Differential absorption of exogenous and endogenous cholesterol in man

Paul Samuel and Donald J. McNamara

The Rockefeller University, New York, NY 10021

Abstract Cholesterol absorption was measured in six patients by a triple-lumen intubation technique: *1*) to determine whether isotopic exchange between radiolabeled lumenal cholesterol and unlabeled mucosal cholesterol occurs during cholesterol absorption measurements, and *2*) to differentiate the rates of absorption of endogenous cholesterol ([1,2-^{3}H]cholesterol marker given intravenously 6 weeks prior to the tests), exogenous cholesterol ([4-^{14}C]cholesterol marker infused into the proximal duodenum), and total cholesterol mass (sitosterol marker incorporated in the infusion mixture). Measurements of endogenous cholesterol absorption during infusion of a cholesterol-free formula produced identical results whether calculated by the [1,2-^{3}H]cholesterol marker (46.2 ± 18.2%) or sitosterol marker (44.3 ± 16.9%) in eight studies. When exogenous cholesterol was administered in liquid formula, its absorption was significantly lower than that of endogenous cholesterol in six out of nine experiments (endogenous = 46.4 ± 15.4%; exogenous = 33.6 ± 8.3%; total = 40.9 ± 10.0%). When exogenous cholesterol was dissolved and administered in triglycerol monooleate, its absorption was higher than that of endogenous cholesterol in four of seven experiments (endogenous = 29.6 ± 14.5%; exogenous = 33.4 ± 9.1%; total = 29.9 ± 12.0%). These comparisons indicate that differential absorption of endogenous and exogenous cholesterol can occur over a 1- or 2-meter segment of the upper small intestine, and that the rate of cholesterol absorption is critically dependent on the physicochemical state of the intralumenal contents. Our results indicate that currently available methods for measurement of cholesterol absorption (most of which are based on the use of radioisotopic sterols for differentiating exogenous from endogenous cholesterol) reliably quantitate the absorption of dietary cholesterol, and that the results of such tests are not significantly confounded by in vivo isotopic exchange.—**Samuel, P., and D. J. McNamara.** Differential absorption of exogenous and endogenous cholesterol in man. *J. Lipid Res.* 1983. **24:** 265–276.

Supplementary key words dietary cholesterol • isotopic exchange • mucosal cholesterol secretion • intestinal intubation

The precise measurement of cholesterol absorption is one of the basic tests required for the quantitation of cholesterol homeostasis in man. There are at present eight methods available to measure cholesterol absorption; papers from this (1, 2) and other laboratories (3) have reviewed and evaluated these techniques in detail. Seven of the eight methods use radioisotopic cholesterol administered to the patient orally, intravenously, or both, in order to distinguish exogenous from endogenous cholesterol (2, 4). The eighth method, which does not require the use of radiolabeled material (5), entails the intestinal intubation of patients with a triple-lumen tube and the measurement of changes in the concentration of intralumenal cholesterol over a segment of the gut using sitosterol as a nonabsorbable marker.

Of the eight methods, the simplest are Method IV (6) and the plasma isotope-ratio method (2, 4). Method IV is based on the oral administration of a single dose of labeled cholesterol and measurement of the amount of excreted fecal radioactivity (appropriate corrections must be made for variations in fecal flow and intralumenal sterol degradation). Even simpler is the plasma isotope-ratio method introduced by Zilversmit and Hughes (4), recently evaluated by us in man (2), which is based on the simultaneous oral and intravenous administration of [^{3}H]- and [^{14}C]cholesterols and measurement of plasma cholesterol isotope ratios.

Grundy and Mok (5) recently reported studies suggesting the occurrence in man of significant isotope exchange between intralumenal radiolabeled cholesterol and unlabeled mucosal cholesterol. If significant isotope exchange does occur during intestinal transit, any results obtained by absorption tests using radioisotopic cholesterol would necessarily be inaccurate.

In the present study we have investigated the extent of isotopic exchange occurring in the human intestine, and the effect such exchange would have on the quantitation of cholesterol absorption. The data demonstrate that, when radiolabeled cholesterol is given in a liquid formula (the usual method of administration), exogenous cholesterol is absorbed at a *lower* rate than endogenous biliary cholesterol. Conversely, when cholesterol in the test meal is dissolved in the detergent triglycerol monooleate (TGM), exogenous cholesterol is absorbed at a *higher* rate than endogenous. In our view, the data of Grundy and Mok (5) can be explained on the basis of their routine use of TGM; thus, there is no need to invoke the possibility of in vivo isotopic exchange. We

Abbreviation: TGM, triglycerol monooleate.

conclude that presently available methods using radioisotopic cholesterol can validly be used to measure the absorption of cholesterol in man.

METHODS

Patients

Studies were carried out in six patients hospitalized on the metabolic ward of the Rockefeller University Hospital. Their age, sex, height, weight, and clinical diagnoses are presented in **Table 1.** Five patients had elevated plasma lipids, five had coronary artery disease, and one had asymptomatic cholelithiasis. The study protocol was approved by the University Institutional Review Board, and informed consent was obtained from each patient.

Diets for steady-state maintenance

Constant body weights were maintained in all patients during the course of the study. All subjects except Patient 3 were fed solely by liquid formula feedings as described elsewhere (7); fat contributed 35% (cottonseed oil), protein 15%, and carbohydrate 50% of total caloric intake. The formula contained 62 mg of sitosterol and 90 mg of cholesterol per 500 kcal. The caloric requirements and dietary sterol intake for each patient are given in Table 1. Minerals and vitamins were given as supplements (7).

Patient 3 was maintained during these experiments on a solid food diet fed in 2-day repetitive cycles. The percentage of energy derived from the major nutrients was determined from standard food tables (8) and was held constant from one day's menu to the next; fat content was 35% of total calories (P/S = 2.0). Cholesterol absorption tests were carried out with a formula similar in fatty acid composition and sterol content to the solid food diet: 35% of calories from soy oil, 13% from protein, and 52% from carbohydrate.

Radioactive sterols

[1,2-^{3}H]Cholesterol and [4-^{14}C]cholesterol were obtained from New England Nuclear Corp., Boston, MA; they were purified by thin-layer chromatography on Florisil (Floridin Co., Tallahassee, FL) developed with ethyl ether–heptane 45:55 (v/v). Only material that chromatographed with the R_f value of a pure cholesterol standard was administered to patients. Doses of radioactivity ranged from 100 to 150 μCi for [^{3}H]cholesterol, and from 1 to 6 μCi for [^{14}C]cholesterol. Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter (Model 3380-3390, Packard Instrument Co., Inc., Downers Grove, IL) with quench corrections performed automatically by means of an absolute activity analyzer (Packard Instruments, Model 544), as previously described (9, 10).

For accurate measurement of cholesterol absorption by the isotope-ratio method it is necessary to determine the radiochemical reliability of each batch of [1,2-^{3}H]cholesterol administered to patients (11); this was assessed by determining the dose-normalized plasma cholesterol ^{3}H/^{14}C ratio 1 to 7 days after simultaneous intravenous infusion of [4-^{14}C]cholesterol and [1,2-^{3}H]cholesterol in six volunteers. The [^{3}H]cholesterol used in the present study was found to give a dose-corrected ^{3}H/^{14}C ratio of 0.884, and this correction was used to calculate all dosages administered.

Experimental procedures

Measurement of cholesterol absorption. Method IV and the isotope-ratio method were performed as previously described (2).

TABLE 1. Clinical and dietary data

Patient: Age (yrs) Sex, Height (cm)	Relative Body Weight[a]	Plasma Lipids[b]			Dietary				Diagnosis[e]
		Cholesterol	Triglyceride	(n)	Regimen[c]	Calories[d]	Cholesterol	Sitosterol	
		mg/dl				*mg/day*			
1. AH: 55, F, 154	106	265 ± 16	154 ± 16	(56)	Formula	1800	324	223	CAD
2. GD: 51, M, 169	97	243 ± 19	291 ± 63	(38)	Formula	2575	464	319	HTG, CAD
3. HS: 66, M, 166	155	235 ± 18	437 ± 69	(24)	Rotating solid	2600	417	225	HTG
4. BE: 53, F, 156	146	275 ± 11	333 ± 42	(22)	Formula	2600	468	322	HTG, CAD Cholelithiasis
5. TF: 69, F, 159	122	473 ± 26	194 ± 16	(19)	Formula	2100	378	260	HC, CAD
6. SG: 54, M, 167	101	344 ± 41	1303 ± 36	(23)	Formula	2600	468	322	CHL, CAD

[a] Relative body weight = $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100$.

[b] Data presented as mean ± standard deviation with number of assays (n) shown in parentheses.

[c] See Methods for description of formula and rotating solid food diets.

[d] Daily calories required to maintain constant body weight.

[e] CAD, coronary artery disease; HTG, hypertriglyceridemia; HC, hypercholesterolemia; CHL, combined hyperlipidemia.

Intubation procedure. Patients were intubated with a single-lumen radiopaque 5-meter tube possessing a mercury bag at the end. When the tube appeared through the anus, the mercury bag was removed, and the tube was drawn up the intestinal tract to be positioned 1 or 2 meters distal to the ligament of Treitz (tube 3). A second tube (double-lumen) was then positioned so that the proximal outlet (tube 1) was adjacent to the ampulla of Vater and the second outlet (tube 2) was 10 cm distal, just past the ligament of Treitz. A surgical thread holding a mercury bag at the end of tube 2 was cut loose when the tubes were judged to be in proper position by fluoroscopy (the bag appeared in the feces 1–3 days later). This procedure achieved a triple-lumen intubation without requiring the presence of mercury bags at the end of the tubes; thus, we minimized (and possibly eliminated) the sleeving of bowel on the tube (12).

Preparation of infusion solutions. In the first series of experiments, 150–500 mg of nonisotopic cholesterol was dissolved in 30 g of TGM (Capital City Products; Columbus, OH), together with 1 μCi of [4-^{14}C]cholesterol in 1 ml of ethanol. The solution was added to 1 liter of water and homogenized for 3 min in a Waring blender. This opaque solution was infused intralumenally through the proximal tube together with the patients's usual formula (formulated without added cholesterol), using a 3-way stopcock for combining the two solutions into one. In a second series of experiments, 1 μCi of [4-^{14}C]cholesterol (in 1 ml of ethanol) was added directly to a 1-day portion of the patient's regular cholesterol-containing formula, was homogenized, and infused through the proximal tube. In a third series of experiments cholesterol-free formula was infused. Sitosterol inherent in the dietary oils (Table 1) served as a nonabsorbable marker in these experiments.

Infusion and aspiration procedure. All solutions of liquid formula were infused at a constant rate through the proximal outlet (tube 1) by a Sigmamotor pump (Model TM 20-4, Sigmamotor, Inc., Middleport, NY). Cholesterol and [4-^{14}C]cholesterol emulsified in TGM and water were infused with a second Sigmamotor pump simultaneously with the fat-containing formula. Formula input was adjusted to deliver approximately one-tenth of the subject's daily caloric requirements per hr. Suspensions of TGM were delivered at a rate of approximately 95 ml per hr. Five-min samples of infusate were collected in triplicate at the beginning and end of each 10-hr experiment for determination of the infusion rate of formula, markers, and sterols.

Constant aspiration of intestinal contents was carried out from the two distal outlets (tubes 2 and 3) with a Harvard pump (Model 940, Harvard Apparatus Co., Inc., Mills, MA). The rate of aspiration from each site was approximately 12 ml per hr, which is less than 5% of the flow of intestinal contents (13); aspirates were transferred to glass tubes containing 12 ml of ethanol and were stored at 4°C until assayed.

Time sequence of experimental procedures. Patients were admitted to the metabolic ward and stabilized on the experimental diets. Chromic oxide was administered to monitor fecal flow (14) and sufficient time (2 to 3 weeks) was allowed for each patient to attain the metabolic steady state (as defined previously) (15) before initiating the study. [1,2-^{3}H]Cholesterol (100–150 μCi) was given intravenously and 6 weeks later the patients were intubated as described above; the tubes remained in situ for a period of 2–4 weeks. Three to six infusion experiments of 10-hr duration were carried out in each patient. In three patients a second cholesterol absorption test (by Method IV) was carried out while the tubes were in situ in the intestines. Patients suffered no ill effects at any time as a result of these procedures.

Analytical Methods

Concentrations of plasma cholesterol and triglycerides were determined by the method of Block, Jarrett, and Levine (16) and of Kessler and Lederer (17), respectively, using the AutoAnalyzer II (Technicon Instruments Corp., Tarrytown, NY). Plasma cholesterol specific activities were measured in identical aliquots as previously described (10). Fecal neutral steroids were isolated from 24-hr stool collections; their mass and radioactivity were measured by methods developed in this laboratory (9, 18). Dietary sitosterol was used as internal standard for neutral sterol breakdown during intestinal transit (19), and chromic oxide was employed (14) as an internal standard to correct for fecal flow variations and stool recovery.

Intestinal sterols in aspirated succus entericus were analyzed by the method of Grundy and Mok (5). It was found that constant biliary output was obtained after 2–3 hr of formula infusion; thus, all calculations of cholesterol absorption by the intubation method represent the mean and standard deviation of the last 7–8 consecutive hourly measurements.

Calculations

Percent cholesterol absorptions were calculated as previously described for Method IV and the plasma isotope-ratio method (2). Cholesterol absorptions by the intubation method were calculated using the equations presented in the Results section.

Statistical analysis

All data are presented as the mean ± standard deviation for (n) number of assays (shown in parentheses or in the legend). Statistical analysis was carried out using a Hewlett-Packard 97 calculator and statistical

programs for *t*-test analysis of paired values included in the Hewlett-Packard Stat Pac I. Significance was determined using a two-tailed *t*-test (20).

RESULTS

There are at least three possible causes for discrepancies between mass measurements and radiolabeled cholesterol tracer measurements of cholesterol absorption: *a*) mucosal secretion of cholesterol into the gut lumen; *b*) isotopic exchange of lumenal and mucosal cholesterol; and/or *c*) differential absorption of exogenous dietary and endogenous biliary cholesterol. In order to investigate these potentialities, a series of studies was carried out in six patients 6 weeks after intravenous infusion of each patient with [1,2-^{3}H]cholesterol; the purpose of this was to achieve uniform specific activities of cholesterol in plasma, bile, and intestinal mucosa (15).

Cholesterol absorption with and without intubation by the triple-lumen tube

The first question addressed in this study was to determine whether the presence of the triple-lumen tube in the small bowel affected the absorption of cholesterol. In three patients (Nos. 3, 4, and 5) cholesterol absorption was measured by Method IV (2) prior to intubation and a second test was carried out after the triple-lumen tube was positioned in the intestine. Percent cholesterol absorption values were 39, 55, and 59% prior to, and 33, 38, and 31% after intubation. Thus, the presence of the intralumenal tubes reduced cholesterol absorption in two of the three patients, suggesting that the values obtained for the rate of cholesterol absorption using the triple-lumen tube intubation method may not represent what one might obtain in the absence of intubation. However, since all succeeding studies were carried out in intubated patients, the rates of cholesterol absorption measured under these conditions permitted us to investigate the questions of isotope exchange and mucosal secretion, even though the absorption rates we obtained may not have represented the true physiological rates obtained in the absence of such tubes.

Cholesterol absorption during infusion of a cholesterol-free formula

If significant secretion of newly synthesized (unlabeled) mucosal cholesterol occurred, one would expect that during infusion of a cholesterol-free formula the absorption of endogenous cholesterol measured with the sitosterol marker would be lower than that measured with the radiolabeled endogenous [1,2-^{3}H]cholesterol marker. In addition, one would expect to find the specific activity of lumenal cholesterol to decrease over the 1-meter test segment between tube 2 and tube 3 due to dilution with unlabeled cholesterol.

To test for possible secretion of mucosal cholesterol during triple-lumen tube intubation studies, we carried out eight experiments in six patients infused with a cholesterol-free formula. The following equations were used to calculate cholesterol inflow (mass and radioactivity inflow at tube 2), cholesterol outflow (mass and radioactivity outflow at tube 3), and cholesterol absorption (mg/hr and percent).

Total inflow (mg/hr) at tube 2 = [cholesterol (mg):sitosterol (mg) ratio at tube 2] × sitosterol input (mg/hr) at tube 1.

Eq. 1

Total cholesterol outflow (mg/hr) at tube 3 = [cholesterol (mg):sitosterol (mg) ratio at tube 3] × sitosterol input (mg/hr) at tube 1.

Eq. 2

Net cholesterol absorption (mg/hr) = total cholesterol inflow (mg/hr) at tube 2 − total cholesterol outflow (mg/hr) at tube 3.

Eq. 3

As seen in **Table 2** (left) total cholesterol inflow averaged 97 ± 32 mg/hr at tube 2 and cholesterol outflow equaled 51 ± 15 mg/hr at tube 3, giving mean cholesterol absorption rates of 46 ± 27 mg/hr and 44.3 ± 16.9% (calculations based on use of the sitosterol mass marker). Since exogenous cholesterol inflow was zero, cholesterol inflow at tube 2 was totally of biliary origin.

In order to calculate cholesterol absorption rates using the endogenous [1,2-^{3}H]cholesterol marker, the following equations were applied.

Endogenous cholesterol inflow (mg/hr) at tube 2 = total cholesterol inflow (mg/hr) at tube 2 (Eq. 1) − exogenous cholesterol inflow (mg/hr) at tube 2 (in this case zero for a cholesterol-free formula).

Eq. 4

Total cholesterol outflow (mg/hr) at tube 3 (based on ^{3}H-marker) = [^{3}H]cholesterol radioactivity outflow (dpm/hr) at tube 3 ÷ [^{3}H]cholesterol specific activity (dpm/hr) at tube 2,

Eq. 5

where ^{3}H-radioactivity outflow (dpm/hr) at tube 3 = [ratio of [^{3}H]cholesterol radioactivity (dpm):sitosterol (mg) at tube 3] × sitosterol input (mg/hr) at tube 1.

Eq. 6

TABLE 2. Endogenous cholesterol absorption during the infusion of a cholesterol-free formula[a]

Patient	Total Cholesterol (Sitosterol Marker)				Total Cholesterol (^{3}H-Marker)		
	Inflow	Outflow	Absorption		Outflow	Absorption	
	mg/hr		*mg/hr*	*%*	*mg/hr*	*mg/hr*	*%*
1A	130 ± 44	54 ± 16	76 ± 20	58.5	52 ± 15	78 ± 22	60.0
1B	116 ± 24	37 ± 4	79 ± 25	68.1	37 ± 7	79 ± 25	68.1
2	56 ± 13	43 ± 2	13 ± 3	23.2	44 ± 10	12 ± 9	21.4
3	90 ± 27	47 ± 6	43 ± 17	47.8	41 ± 8	49 ± 10	54.4
4A	64 ± 6	45 ± 2	19 ± 5	29.7	44 ± 4	20 ± 8	31.2
4B	66 ± 8	47 ± 1	19 ± 8	28.8	48 ± 4	18 ± 8	27.3
5	112 ± 39	44 ± 6	68 ± 27	60.7	39 ± 7	73 ± 18	65.2
6	139 ± 28	87 ± 7	52 ± 18	37.4	81 ± 10	58 ± 11	41.7
Mean ± SD	97 ± 32	51 ± 15	46 ± 27	44.3 ± 16.9	48 ± 14	48 ± 28	46.2 ± 18.2

[a] All measurements were carried out over a 1-meter segment of the intestine except in Patient 1B where a 2-meter segment was studied. Data are presented as mean ± standard deviation for seven or eight determinations.

Endogenous cholesterol absorption (mg/hr) = cholesterol inflow (mg/hr) at tube 2 (Eq. 4) − [1,2-^{3}H]cholesterol outflow (mg/hr) at tube 3 (Eq. 5). *Eq. 7*

Endogenous cholesterol absorption values calculated with [1,2-^{3}H]cholesterol as marker are shown in Table 2 (right). In each of the eight experiments, the cholesterol outflow rate at tube 3, the rate of cholesterol absorption, and the percent absorption values were essentially the same, whether calculated with the sitosterol or the [^{3}H]cholesterol markers (**Fig. 1**). Furthermore, as shown in **Table 3,** the specific activity of biliary cholesterol did not change over the test segment of the intestine; the ratio of the cholesterol specific activity at tube 3:tube 2 remained constant during the 10-hr infusion with a mean value of 0.96 ± 0.07.

This series of studies suggests that no significant secretion of newly synthesized (unlabeled) mucosal cholesterol occurred during infusion of a cholesterol-free formula. Secondly, the uniformity of the cholesterol absorption results measured by the mass and [^{3}H]cholesterol markers and the constancy of the cholesterol specific activity at the two sampling sites support the contention that there was no significant exchange of radiolabeled lumenal cholesterol with unlabeled mucosal cholesterol. It is possible, however, that exchange of equilibrated radiolabeled mucosal cholesterol may have occurred.

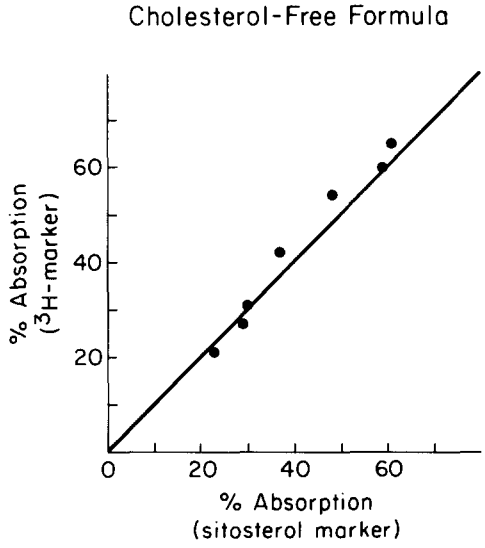

Fig. 1. Cholesterol absorption during infusion of a cholesterol-free formula. Percent cholesterol absorption calculated by the sitosterol marker versus endogenous [^{3}H]cholesterol marker plotted against the line of identity.

Cholesterol absorption during infusion of a cholesterol-containing formula

If isotopic exchange and mucosal secretion of newly synthesized cholesterol do not interfere in the measurement of cholesterol absorption, the discrepant re-

TABLE 3. Ratio of cholesterol specific activities at Tubes 2 and 3: cholesterol-free formula infusion

Patient	[1,2-^{3}H]Cholesterol Specific Activity Ratio[a] (Tube 3/Tube 2)
1A	0.98 ± 0.04 (7)
1B	0.98 ± 0.10 (7)
2	1.06 ± 0.20 (8)
3	0.85 ± 0.12 (7)
4A	0.96 ± 0.08 (8)
4B	1.03 ± 0.07 (8)
5	0.89 ± 0.09 (8)
6	0.96 ± 0.07 (7)
Mean ± SD	0.96 ± 0.07

[a] All measurements were carried out over a 1-meter segment of the intestine except in Patient 1B where a 2-meter segment was studied. Data are presented as mean ± standard deviation for number of assays shown in parentheses.

TABLE 4. Endogenous and exogenous cholesterol absorption: exogenous cholesterol (^{14}C-marker) dissolved in formula free of triglycerol monooleate[a]

Patient	Total Cholesterol (Sitosterol Marker)				Total Cholesterol (^{14}C-Marker)			Total Cholesterol (^{3}H-Marker)			Δ[b]	P[c]
	Inflow	Outflow	Absorption		Outflow	Absorption		Outflow	Absorption			
	mg/hr		mg/hr	%	mg/hr	mg/hr	%	mg/hr	mg/hr	%		
1A	126 ± 28	66 ± 17	60 ± 20	47.6	106 ± 13	20 ± 4	15.9	45 ± 19	81 ± 25	64.3	303	<0.001
1B	181 ± 36	73 ± 21	108 ± 36	59.7	106 ± 24	35 ± 22	41.4	48 ± 11	133 ± 46	73.5	78	<0.005
2	95 ± 23	61 ± 3	34 ± 16	35.8	64 ± 21	31 ± 10	32.6	59 ± 15	36 ± 8	37.9	16	NS
3A	135 ± 21	81 ± 8	54 ± 13	40.0	90 ± 15	45 ± 15	33.3	61 ± 19	74 ± 30	54.8	65	<0.05
3B	127 ± 23	76 ± 8	51 ± 16	40.2	93 ± 14	34 ± 12	26.8	57 ± 15	70 ± 33	55.1	106	<0.02
4A	120 ± 18	70 ± 7	50 ± 17	41.7	73 ± 30	47 ± 14	39.2	71 ± 24	49 ± 12	40.8	4	NS
4B	118 ± 12	74 ± 3	44 ± 11	37.3	74 ± 12	44 ± 6	37.3	68 ± 6	50 ± 15	42.4	14	NS
5	93 ± 17	52 ± 6	41 ± 16	44.1	57 ± 8	36 ± 10	38.7	42 ± 8	51 ± 51	54.8	42	<0.02
6	102 ± 8	80 ± 5	22 ± 12	21.6	65 ± 12	37 ± 8	36.3	87 ± 8	15 ± 10	14.7	−59	<0.02
Mean ± SD	122 ± 27	70 ± 9	52 ± 24	40.9 ± 10.0	81 ± 18	41 ± 15	33.4 ± 8.3	53 ± 22	62 ± 33	48.7 ± 17.0	63 ± 102	

[a] All measurements were carried out over a 1-meter segment of the intestine except in Patient 1B where a 2-meter segment was studied. Data are presented as mean ± standard deviation for seven or eight assays.
[b] Percent difference between total cholesterol absorption using the exogenous [^{14}C]- and endogenous [^{3}H]cholesterol markers.
[c] Significance of differences in cholesterol absorption calculated by means of the two radiolabeled cholesterol markers; NS, not significantly different.

sults obtained by Grundy and Mok (5) with the triple-lumen tube intubation procedure must have arisen from one or more of three sources. *a*) If secretion into the lumen of already equilibrated mucosal cholesterol occurred, then the rate of cholesterol absorption calculated with the endogenous [1,2-^{3}H]cholesterol marker would have been lower than that calculated by either the sitosterol mass marker or the exogenous [4-^{14}C]cholesterol marker. *b*) If isotopic exchange of lumenal and equilibrated mucosal cholesterol occurred, then the rate of cholesterol absorption calculated with the endogenous marker would have been lower, and with the exogenous cholesterol marker higher, than that calculated with the sitosterol mass marker. *c*) If differential absorption of endogenous and exogenous cholesterol occurred, then the rate of cholesterol absorption measured by the sitosterol marker should fall between the two values calculated by the exogenous and endogenous cholesterol markers.

To test these possibilities nine further studies were carried out in the same six patients during infusion with a formula containing known amounts of exogenous [4-^{14}C]cholesterol. In comparable experiments, Grundy and Mok (5) solubilized their exogenous cholesterol with TGM; in the studies now to be presented, we omitted TGM. Total cholesterol flow and absorption values were calculated using the mass sitosterol marker (Eq. 1–3) and the endogenous [1,2-^{3}H]cholesterol marker (Eq. 4–7). For measurement of cholesterol absorption with exogenous [4-^{14}C]cholesterol marker, the following equations were used.

Exogenous cholesterol inflow (mg/hr) at tube 2 = infusion rate of cholesterol-containing formula (mg/hr).

Eq. 8

[^{14}C]Cholesterol outflow (mg/hr) at tube 3 is calculated as previously described for [^{3}H]cholesterol outflow (Eq. 5 and 6).

Total cholesterol absorption (mg/hr) (based on ^{14}C-marker) = cholesterol inflow (mg/hr) at tube 2 (Eq. 4) − [^{14}C]cholesterol outflow (mg/hr) at tube 3 (Eq. 5).

Eq. 9

The data presented in **Table 4** compare the measurements of cholesterol absorption rates calculated with the mass, endogenous, and exogenous cholesterol markers in patients infused with [^{14}C]cholesterol-containing formula. We found large and significant differences in the rate of cholesterol outflow at tube 3 when calculations were based on the sitosterol mass marker (70 ± 9 mg/hr), the [4-^{14}C]cholesterol marker (81 ± 18 mg/hr), and the [1,2-^{3}H]cholesterol marker (53 ± 22

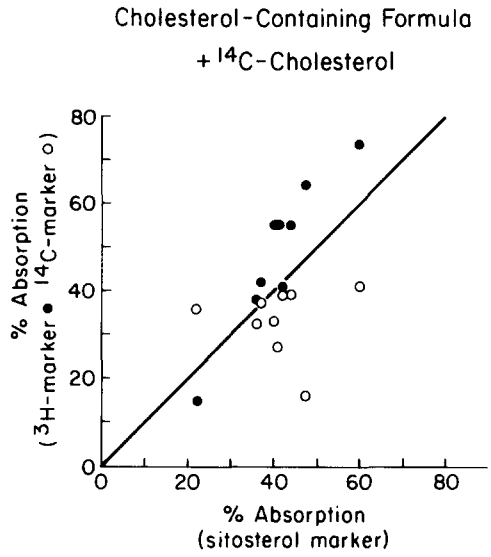

Fig. 2. Cholesterol absorption during infusion of a cholesterol-containing formula free of TGM. Percent cholesterol absorption calculated by the sitosterol marker versus endogenous [^{3}H]cholesterol (●) and exogenous [^{14}C]cholesterol (○) markers plotted against the line of identity.

mg/hr). These outflow rates resulted in absorption values of 40.9, 33.4, and 48.7%, respectively. In five of the nine studies (Nos. 1A, 1B, 3A, 3B, and 5) the rate of cholesterol absorption by the endogenous [1,2-^{3}H]cholesterol marker was significantly greater than that calculated by the exogenous [4-^{14}C]cholesterol marker; in one patient (No. 6) the opposite result was found; and in the remaining three studies the differences were not significant (**Fig. 2**).

Since the absorption values were higher when based on the endogenous [1,2-^{3}H]cholesterol marker than when based on the exogenous [4-^{14}C]cholesterol marker, it is unlikely that either mucosal secretion or isotopic exchange of equilibrated cholesterol occurred over the test segment of the gut; either would have resulted in results opposite to those we obtained. The data suggest a differential absorption of exogenous and endogenous cholesterol; the values for absorption calculated with the sitosterol mass marker routinely fell between the values obtained with the two radiolabeled cholesterol markers (Table 4).

Additional evidence for such a conclusion is obtained from comparisons of the ratios of the specific activities of the two labeled cholesterols at tube 2 and tube 3 (**Table 5**). The tube 3:tube 2 specific activity ratios for [4-^{14}C]cholesterol increased while those of [1,2-^{3}H]cholesterol decreased over the test segment of gut. This could occur only if endogenous [1,2-^{3}H]cholesterol was absorbed at a faster rate than exogenous [4-^{14}C]cholesterol.

Since the specific activities of the exogenous and endogenous cholesterols are known and there is no evidence for secretion or isotopic exchange of mucosal cholesterol, it is possible to calculate the flow and absorption rates of endogenous and exogenous cholesterol independently. The results of these calculations are presented in **Table 6;** the mean absorption of exogenous cholesterol equaled 33.6 ± 8.3% and of endogenous cholesterol equaled 46.4 ± 15.6%. Endogenous cholesterol absorption exceeded that of exogenous cholesterol in six of nine experiments; the results were very similar in Patients 2 and 4A; and only in Patient 6 was exogenous cholesterol absorbed at a faster rate than endogenous cholesterol.

The data reported support the concept that over a 1- or 2-meter test segment of the gut endogenous cholesterol is absorbed faster than exogenous cholesterol, and that there is no significant secretion of mucosal cholesterol or isotopic exchange of lumenal and mucosal cholesterol in such studies.

Cholesterol absorption during infusion of a formula containing cholesterol in TGM

The results cited above stand in striking contrast to those reported by Grundy and Mok (5) who found that infusion of exogenous [4-^{14}C]cholesterol suspended in the detergent TGM resulted in significant differences in absorption rates calculated with sitosterol versus exogenous [4-^{14}C]cholesterol markers; these differences were explained by them on the basis of the occurrence of significant isotope exchange.

To explore these differences, we carried out seven further studies in five of the same patients by the triple-

TABLE 5. Ratio of cholesterol specific activities at Tubes 2 and 3: exogenous cholesterol (^{14}C-marker) dissolved in formula free of TGM[a]

Patient	[4-^{14}C]Cholesterol Specific Activity Ratio[b] (Tube 3/Tube 2)	[1,2-^{3}H]Cholesterol Specific Activity Ratio[b] (Tube 3/Tube 2)	P[c]
1A	1.49 ± 0.49 (8)	0.68 ± 0.16 (8)	<0.001
1B	1.54 ± 0.38 (8)	0.70 ± 0.11 (7)	<0.001
2	0.94 ± 0.34 (8)	0.83 ± 0.31 (8)	NS
3A	1.09 ± 0.19 (8)	0.73 ± 0.25 (8)	<0.05
3B	1.19 ± 0.23 (7)	0.71 ± 0.14 (7)	<0.01
4A	0.81 ± 0.27 (8)	0.98 ± 0.19 (8)	NS
4B	0.97 ± 0.13 (8)	0.93 ± 0.07 (8)	NS
5	1.09 ± 0.17 (8)	0.78 ± 0.12 (8)	<0.005
6	0.78 ± 0.17 (8)	1.12 ± 0.14 (8)	<0.01
Mean ± SD	1.10 ± 0.27	0.83 ± 0.15	

[a] All measurements were carried out over a 1-meter segment of the intestine except in Patient 1B where a 2-meter segment was studied.
[b] Mean ± standard deviation for (n) determinations.
[c] Significance of differences between the two sets of specific activity ratios; NS, not significantly different.

TABLE 6. Flow rates and absorption rates of exogenous and endogenous cholesterol during infusion of a cholesterol-containing formula free of TGM[a]

Patient	Exogenous [4-^{14}C]Cholesterol				Endogenous [1,2-^{3}H]Cholesterol			
	Inflow	Outflow	Absorption		Inflow	Outflow	Absorption	
	mg/hr		*mg/hr*	*%*	*mg/hr*		*mg/hr*	*%*
1A	23	19	4	17.4	103	38	65	63.1
1B	27	16	11	40.7	154	53	101	65.6
2	29	19	10	34.5	66	46	20	30.3
3A	42	29	13	31.0	93	44	49	52.7
3B	42	31	11	26.2	85	40	45	52.9
4A	35	19	16	45.7	85	50	35	41.2
4B	37	23	14	37.8	81	47	34	42.0
5	27	17	10	37.0	66	31	35	53.0
6	25	17	8	32.0	77	64	13	16.9
Mean ± SD	32 ± 7	21 ± 5	11± 3	33.6 ± 8.3	90 ± 27	46 ± 9	44 ± 26	46.4 ± 15.4

[a] All measurements were carried out over a 1-meter segment of the intestine except in patient 1B where a 2-meter segment was studied.

lumen tube intubation method using exogenous [4-^{14}C]cholesterol dissolved in TGM. The results of these studies are presented in **Table 7.** Percent cholesterol absorption averaged 29.9 ± 12.0% (sitosterol marker), 34.2 ± 9.7% (exogenous [^{14}C]cholesterol marker), and 30.3 ± 9.7% (endogenous [^{3}H]cholesterol marker). In three of the seven studies exogenous cholesterol was absorbed at a faster rate than endogenous cholesterol; in one patient (No. 5) endogenous cholesterol was absorbed faster than exogenous cholesterol; and in the remaining three subjects the differences were not significant (**Fig. 3**). The mean difference between the calculated absorption values by the sitosterol marker and the [4-^{14}C]cholesterol marker was 23% (range, −23 to +89%).

The tube 3:tube 2 specific activity ratios for [4-^{14}C]cholesterol decreased while those of [1,2-^{3}H]cholesterol remained unchanged over the test segment of gut (**Table 8**). This is in striking contrast to the findings obtained when exogenous cholesterol was dissolved in formula free of TGM (Table 5) and suggests that exogenous [4-^{14}C]cholesterol was absorbed at a faster rate than endogenous [1,2-^{3}H]cholesterol. Calculations of the flow and absorption rates of endogenous cholesterol and exogenous cholesterol dissolved in TGM are presented in **Table 9;** the mean absorption of endogenous cholesterol equaled 29.6 ± 14.5% and of exogenous cholesterol equaled 33.4 ± 9.1%. In three patients cholesterol dissolved in TGM was absorbed at a faster rate than endogenous cholesterol; in three patients the two cholesterols were absorbed equally, and in one subject endogenous cholesterol was absorbed at a greater rate than exogenous.

These studies suggest but do not conclusively prove that the detergent TGM causes a preferential absorption of the exogenous [4-^{14}C]cholesterol, a result strikingly in contrast to that obtained in the absence of TGM. Comparison of the results of all three sets of studies (**Fig. 4**) suggests that the TGM actually caused a reduced rate of endogenous cholesterol absorption, since the endogenous absorption equaled 46.2 ± 18.2% (n = 8) and 46.4 ± 15.6% (n = 9) in studies 1 and 2, respectively, but only 29.6 ± 14.5% (n = 7) in study 3 using TGM. By contrast, exogenous cholesterol absorptions in studies 2 and 3 were 33.6 ± 8.3% (n = 9) and 33.4 ± 9.1% (n = 7), respectively.

DISCUSSION

Cholesterol absorption from the gastrointestinal tract is one of the many factors that regulate plasma cholesterol levels and the size of the body pools of cholesterol; we also have shown that it affects the rate of deposition of cholesterol in various body storage sites (21). Since the quantitation of cholesterol absorption depends on availability of reliable and reproducible methods, it is worrisome that there exist a number of uncertainties regarding the theoretical bases upon which these measurements are based. Some of these uncertainties arise from the fact that the mechanisms by which cholesterol is absorbed remain unclear, despite the research efforts of many laboratories over the past three decades.

Seven of the eight currently available methods for measurement of cholesterol absorption in man (1–3) depend on the use of radiolabeled cholesterol to differentiate exogenous from endogenous cholesterol. The present study was undertaken to investigate the relative absorption rates of exogenous and endogenous cholesterol over a 1- to 2-meter segment of the proximal small bowel, in the course of which we tested the validity of using radiolabeled cholesterol as a tracer in absorption measurements.

Isotope exchange and/or mucosal secretion

Simmonds, Hofmann, and Theodor (22) measured cholesterol absorption in patients intubated with a triple-lumen tube during infusion of a micellar solution of bile salts, 1-monoglyceride, and [^{14}C]cholesterol. These investigators reported that the specific activity of the infused cholesterol decreased over a 50-cm test segment of the proximal jejunum; they concluded that the secretion of nonlabeled cholesterol into the lumen of the intestine resulted in the reported decrease in radioactivity of the infused cholesterol.

Grundy and Mok (5) carried out similar studies except that radiolabeled exogenous cholesterol was dissolved in the detergent TGM, and in agreement with Simmonds et al. (22) found that the specific activity of exogenous cholesterol decreased over a 1-meter test segment. They concluded that a significant degree of exchange of radiolabeled lumenal cholesterol with unlabeled mucosal cholesterol had taken place.

In the rat it is claimed that there is a significant secretion of newly synthesized mucosal cholesterol into the lumen (23, 24); however, in man the only data supporting this possibility have been obtained in patients given cholestyramine and in others subjected to partial ileal bypass (25). If intralumenal secretion does not occur in man, the data of Simmonds et al. (22) and Grundy and Mok (5) are consistent with the possibility that isotopic exchange occurs; but in this event we are forced to conclude that all methods for measurement of cholesterol absorption that rely on the use of radiolabeled cholesterol are subject to serious error.

For these reasons the results we obtained in the first set of studies, using a cholesterol-free formula, are critically important; there was no dilution of endogenous [^{3}H]cholesterol over a 1-meter length of the test segment in any of the eight studies, showing conclusively that mucosal secretion of unlabeled cholesterol had not occurred. And in the same studies the absorption of endogenous cholesterol ([^{3}H]cholesterol-labeled) was the same as mass cholesterol absorption (sitosterol-labeled), showing that isotopic exchange between radiolabeled lumen and newly synthesized mucosa cholesterol had not taken place.

Differential absorption of endogenous and exogenous cholesterol

However, the findings of these two laboratories also can be explained by a third possibility: preferential absorption of exogenous cholesterol (either from a bile salt micellar solution or from a lumenal phase containing the detergent TGM). If exogenous cholesterol is preferentially absorbed, then its specific activity will decrease as the test bolus passes down the 1-meter segment of intestine, and there will be no need to invoke

TABLE 7. Endogeous and exogenous cholesterol absorption: exogenous cholesterol (^{14}C-marker) dissolved in triglyceride monooleate[a]

Patient	Total Cholesterol (Sitosterol Marker)				Total Cholesterol (^{14}C-Marker)				Total Cholesterol (^{3}H-Marker)				Δ^b	P^c
	Inflow	Outflow	Absorption		Outflow		Absorption		Outflow		Absorption			
	mg/hr		mg/hr	%	mg/hr		mg/hr	%	mg/hr		mg/hr	%		
2A	106 ± 26	64 ± 5	42 ± 16	39.6	63 ± 14		43 ± 16	40.5	66 ± 14		40 ± 9	37.7	−7	NS
2B	49 ± 7	40 ± 2	9 ± 2	18.4	32 ± 15		17 ± 3	34.7	43 ± 5		6 ± 1	12.2	−65	<0.005
3	104 ± 17	54 ± 5	50 ± 13	48.1	54 ± 8		50 ± 12	48.1	51 ± 13		53 ± 15	50.9	6	NS
4A	86 ± 10	62 ± 5	24 ± 5	27.9	56 ± 9		30 ± 4	34.9	56 ± 8		30 ± 7	34.9	0	NS
4B	82 ± 8	59 ± 3	23 ± 6	28.1	52 ± 8		30 ± 7	36.6	59 ± 5		23 ± 10	28.0	−23	<0.01
5	76 ± 13	50 ± 2	26 ± 8	34.2	56 ± 12		20 ± 6	26.3	46 ± 8		30 ± 10	39.5	50	<0.01
6	100 ± 18	87 ± 7	13 ± 3	13.0	82 ± 13		18 ± 5	18.0	91 ± 11		9 ± 2	9.0	−50	<0.01
Mean ± SD	86 ± 20	59 ± 15	27 ± 15	29.9 ± 12.0	56 ± 15		30 ± 13	34.2 ± 9.7	59 ± 16		27 ± 16	30.3 ± 15.1	−13 ± 38	

[a] Data are presented as mean ± standard deviation for seven or eight assays.
[b] Percent difference between total cholesterol absorption using exogenous [^{14}C]- and endogenous [^{3}H]cholesterol markers.
[c] Significance of differences in cholesterol absorption calculated by means of the two radiolabeled cholesterol markers: NS, not significantly different.

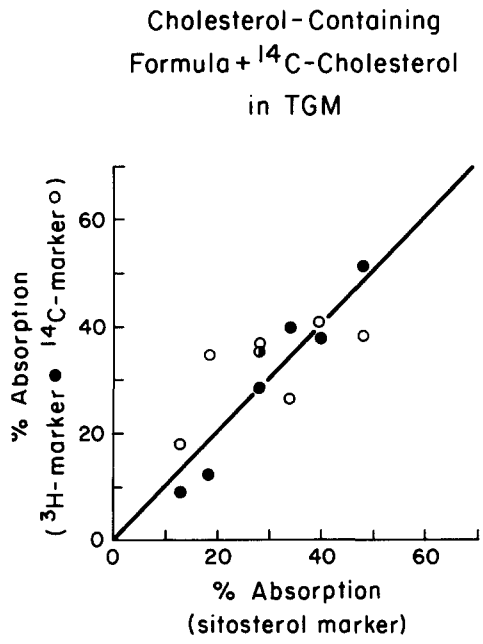

Fig. 3. Cholesterol absorption during infusion of a cholesterol-containing formula plus [^{14}C]cholesterol dissolved in TGM. Percent cholesterol absorption calculated by the sitosterol marker versus endogenous [^{3}H]cholesterol (●) and exogenous [^{14}C]cholesterol (○) markers plotted against the line of identity.

lumenal secretion of cholesterol and/or isotopic exchange as explanations for the findings.

The results of the present study strongly suggest that differential absorption of exogenous and endogenous cholesterol does occur, the degree of which is dependent upon the physicochemical state of intralumenal cholesterol. Measurements of the absorption of total, endogenous, and exogenous cholesterol during infusion of exogenous cholesterol in liquid formula demonstrated significant differences between the absorption values. The absorption of endogenous cholesterol was greater than that of exogenous cholesterol in six of nine experiments, and the absorption rates calculated by means of the sitosterol mass-marker routinely fell between those obtained by means of the two radiolabeled cholesterol markers. If secretion of radiolabeled mucosal cholesterol had occurred, then the calculated absorption rates using the endogenous cholesterol marker should have been lower than those derived by use of the exogenous cholesterol marker; this was not the case. If exchange of mucosal cholesterol with lumenal cholesterol (either exogenous or endogenous) had occurred, then again one would expect to find a lower rate of absorption calculated by means of the endogenous marker, a higher rate calculated with the exogenous cholesterol marker, and an intermediate value when the rate of absorption is calculated by means of the sitosterol marker. During the infusion of exogenous cholesterol in liquid formula, the data found were opposite to these predictions; endogenous cholesterol was absorbed at a greater rate than exogenous, suggesting that differential absorption rather than exchange or secretion had occurred.

When the cholesterol-containing test meal contained TGM, the rate of absorption of exogenous cholesterol was greater than that of endogenous cholesterol, suggesting either exchange of lumenal and endogenously radiolabeled mucosal cholesterol, secretion of endogenously radiolabeled mucosal cholesterol, and/or differential absorption. It would appear that the discrepancies in the two sets of experiments are due to presence of TGM. It should be noted that during infusion of the exogenous cholesterol in TGM the biliary secretion rate of cholesterol was decreased from 97 ± 32 mg/hr (study 1) and 90 ± 27 mg/hr (study 2) to 54 ± 12 mg/hr (study 3). Why infusion of TGM containing-solutions should decrease endogenous cholesterol flow and to what extent this decreased output affects endogenous cholesterol absorption cannot be explained from the present study. The data do support, however, the suggestion that the presence of TGM in the infusion mixture does significantly alter the processes of endogenous cholesterol output and readsorption in vivo.

Modifications in the intubation procedure

It should be noted that a number of procedural modifications were introduced in order to optimize the conditions under which cholesterol absorption was measured by the triple-lumen tube procedure. First, all patients in the study were intravenously infused with [1,2-^{3}H]cholesterol 6 weeks prior to the absorption studies. We have previously demonstrated that in that period of time the mucosal pool of cholesterol attains the same specific activity as plasma cholesterol and biliary

TABLE 8. Ratio of cholesterol specific activities at Tubes 2 and 3: exogenous cholesterol (^{14}C-marker) dissolved in triglycerol monooleate

Patient	[4-^{14}C]Cholesterol Specific Activity Ratio[a] (Tube 3/Tube 2)	[1,2-^{3}H]Cholesterol Specific Activity Ratio[a] (Tube 3/Tube 2)	P^{b}
2A	0.86 ± 0.13 (8)	0.96 ± 0.17 (8)	NS
2B	0.71 ± 0.12 (8)	0.98 ± 0.21 (8)	<0.001
3	0.84 ± 0.16 (8)	0.91 ± 0.09 (8)	NS
4A	0.87 ± 0.14 (8)	0.90 ± 0.13 (8)	NS
4B	0.87 ± 0.11 (8)	1.00 ± 0.08 (8)	<0.025
5	1.09 ± 0.20 (8)	0.90 ± 0.12 (8)	NS
6	0.85 ± 0.11 (8)	1.18 ± 0.14 (8)	<0.01
Mean ± SD	0.87 ± 0.11	0.98 ± 0.10	

[a] Mean ± standard deviation for (n) determinations.
[b] Significance of differences between the two sets of specific activity ratios; NS, not significantly different.

TABLE 9. Flow rates and absorption rates of exogenous and endogenous cholesterol during infusion of exogenous cholesterol in TGM

Patient	Exogenous [4-^{14}C]Cholesterol				Endogenous [1,2-^{3}H]Cholesterol			
	Inflow	Outflow	Absorption		Inflow	Outflow	Absorption	
	mg/hr		*mg/hr*	*%*	*mg/hr*		*mg/hr*	*%*
2A	49	30	19	38.8	49	39	18	31.6
2B	12	8	4	33.3	37	33	4	10.8
3	36	19	17	47.2	68	35	33	48.5
4A	29	19	10	34.5	57	38	19	33.3
4B	31	20	11	35.5	51	32	19	37.3
5	35	36	9	25.7	41	26	15	36.6
6	32	26	6	18.8	68	62	6	8.8
Mean ± SD	32 ± 11	21± 7	11 ± 5	33.4 ± 9.1	54 ± 12	38 ± 11	16 ± 10	29.6 ± 14.5

cholesterol (15). This approach allowed us to measure the absorption of endogenous cholesterol and to compare it to that of exogenous cholesterol. Further, it allowed us to distinguish between secretion of newly synthesized mucosal cholesterol and isotopic exchange.

The second modification was our avoidance of intestinal sleeving during the prolonged intubation experiments; our intralumenal tubes were not weighted with mercury bags, and thus the tubes lay freely in the intestinal lumen (12). For these reasons the length of test segment of the intestine was precisely controlled, and patient-to-patient variations in the length of test segment under study were minimized.

Third, and most important, intralumenal cholesterol was presented to the mucosa in various physicochemical states: as endogenous biliary micelles, as exogenous cholesterol suspensions in the fat of the infused formula, and as exogenous cholesterol solutions in TGM. Fourth, we measured endogenous cholesterol absorption during the infusion of a formula free of exogenous cholesterol; this allowed us to rule out the secretion and/or exchange of newly synthesized mucosal cholesterol. These studies also served as tests of the analytical accuracy of this rather complicated procedure.

The validity of cholesterol absorption measurements in man

For many years we and other investigators have assumed that the absorption rates of endogenous and exogenous cholesterol are equal throughout the length of the small intestine. The present study does not disprove this assumption, since we measured the absorption of cholesterol only over the proximal 1- or 2-meters of jejunum, and not over the entire length of the small bowel. Indeed, the studies in Patient 1 (Tables 2, 3, and 6) suggest that the absorption of exogenous cholesterol was twice as great over a test segment length of 2 meters as over a 1-meter length. Further experiments are required in order to test the generality of this finding.

It should also be noted that these studies were carried out with a triple-lumen tube indwelling in the upper intestine for several weeks. As shown in the first part of the results section, this in itself significantly decreased percent cholesterol absorption, possibly by an alteration in the unstirred water layer (26). Whether the presence of an intestinal tube affects the absorption of exogenous and endogenous cholesterol differentially remains a question.

In the present study and in that of Grundy and Mok (5) a wide range in patient-responses to the infusion systems was noted; for instance, Patient 5 absorbed endogenous cholesterol at a faster rate than exogenous cholesterol, irrespective of the physicochemical state of the exogenous cholesterol, and Patient 6 did just the opposite. In the studies of Grundy and Mok (5), the discrepancies in absorption of exogenous cholesterol ranged from 0 to 65% in 20 experiments when the data were compared between rates obtained by means of sitosterol and exogenous [^{14}C]cholesterol markers. The

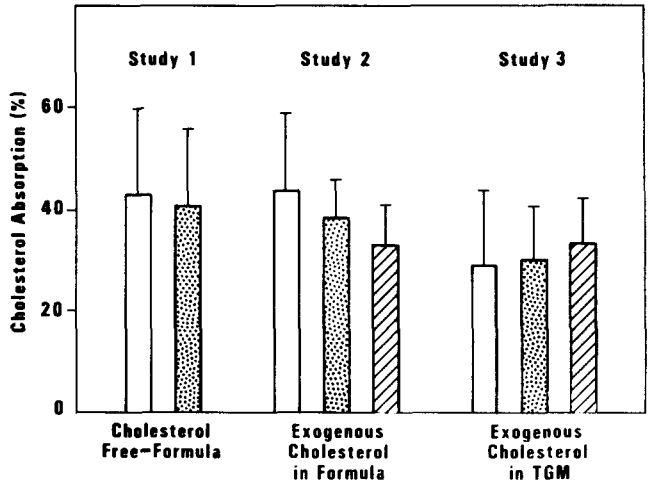

Fig. 4. Endogenous, exogenous, and total cholesterol absorption over a 1-meter segment of the intestine during infusion of a cholesterol-free formula (n = 7), exogenous cholesterol in formula (n = 8), and exogenous cholesterol in triglycerolmonooleate (n = 8). Endogenous cholesterol, open bars; total cholesterol, striped bars; exogenous cholesterol, hatched bars.

causes of these patient-to-patient variations remain unknown.

While numerous uncertainties exist regarding the molecular basis for the absorption of cholesterol, the present study lends support to the continued use of radiolabeled cholesterol in measurements of exogenous cholesterol absorption in man, since neither mucosal secretion of newly synthesized cholesterol nor exchange of lumenal and mucosal cholesterol appear to occur in vivo.

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