Origin of cholesterol transported in intestinal lymph: studies in patients with filarial chyluria

Eder C. R. Quintão, Abram Drewiacki, Karin Stechhahn, Eliana C. de Faria, and Aytan M. Sipahi

Department of Internal Medicine, Hospital das Clínicas, The University of São Paulo Medical School, São Paulo, Brazil

Abstract In subjects fed a cholesterol-free diet there are three possible sources of intestinal lymph cholesterol: *a*) mucosal synthesis; *b*) absorption of endogenous (biliary) cholesterol; and *c*) transudation of plasma lipoproteins into the lacteals of the intestinal wall. To test these possibilities, the extent of transudation was measured by means of [^{3}H]β-sitosterol administered intravenously as a marker. Absorption of biliary cholesterol was reduced by oral administration of β-sitosterol (9 g/day), and mucosal synthesis of cholesterol was evaluated by comparisons of plasma/lymph [^{14}C]cholesterol specific activity ratios after intravenous administration of a single dose of labeled cholesterol. Studies were carried out on six patients with filarial chyluria. In five patients fed a cholesterol-free diet the results indicated that lymph cholesterol was largely derived by transudation of plasma lipoproteins into the lacteals from the intestinal blood supply, without contribution from de novo mucosal synthesis or from absorption of endogenous cholesterol. The intestinal lymph of one patient fed cholesterol (2 g/day) contained cholesterol originating mostly from plasma transudation and from dietary absorption, with little contribution from absorbed endogenous cholesterol. In all experiments the larger part of the cholesterol transported away from the intestine in the lymph was carried in chylomicrons, even though it had its origin in plasma lipoproteins.—**Quintão, E. C. R., A. Drewiacki, K. Stechhahn, E. C. de Faria, and A. M. Sipahi.** Origin of cholesterol transported in intestinal lymph: studies in patients with filarial chyluria. *J. Lipid Res.* 1979. **20:** 941–951.

Supplementary key words intestinal cholesterol synthesis · plasma cholesterol transudation to intestinal lymph · β-sitosterol feeding · cholesterol and fat in lymph lipoproteins · cholesterol absorption

It is generally accepted that on a regular American diet plasma cholesterol in man originates mostly from endogenous synthesis (1–5). Several animal tissues make cholesterol (6), but the liver and the intestine represent the largest body pools rapidly exchanging with the plasma (7) and are potentially the main sources of the plasma cholesterol. In fact, several animal studies support the concept that the bulk of plasma cholesterol represented by synthesis originates from the intestinal wall (8–11). However, because there are marked differences in laboratory animals and man in rates of intestinal absorption of dietary cholesterol (2–5) and conversion to bile acids (3, 4), it is important to define the role of the intestinal mucosa in man.

Filarial chyluria offers a rare clinical research opportunity to study mucosal cholesterol metabolism. Consequent to the existence of a fistula between the intestinal lymph system and the renal pelvis, small amounts of cholesterol and lipoproteins originating in the intestinal wall are diverted into the urine; yet the reasonably constant urine losses of dietary fat and of cholesterol do not necessarily interfere with the maintenance of a metabolic steady state. Furthermore, the total cholesterol mass transported to the blood stream via the intestinal lymphatics can be easily calculated if fat intake is used as a marker (12, 13).

Methods were developed to compare the three possible sources of intestinal lymph cholesterol, namely, mucosal synthesis, absorption of bile, and transudation of plasma lipoproteins, in experiments where patients were maintained on a cholesterol-free diet. Plasma cholesterol transudation was quantified in four experiments in three patients by means of [^{3}H]β-sitosterol administered intravenously as a marker. The absorption of endogenous (biliary) cholesterol was diminished by means of oral administration of β-sitosterol (9 g/day) for three patients. The contribution to lymph cholesterol of cholesterol newly synthesized in the mucosa, or absorbed from the diet, was measured by defining the specific activities of plasma and urinary lymph cholesterol after giving a single intravenous dose of labeled cholesterol, respectively, to five patients fed a cholesterol-free diet, and to one patient fed cholesterol, 2 g/day. In all

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH

experimental situations plasma cholesterol transudation appeared to be the major contributor to the lymph cholesterol.

METHODS

Patients

Metabolic studies were carried out in six patients admitted to the Metabolic Ward of the Department of Internal Medicine at the University of São Paulo Medical School in Brazil. All subjects were in good clinical condition and had been previously treated for filariasis in endemic areas of the northeast section of the country. They joined the research program after formal written consent was obtained.

Recurrent chyluria is a known sequel of filariasis, although it is less common than, and never associated with, lymphedema. Several patients proved unsuitable for long-lasting metabolic studies, because their chyluria vanished suddenly for unknown reasons in seven out of ten cases originally admitted to the program. For these reasons, two or more related studies could be performed in any one patient only rarely. After experimental observations were completed, three patients were submitted to surgical ligation of their intestinal–urinary lymph fistula.

The age, sex, body build, mean serum cholesterol measured during cholesterol-free diets, and clinical course of the patients are presented in **Table 1.**

Diets

Upon admission to the hospital all patients were maintained at constant body weight with diets of known composition and fixed caloric intake (**Table 2**). Dietary fat contributed 42%, carbohydrate 46%, and protein 12% of the total caloric intake, in conformance with dietary habits. Cases 1, 2, and 3 were fed a liquid formula diet supplemented with mineral salts and vitamins according to methods already described (15): soybean was the main protein source, to which cottonseed oil and sucrose were added. Cases 4, 5, and 6 did not tolerate this diet and were given a weighed, repetitive solid food diet with egg white as the main protein source, in addition to vegetables, fruits, and starch. In these patients cottonseed oil represented 95% of the fat intake. Phytosterols inherent in cottonseed oil served as a marker for the recovery of fecal neutral steroids (16). However, in the case of solid-food diets, phytosterol intakes were not measured, because raw vegetables were fed ad libitum; thus fecal neutral steroid recovery was corrected only by the intake of 300 mg/day of chromic oxide (17, 18). Because the solid-food diets contained cellulose, fecal steroid degradation was assumed to be negligible (19).

Techniques of sample collection and analysis

Serum cholesterol concentrations and specific activities were measured twice a week. Analyses were carried out by GLC (C&G gas chromatograph, São Paulo, Brazil) after mild alkaline hydrolysis, extraction with hexane, and purification by TLC on Florisil according to methods already described (20).

Bile cholesterol and specific activities were measured as described above. After an overnight fasting period, about 5 ml of bile were collected after duodenal intubation and intravenous administration of cho-

TABLE 1. Clinical data and hospital course

Patient	Initials	Age	Sex	Height	Weight	% of Ideal Body Weight[a]	Serum Cholesterol	Clinical Course
		yr		*cm*	*kg*		*mg/dl*[b]	
1	I.R.M.	53	F	160	67	105	193 ± 17 (9)	Discharged from the hospital with slight chyluria on a low-fat diet.
2	A.A.S.	22	M	172	66	98	141 ± 20 (15)	Spontaneous remission of chyluria.
3	A.F.N.	20	M	169	48	73	150 ± 32 (22)	Spontaneous remission of chyluria.
4	M.L.S.M.	22	F	154	49	98	119 ± 31 (12)	Successful surgical ligation of a left renal–intestinal lymph fistula.
5	M.G.P.P.	17	F	158	44	86	93 ± 21 (12)	Successful surgical ligation of a left renal–intestinal lymph fistula.
6	E.R.S.	33	M	181	66	97	120 ± 5 (4)	Spontaneous remission of intermittent chyluria. Surgical ligation of right renal–intestinal lymph fistula.

[a] Measured by American standards, according to life insurance tables (14).
[b] Mean ± SD (no. of determinations) measured during the first experimental period in each patient.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

TABLE 2. Diet composition and urine fat balance

Patients	Diet[a]	% of Fat Fed as Cottonseed Oil	Cholesterol:β-Sitosterol Intake	Caloric Intake	Fat Intake	Urine Fat	
			mg/1000 Kcal[b]	*Kcal/day*	*g/day*	*g/day ± SD*	*% of intake*
1	Liquid formula	100	0:98	1250	65	5.5 ± 1.7	8.4
2	Liquid formula	100	0:98	2560	118	7.1 ± 0.5	6.0
3A[c]	Liquid formula	100	0:98	2700	109	9.8 ± 2.2	9.0
B[c]	β-Sitosterol 12 g/d[d]	100	0:98	2700	114	9.4 ± 2.2	8.2
4A	Solid food	95	10:Unknown[e]	1600	43	10.1 ± 5.3	23.5
B	Solid food + β-sitosterol 9 g/d	95	10:Unknown	1600	46	6.4 ± 1.3	13.9
5B	Solid food + β-sitosterol 9 g/d	95	10:Unknown	1450	50	1.6 ± 1.1	3.2
A	Solid food	95	10:Unknown	1450	50	3.5 ± 2.0	7.1
6A	Solid food	95	10:Unknown	3000	60	0.24 ± 0.15	0.40
Chol	Solid food + cholesterol 2 g/d[f]	95	667:Unknown	3000	62	0.35 ± 0.26	0.56

[a] Formulas and solid diets contained 12% of calories as protein, 42% as fat, and 46% as carbohydrate.
[b] Cholesterol and β-sitosterol were inherent in the dietary fat.
[c] Sequential study periods, where A = control period, and B = β-sitosterol added.
[d] β-Sitosterol (Eli Lilly Co., Indianapolis, IN) added to the regular solid diet and to liquid formula during its preparation.
[e] The amount of phytosterols originating mostly from raw vegetables was not controlled; it varied approximately from 90–400 mg/day according to GLC analysis of occasional whole-day food intakes.
[f] Cholesterol admixed to the oil of the regular solid diet.

lecystokinin (Pancreozymin, The Boots Co., Nottingham, England).

Radioactive sterols were obtained from New England Nuclear Corp., Boston, MA and purified by Florisil TLC in the solvent system ethyl ether–heptane 55:45 (20). Approximately 100 μCi each of [4-^{14}C]cholesterol or [22,23-^{3}H]β-sitosterol dissolved in 1 ml of ethanol were administered intravenously in a saline infusion. Radioactivity was measured in the Beckman LS-100 beta scintillation counter as previously described (20).

Urine was collected in 24-hr pools under refrigeration at 4°C and submitted daily to all analytical procedures. Fresh urine samples collected at midday (patient 2) were immediately submitted to ultracentrifugal separation, and the fractions obtained were extracted with chloroform–methanol 2:1. Aliquots were taken for the determination of fat by a gravimetric method (12, 13) and for sterol analysis by GLC as described for serum cholesterol.

Fecal collections were carried out either daily (patient 5) or in 3- to 4-day pools and were stored frozen. Determinations of neutral steroids, bile acids, and chromic oxide were performed according to methods already described (17, 20, 21).

Ultracentrifugation of serum and urine samples was carried out in the preparative Beckman L3-50 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) according to the methods of De Lalla and Gofman (22). For the separation of chylomicrons, urine density was adjusted to 1.006 g/ml, either with distilled water or saline, and spun at 10°C in the Beckman model 30 fixed-angle rotor at 105,000 *g* for 40 min. In experiments for the separation of VLDL, samples were submitted to further ultracentrifugation at 105,000 *g* for 11 hr. LDL was obtained by spinning at d 1.063 g/ml at 143,000 *g* in the model 40.3 fixed-angle rotor for 24 hr. All fractions obtained from ultracentrifuged urine samples were dialyzed overnight at 4°C against saline solution. They were submitted to electrophoresis in Cellogel (Chemetron, Milan, Italy) simultaneously with a serum sample of the same patient to monitor the urine lipoprotein separation.

Calculations

Cholesterol synthesis in intestinal mucosa. After labeling patients 1–6 intravenously with a single dose of [4-^{14}C]cholesterol, the serum/lymph cholesterol specific activity ratio was measured during the log-linear phase of decay of the two curves. In patients fed a cholesterol-free diet any dilution of the lymph cholesterol specific activity in relation to that of the serum was considered to represent newly made intestinal mucosal cholesterol delivered into the lymph, provided that absorbed biliary and serum cholesterol specific activities were identical. To insure this identity, cholesterol specific activity was measured in serum and bile samples drawn simultaneously before breakfast during the log-linear phase of the serum cholesterol decay curve in patients 1–4 and found to be the same. On the other hand, lymph cholesterol specific activity should be lower than that of plasma whenever unlabeled dietary cholesterol is provided.

Total daily lymph cholesterol transport. The total amount

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

of cholesterol transported through the lymphatics from the intestine can be calculated if the shunt size of the chyle fistula is determined, as described previously (13), and this is demonstrated in the far-right column of Table 2. Patients 1–6 had shunts of 0.6–24% for dietary fat under the various experimental conditions. Total daily cholesterol transport via the lymph is equal to the urine cholesterol in mg/day divided by the fraction of dietary fat diverted into the urine per day (i.e., by shunt size).

Transudation of plasma lipoproteins in lymph. Intravenous labeling with a single dose of radioactive β-sitosterol provided a marker for transudation of lipoproteins from intestinal arterial blood to intestinal lymph. Use of this marker was based on the facts that *1*) the mass ratio of cholesterol/β-sitosterol was found to be identical in all serum lipoproteins in β-sitosterolemia, an unusual metabolic defect in man in which considerable intestinal absorption of this sterol occurs (23) and *2*) β-sitosterol is normally absorbed only poorly (less than 5%) by the intestine (24); therefore, any radioactive β-sitosterol excreted in bile would not appear in intestinal lymph.

Additional experiments were carried out to validate the use of labeled sitosterol. *1*) Two weeks after intravenous co-administration of a single dose of [4-^{14}C]-cholesterol and [22,23-^{3}H]β-sitosterol (patient 4A), the ^{14}C/^{3}H ratio was compared in (chylomicrons + VLDL) vs. (LDL + HDL). **Table 3** shows that these ratios were similar at four different times. *2*) Serum and urine [4-^{14}C]cholesterol/[22,23-^{3}H]β-sitosterol ratios were similar in free and esterified sterol fractions (patient 6 Chol). *3*) The urines of patients 3B, 4B, and 5B remained free of GLC-detectable β-sitosterol even when 9–12 g/day of this sterol was given orally. *4*) A single oral dose of 20 mg of [22,23-^{3}H]β-sitosterol (3 μCi) in a liquid formula was given to patient 3A, and urines were subsequently examined for radioactivity. Total labeled β-sitosterol transported in lymph during a 3-day collection period was corrected for the urinary lymph shunt size: urine [22,23-^{3}H]β-sitosterol/day divided by the fraction of dietary fat lost in the urine/day. Only 2.8% of administered labeled β-sitosterol could have been transported via the lymphatics.

Having validated the use of [^{3}H]β-sitosterol as a marker for transudation of arterial plasma lipoprotein into intestinal lymph, calculations of the amount of cholesterol thus transported from blood to lymph (T) were made as follows in patients 4, 5 and 6. (T) = (U ÷ P) × (C), where (U) = urine [^{3}H]β-sitosterol in dpm ÷ mg of urine cholesterol; (P) = serum [^{3}H]β-sitosterol in dpm ÷ mg of serum cholesterol; (C) = total daily cholesterol transport via the lymph. If the ratio (U)/(P) = 1, lymph cholesterol mass (C) would originate solely from plasma transudation (T). The other possibility is that (U)/(P) < 1, thus (C) > (T); this would indicate that the intestinal lymph included cholesterol from sources other than plasma transudation, namely: *1*) mucosal synthesis and/or absorption of endogenous (biliary) cholesterol on a cholesterol-free diet; or *2*) mucosal synthesis, absorption of endogenous and of dietary cholesterol on a cholesterol-containing diet.

RESULTS

After intravenous pulse labeling with [4-^{14}C]-cholesterol, serum cholesterol specific activities were similar to those in lymph during the log-linear phase of the serum radiocholesterol decay curve in patients 1–6A fed a cholesterol-free diet (**Fig. 1**). Bile cholesterol specific activities determined in patients 1–4 were almost identical to those in serum in three of four examinations.

It must be cautioned that similarity of lymph and serum cholesterol specific activities supports but does not prove that intestinal cholesterol synthesis is absent,

TABLE 3. Distribution of [22,23-^{3}H]β-sitosterol and of [4-^{14}C]cholesterol in serum lipoprotein classes after radioactive sterols were coadministered intravenously 2 weeks previously in patient 4A

	Specific Activity Ratios (^{14}C/^{3}H)[a]			
	8 AM (fasting)	3 PM (nonfasting)	8 PM (nonfasting)	7 AM (fasting)
Density <1.006	2415/853 (2.83)	2558/1056 (2.42)	2613/924 (2.83)	2271/846 (2.62)
Density >1.006	2667/1290 (2.06)	2363/898 (2.83)	2602/917 (2.84)	2371/984 (2.40)

[a] Expressed as dpm of each radioisotopic sterol per mg serum cholesterol in the two broad lipoprotein groups.

Downloaded from www.jlr.org by guest, on June 19, 2012

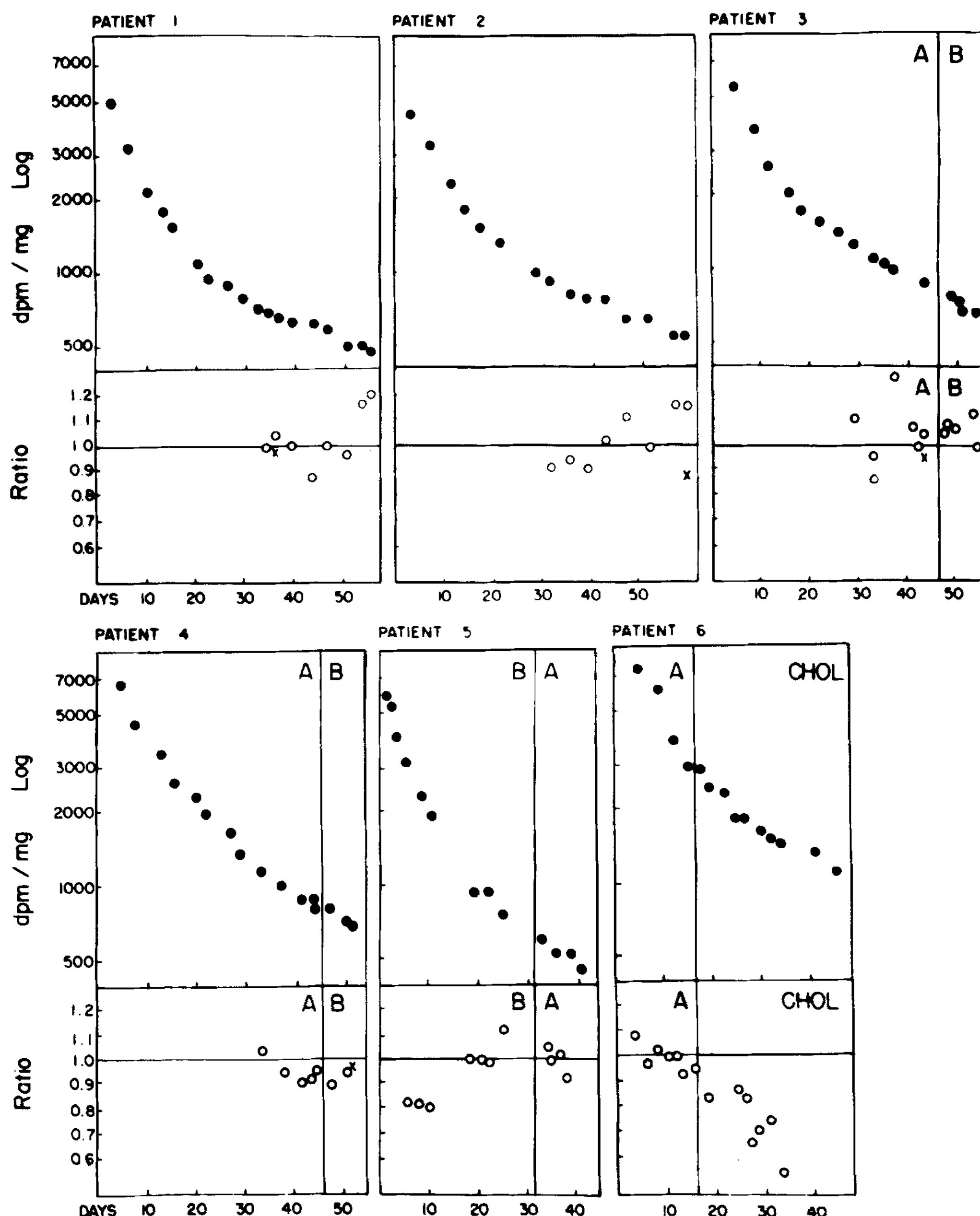

Fig. 1. *Upper panel:* [4-^{14}C]cholesterol specific activity (dpm/mg) decay curve in serum (●). *Lower panel:* [4-^{14}C]cholesterol specific activity ratios bile/serum (x), and urine lymph/serum (○). Urine specific activity of patient 2 refers solely to the chylomicron fraction obtained by ultracentrifugation from recently voided specimens; this was free from lipoprotein contaminants that might originate from plasma transudation into the intestinal lymph. Cholesterol was absent from the diet in experiments 1–6A. In period B patients were fed 9–12 g/day of β-sitosterol in order to interrupt the intestinal absorption of endogenous (biliary) cholesterol. Patient 6 Chol was fed cholesterol, 2 g/day, with an aim to decrease the cholesterol specific activity ratio lymph/plasma by unlabeled absorbed intestinal cholesterol. Similarity of serum and lymph cholesterol specific activities suggests that there had been no dilution of the lymph cholesterol specific activity by newly synthesized (unlabeled) mucosal cholesterol (patients 1–6A).

because exchange of labeled cholesterol may have occurred between plasma, biliary, and mucosal cholesterol (25, 26). However, cholesterol specific activity was significantly lower in urine than in serum during cholesterol feeding (patient 6 Chol), which is a strong indication that such an exchange mechanism must not have interfered with the determination of intestinal cholesterol synthesis in experiments 1–6A.

Table 4 shows the effect of adding large amounts of β-sitosterol to the diets of patients 3–5 in an effort

Downloaded from www.jlr.org by guest, on June 19, 2012

JOURNAL OF LIPID RESEARCH ASBMB

JOURNAL OF LIPID RESEARCH ASBMB

TABLE 4. Sterol balance and lymph transport of cholesterol

Patient[a]	1	2	3A	3B	4A	4B	5B	5A	6A	6 Chol
No. of days of experiment after ^{14}C i.v. pulse labeling	65	62	66		67		42		32	
No. of days on log-linear decay curve:no. of fecal analyses[b]	25:6	24:6	17:4	7:2	26:6	6:2	10:6	10:4	0:4	18:4
Cholesterol:β-sitosterol intake in mg/day	0:146	0:260	0:292	0:12000[c]	12:[d]	12:9000[c]	14:9000[c]	14:[d]	0:[d]	2000:[d]
a. Fecal steroids[e] (mg/day ± SD)										
Neutral	274 ± 35	383 ± 60	278 ± 31	558 ± 37	214 ± 67[i]	267 ± 12[f]	514 ± 159[f]	457 ± 185[f]	563 ± 217[f]	1928 ± 424[f]
Acidic	158 ± 22	124 ± 50	178 ± 46	121 ± 2	87 ± 37	90 ± 31	57 ± 28	106 ± 44	54 ± 34	54 ± 21
b. Urinary cholesterol (mean mg/day ± SD)	69 ± 27	98 ± 7	58 ± 22	83 ± 11	125 ± 72	60 ± 16	3.8 ± 2.3	7.4 ± 5.3	7.2 ± 4.5	18.3 ± 6.8
a + b. Whole body cholesterol synthesis (mean mg/day ± SD)	501 ± 84	605 ± 117	514 ± 71	762 ± 3	426 ± 176	417 ± 59	574 ± 78	570 ± 154	621 ± 244	−15 ± 440[i]
Total daily lymph cholesterol transport[g] mg/days ± SD	806 ± 134	1628 ± 55[h]	647 ± 177	1014 ± 164	536 ± 58	429 ± 30	196 ± 47	103 ± 41	2021 ± 857	3552 ± 2575
days of analysis	4	4	4	4	5	2	5	6	5	6

[a] Study periods presented according to the sequential order of investigation, where A = control period, B = added β-sitosterol to the diet; Chol = added cholesterol to the diet.
[b] Feces analyzed daily (patient 5) and in 3–4 day pools in the other patients.
[c] β-Sitosterol (Eli Lilly Co., Indianapolis, IN) added to the regular solid diet (patients 4B and 5B) and to liquid formula (patient 3B).
[d] β-Sitosterol inherent to the regular solid diet varied approximately from 90 to 400 mg/day.
[e] Values corrected for the intake of β-sitosterol and chromic oxide (16, 17). All patients were ideal for fecal metabolic studies since fecal recoveries of chromic oxide were close to 100% (17).
[f] Fecal neutral sterols of patients 4–6 on regular diet were corrected only by chromic oxide since the intake of β-sitosterol was not precisely known.
[g] Calculation shown in Methods.
[h] Urine cholesterol measured in midday, freshly collected, occasional samples may not represent a 24-hr lymph cholesterol transport.
[i] Calculated by the combined isotopic and fecal steroid mass measurements (2).

Downloaded from www.jlr.org by guest, on June 19, 2012

to reduce the amount of luminal cholesterol available for absorption and transport through the lymphatics (27). This experimental condition is known to elicit a marked increment in the body synthesis of cholesterol (1). In patient 3B the drug caused considerable interruption of the endogenous cholesterol absorption as shown by the increment of the excretion of fecal neutral steroids. In patients 4 and 5 differences in fecal neutral steroids between the β-sitosterol feeding period and the control diet were negligible. This may have occurred only because the β-sitosterol period in patient 4B and the control phase in patient 5A were very brief, due to the sudden disappearance of chyluria. However, according to the literature, the interruption of the intestinal absorption of cholesterol must have occurred in patient 5B since the drug was administered for 35 days (27). Nevertheless, in the present studies the specific activities of the lymph and serum cholesterol were rather similar (Fig. 1). Therefore, decreased availability of lumen cholesterol to the intestinal mucosa did not trigger "de novo" synthesis of cholesterol in the mucosa by setting free a feedback control mechanism.

Having validated the usefulness of labeled β-sitosterol as a marker for transudation of plasma lipoproteins into the intestinal lymphatics (see Methods), we proceeded to measure the contribution of transudated lipoprotein cholesterol to total lymph cholesterol in patients 4, 5, and 6. **Table 5** shows that in the absence of dietary cholesterol almost all lymph cholesterol could be accounted for on the basis of transudation. This result was obtained because the ratio of labeled β-sitosterol to cholesterol (dpm β-sitosterol/mg cholesterol) was nearly the same in serum and in urine in experiments 4A, 4B, and 5B. On the other hand, the ratio labeled β-sitosterol to cholesterol (dpm/mg) was considerably lower in urine than in serum during cholesterol feeding (patient 6 Chol). This result also indicates that isotope exchange between plasma, bile, and mucosa is not a relevant process, and therefore cannot interfere with the use of labeled β-sitosterol as a marker for transudation.

Urine lipoproteins were separated by preparative ultracentrifugation as described in Methods. After an overnight dialysis against saline all fractions obtained had electrophoretic mobilities on Cellogel identical to simultaneously run serum samples. Cross contamination of lymph chylomicrons and VLDL represented less than 10%, according to quantitative densitometry of electrophoretic strips. Furthermore, urine lipoproteins isolated by ultracentrifugation either from freshly collected or from 24-hr specimens stored at 4°C, disclosed identical electrophoretic patterns.

Fig. 2 shows the percentage distribution of urine lipoproteins obtained by preparative ultracentrifugation; the largest part of lymph cholesterol and fat was transported in chylomicrons, although cholesterol originated largely by transudation of plasma lipoproteins.

DISCUSSION

The literature on the role of the animal intestine in the production of cholesterol deserves a critical

TABLE 5. Quantitative measurement of the contribution to the intestinal lymph of cholesterol originating from plasma transudation, intestinal mucosal synthesis, and biliary or dietary cholesterol absorption

Patient[a]	Lymph Cholesterol Transport (C)[b] (mg/day ± SD)	Lymph Cholesterol Originating from Plasma Transudation (T)[b] (mg/day ± SD)	Lymph Cholesterol Not Accounted for by Transudation from Plasma (C) − (T)	Dietary Cholesterol Absorption		
				Lymph Balance Method ($C_{Chol} - C_A$)	Isotopic Method I[c] (mg/day ± SD)	Isotopic Ratio Lymph/Plasma[d] (mg/day ± SD)
4A (5)	536 ± 58	528 ± 54	+8[g]			
4B (2)[e]	429 ± 30	428 ± 60	+1[g]			
5B (5)[e]	196 ± 47	173 ± 51	+23[g]			
6A (5)	2021 ± 857					
6 Chol (5)[f]	3552 ± 2575	2282 ± 1763	1268 ± 888[h]	1531[g]	804 ± 383[i]	1119 ± 940[i]

[a] Number of determinations. Patient 5A excluded because of insufficient β-sitosterol radioactivity in the samples. Patient 6A was not administered labeled β-sitosterol.
[b] Calculation shown in Methods.
[c] Cholesterol absorption measured after i.v. pulse with radioactive cholesterol (2) = daily intake of cholesterol − (total fecal neutral steroids − endogenous fecal neutral steroids).
[d] Dietary cholesterol absorption = (1 − cholesterol S.A. lymph/plasma) × C.
[e] β-Sitosterol feeding 9 g/day to impair the intestinal absorption of biliary cholesterol.
[f] Cholesterol feeding 2 g/day. [^{3}H]β-Sitosterol administered i.v. simultaneously to starting cholesterol feeding.
[g] Difference not significant according to Student's *t* test.
[h] Difference significant according to Student's *t* test ($0.01 < P < 0.02$).
[i] Difference of absorption between the two methods not significant according to Student's *t* test.

Downloaded from www.jlr.org by guest, on June 19, 2012

JOURNAL OF LIPID RESEARCH ASBMB

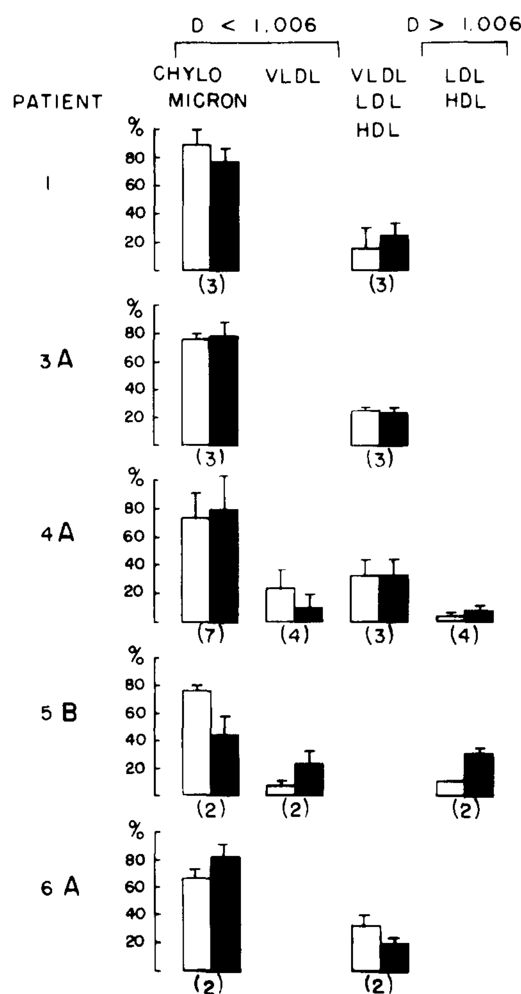

Fig. 2. Percentage distribution and range (I) of cholesterol (open bars) and fat (dark bars) of urine lipoproteins separated by preparative ultracentrifugation. Number of urine samples analyzed in brackets. Diet was cholesterol-free. Absorption of biliary cholesterol was interrupted in patient 5B being fed 9 g/day of β-sitosterol; nevertheless, as in the other patients, the largest fraction of lymph cholesterol and fat was transported in chylomicrons.

analysis. According to Hotta and Chaikoff (28), experiments in rats support the role of the liver as the main source of the plasma cholesterol, because the serum cholesterol specific activity decay curve in rats that were pulse labeled intravenously with radiocholesterol was considerably slowed down after hepatectomy but not after evisceration. On the other hand, several papers dealing with animal experiments support the fact that plasma cholesterol originates mostly from intestinal mucosal synthesis. Accordingly, feeding cholesterol to men, rats, and squirrel monkeys elicited an interruption of the incorporation of [^{14}C]-acetate into radiocholesterol in incubated liver slices (11, 29–33) but not in the intestinal mucosa (8, 32). Nevertheless, radiocholesterol appeared in the lymph but not in plasma after the intravenous administration of labeled acetate to the intestinal lymph duct-cannulated animals (9, 10).

In man and animals it is known that plasma cholesterol specific activity reaches a plateau after a few weeks on a constant high intake of radiocholesterol (18, 34, 35). However, in this circumstance plasma cholesterol specific activity is equal to, or remains at most only slightly below, the dietary cholesterol specific activity in rats and dogs (34, 36), and is 40–60% lower in monkeys and man. Simultaneously measured hepatic cholesterol synthesis is shut off during such elevated cholesterol feeding (29–31). These findings might result from the continuous dilution of the plasma radiocholesterol by unlabeled cholesterol originating from extrahepatic synthesis, for instance in the intestine, where supposedly synthesis is not controlled by exogenous cholesterol (11).

The work of Pertsemlidis, Kirchman, and Ahrens (36) in dogs with bile fistula is a clear, quantitative demonstration of the mucosal origin of cholesterol mass which, nevertheless, was diverted into the intestinal lumen. The work of Simmonds, Hofman, and Theodor (25) and a report by Grundy and Mok (26) also suggest that there is some exchange of luminal and mucosal cholesterol in man. Previous work in cholestyramine-treated hyperlipemic patients supports the conclusion that there is local intestinal mucosal cholesterol synthesis; mucosal cholesterol specific activity was lower than the serum sampled during the log-linear phase of the plasma cholesterol specific activity decay curve after pulse labeling intravenously with radiocholesterol (37). Obviously the intestinal mucosa is capable of synthesizing the membrane cholesterol of the sloughing-off cells (38, 39). However these findings do not assure that locally made mucosal cholesterol reaches the blood stream.

Evidence that the human intestine does not contribute newly made cholesterol to the plasma was based on the similarity of serum and lymph cholesterol specific activities in patients maintained on a cholesterol-free diet. Feeding β-sitosterol enhances the body synthesis of cholesterol (1), and yet in at least two reliable studies out of three conducted (patients 3B and 5B) the lymph and plasma cholesterol specific activities were identical. Furthermore, in patient 5B where the absorption of endogenous cholesterol was impaired by β-sitosterol, and plasma cholesterol transudation was simultaneously measured, the latter accounted for nearly all the lymph cholesterol mass transported.

Taking into account that bile cholesterol reaching the intestinal lumen is partially absorbed by the intestine, it should have been found in the intestinal

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

lymph. Our failure to recognize its presence there stemmed in part from the lack of an ideal marker for the absorption of bile cholesterol. To some extent the negligible contribution of endogenously absorbed cholestrol to the intestinal lymph is drawn from incidental findings. Thus, as shown in Table 4, the fecal neutral steroid mass on a cholesterol-free diet (patients 1–6A, excluding β-sitosterol feeding experiments) ranged from 214 to 563 mg/day (average 361 mg) as compared to 103 to 2021 mg/day (average 957 mg) of cholesterol transported in lymph. The largest proportion of cholesterol in the lumen is not absorbed by the intestine (2, 3, 18). Therefore, there is apparently much more cholesterol transported in the intestinal lymph than could be accounted for by absorption of endogenous cholesterol from the intestinal lumen.

In order to know precisely the mass of endogenous cholesterol absorbed, direct measurement methods must be devised to replace present indirect procedures based on the simultaneous determination of the plasma cholesterol transudation in lymph. Nevertheless, in the case of patient 6 Chol, where 2 g/day of cholesterol were fed and lymph cholesterol transudation was simultaneously measured, the absorbed endogenous cholesterol was identified in the lymph. In this experiment (Table 5) 2282 mg out of 3552 mg of cholesterol transported in lymph was attributable to plasma transudation, and the difference between these values (1268 mg) represents cholesterol originating from intestinal absorption, namely, absorbed endogenous and dietary cholesterol. Dietary cholesterol absorption was not statistically different when the isotopic method I (804 mg/day) was compared to the value derived from the ratio lymph/plasma cholesterol specific activity (1119 mg/day). Accordingly, the difference between 1268 mg and either 804 or 1119 mg represents endogenous cholesterol mass absorbed, i.e., 464 or 149 mg, respectively. These values are, respectively, 64 and 20% of 732 mg/day of endogenous cholesterol excreted in feces according to isotopic method I. Stating this in a different way, the fractional absorption of the total endogenous cholesterol mass reaching the intestinal lumen per day was relatively small, namely, 39% according to one calculation (percent of 464 mg + 732 mg), or 17% as obtained from the other available data (percent of 149 mg + 732 mg). Such values of endogenous cholesterol absorption are, respectively, 13% and 4% of the total mass of cholesterol transported in the lymph per day. Eventually the low mass of endogenous cholesterol absorbed could have been due to unusually small biliary output of cholesterol in these patients.

The possibility of plasma transudation was not raised in the initial protocol of this investigation. It was suggested in the course of the experiments mostly because a considerable mass of cholesterol was found to be transported in lymph in spite of the impairment of the biliary cholesterol absorption by dietary β-sitosterol in at least two experiments (patients 3B and 5B). Particularly in patient 3, lymph cholesterol and fecal neutral steroids increased, respectively, 367 and 280 mg, whereas in patient 5 there was a trend for cholesterol to increase simultaneously in feces and lymph when β-sitosterol was fed (Table 4). Although no clear-cut explanation can be offered for these findings, they do suggest that the flow of cholesterol in the intestinal lymph is rather independent of the variations in the luminal content of cholesterol that is absorbed by the intestinal mucosa.

The finding that about 75% of the lymph cholesterol was in chylomicrons was rather surprising since these particles are made only by the intestinal mucosa. Plasma lipoproteins carried by the blood that nourishes the intestinal wall must escape the capillary bed into the lacteals of the intestinal mucosa and attach to the chylomicrons. This possibility is currently being investigated in chyluria patients and in intestinal lymph fistula animals through the intravenous infusion of labeled lipoproteins. Preliminary investigations in two dogs showed that intravenously infused HDL, where apoprotein was labeled with ^{125}I, appeared in the intestinal lymph attached to chylomicrons. Furthermore in patient 5B lymph cholesterol was mostly in chylomicrons (Fig. 2) and must have had its origin in transudated plasma because bile cholesterol absorption was impaired by β-sitosterol feeding.

There are gaps in the lacteal wall that allow for the leakage of particles as big as red blood cells (40). The spontaneous clotting of the intestinal lymph at least indicates that it contains plasma fibrinogen. Since fat and cholesterol are absent from the urine chyle during the fasting state, it is likely that during absorption of diet the lacteal gaps open wide to allow for the simultaneous flow of the absorbed fat and of plasma lipoproteins. This possibility is in agreement with *1*) a report where lipoproteins of unknown origin were found in intestinal lymph of rats fed medium chain triglycerides, which are known to be transported solely by the portal system (41); *2*) previous work in rats suggesting that plasma lipoproteins contribute to the composition of the intestinal lymph lipoproteins (42); *3*) the fact that chylomicrons expand in the extracellular space by formation of clusters and attachment by smaller lipoproteins (43); *4*) the transfer of blood serum lipoprotein apoproteins to lymph chylomicrons, which has been shown to occur in vitro (44); and *5*) the suggestion that in lymph duct-cannulated

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH

squirrel monkeys 70–90% of the lymph cholesterol originated from plasma (10).

The lack of contribution of de novo mucosal synthesis to the intestinal lymph and the significant fraction of lymph cholesterol represented by plasma transudation are the major results of this investigation. Nonetheless, these findings may be peculiar to man, in as much as there are basic differences in cholesterol metabolism between man and animals. The present research protocol was utilized in lymph duct-cannulated rats fed a cholesterol-free diet.[1] The origin of the lymph cholesterol mass was approximately 30% from mucosal synthesis, 30% from plasma transudation, and 40% from endogenous cholesterol absorbed by the intestine.

Finally, although there is evidence indicating that the animal intestine makes lipoproteins other than chylomicrons, human chyluria would be a useful model to study this subject.

This work was supported by grants from the National Institutes of Health (Allergy and Infectious Diseases, grant 1 R22 AI 10849-10) and by CNPq (Brazilian National Research Council, grant 13332/70). The authors are grateful to Professor E. H. Ahrens, Jr. for his criticism and valuable contribution to the text; to Dr. Salvador V. C. Lima, Mrs. Anna C. K. Goldberg, Mrs. Marcia A. L. Aguiar, and Miss Eunice A. Ferreira for their technical assistance; and to Professors E. Mattar and A. B. Ulhoa Cintra for their continuous encouragement. Previous grants from the São Paulo State Research Foundation (FAPESP) provided our laboratory with basic equipment required for the research.

Manuscript received 16 November 1978; accepted 20 May 1979.

REFERENCES

1. Grundy, S. M., E. H. Ahrens, Jr., and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. *J. Lipid Res.* **10:** 304–315.
2. Quintão, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. An evaluation of four methods for measuring cholesterol absorption by the intestine in man. *J. Lipid Res.* **12:** 221–232.
3. Quintão, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12:** 233–247.
4. Wilson, J. D., and C. A. Lindsey, Jr. 1965. Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady states in man. *J. Clin. Invest.* **44:** 1805–1814.

[1] Quintão, E. C. R. et al. Unpublished data.

5. Borgstrom, B. 1969. Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope labeled meal. *J. Lipid Res.* **10:** 331–337.
6. Srere, P. A., J. L. Chaikoff, S. S. Treitman, and L. S. Burstein. 1950. The extrahepatic synthesis of cholesterol. *J. Biol. Chem.* **182:** 629–634.
7. Goodman, D. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. *J. Clin. Invest.* **47:** 231–241.
8. Dietschy, J. M., and M. D. Siperstein. 1965. Cholesterol synthesis by the gastrointestinal tract: Localization and mechanism of control. *J. Clin. Invest.* **44:** 1311–1327.
9. Lindsey, C. A., Jr., and J. D. Wilson. 1965. Evidence for a contribution by the intestinal wall to the serum cholesterol of the rat. *J. Lipid Res.* **6:** 173–181.
10. Wilson, J. D. 1968. Biosynthetic origin of serum cholesterol in the squirrel monkey: evidence for a contribution by the intestinal wall. *J. Clin. Invest.* **47:** 175–187.
11. Dietschy, J. M., and J. D. Wilson. 1968. Cholesterol synthesis in the squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. *J. Clin. Invest.* **47:** 166–174.
12. Blomstrand, R., N. A. Thorn, and E. H. Ahrens, Jr. 1958. The absorption of fats studied in a patient with chyluria. I. Clinical investigation. *Am. J. Med.* **24:** 958–966.
13. Blomstrand, R., and E. H. Ahrens, Jr. 1958. Absorption of fats studied in a patient with chyluria. III. Cholesterol. *J. Biol. Chem.* **233:** 327–330.
14. Metropolitan Life Insurance Co. Statistical Bulletin 40, 1959.
15. Ahrens, E. H., Jr., V. P. Dole, and D. H. Blankenhorn. 1954. The use of orally fed liquid formulas in metabolic studies. *J. Clin. Nutr.* **2:** 336–342.
16. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1968. Dietary β-sitosterol as an internal standard to correct for cholesterol losses in sterol balance studies. *J. Lipid Res.* **9:** 374–387.
17. Davignon, J., W. J. Simmonds, and E. H. Ahrens, Jr. 1968. Usefulness of chromic oxide as an internal standard for balance studies in formula fed patients and for assessment of colonic function. *J. Clin. Invest.* **47:** 127–138.
18. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurement of cholesterol turnover, synthesis, and absorption in man carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
19. Denbesten, L., W. E. Connor, T. H. Kent, and D. Lin. 1970. Effect of cellulose in the diet on the recovery of dietary plant sterols from feces. *J. Lipid Res.* **11:** 341–345.
20. Miettinen, T. A., E. H. Ahrens, Jr., and S. M. Grundy. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6:** 411–424.
21. Grundy, S. M., E. H. Ahrens, Jr., and T. A. Miettinen. 1965. Quantitative isolation and gas–liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* **6:** 397–410.
22. De Lalla, O., and J. W. Gofman. 1954. Ultracentrifugal analysis of serum lipoproteins and proteins in man. *In* Methods of Biochemical Analysis, Vol. 1. D. Glick, editor. Interscience Pub. Inc., New York. 459–478.
23. Schulman, S. R., A. K. Bhattacharyya, W. E. Connor,

Downloaded from www.jlr.org by guest, on June 19, 2012

and D. S. Fredrickson. 1976. β-Sitosterolemia and xanthomatosis. *N. Engl. J. Med.* **294:** 482–483.

24. Salen, G., E. H. Ahrens, Jr., and S. M. Grundy. 1970. Metabolism of beta-sitosterol in man. *J. Clin. Invest.* **49:** 952–967.
25. Simmonds, W. J., A. F. Hofmann, and E. Theodor. 1967. Absorption of cholesterol from micellar solution: intestinal perfusion studies in man. *J. Clin. Invest.* **46:** 874–890.
26. Grundy, S. M., and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *J. Lipid Res.* **18:** 263–271.
27. Grundy, S. M., and H. Y. I. Mok. 1976. Effects of low dose phytosterols on cholesterol absorption in man. *In* Lipoprotein Metabolism. H. Greten, editor. Springer-Verlag, Berlin. 112–118.
28. Hotta, S., and I. L. Chaikoff. 1955. The role of the liver in the turnover of plasma cholesterol. *Arch. Biochem.* **56:** 28–37.
29. Tomkins, G. K., H. Sheppard, and I. L. Chaikoff. 1953. Cholesterol synthesis by the liver III. Its regulation by ingested cholesterol. *J. Biol. Chem.* **201:** 137–141.
30. Frantz, I. D., Jr., H. S. Schneider, and B. T. Hinkelman. 1954. Suppression of hepatic cholesterol synthesis in rat by cholesterol feeding. *J. Biol. Chem.* **206:** 465–469.
31. Siperstein, M. D. 1960. The homeostatic control of cholesterol synthesis in the liver. *Am. J. Clin. Nutr.* **8:** 645–850.
32. Siperstein, M. D., and M. J. Guest. 1960. Studies on the site of feedback control of cholesterol synthesis. *J. Clin. Invest.* **39:** 642–652.
33. Bhattathirry, E. P. M., and M. D. Siperstein. 1963. Feedback control of cholesterol synthesis in man. *J. Clin. Invest.* **42:** 1613–1618.
34. Wilson, J. D. 1964. The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: The influence of dietary cholesterol. *J. Lipid Res.* **5:** 409–417.
35. Wilson, J. D. 1970. The measurement of the exchangeable pools of cholesterol in the baboon. *J. Clin. Invest.* **49:** 655–665.
36. Pertsemlidis, D., E. H. Kirchman, and E. H. Ahrens, Jr. 1973. Regulation of cholesterol metabolism in the dog. I. Effects of complete bile diversion and of cholesterol feeding on absorption, synthesis, accumulation, and excretion rates measured during life. *J. Clin. Invest.* **52:** 2353–2367.
37. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: Comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
38. Dietschy, J. M., and W. G. Gamel. 1971. Cholesterol synthesis in the intestine in man: regional differences and control mechanism. *J. Clin. Invest.* **50:** 872–880.
39. Lipkin, M., and B. Bell. 1968. Cell proliferation. *In* Handbook of Physiology, Section G: Alimentary Canal. C. F. Code and W. Heidel, editors. American Physiol. Soc., Williams & Wilkie Co., Baltimore, Maryland. **5:** 2861–2879.
40. Casley Smith, J. R. 1962. The identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals. *J. Cell Biol.* **15:** 259–277.
41. Barrowman, J. A., and S. G. Turner. 1976. The effect of feeding medium chain triglycerides on the flow and protein content of small intestinal lymph. (Abstract). *Gastroenterology.* **70:** 861.
42. Shrivastava, B. K., T. G. Redgrave, and W. J. Simmonds. 1967. The source of endogenous lipid in the thoracic duct lymph of fasting rats. *Quart. J. Exp. Physiol.* **52:** 305–312.
43. Tytgat, G. M., C. E. Rubin, and D. R. Saunders. 1971. Synthesis and transport of lipoprotein particles by intestinal absorptive cells in man. *J. Clin. Invest.* **50:** 2065–2078.
44. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.

ASBMB

JOURNAL OF LIPID RESEARCH

JLR

Downloaded from www.jlr.org by guest, on June 19, 2012