whom the intake of plant sterols was accurately known, as has been suggested in previous publications (16, 17). The plant sterol content of the diet and of the stool was determined by a combination thin-layer chromatography and gas chromatography method (14). Based on the recoverage of β-sitosterol, the loss of neutral sterol was corrected, and in turn its absorption of cholesterol was calculated with the same method as described in the report by Quintao, Grundy, and Ahrens (17).

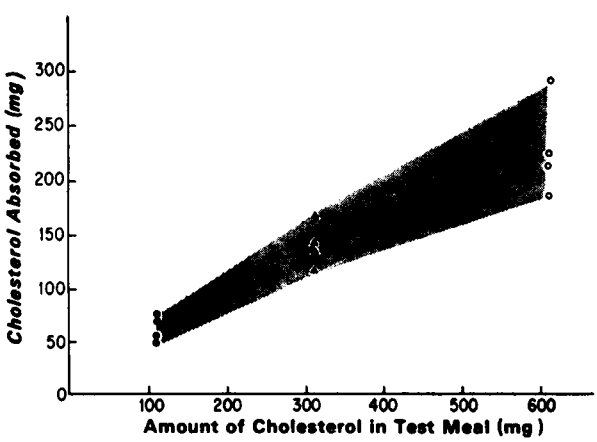

FIGURE 2 The cholesterol absorption (in milligrams per day) of six normal subjects after the ingestion of a test breakfast containing different amounts of cholesterol. Two men received 110 mg of cholesterol; two men, 310 mg; and two men, 610 mg. The background diets contained 10–610 mg of cholesterol.

RESULTS

The serum cholesterol specific activities of all 21 experimental subjects usually reached a peak at 48 h after the test breakfast containing isotopic cholesterol and then declined gradually. The peak serum cholesterol radioactivity was at 48 h in 32 of 34 total tests and was similarly attained for both normal and hypercholesterolemic subjects. In the other two tests the peak was at 72 h. The serum cholesterol specific activity curves of the six normal subjects studied (subjects 4–9) were representative and are depicted in Fig. 1. Radioactivity appeared in the blood as soon as 4 h after the test meal.

The intestinal absorption of dietary cholesterol by normal subjects range from 25.9 to 60.3% of the test meal with a mean of 44.5±9.3% (Table III). The hypercholesterolemic (type II) patients absorbed cholesterol similarly ($P < 0.6$). The range was from 29.3 to 67.5%, and the mean was 47.6±12.6. Subject 1 had three test meals at different times. Three meals contained similar amounts of radioactivity (11.4, 8.3,

TABLE V

Cholesterol Absorption of Subjects Fed Different Form of Labeled Cholesterol (Cholesterol from Labeled Egg Yolk and Labeled Crystalline) in the Test Meal

Subjects	Egg yolk labeled cholesterol		Crystalline labeled cholesterol		Egg yolk cholesterol vs. crystalline cholesterol
	No. of test	Absorption	No. of test	Absorption	
		%		%	
Normal	10	42.9±8.1	7	44.7±8.8	$P < 0.7$
Type II	5	40.4±12.1	5	54.8±8.8	$P < 0.1$
Normal +.type II	15	42.1±9.3	12	48.9±9.8	$P < 0.1$

and 13.0 μCi) but different amounts of cholesterol (398, 308, and 2,799 mg, respectively). The cholesterol absorption was 25.9, 35.7, and 23.9%, respectively, for these different amounts of cholesterol in the test meal.

Cholesterol absorption was determined in six normal subjects whose previous background diets had differing intakes of cholesterol (Table IV). The mean cholesterol absorption after 4 wk of a very low cholesterol diet (10 mg/day) was 42.3±6.0%. After 4 wk of diets containing higher amounts of cholesterol (110–610 mg/day), the cholesterol absorption was similar, 45.4±8.3%. Furthermore, the cholesterol content of the test meal (110, 310, or 610 mg) did not consistently affect the percentage absorption of cholesterol. The amount of cholesterol absorbed in milligrams was linearily related to the dose fed over the range from 110 to 610 mg (Fig. 2).

In the human diet the cholesterol contained in foodstuffs is in the form of lipoprotein in membranes or else dissolved in fat droplets. It was considered crucial to measure the absorption of cholesterol as it exists in a natural food product, egg yolk, and to compare its absorption when added as a crystalline isotope to a natural food product. Accordingly, considerable effort was expended to produce naturally labeled egg yolks by giving laying hens [4-^{14}C]cholesterol (11). This ideal system was then compared with the perhaps less ideal (but more practical) system of mixing oil containing cholesterol isotope with egg yolk to make up the test breakfast. For both normal and hypercholesterolemic subjects cholesterol was absorbed similarly with either the naturally labeled egg yolk or the cholesterol isotope mixed with egg yolk, 42.1% vs. 48.9, $P < 0.1$ (Table V).

Information about cholesterol absorption in this study was obtained by the single isotope meal technique. This technique has a small, perhaps completely insignificant intrinsic error produced by the recirculation of absorbed radioactive cholesterol, which mingles indistinguishably with the excreted cholesterol of the test meal. To validate the method further, we collected daily stool from two subjects (Nos. 1A and 2A) for 13 days after the labeled meal. The radioactivity in both the neutral sterol and bile acid fractions of stool was measured. As indicated in Fig. 3, the radioactivity excreted in neutral sterol fraction after 6–8 days, which was a representation of the endogenous cholesterol excretion in subjects receiving a cholesterol-free diet, was extremely small (about 0.1 μCi). The bile acid radioactivity was always low (about 0.1 μCi). Since the amounts of fecal cholesterol and bile acids derived from endogenous sources are roughly equivalent, the radioactivity contributed by the recirculated isotopic cholesterol to the total excretion of radioactive cholesterol during the 6-day period was therefore small (less than 1% of the amount in the test breakfast). The fact that the excretion per day of cholesterol absorbed into the body is minute is supported also by the kinetic data of cholesterol turnover. Absorbed cholesterol is diluted by the larger exchangeable pools of the body. The daily excretion of any given dose is small since the turnover is a matter of months.

To take cognizance of possible sterol degradation in the gut that might provide an erroneously high figure for cholesterol absorption, we corrected the fecal sterol results and then cholesterol absorption according to the recovery of dietary β-sitosterol in the stool. The analyses indicated a mean value for recovery of 91.5% (range 70–100%). The corrected mean absorption in six tests in six normal subjects in whom dietary plant sterols were known was 43.7±8.4%. For seven absorption tests for four type II patients, the corrected absorption was 42.3±13.4%. Thus, there was no statistical difference between these two groups ($P < 0.9$), which was the same conclusion drawn from a consideration of all of the uncorrected data (Table III). The recovery of β-sitosterol of the six men fed low and moderate cholesterol diet in study II was a mean of 85.6% with a range from 75 to 94% (Table IV). The conclusions of that study also were not altered by plant sterol degradation. In all of our data, there is no strangely high figure of cholesterol absorption which

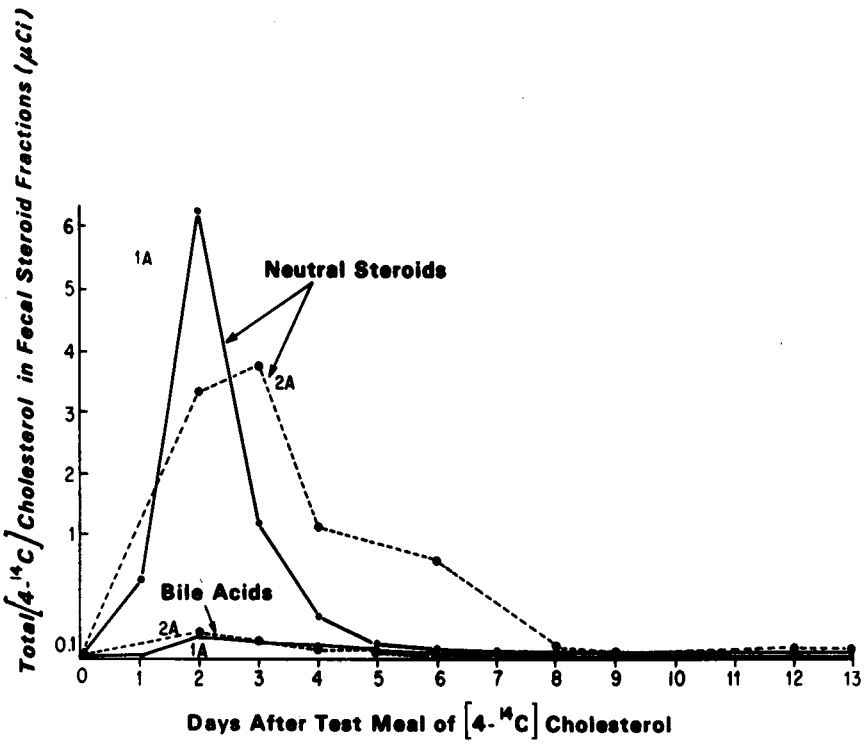

FIGURE 3 The excretion of radioactivity in the fecal neutral steroids and bile acids in two subjects after the ingestion of the test breakfast containing [4-^{14}C]cholesterol.

would have resulted if there had been extensive loss of sterols in the gut from degradation.

DISCUSSION

The most remarkable and most consistent characteristic of cholesterol absorption in man was its incompleteness. In this study, on the average, only 45% of the intake of cholesterol was absorbed. At first glance, then, the intestinal mucosa would seem to present a barrier of considerable importance in resisting the accumulation of excessive amounts of cholesterol in the blood and tissues. Yet hypercholesterolemic type II patients, some with xanthoma and atherosclerosis, who already had excessive total body cholesterol, absorbed cholesterol at a rate similar to normocholesterolemic subjects. Apparently, the intestine does not have the capacity to restrict cholesterol absorption further when plasma cholesterol levels are abnormally high, 295–862 mg/100 ml. The metabolic defect in type II hypercholesterolemia was clearly *not* exaggerated cholesterol absorption. Nor was absorption more rapid in the hypercholesterolemic patients since their plasma radioactivity curves peaked at 48 h, as did those of our normal subjects and those of the subjects of other studies (18, 19). In the recent paper by Quintao, Grundy, and Ahrens, the measurement of the intestinal absorption of five hypercholesterolemic patients and one normocholesterolemic subject also indicated a similarity (6).

The reason for the delay in the plasma peak level after cholesterol absorption as compared to most other absorbed nutrients may be a holdup in the liver. Biggs found that intravenously injected chyle [^{3}H]cholesterol was selectively taken up by rat liver and pointed to this organ as one site responsible for the delay in the appearance of newly absorbed cholesterol (20). In rats with lymph fistulas, the peak of [4-^{14}C]cholesterol absorption as measured in the lymph was 6–8 h, but the absorbed cholesterol continued to appear in lymph up to 48 h, thus pointing to a sluggish absorption per se (21). Studying the distribution of radioactivity in different organs after feeding [4-^{14}C]cholesterol to rats, Borgstrom, Lindhe, and Wlodawer concluded that the late peak of cholesterol specific activity is caused by a holdup of newly absorbed cholesterol, first in the intestinal wall and later in the liver before its reappearance in the circulation (22). In humans, Hellmann, Frazell, and Rosenfeld (23) also found the maximum radio-specific activity in the lymph at 8 h after the [4-^{14}C]cholesterol meal. Similar delaying mechanisms may operate in humans as in the rat. It is conceivable that the sluggish absorption of labeled dietary choles-

terol may be caused by a mixing of the label with a large pool of metabolically active "cold" cholesterol contained in the mucosal cell. Each increment of time a certain percentage of the total pool (cold plus labeled cholesterol) moves into the lymph in the form of chylomicrons whose formation may well be influenced more by dietary fat intake than by dietary cholesterol.

We were concerned more about the absorption of cholesterol as present in a natural food such as egg yolk rather than about the absorption of isotopic crystalline cholesterol added to the test meal. Cook, Edwards, and Riddell had previously found that crystalline cholesterol was poorly absorbed compared with egg yolk cholesterol, 14 vs. 53% (24). Commonly, most investigators have used crystalline labeled cholesterol dissolved in oil for the test meal to measure intestinal absorption of cholesterol in man. However, there has been no direct evidence to indicate whether the degree of absorption of cholesterol in this form is the same as that of egg yolk labeled cholesterol, the form actually consumed in the diet. In an earlier report, Wilson and Lindsay had fed cholesterol in both crystalline and egg yolk form to two subjects to establish an isotopic steady state. The calculated maximal net cholesterol absorption was similar whether the source of cholesterol was crystalline or egg yolk (23). Our results indicated equal absorption of naturally labeled egg yolk cholesterol and tracer doses of labeled cholesterol dissolved in oil mixed with unlabeled egg yolk. A practical test breakfast, verified now for its accuracy, makes possible the performance of a standardized test of cholesterol absorption.

As stated previously, the intestinal absorption of cholesterol in this study was measured by the single isotope meal technique, in which unabsorbed cholesterol is calculated by measuring its amount in the stools passed over the next 7 days. The accuracy of this test is clearly dependent upon the test subject having regular, daily, or almost daily bowel movements. Reliability is jeopardized in constipated subjects. This technique has been evaluated and used for measuring cholesterol absorption in man by our laboratory and others (25). In a previous study, Quintao, Grundy, and Ahrens measured the cholesterol absorption of human subjects by four different techniques. They found that similar results were obtained by single meal technique (their method IV) and isotope-chromatographic techniques (methods I and II) (17). Therefore, the technique used in our study had been verified as one of the reliable methods to estimate cholesterol absorption in man.

The cholesterol absorption in a single test meal was not affected by background diets that provided 10–610 mg of cholesterol/day for the preceding 4 wk. These are amounts of cholesterol commonly contained in the diets of human beings the world over, with the upper amounts being typically consumed by Americans. It must be considered that the cholesterol of the diet mingles in the intestine with cholesterol excreted in the bile or from the intestinal mucosa. This endogenous cholesterol may comprise up to 1,400 mg/day (5) and might to a certain extent minimize the effects of added dietary cholesterol. However, Quintao, Grundy, and Ahrens recently found that the percentage of absorption in one patient decreased from 44% in a cholesterol-free diet to 30% in the high cholesterol diet (17). This variation may result from the different amounts of cholesterol given in the two experiments. The cholesterol content of their diet was 2,936 mg/day, which is about 5–30 times higher than ours (110–610 mg/day) and is an amount not usually consumed by Americans.

The amount of cholesterol absorbed was roughly linear for different quantities of dietary cholesterol between 110 and 610 mg, amounts compatible with the intakes contained in typical American meals. Borgstrom fed different amounts of cholesterol (150–1,910 mg) to 20 outpatients and observed similar linear relationships (26). Using different technique (isotope-chromatography), Ahrens and associates and Horlick and colleagues also found increased cholesterol absorption when dietary cholesterol was increased (6, 25, 26). One of our subjects (No. 1A-C) was fed test meals containing different amounts of cholesterol (308, 398, and 2,799 mg). The absorption was increased from 103 mg and 110 mg for the two lower doses to 662 mg when highest amount of cholesterol was given.

Thus, the absorption of cholesterol appears to increase proportionately to increase in the amount of dietary cholesterol, with no absolute upper ceiling clearly defined. The percentage absorption of cholesterol apparently remains constant as the amount of cholesterol in the diet increases up to amounts that are much greater than Americans usually consume. Above 1,000 mg/day the percentage absorbed apparently decreased. A similar phenomenon was found in the rat (27). In an earlier study, Karvinen, Lin, and Ivy measured the cholesterol absorption of the subjects fed cholesterol in the amounts of 1, 3, 6, and 9 g/day by the balance technique (28). They found that the average maximum intestinal capacity of their subjects to absorb cholesterol was 2.0 g/day. Results derived from two steady-state isotopic experiments indicated the maximum daily absorption of no more than 200–300 mg of dietary cholesterol (2, 3). We do not have a proper explanation of these different experimental results at this time. Certainly, the more recent data from direct techniques indicates absorption of up to a maximum

of 600–700 mg of cholesterol when calculated by either single meal or daily isotopic dose method (our Table III and reference 17).

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