Long-Term Decay of Serum Cholesterol Radioactivity: Body Cholesterol Metabolism in Normals and in Patients with Hyperlipoproteinemia and Atherosclerosis

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ABSTRACT After the intravenous injection of labeled cholesterol, the decay of specific radioactivity of total serum cholesterol was studied in 12 patients for 15–63 wk (average, 45 wk). In some, but not all of the patients studied, the slow slope of the decay curves suggested a deviation from monoexponential behavior, and the data of the slow period of the decay of specific activity were curve fitted by two exponentials. Six patients had serum lipid values regarded as normal and six had hyperlipoproteinemia. The data were analyzed by input-output analysis and yielded the following results. Values for the input rate of cholesterol (I_T) (the sum of dietary and biosynthesized cholesterol) showed no difference betwen the normals and patients with hypercholesterolemia. The size of the rapidly miscible pool of cholesterol (M_1) was significantly higher in the group of hypercholesterolemic patients partly due to increased serum cholesterol levels. The size of the total exchangeable body mass of cholesterol (M) was higher by an average of 49 g in the patients with hypercholesterolemia as compared to normals. The remaining exchangeable mass of cholesterol ($M - M_1$) of the hypercholesterolemic subjects was higher by an average of 29 g as compared to normals. These differences were statistically significant.

INTRODUCTION

The metabolism of cholesterol in man has been extensively studied by the use of radioactive tracers. The information obtained by the chemical determination of serum cholesterol levels is restricted to a comparatively small fraction of body masses of cholesterol. However, the analysis of the serum decay curves of labeled cholesterol may yield more detailed information of the size and kinetics of body pools of cholesterol. The possible relationship between the level of serum lipids and the size and kinetics of body masses of cholesterol is an important problem in this field. Of particular interest is the estimation of the size of body masses of cholesterol in patients with hyperlipoproteinemia as compared to patients with normal serum lipid levels.

The application of input-output analysis was proposed recently in the study of the decay curves of radioactive cholesterol (2). This method permits the calculation of the total input rate (sum of dietary and biosynthesized cholesterol) and the total exchangeable mass of body cholesterol from the area and the first time moment of the decay curves. The method is independent of the more detailed shape of the curves, in particular, of whether or how many exponentials can be fitted to them. The analysis was motivated by the experimental finding (1) that in some patients the decay curves required more than two exponentials for curve fitting, so that existing compartmental analyses based on a 2-exponential curve fit (3, 4) could not be applied. A total of 12 patients were studied in this laboratory, after the intravenous injection of labeled cholesterol. The decay curves and some of the kinetic data in five of these patients were published previously (4). 6 of the 12 patients had serum lipid values considered as normal and normal serum lipoprotein electrophoresis, and six had hyperlipoproteinemia. Data are presented in the present paper on different

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parameters of cholesterol metabolism in these 12 patients, and values obtained in patients with normal serum lipids were compared to patients with hyperlipoproteinemias.

METHODS

12 patients were studied. The age, sex, and clinical diagnoses are included in Table I. All patients were ambulatory, their respective diseases under good control, and 7 of the 12 were working at their regular jobs during the study. Medications, if any, were kept constant and substances known to influence serum cholesterol levels were not given. The diet was uncontrolled, but the patients were instructed to adhere to their customary diets. The patients were seen weekly, with the exception of a few appointments that were missed (vacations, family and job engagements, or other reasons). They were weighed weekly. Physical examination, complete blood count, urinalysis, blood urea nitrogen, blood sugar, serum bilirubin, serum glutamic oxalacetic transaminase, and cephalin flocculation were carried out periodically.

Cholesterol-7α-^{3}H [1] (225 μCi/mmole) and cholesterol-4-^{14}C (50 mCi/mmole)[1] were purified by thin-layer chromatography (5). About 20 ml of blood was drawn from each subject 4–6 days before the injection of the tracer, and the serum was separated aseptically and stored at 4°C. An appropriate amount of labeled cholesterol was dissolved in 0.5 ml of ethanol, and 0.5 ml of sterile normal saline solution was added. The serum of each individual patient was mixed aseptically with the solution of the tracer, and was incubated at 37°C for 24 hr. After ultrafiltration (size 0.2 μ filter) and radioassay 31–43 μCi of cholesterol-7α-^{3}H or 30–83 μCi of cholesterol-4-^{14}C was given intravenously to each patient. The tube containing the radioactive serum was washed twice with sterile normal saline which was then mixed with the material injected. There were no reactions to the injection of the tracers.

After the injection of the tracer, blood was drawn serially during the 1st week and weekly thereafter in the fasting state. Total serum cholesterol concentrations were determined in each sample by the method of Abell, Levy, Brodie, and Kendall (6). Serum triglyceride levels were done by the method of Van Handel and Zilversmit (7). Serum lipoprotein electrophoresis was carried out by the method of Fredrickson and Lees (8) on at least five fasting blood samples in each subject. A separate 3 ml aliquot of the serum was saponified by the addition of 28 ml of ethanol and 2 ml of 33% KOH solution. After the addition of an equal volume of water, neutral steroids were extracted four times with equal volumes of hexane. There was no hexane-extractable radioactive material left in the ethanol-water layer. The combined hexane extracts were washed with water, the hexane was evaporated, and the residue was dissolved in toluene. Parallel determinations of specific radioactivity were identical when this procedure was compared to a procedure involving digitonide precipitation of sterols. Radioactivity was measured in a Tri-Carb liquid scintillation spectrometer, with toluene solution containing 4 mg of 2,5-diphenyloxazole and 0.3 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene per ml. The samples were recounted after the addition of known internal standards for ^{3}H or ^{14}C activity in 0.1 ml toluene each (internal standard method). After correction for quenching, radioactivity was calculated in disintegrations per minute. The results were expressed as the specific activity (dpm per gram total serum cholesterol), divided by the injected dose (dpm) of radioactive cholesterol (per cent of dose of radioactivity given per 1 g of total serum cholesterol).

As explained elsewhere (2), although the input-output method does not require curve fitting, it is convenient for computational purposes to fit the decay curves with a sum of exponential functions. Accordingly, the semilogarithmic plots of the experimental decay curves were fitted graphically (peeling-off process) by a sum of exponential decay functions. The resulting exponential parameters were the amplitudes a_j (1/100 g) and the half-lives $(t_{\frac{1}{2}})_j$ (days), where $j = 1,2,3$ for a 3-exponential curve fit and $j = 1,2$ for a 2-exponential curve fit (2). The input-output analysis then yielded, in terms of the preceding exponential parameters:

(*a*) The total input rate I_T (g/day) of body cholesterol as the reciprocal of the area under the decay curve, or (Σ_j denotes summation over the values of j)

$$I_T = 69.3/\Sigma_j a_j (t_{\frac{1}{2}})_j \quad (1)$$

The total input rate I_T is defined as the sum of all the component rates of input of cholesterol into the body. These component rates include rates of biosynthesis and rate of dietary intake. It is assumed that all entering cholesterol becomes mixed with labeled cholesterol at entrance (at synthesis and dietary intake).

(*b*) The mean transit time $\bar{t}_p$ (days) of tracer cholesterol as the first time moment of the decay curve, or

$$\bar{t}_p = \Sigma_j a_j (t_{\frac{1}{2}})_j^2 / 0.693 \Sigma_j a_j (t_{\frac{1}{2}})_j \quad (2)$$

The mean transit time ($\bar{t}_p$) is defined as the average time of traversal of a labeled cholesterol molecule from the venous blood inlet to the cholesterol end product, fecal outlet.

(*c*) The total exchangeable mass M(g) of body cholesterol, as the product of total input rate and mean transit time, or

$$M = I_T \bar{t}_p \quad (3)$$

The mass (M) is defined as the sum of all body cholesterol from which labeled cholesterol is contributed to the blood in an experimentally detectable rate.

(*d*) an additional quantity of interest yielded by two-compartment analysis (3, 4) but not by input-output analysis, is the mass M_a(g) of body cholesterol in the rapidly miscible pool (denoted pool A in reference 3 and pool a in reference 4) and calculated as

$$M_a = 100/\Sigma_j a_j \quad (4)$$

The summation was over $j = 1,2$ in references 3 and 4. In the present application the summation is also over $j = 1,2,3$ in the 3-exponential cases. The reason that the same formula and physical interpretation hold in the 3(or more)-exponential case as in the 2-exponential case is that equation 4 can be written more generally as

$$M_a = 100/w(0) \quad (5)$$

where w(0) is the value at time zero of the sum of exponential representation of the data

$$w(t) = \Sigma_j a_j e^{-\alpha_j t} \quad (6)$$

Equation 5 can be interpreted independently of equation 6. If a smooth curve w(t) is drawn through the experimental points by any means, from time t_1, to time t_2, and this curve

[1] New England Nuclear Corp., Boston, Mass.

TABLE I
Experimental Data of

Patient, age, sex	Diagnosis	Weight	Duration of experiment	Average total serum cholesterol
		kg	*wk*	*mg/100 ml*
A. F., 45, F	No clinical disease	58.1	28	249 ±15§
S. K., 67, F	No clinical disease‖	52.2	60	234 ±12
E. A., 60, F	Pulmonary emphysema	52.2	50	224 ±17
D. B., 43, F	Essential hypertension	76.2	55	224 ±15
N. S., 72, M	Coronary artery disease	77.1	58	197 ±23
E. D., 52, M	Coronary artery disease	78.9	41	240 ±18
Average (I)				
H. F., 47, M	Coronary artery disease; CVA	84.8	30	313 ±21
J. L., 49, M	Coronary artery disease	73.5	63	318 ±19
M. R., 53, F	Peripheral vascular insufficiency	64.4	54	289 ±21
S. P., 49, F	Essential hypertension	83.0	58	272 ±32
H. Z., 43, F	Xanthomatosis; coronary artery disease	52.2	15	551 ±28
Average (II)				
Difference of (I) and (II)				
S. S., 62, M	Coronary artery disease, diabetes mellitus	82.6	34	236 ±17

M_a = rapidly miscible pool of cholesterol; M = total exchangeable mass of cholesterol; $M - M_a$ = remaining exchangeable mass of cholesterol; $\bar{t}_p$ = mean transit time; for further definitions see text. I_T = total input rate; NS = not significant statistically.

* See reference 8.

‡ Total cholesterol content of serum, calculated from serum cholesterol concentrations, body weight, and assumed blood volume (Handbook of Physiology. The American Physiological Society, Washington, D. C. 1:52).

§ Standard deviation.

‖ Developed coronary artery disease 1½ yr after termination of experiment.

¶ $P < 0.01$.

** $P < 0.05$.

is back extrapolated to the value w(0) at time zero, then this w(0) in equation 5 defines a single pool or compartment into which the injected tracer may be regarded as distributing uniformly and relatively quickly subsequent to injection. This "initially dilutional" or rapidly miscible pool M_a exists independently of the distribution or exchange characteristics of the remaining body cholesterol.

(*e*) Since the total exchangeable cholesterol M is given by equation 3 and the rapidly miscible pool M_a is given by equation 4, it is convenient to denote the difference $M - M_a$ as the remaining exchangeable mass of body cholesterol. This mass need not be given a compartmental interpretation (2).

In the 12 patients the duration of the study varied from 15 to 63 wk, and the average for the group was 43.7 wk. In 7 of the 12 subjects the decay curves were followed for 50–63 wk (average, 56.9 wk). In these seven patients a study of the long-term decay of serum cholesterol radioactivity was done (Fig. 1–7). The remaining five subjects were not included in the long-term study because of the comparatively shorter follow-up of radioactive decay curves (15–41 wk).

6 of the 12 patients had serum lipid levels regarded as normal and a normal serum lipoprotein electrophoresis pattern. Three of these had no demonstrable clinical disease due to atherosclerosis, two had coronary disease, and one had essential hypertension (Table I). Five additional patients were classified as having hypercholesterolemia (type II hyperlipoproteinemia) (8). One had xanthomatosis and each had clinical evidence of vascular disease (Table I). The remaining patient had hypertriglyceridemia (type IV hyperlipoproteinemia) (8), diabetes mellitus, and coronary artery disease. The normal and the hypercholesterolemic (type II) patients were instructed to eat a low-fat, low-cholesterol diet, and the patient with hypertriglyceridemia (type IV) consumed a low-carbohydrate diabetic diet throughout the study.

RESULTS

During the study there were no major changes in the over-all level of total serum cholesterol concentration in any of the patients. Figs. 1–7 include the concentra-

the 12 Patients Studied

Average serum triglyceride	Type*	I_T	M_s‡	M_a	M	M − M_a	$\bar{t}_p$
mg/100 ml		*g/day*	*g*	*g*	*g*	*g*	*days*
79 ±14§	—	1.18	5.26	25.1	72.7	47.6	61.6
122 ±14	—	0.66	4.44	25.6	60.6	35.0	91.4
86 ±11	—	1.06	4.25	20.2	73.8	53.6	69.6
94 ±19	—	1.02	6.20	27.4	80.5	53.1	78.9
100 ±17	—	0.89	6.49	33.7	87.7	54.0	97.7
110 ±8		1.26	8.09	35.0	93.5	58.5	74.2
		1.01	5.79	27.8	78.1	50.3	78.9
133 ±14	II	1.63	11.3	38.5	97.8	59.3	60.0
99 ±20	II	1.23	9.99	76.9	118.0	41.1	96.0
104 ±19	II	0.99	6.77	18.6	122.0	103.0	124.0
84 ±12	II	1.25	8.21	45.7	125.0	79.3	100.0
103 ±19	II	1.36	10.5	58.8	173.0	114.0	127.0
		1.29	9.35	47.7	127.2	79.3	101.4
		NS	3.56¶	19.9**	49.1¶	29.0**	NS
227 ±63	IV	3.29	8.33	40.1	112.0	71.9	34.2

tions of serum cholesterol throughout the study in seven patients. The weight of the subjects remained constant to within 3–4 lb., and the results of the physical examinations and the monitoring laboratory tests remained unchanged.

Long-term decay of serum cholesterol radioactivity. The specific activity of total serum cholesterol decayed rapidly at decreasing exponential rates during the initial 3–8 wk after injection of the tracer (Figs. 1–7). For the remainder of the experiment (the "slow" period) in three patients (Figs. 1–3) the semilogarithmic plot of the data suggested nonlinearity and required two exponentials for data fitting, of which the "slower" exponential seemed to appear after 20–30 wk. In two patients (Figs. 4 and 5) the data in the slow period also suggested nonlinearity but not to the extent of the preceding three patients. In the remaining two patients of this group (Figs. 6 and 7) the semilogarithmic plot of the slow period was linear (monoexponential). Of the exponential curve-fitting half-times ($t_{\frac{1}{2}}$) for all seven subjects, the slowest half-time $(t_{\frac{1}{2}})_3$ ranged from 76 to 110 days, the next faster half-time $(t_{\frac{1}{2}})_2$ ranged from 7 to 32 days, and the fastest half-time $(t_{\frac{1}{2}})_1$, where fittable, ranged from 1 to 8 days (Table II). The experimental data for the seven patients yielded the calculated kinetic parameters and were included in Tables I and II. Table III includes the accumulated net counts (total minus background) of radioactivity of the serum samples at 40 wk, 45 wk, and at the end of the follow-up period in the seven patients. The coefficients of variation of the observed sample counting rate (V_s) (9) are also included in the table.

Analysis of data in normals and in patients with hyperlipoproteinemia. The results of the data analysis obtained in the 12 patients are included in Table I. The exponential parameters of the experimental data are

shown in Table II. The calculated average value for input rates (I_T) was 1.01 g/day for the patients with normal serum lipids, and 1.29 g/day for patients with hypercholesterolemia. The difference was not statistically significant. The average size of M_a (rapidly miscible pool) was 27.8 g for patients with normal serum lipids and 47.7 g for the subjects with hypercholesterolemia. The difference, 19.9 g, was satistically significant at the 5% level. The average values for M (total exchangeable body mass of cholesterol) were 78.1 and 127.2 g, respectively, for the two groups. The difference, 49.1 g, was significant at the 1% level. The average value for $M - M_a$ was 50.3 g in normals and 79.3 g in patients with hypercholesterolemia. The difference, 29.0 g, was statistically significant at the 5% level. The average value for mean transit times ($\bar{t}_p$) was 78.9 and 101.4 days, respectively, for the two groups. The difference was not statistically significant.

The single patient with hypertriglyceridemia (type IV hyperlipoproteinemia) showed values comparable to patients with hypercholesterolemia with the exception of I_T (input rate) which was unusually large (3.29 g/day).

DISCUSSION

The present data suggest that the long-term decay of serum cholesterol specific activity may proceed at more than a single exponential rate in man. In three of the seven patients studied, where two exponentials were used to fit the data of the slow period, the slowest slope became evident after 20–30 wk (Figs. 1–3). In two subjects a similar situation is suggested (Figs. 4 and 5). In the additional two patients, studied for 58 and 55 wk, the semilogarithmic plot was linear after the initial rapid decay (Figs. 6 and 7). It is not known whether these last two sets of data would exhibit a further flattening with a longer period of observation. Whether these data indicate the existence of several types of long-term handling of cholesterol is not quite clear at present. To be sure, the two types of patients failed to show any clear differentiation on the basis of age, sex, clinical diagnoses, or serum lipid levels (Table I). The further flattening resulted in an increase of the area under the decay curves by a small amount. Since I_T (input rate) is the reciprocal of the area under the curves (equation 1), the effect of the flattening is a slight decrease in the value of I_T. The value of $\bar{t}_p$ (mean transit time of tracer cholesterol) is slightly increased due to the further flattening of the curves. Since the calculated size of M (total exchangeable mass of body cholesterol) is the product of the two former values (equation 3), the flattening of the curves may either increase or decrease the value of M (2).

In scrutinizing the data for possible error, several points must be mentioned. Exponential curve fitting is a

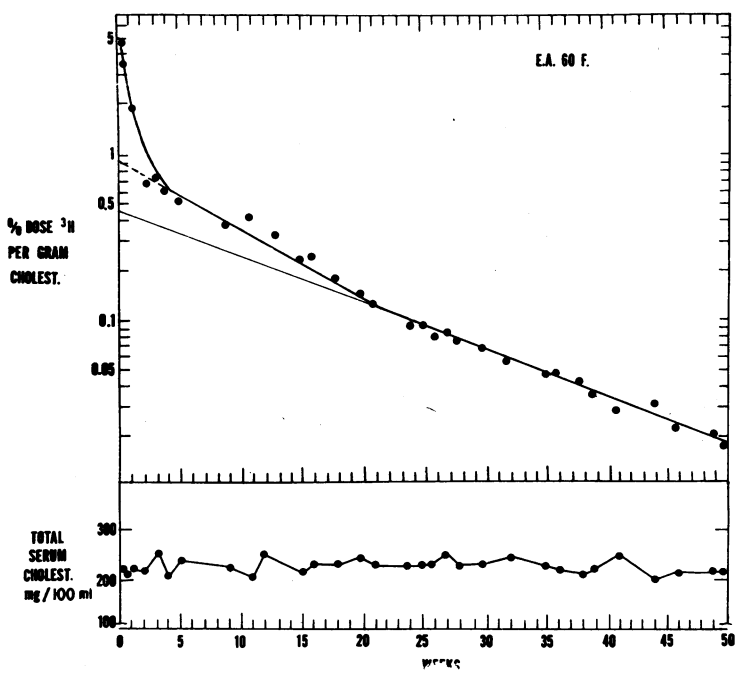

FIGURE 1 Semilogarithmic plot of total serum cholesterol specific activity (upper portion) and plot of total serum cholesterol concentrations (lower portion) throughout the experiment.

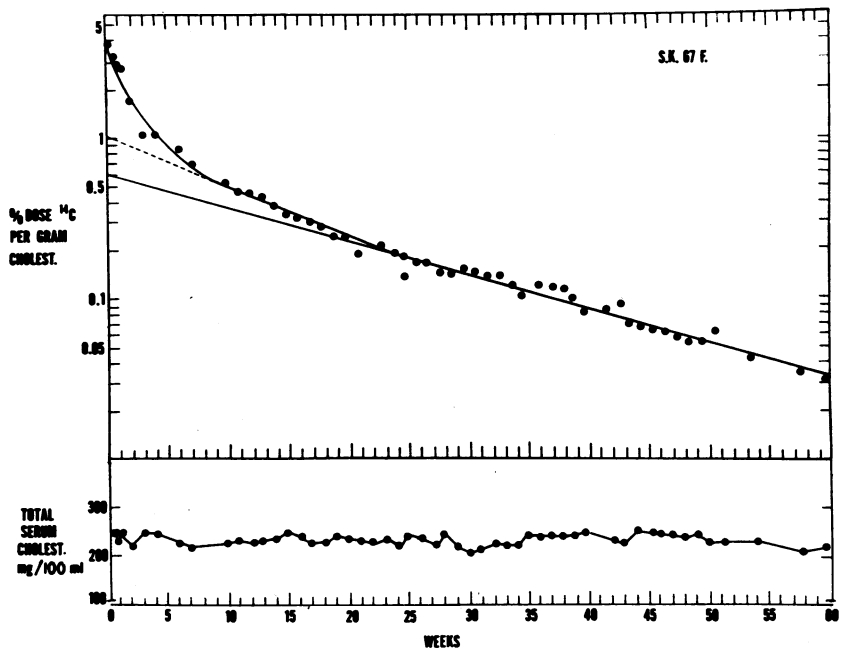

FIGURE 2 See legend to Fig. 1.

graphic method, subject to human error or bias of the investigator. The inspection of Figs. 1, 2, and 3 gives the impression of clear deviation from monoexponential behavior, whereas the data in Figs. 4 and 5 are at best equivocal. Drawing of these lines is subject to considerable error and thus any number of extra components may be obscured. Nonetheless, the decay curves in Figs. 6 and 7 give the impression of monoexponential behavior. An additional difficulty in drawing lines of exponentials is the variation and occasional scatter of the data points of specific radioactivity (Figs. 1–7). The reproducibility of the recovery of radioactive cholesterol from human serum in this laboratory was periodically checked by adding known amounts of labeled cholesterol to a pooled serum sample and was found to be within ±5% of error. The internal standard method for quenching correction ruled out the size of the extracted aliquot of serum as the cause of significant deviations. The character and the

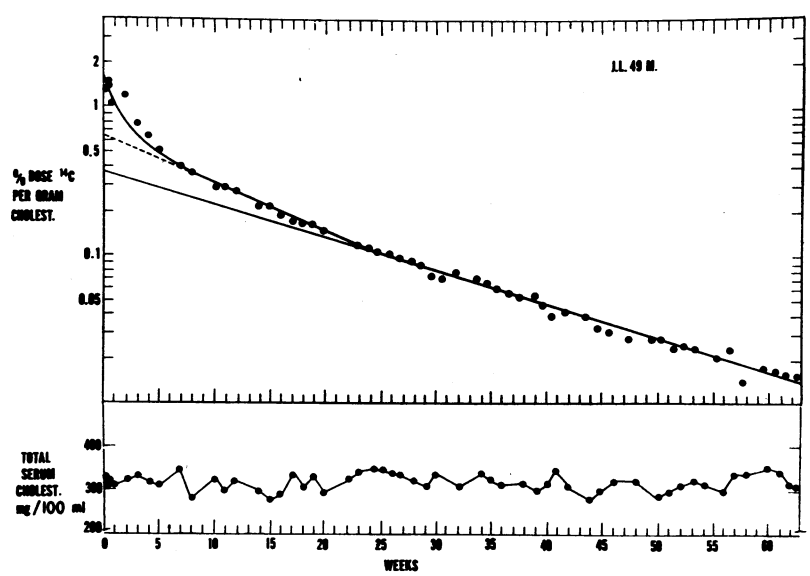

FIGURE 3 See legend to Fig. 1.

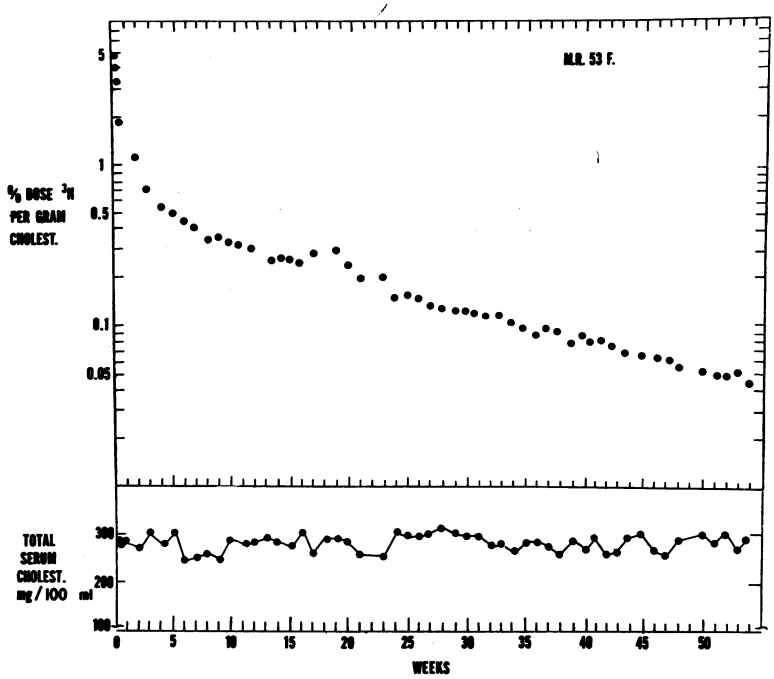

FIGURE 4 See legend to Fig. 1.

extent of the variations of our data are, in general, comparable with data reported from other laboratories (10–16). One of the critically important points in the evaluation of the shape of the curves is the magnitude of the counting error of the radioactive samples, especially at the end of the follow-up periods. Table III includes the accumulated net counts of radioactivity (total minus background) of the last and least radioactive samples in the seven patients, together with the coefficient of variation of the observed net sample counting rate (V_s) which reflects the magnitude of the counting error (9). At the end of the follow-up periods the coefficient of

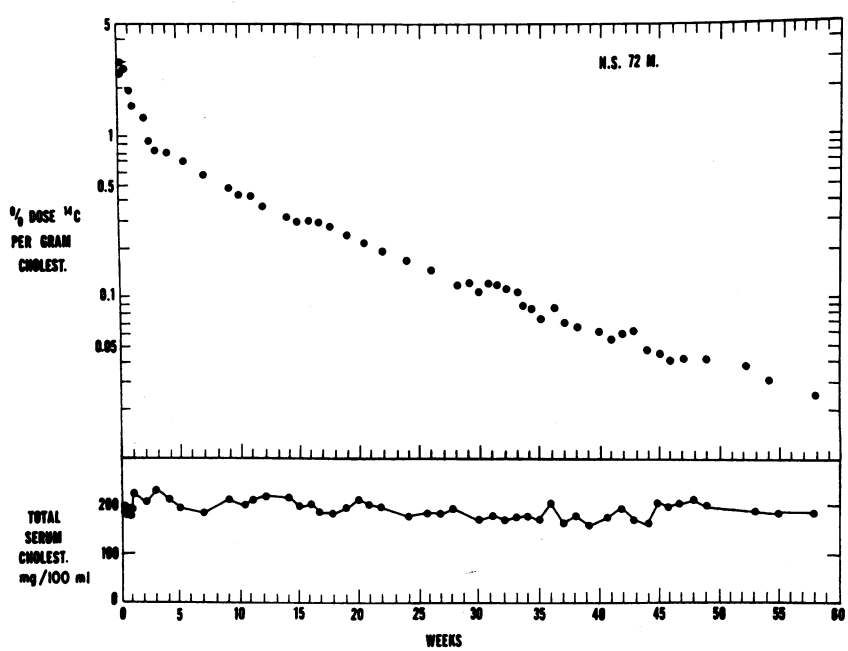

FIGURE 5 See legend to Fig. 1.

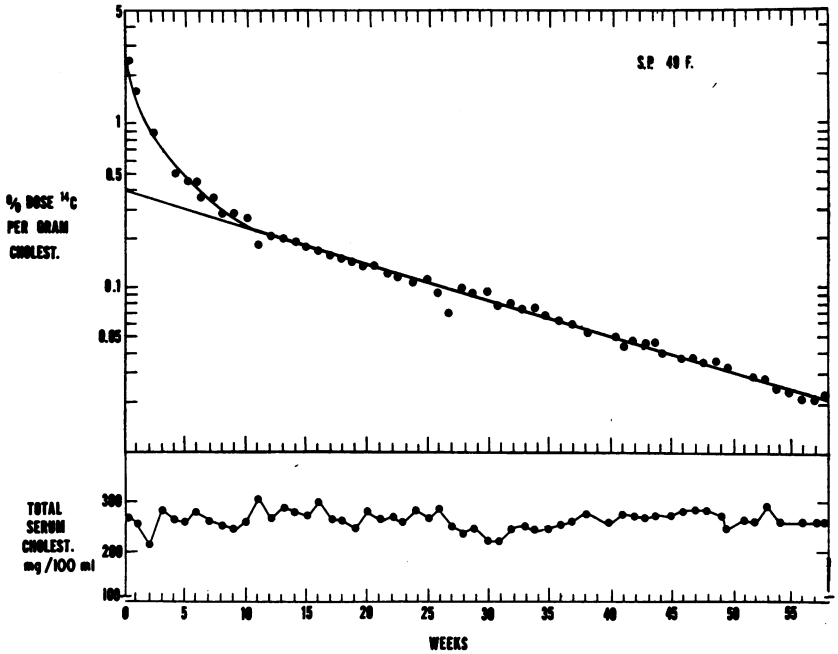

FIGURE 6 See legend to Fig. 1.

variation of the counting rate ranged from 0.79% to 2.19% (average 1.17%) which is of a negligible magnitude for present purposes. Should, however, these counts be considered appreciably uncertain from a statistical point of view, the following points should be considered. On inspection of the figures (Figs. 1–5) one can postulate that the flattening of the slow slopes could become evident after a 45 wk, or even a 40 wk follow-up, by neglecting the data points beyond these time periods. Table III includes the radioactivity counts at these two time periods. The average coefficients of variation were 0.69 and 0.78%, respectively, for 40 and 45

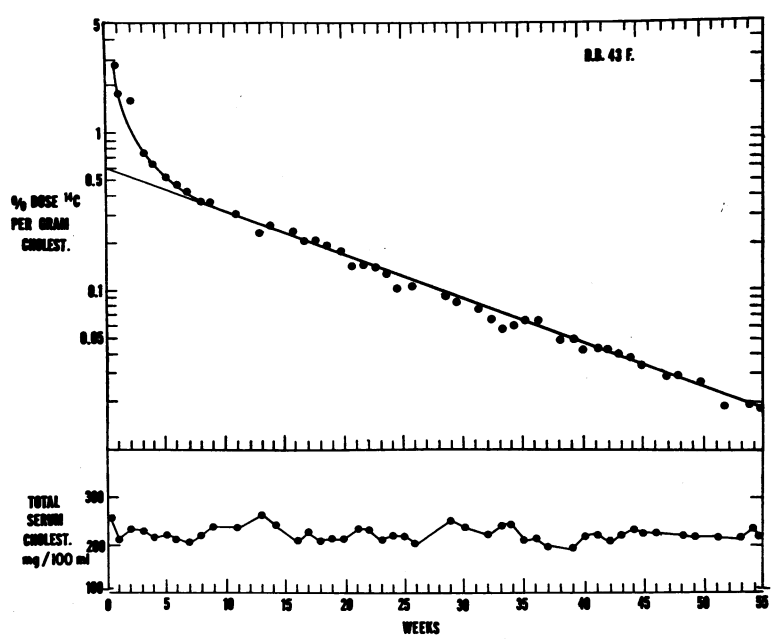

FIGURE 7 See legend to Fig. 1.

TABLE II

Exponential Parameters of the Experimental Data in 12 Patients

Patient	a_1	a_2	a_3	$(t_{\frac{1}{2}})_1$	$(t_{\frac{1}{2}})_2$	$(t_{\frac{1}{2}})_3$
		$(100\ g)^{-1}$			*days*	
A. F.	3.3	0.69	—	5.4	59.0	—
S. K.	2.0	1.3	0.60	8.19	22.2	98.7
E. A.	3.9	0.60	0.44	3.40	31.5	75.6
D. B.	0	3.05	0.60	—	7.12	77.0
N. S.	1.5	0.78	0.69	3.85	16.2	85.1
E. D.	2.4	0.46	—	8.3	76.0	—
H. F.	2.2	0.40	—	7.7	64	—
J. L.	0	0.90	0.40	—	22.4	91.0
M. R.	3.7	1.2	0.49	1.05	10.5	110
S. P.	0	1.8	0.39	—	9.66	97.1
H. Z.	1.3	0.40	—	6.9	105	—
S. S.	2.3	0.19	—	5.2	48.0	—

wk. It is thus highly improbable that counting error was a significant source of deviation in the present study. Furthermore, since the total number of data points was sufficiently large, one could logically conjecture that the counting error was randomly distributed and did not occur always in the same direction. If this was not so, then some of the curves should be flattened and some have an increase in slope. Since all deviated curves have flattened, in the context of this finding and of the foregoing data, the hypothesis of the significance of counting error can be safely rejected.

Each of the patients were in the apparent steady state throughout 50–63 wk of the sutdy. Any significant change from the steady-state condition could, of course, explain the deviation of the decay curves from linearity. For example, an increase in the size of body pools or a decrease in the rate of synthesis of cholesterol will cause the slope for decay of specific activity of serum cholesterol to decrease (15). The variations of the level of total serum cholesterol in each patient are shown in Figs. 1–7 and values of standard deviations are included in Table I. The variations were totally erratic and were entirely comparable to data reported by a large number of investigators (17–20) in long-term longitudinal studies in patients in the apparent steady state. It is highly improbable that the steady-state conditions were significantly altered in the present study, and thus caused the modification of the rate of the decay of specific activity. In the two patients who exhibited a monoexponential behavior of the long-term decay curves,

TABLE III

Net Counts of Radioactivity Accumulated on Serum Samples and Coefficient of Variation of the Observed Sample Counting Rates

		40 Wk		45 Wk		Last point		
Patient	Isotope	Counts/ 100 min	V_s*	Counts/ 100 min	V_s*	Weeks	Counts/ 100 min	V_s*
			%		%			%
J. L.	^{14}C	52,400	0.45	38,400	0.53	63	15,400	0.87
S. K.	^{14}C	48,000	0.47	39,100	0.52	60	14,700	0.89
D. B.	^{14}C	24,500	0.67	19,900	0.75	55	10,500	1.08
N. S.	^{14}C	28,300‡	0.62	24,500	0.67	58	11,400	1.03
S. P.	^{14}C	42,900	0.49	36,200	0.54	58	17,800	0.79
E. A.	^{3}H	7700‡	1.29	6700§	1.49	50	3500	2.19
M. R.	^{3}H	17,400	0.81	12,500	0.98	54	7100	1.37
Average			0.69		0.78			1.17

Net counts (total minus background) of radioactivity accumulated on serum samples (rounded to next hundred) and coefficient of variation of the observed sample (net) counting rate in seven patients at 40 wk, 45 wk, and at the end of follow-up period. The average background was 1200 counts/100 min.

* Coefficient of variation of observed sample (net) counting rate (%) (9).

$$V_s = 100\,\frac{(N_c t_b^2 + N_b t_c^2)^{\frac{1}{2}}}{N_c t_b - N_b t_c}$$

N_c = counts accumulated during time t_c in the measurement of background and sample combined; N_b = counts accumulated during time t_b in the measurement of background; t_b = counting time when background is measured; t_c = counting time when sample and background together are measured.

‡ 39 wk.

§ 44 wk.

variations of serum cholesterol levels were comparable to the subjects who exhibited multiexponential decays (Table I and Figs. 1–7). The physiological explanation of the further flattening of the curves remains a matter of speculation. One possibility is that this may represent slow exchange of cholesterol in atheromas or in mesenchymal tissue (adipose tissue, muscle, connective tissue). Another possibility is that cholesterol in the central nervous system may exchange very slowly with serum although specific activity of brain cholesterol was found to be minimal of nil 226 days after injection of tracer (21). The appearance of a "very very slowly" exchangeable pool in some patients may indicate that the slowly exchangeable pool may be composed of more than a single compartment.

Of the 12 patients studied, six had serum lipid values regarded as normal (Table I). Of the five patients classified as type II hypercholesterolemia (8), four had a comparatively modest elevation of total serum cholesterol levels. It is the impression of the authors on the basis of long time knowledge of these patients, that each of the subjects in this group had genetically determined hypercholesterolemia. Regardless, however, whether the hypercholesterolemia was due to genetic or environmental factors, the two groups of patients represent clearly a group of "normals" and a group with hyperlipoproteinemia, taking 250 mg/100 ml for serum cholesterol and 150 mg/100 ml for serum triglycerides as cutting points.

10 of the 12 patients were within their desirable weight according to standard tables (Metropolitan Life Insurance Company, Statistical Bureau). One patient (D. B.) was above her ideal weight by 18% and another (S. P.) by 33%. It has been reported that M_B (as calculated by compartmental analysis) (3), corresponding to the remaining exchangeable mass of cholesterol ($M - M_A$), may be increased in the obese due to obesity per se (22). Since one of these subjects (D. B). was in the group of normals, and the other (S. P.) in the group of patients with hypercholesterolemia, it is highly improbable that the end results of this study were significantly influenced by this phenomenon. When the value of M (total exchangeable body mass of cholesterol) was calculated in grams per kilograms of body weight, the average for the group of normals was 1.19 g/kg and for patients with hypercholesterolemia 1.89 g/kg. The per cent difference between the two values of M is practically identical (Table I) whether or not M is divided by the value of body weight.

There was no statistically significant difference in input rates (I_T) (the sum of dietary and biosynthesized cholesterol) between the normal and hypercholesterolemic subjects. Several authors have compared (or their published data could be used for comparison) the rate of turnover, rate of biosynthesis or the production rate of cholesterol (corresponding to I_T in the present paper) in normals and in patients with hypercholesterolemia (3, 13, 22–25). The methods used were varied: direct chemical measurement of fecal end products of cholesterol metabolism (24, 25), analysis of specific activity decay curves (13, 23), or compartmental analysis (3, 22). There was no significant difference found in these studies between normals and patients with hypercholesterolemia. The present data are in agreement. In a recent study Grundy and Ahrens (26) compared turnover rates of cholesterol obtained by isotopic decay curves using the two pool model, with those based on sterol balance data using chemical analysis, in the same patients. They reported good agreement between the two methods, although the calculated production rates were by an average of 15% higher than values obtained by sterol balance. As they pointed out, a possible explanation for this difference was the increment of area under the specific activity decay curves for the first few days after injection, which would be missed by curve fitting by two exponentials (4). An additional source of explanation of this discrepancy is indicated by the present long-term results which show the flattening effect. The long-term flattening yields an increment of area which, by equation 1, would reduce the total input rate by approximately the discrepancy noted by Grundy and Ahrens (26). In the patients of the present study, followed for more than 50 wk, the average value of I_T for the subjects who exhibited a monoexponential behavior of the slow slopes of decay curves (Figs. 6 and 7) was 1.14 g/day. The average input rate (I_T) of patients who followed a multiexponential decay (Figs. 1–5) was 0.97 g/day, or 15% lower.

The average size of the rapidly miscible pool of cholesterol M_A in the group of normocholesterolemic patients was 27.8 g. This figure is in good agreement with data of others (3, 22) for their normal subjects. The average size of M_A was 47.7 g in the five patients with hypercholesterolemia, an increase of about 20 g over the values of the normal group (Table I). Part, but not all of this cholesterol is accounted for by increased serum cholesterol concentrations (3, 4) (Table I). The difference in the average size of the total exchangeable body mass of cholesterol (M) was 49 g between the group of normal and hypercholesterolemic subjects, a rather impressive figure. Although there were marked variations in the individual values (Table I), the value for M was higher in each hypercholesterolemic patient than in any of the subjects with normal cholesterol. The highest value of M (173 g) was that of a patient with the highest cholesterol level, diffuse tendon xanthomas and severe coronary artery disease (H. Z.) (Table I). The average value for the remain-

ing exchangeable mass of cholesterol ($M - M_a$) was 29 g higher for the group of hypercholesterolemic patients than for normals, again a rather sizable mass. In a group of patients, about half of whom were overweight, hypercholesterolemia was not associated with any alteration in the amount and distribution of exchangeable cholesterol (22), when the size of the slowly exchangeable pool (M_B) was estimated by compartmental analysis. In the present group of patients, who were by and large of a normal weight, hypercholesterolemic subjects had significantly higher masses of body cholesterol than patients with normal serum cholesterol levels. Since obesity increased the size of pool M_B by 0.9 g of cholesterol per 1 kg of extra body weight (22), the absence of a significant relationship between serum cholesterol levels and body cholesterol masses in this group of patients can possibly be explained on the basis of this extra amount of added cholesterol.

The data of the patient with hypertriglyceridemia (type IV) were comparable to the group of patients with hypercholesterolemia (type II), in spite of a relatively low serum cholesterol level (Table I). On the basis of a single patient, no conclusions can be reached, of course. The reason for the unusually high input rate (3.29 g/day) in this patient is not quite clear. Goodman and Noble (3) reported data on four patients with hypertriglyceridemia, in whom the production rate of cholesterol (equivalent to input rates) seemed higher than the rest of their subjects. However, each of these patients had hypercholesterolemia as well.

It must be emphasized that the total exchangeable mass M as calculated in this paper does not logically require M to be apportioned into compartments. That is, the multiexponential curve fits of the data were used only as computational aids and not as the required expression of a particular mathematical model. The present analysis, however, is not without its own set of assumptions (2). Possibly the most restrictive assumption is that tracer cholesterol, upon injection, labels body cholesterol in proportion to local input rate, wherever body cholesterol is entering the system, whether by biosynthesis or by dietary intake. In a compartmental model (3, 4) the analogous assumption would be that the input rate of body cholesterol is zero in all compartments other than the one in which tracer is considered to be injected. If this assumption is not satisfied, that is, if the injected tracer does not label all input channels for body cholesterol, then the present derived M underestimates the true total body mass of cholesterol (γ_∞ is less than unity in reference 2). Whether or not this assumption is satisfied is, however, independent of whether or not a compartmental model validly represents the system. Thus, agreement of the present derived M with an independently measured total body cholesterol would not of itself constitute evidence in favor of a compartmental model. As noted in reference 2, the present values of M can also be derived from compartmental analysis for the 2-exponential case, if analogous assumptions are made (see limit case 1 of reference 4). It must be further emphasized that the masses of cholesterol as described in this study (M_a, M, and $M - M_a$) are conceptual rather than well defined anatomical eintities. At present, there are no data available in man or in any experimental animal species to demonstrate their exact morphologic boundaries or precise physical distribution. Since there are no independent data available to confirm the results on the calculated size of M, M_a, or $M - M_a$ (I_T can be verified by chemical measurements [26]), the acceptance of the present findings (or that of all compartmental analyses) depends on the model. At the present level of plausibility of the model, the data in the present paper indicate that the level of serum lipids and the masses of body cholesterol seem to have some relationship between two groups of patients studied, one with normal serum lipids the other with hypercholesterolemia. The need for independent verification of these inferences by direct chemical analysis in humans is evident and is open to future inquiry.

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