Three-pool model of the long-term turnover of plasma cholesterol in man

DeWitt S. Goodman, Robert P. Noble, and Ralph B. Dell

Departments of Medicine and Pediatrics, Columbia University College of Physicians and Surgeons, New York 10032, and the Sharon Research Institute, Sharon Hospital, Sharon, Connecticut 06069

Abstract The long-term turnover of plasma cholesterol was examined in six men injected intravenously with [4-^{14}C]cholesterol. The specific radioactivity–time curves were determined for periods of 32–41 wk and were analyzed by computer according to a two-pool and to a three-pool model. In each subject, the three-pool model provided a significantly better description of the long-term turnover curve than did the two-pool model. No further improvement in fit between observed and computed curves was obtained with a four-pool model. The results indicate that the turnover curves of all six subjects conformed to, and could be satisfactorily described by, a three-pool model, as expressed by the equation: specific activity = $A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + A_3e^{-\alpha_3 t}$.

The assumption was made that exit of cholesterol from the body occurs only by way of the tissue pools which comprise the rapidly exchangeable compartment, pool 1. With this assumption, the production rate in pool 1 (*PR*) is equivalent to the total body turnover rate. The parameters of the three-pool model which can be calculated include *PR*, the size of pool 1 (M_1), and the rate constants for transfer between pools. Unique values cannot be obtained for the sizes of the more slowly exchangeable pools of body cholesterol, but their upper and lower limiting values can be determined. The observed values for *PR* and M_1 (means ± SEM) were 1.13 ± 0.09 g/day and 23.4 ± 1.1 g, respectively.

When only the first 12 wk of data were analyzed, the turnover curves in all subjects conformed to the two-pool model. The results so obtained were compared with those obtained with the long-term data. The medium-term data provided a valid estimate for M_1, a slightly (8–9%) elevated value for *PR*, and a quantitatively unreliable (low) estimate of total exchangeable body cholesterol, as compared with the long-term data. Previous estimates of the production rate from studies of 10–12 wk duration can be considered valid if reduced by 8–9%.

Supplementary key words compartmental analysis · computer model · cholesterol, exchangeable · cholesterol, tissue pools · production rate, cholesterol · isotopically labeled cholesterol

During the past few years a considerable number of studies of the turnover of plasma cholesterol have been conducted in order to obtain information about cholesterol metabolism in normal and hyperlipidemic humans. It has been known for several years that when isotopically labeled cholesterol is injected intravenously, the semilogarithmic plot of cholesterol specific activity vs. time describes a curve during the first 5–6 wk, whereas beyond this time the plot appears to be linear. In 1968 we reported (1) that the plasma cholesterol specific radioactivity–time curves obtained in experiments of about 10 wk duration could be resolved precisely into two exponential functions, and that the turnover of plasma cholesterol hence conformed to a simple two-pool model. Subsequent reports from a number of laboratories demonstrated that this model, and the corresponding two-term exponential equation, appeared to characterize satisfactorily turnover curves of plasma cholesterol in man (2–6) and also in subhuman primates (7).

Most of the parameters of the two-pool model can be calculated readily by analysis of the plasma cholesterol turnover curve. These parameters include several rate constants, the size of the rapidly turning over first pool (which includes plasma), and the production rate in the first pool. The production rate is equivalent to the total body turnover rate, if we assume that all of cholesterol catabolism and excretion occurs via the tissues which comprise the first pool. Strong evidence for the validity of this interpretation has been presented by Grundy and Ahrens (4). Although the size of the second pool cannot be determined uniquely, it is possible to calculate upper and lower limiting values for the size of the slowly turning over pool (3). This approach has been usefully applied to a study of the relationship between body weight (particularly excess body weight) and the parameters of cholesterol metabolism (3).

TABLE 1. Characteristics of the six male subjects

Subject	Duration of Study	Age	Ht	Wt	% of Ideal Body Wt[a]	Plasma		LP Pattern (Type)
						Cholesterol[b]	TG[b]	
	wk	*yr*	*cm*	*kg*		*mg/100 ml*		
F.C.	41	51	175	76.2	112	220 ± 2	85 ± 4	NL[c]
R.M.	32	53	183	76.2	102	231 ± 3	102 ± 3	NL
R.N.	39	54	183	79.4	108	211 ± 2	69 ± 3	NL
J.B.	36	61	145	63.5	100	223 ± 3	294 ± 13	IV
G.F.	41	54	180	73.9	104	240 ± 2	216 ± 11	IV
H.L.	37	40	173	79.4	115	295 ± 5	280 ± 24	IV

[a] Metropolitan Life Insurance Co., Statistical Bulletin 40, 1959.
[b] Mean ± SEM during the period of the study; TG, triglyceride.
[c] NL, normal.

In 1970, Samuel and Perl (8) reported that in some patients studied for very long periods the slow slope of the decay curves deviated from monoexponential behavior after approximately 20–25 wk. In seven patients whom they studied for very long periods (50–63 wk), three patients showed a definite flattening of the slope of the semilogarithmic plot after 20–25 wk; in two other patients there was an equivocal change of slope and the data "suggested nonlinearity," whereas in two patients no change in the semilogarithmic slope was detectable (8). These findings indicated that in some patients the long-term turnover curve could not be described fully by a two-term exponential equation. Accordingly, a different method of data analysis ("input–output analysis") was developed and utilized (8, 9) that does not require curve fitting (although multiexponential equations were fitted for "convenience") and does not involve the use of a compartmental model.

We now report the results of studies carried out to determine whether a multicompartmental model containing more than two pools is necessary to describe the long-term turnover of plasma cholesterol in man. These studies indicate that a three-pool model is required for an adequate fit of long-term turnover curves.

METHODS

Six volunteer male subjects participated in this study; their ages are listed in Table 1. Three of the subjects were normal controls and three subjects had hypertriglyceridemia and a type IV pattern on lipoprotein electrophoresis (10). All subjects ate their usual diets, and none of the subjects lost or gained significant amounts of weight during the study. Plasma lipid concentrations were reasonably stable during the period of the study (see Table 1).

[4-^{14}C]Cholesterol (specific radioactivity 59.2 μCi/μmole, New England Nuclear Corp., Boston, Mass.) was complexed with serum lipoprotein, and the labeled serum prepared for injection as described previously (1). Serum containing approximately 22 μCi of ^{14}C-labeled cholesterol was injected intravenously into each subject. The amounts injected were measured precisely, and the data were later adjusted to an injected dose of 25 μCi for each subject. Samples of venous blood were collected before breakfast after 1, 3, 5, 10, and 15 days and at approximately weekly intervals thereafter for a total of 36–41 wk in five subjects and for 32 wk in the sixth subject (see Table 1). Serum was separated from blood cells and stored at −20°C. The serum was later used for measurement of the concentration of serum cholesterol and triglyceride with a Technicon AutoAnalyzer I, using the method N-24a for cholesterol (11) and a modification (12) of the Kessler and Lederer technique (13) for triglyceride. A portion (5 ml) of each serum sample was saponified, and the nonsaponifiable lipids were collected and dissolved in 5.00 ml of benzene, as described (1). Measured portions of each benzene solution were assayed for cholesterol by the same method. The standard deviation of the cholesterol assay, as determined by repeated analysis of standard solutions, was 1.5% of the mean value, for a series of assays. Other portions of each benzene solution were assayed for ^{14}C in a Packard liquid scintillation counter model 3365, using 0.5% diphenyloxazole in toluene as scintillation solvent. Each radioassay was corrected for the small amount of quenching present by use of an automatic external standard of radium-226, together with an appropriate quench correction curve prepared for plasma nonsaponifiable lipids. The amounts of radioactivity assayed were such that the coefficient of variation of the radioassay was approximately 0.25% for the first samples drawn and 2.0% for the last samples collected at the end of the study. The specific radioactivity of cholesterol in each sample was determined from the measured concentration of ^{14}C and of cholesterol in each benzene solution of nonsaponifiable lipids.

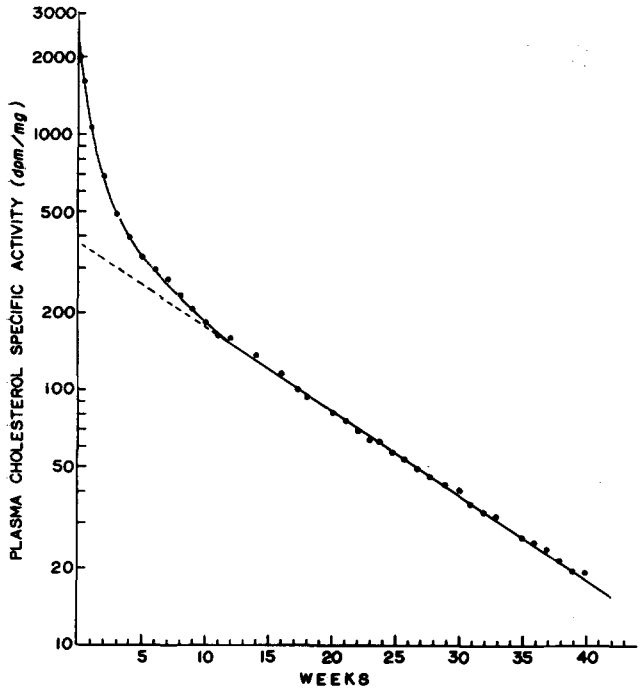

FIG. 1. Turnover of plasma cholesterol in normal subject F.C. The curve was drawn by hand to fit the data and has no other special significance. The plot was completely linear from approximately 10–12 wk on, as shown by the straight line drawn with a ruler and extrapolated back (as a broken line) to zero time.

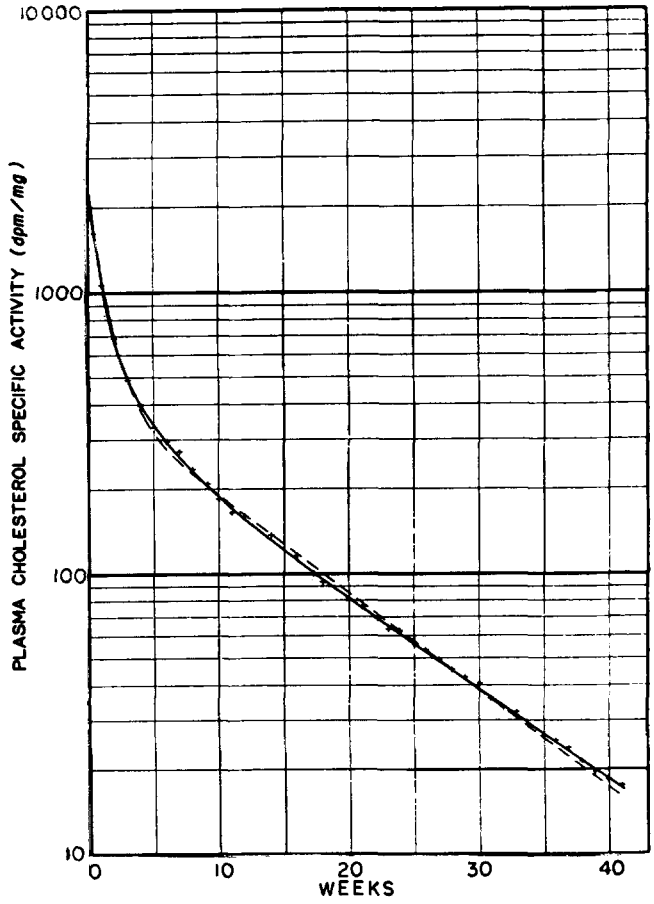

FIG. 2. Computer analysis of the turnover of plasma cholesterol in subject F.C. Observed data points are represented by crosses. The solid curve represents the best fit to the data which can be obtained with a three-pool model, whereas the broken curve represents the best fit obtained with a two-pool model.

The data for each subject were analyzed by digital computer in order to determine the parameters which would provide the best fit obtainable with a two-pool model and with models containing three or more pools. This was done according to the following considerations.

The decline in tracer concentration in the compartment into which the tracer was injected is described by a multiexponential equation. This equation contains as many exponential terms as there are compartments in the system. Thus, the equation is of the form:

$$y = \sum_{i=1}^{nc} A_i e^{-\alpha_i t} \qquad \text{Eq. 1}$$

where y is the tracer concentration, the A_i are the zero-time intercepts, the α_i are the rate constants, and nc is the number of compartments. Since the number of compartments needed to describe cholesterol kinetics optimally was not known, nc became a parameter with an unknown value. Thus, analysis of the data required finding values not only for the parameters A_i and α_i but also for nc. We used in the present study a weighted least squares nonlinear regression technique.

The least squares technique involves finding values for the parameters of the equation that minimize the sum of the squared distances between the data points and the points predicted by the equation. When the equation is nonlinear the minimization process leads to equations (called normal equations) that cannot be solved by ordinary algebraic means. Therefore, one starts with an initial guess for the parameters and then successively computes changes in the initial guesses until the sum of squared differences between the actual and calculated data points is a minimum. The technique used for this process was a modification[1] of the Marquardt technique (14).

As noted above, not all the data points were equally accurate but, rather, as plasma concentration of radioactive cholesterol declined the relative variance increased. Thus, the squared differences between the actual and calculated data points were weighted by the

[1] A manuscript completely describing the mathematical analysis is in press (R. Dell, R. Sciacca, K. Lieberman, D. Case, and P. Cannon. A weighted least squares technique for the analysis of kinetic data and its application to the study of renal ^{133}xenon washout in dogs and man. *Circ. Res.* In press).

reciprocal of the variance of each data point. Since the data points represent specific radioactivity, the variance was calculated by summing the relative errors of the cholesterol assay procedure and the radioactivity assay procedure. The effect of the weighting procedure is to give more weight to data points which are more accurate (i.e., those determined early in the study) so that the estimated parameter values are more heavily dependent upon the more accurately determined experimental points. It can be shown mathematically (15) that if the data do not have equal variances some weighting procedure must be adopted to yield valid parameter estimations.

The method of residual error testing was used to find the number of terms needed to describe best the specific radioactivity–time curves. The residual error is the sum of the squared differences between the given and calculated data points. As the number of parameters in the model increases, the residual error becomes smaller until the number of parameters equals the number of data points, at which point the residual error becomes zero. However, there is, for each set of data, a point at which addition of parameters does not significantly reduce the residual error. A method has been derived (Gauss-Markov theorem [16]) which tests, by an F test, the significance of the reduction in residual error upon addition of parameters. The test statistic is computed as follows:

$$F_{(np_i - np_{i-1}),(nx - np_i)} = \frac{(Q_{i-1} - Q_i)/(np_i - np_{i-1})}{Q_i/(nx - np_i)} \quad \text{Eq. 2}$$

where np_i is the number of parameters included in the current model, np_{i-1} is the number of parameters in the previous model, nx is the number of experimental points, Q_i and Q_{i-1} are the residual errors for the current and previous model, respectively, and F is the test statistic with $np_i - np_{i-1}$ and $nx - np_i$ degrees of freedom. The method employed in the present study was to fit two-, three-, and four-term exponential equations to the data and to test for significance of reduction in residual error by Eq. 2 after the parameters of the three- and four-term equations had been determined.

RESULTS

Fig. 1 shows the results obtained with normal subject F.C. The final slope of the turnover curve was achieved after approximately 10–12 wk of study and did not deviate from linearity thereafter. A very similar curve was obtained for each of the six subjects studied. In no instance was a change in slope observed near or beyond 20 wk of study.

TABLE 2. Comparison of the closeness of fit obtained by analysis of the data with a two-pool vs. a three-pool model

Subject	% Reduction in Residual Error[a]	P
F.C.	117	<0.01
R.M.	45	<0.025
R.N.	51	<0.01
J.B.	80	<0.01
G.F.	58	<0.01
H.L.	56	<0.01

[a] The residual error, a measure of the closeness of fit, was calculated for the best fit obtained with each model. The % reduction in residual error obtained with the three-pool model was calculated as: (residual error 2-pool − residual error 3-pool/residual error 3-pool) × 100. The statistical significance of the calculated reductions in residual error was assessed as indicated under Methods.

Fig. 2 shows the best fit obtained with the data of subject F.C. using a two-pool model (broken curve) and a three-pool model (solid curve). When a two-pool model was fitted to the data without weighting for the relative accuracy of each data point (i.e., a weight of one was assigned to each data point), a much poorer fit was obtained than the one shown by the broken curve in Fig. 2. When the data were weighted by the reciprocal of the variance of each data point, however, a fair fit was obtained with the two-pool model (see Fig. 2). The fit was, however, substantially improved when the data were analyzed according to a three-pool model.

Table 2 quantitatively describes the improvement in fit obtained with the three-pool as compared with the two-pool model. In each subject a statistically highly significant improvement in fit was obtained with the three-pool model. The data for each subject were also analyzed according to a four-compartment model. No further improvement in fit was obtained with a four-compartment model, as compared with the three-pool model. We therefore conclude that the three-pool model is required for an adequate fit of the data and also provides as good a fit as can be obtained in these long-term turnover studies.

The three-pool model for cholesterol turnover proposed for these studies is shown in Fig. 3. In this model the following symbols are used:[2]

(*a*) The pools (formerly designated by letters [Ref. 1]) are denoted by arabic numbers: 1, 2, 3, ...

[2] The symbols used generally adhere to published recommendations on nomenclature for tracer kinetics (17), except for the continued use of M for pool size.

(b) Rate constants are denoted by k; k_{ij} is the rate constant (in units of days^{-1}) for transfer into pool i from pool j, k_{11} is the rate constant for the total rate of removal of cholesterol from pool 1.

(c) The rate of transfer of cholesterol mass (in grams/day) into or out of a pool is denoted by R; R_{01} refers to rate of mass transfer out of the system (to "pool zero") from pool 1.

(d) Pool sizes (in grams) are designated M_1, M_2, and M_3.

For this model, the equation for the specific activity of pool 1 (a_1) is:

$$\frac{da_1}{dt} = k_{11}a_1 + \frac{M_2}{M_1}k_{12}a_2 + \frac{M_3}{M_1}k_{13}a_3 \qquad \text{Eq. 3}$$

or:

$$a_1 = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + A_3e^{-\alpha_3 t} \qquad \text{Eq. 4}$$

In Eq. 4, A_1, A_2, and A_3 are constants whose sum equals the y intercept of the specific activity–time curve when extrapolated back to zero time. The constants α_1, α_2, and α_3 are the rate constants of the three exponentials that describe the turnover curve. The half-times of the exponential terms were derived from the α values, since $t_{1/2} = \ln 2/\alpha$.

In the model shown, movement of cholesterol out of the body (i.e., catabolism and excretion) is assumed to occur only by way of the tissue pools which comprise the rapidly turning over pool 1. A comparable assumption was previously made for shorter-term studies with the two-pool model (1, 3, 4). This assumption permits the interpretation that production rate in pool 1 (PR) is equivalent to the total body turnover rate. Evidence in support of this interpretation has been reported from other laboratories (4, 5, 7). It should be noted that the PR comprises exogenous cholesterol absorbed into the body as well as newly biosynthesized cholesterol. For a turnover curve described by an equation of the form of Eq. 1, PR is described by the general equations:

$$PR = \frac{R^*}{\int_0^\infty \sum_{i=1}^{nc} A_ie^{-\alpha_i t}dt} = \frac{R^*}{\sum_{i=1}^{nc} \frac{A_i}{\alpha_i}}$$

where R^* is the amount of tracer injected.

Analysis of the turnover data in terms of this three-pool model results in values being calculated for each of the six constants of Eq. 4. From these constants, unique values can be calculated for each of the rate constants for transfer between pools (k_{21}, k_{12}, k_{31}, and k_{13}); for PR (which equals R_{01}); and for M_1, the size of the rapidly turning over pool. (See Appendix for a detailed description of the equations used.) Unfortunately, unique values cannot be obtained for the sizes of the more slowly turning over pools 2 and 3. Upper and lower limiting values for M_2 and for M_3 can, however, be calculated by making certain assumptions about the relative extent of synthesis of cholesterol in the tissues which comprise pools 1, 2, and 3, i.e., about the relative values of R_{10}, R_{20}, and R_{30}. Thus, the lower limiting value of M_2 is obtained when R_{20} is zero, and the upper limiting value for M_2 when R_{20} is at its maximal value. Since $R_{10} + R_{20} + R_{30}$ equals PR, and since PR represents the sum of endogenously synthesized cholesterol plus that derived from the diet, the maximal value for R_{20} can be estimated as $PR - 0.2$. This estimate depends on the assumptions previously employed (3) that daily cholesterol absorption is about 200 mg and that all newly absorbed cholesterol enters pool 1 (i.e., is part of R_{10}). Similarly, the lower limiting value of M_3 is obtained when R_{30} is zero, and the upper limiting value of M_3 when $R_{30} = PR - 0.2$. The minimal value for the total body exchangeable cholesterol ($M_1 + M_2 + M_3$) is obtained when $PR = R_{10}$ (R_{20} and R_{30} both equal zero). The maximal value for total body exchangeable cholesterol is represented by the case where pool 3, the largest of the three compartments, is at its upper limiting value.

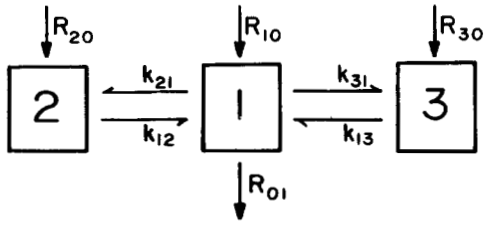

FIG. 3. Three-pool model of cholesterol turnover in man (see text for explanation of symbols and assumptions).

Table 3 presents the values for each of the several parameters discussed above for each of the six long-term studies reported here. Generally, similar values were obtained in all six subjects for each of the parameters; moreover, the three hypertriglyceridemic subjects did not differ significantly from the normal subjects with regard to any of the parameters. The results for the six subjects were therefore pooled to obtain a mean (± SEM) value for each parameter, as listed in the table. Future studies, with larger numbers of subjects, may of course reveal significant differences between normal and hypertriglyceridemic subjects with regard to some parameters of cholesterol metabolism.

Fig. 4 shows the three-pool model with the mean parametric values obtained for this group of subjects. It is clear from the relative rate constants to and from pools 2 and 3 that pool 2 is smaller, and pool 3 larger, than pool 1.

Calculations were then carried out to determine how the results obtained by three-pool model analysis of these

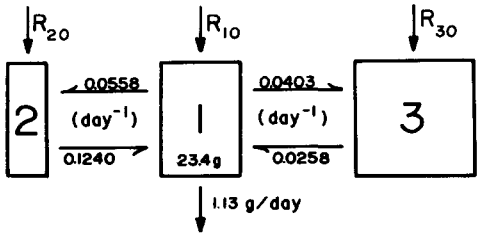

FIG. 4. Three-pool model of cholesterol turnover in man, with mean parameter values as calculated from the six turnover studies reported here. The mean minimum and maximum values for M_2 were 11.3 and 20.2 g, respectively, and for M_3 were 35.7 and 72.1 g, respectively (see Table 3).

long-term studies (Table 3) compared with the results which would have been obtained in shorter-term studies, such as those previously carried out (1, 3). For each subject, the first 12 wk of data were separately analyzed according to the two-pool model to obtain the values for the parameters of cholesterol metabolism shown in Table 4. Analyses of these data (first 12 wk) by the three-pool model demonstrated that no improvement of fit was obtained, as compared with the two-pool model. The results of medium-term (10–12 wk) studies are therefore satisfactorily described by the two-pool model.

Table 5 compares the production rate as calculated from the two-pool model, using the first 12 wk of data, with the *PR* calculated from the three-pool model in the complete long-term studies. For every subject, the *PR* calculated with the three-pool model for the long-term study was slightly less than that calculated with the two-pool model for 12 wk of data. The mean percentage difference in *PR* was 8.4%, and this difference was statistically highly significant ($P < 0.001$ by the paired t test).

Table 6 compares the values of several other parameters calculated from the first 12 wk of data with the two-pool model with the values obtained by analysis of the long-term data with the three-pool model. Similar values of M_1 were obtained in both analyses. In contrast, very large differences were obtained for the estimates of the upper and lower limiting values of the slowly turning over compartments (Tables 3 and 4) and hence for the minimal and maximal values calculated for total exchangeable body cholesterol (Total M, Table 6). It is apparent that previous estimates of the size of the slowly turning over pool of body cholesterol, obtained by analysis of medium-term turnover studies, cannot be considered quantitatively valid.

DISCUSSION

These studies were conducted in order to examine in detail the long-term turnover of plasma cholesterol in

TABLE 3. Turnover of plasma cholesterol: parameters of the three-pool model in six men

Subject	A_1	A_2	A_3	Half-time of Exponential 1st	Half-time of Exponential 2nd	Half-time of Exponential 3rd	α_1	α_2	α_3	k_{11}	k_{12}	k_{13}	k_{21}	k_{31}	PR	M_1	Minimum M_2	Minimum M_3	Maximum M_2	Maximum M_3	Total M Min.	Total M Max.
	dpm/mg			*days*			*days*$^{-1}$					*days*$^{-1}$			*g/day*		*g*		*g*		*g*	
F.C.	1501	400	355	4.09	16.06	65.6	0.1695	0.0432	0.0106	−0.122	0.079	0.023	0.050	0.029	1.07	24.6	15.6	31.3	26.7	70.0	71.5	110.2
R.M.	1445	513	336	3.80	10.87	55.9	0.1824	0.0638	0.0124	−0.131	0.101	0.027	0.036	0.042	1.29	24.2	8.6	37.6	19.4	78.1	70.4	110.9
R.N.	1284	419	404	3.92	13.61	68.5	0.1768	0.0509	0.0101	−0.120	0.092	0.026	0.047	0.035	1.00	26.4	13.4	35.2	22.2	66.0	75.0	105.8
J.B.	1183	1018	511	2.55	9.71	67.4	0.2718	0.0714	0.0103	−0.147	0.178	0.028	0.063	0.045	0.81	20.5	7.2	32.3	10.7	54.0	60.0	81.7
G.F.	1387	1119	328	2.02	8.57	59.5	0.3431	0.0809	0.0117	−0.201	0.209	0.025	0.088	0.052	1.21	19.6	8.2	40.3	13.0	80.3	68.1	108.1
H.L.	1581	335	305	3.60	13.18	55.6	0.1925	0.0526	0.0125	−0.147	0.085	0.026	0.051	0.039	1.42	25.0	14.9	37.6	29.4	84.2	77.5	124.1
Mean	1397	634	373	3.33	12.00	62.1	0.2226	0.0604	0.0112	−0.145	0.124	0.026	0.056	0.040	1.13	23.4	11.3	35.7	20.2	72.1	70.4	106.8
SEM	59	140	31	0.34	1.14	2.4	0.0285	0.0057	0.0004	0.012	0.023	0.001	0.007	0.003	0.09	1.1	1.5	1.4	3.0	4.6	2.5	5.7

TABLE 4. Parameters of the two-pool model from the data for the first 12 wk of study in six men

Subject	A_1	A_2	Half-time of Exponential 1st	Half-time of Exponential 2nd	k_{11}	PR	M_1	Min. M_2	Max. M_2
	dpm/mg		*days*		*days*$^{-1}$	*g/day*		*g*	
F.C.	1651	567	4.72	44.2	−0.113	1.17	24.8	33.5	53.2
R.M.	1751	479	4.76	40.9	−0.118	1.38	24.7	34.8	61.3
R.N.	1487	589	4.63	46.6	−0.112	1.12	26.5	34.7	52.0
J.B.	1801	724	4.83	46.9	−0.107	0.91	21.8	27.6	41.3
G.F.	1972	504	4.62	40.0	−0.123	1.31	22.2	32.3	57.3
H.L.	1718	441	4.25	41.3	−0.133	1.51	25.5	40.6	68.7
Mean	1730	551	4.65	43.3	−0.118	1.23	24.2	33.9	55.6
SEM	66	41	0.08	1.2	0.004	0.09	0.8	1.7	3.8

man. Although the number of subjects studied was small, the fact that very similar results were obtained with every one of the six subjects suggests that these findings may have more general validity. In each subject, a three-pool model provided a significantly better description of the long-term turnover curve than did a two-pool model. No further improvement in fit between observed and computed curves was obtained by analysis according to a four-compartment model. The results indicate that the turnover curves of all of these subjects conformed to and could be satisfactorily described by a three-pool model such as that illustrated in Fig. 3.

We were stimulated to conduct this study because of the report by Samuel and Perl (8) that in three of seven patients the long-term slow slope of the turnover curve deviated from monoexponential behavior and exhibited an obvious change in slope, apparent on visual inspection, after approximately 20–25 wk. This phenomenon was not observed with any of our subjects. It should be noted, however, that in our subjects the final linear slope of the semilogarithmic decay curve was not attained until approximately 9–15 wk after pulse-labeling. The occurrence of a later change in slope (flattening) of the turnover curve would presumably depend upon the existence of a pool of significant size of very slowly turning over cholesterol in poorly exchanging tissue sites (e.g., arteries, connective tissue [7, 18, 19]). These conditions may exist in some persons (such as the three subjects reported by Samuel and Perl [8]) and not in others. More extensive studies of a much larger series of subjects will be required in order to define the frequency with which late flattening of the turnover curve may occur and the characteristics of the patients in whom this phenomenon is observed. If and when this phenomenon occurs, a multicompartmental model containing more than three pools might possibly be required for optimal analysis of the data.

TABLE 5. Comparison of production rates calculated from 12 wk of data with the two-pool model and from 32–41 wk of data with the three-pool model

Subject	PR 2-Pool	PR 3-Pool	% Difference
	g/day		
F.C.	1.17	1.07	−8.5
R.M.	1.38	1.29	−6.5
R.N.	1.12	1.00	−10.7
J.B.	0.91	0.81	−11.0
G.F.	1.31	1.21	−7.6
H.L.	1.51	1.42	−6.0
Mean	1.23	1.13	−8.4
SEM	0.09	0.09	0.9

As discussed previously with regard to the two-pool model (1), it must be recognized that the three pools in the proposed model (Fig. 3) represent mathematical constructs and do not have precise physical meaning. The finding that the long-term turnover of plasma cholesterol conforms to a three-pool model means that the various tissue pools of exchangeable body cholesterol fall into three groups in terms of the rates at which they equilibrate with plasma cholesterol. The first compartment consists of cholesterol in fairly rapid equilibrium with plasma cholesterol, and probably includes plasma, red blood cell, and liver cholesterol, together with much of the cholesterol in several other viscera (e.g., intestines, pancreas, spleen, kidney, lung) (7, 18, 19). A portion of the cholesterol in pool 1 is probably also located in peripheral tissues (adipose tissue, muscle, skin) (7). The second compartment (pool 2) consists of cholesterol which equilibrates at an intermediate rate with plasma cholesterol, and probably includes some of the cholesterol in viscera, together with some of the cholesterol in peripheral tissues. Most of the cholesterol in peripheral tissues (particularly skeletal muscle [18, 19]) equilibrates more slowly with plasma cholesterol and comprises the major portion of the most slowly turning over pool, pool 3.

In addition to the three pools of exchangeable cholesterol (Fig. 3), a complete model of body cholesterol metabolism would require the addition of a fourth pool representing nonexchangeable (or exceedingly slowly exchangeable) cholesterol. The addition of such a pool

TABLE 6. Comparison of parameter values calculated from 12 wk of data with the two-pool model and from 32–41 wk of data with the three-pool model

Parameter	2-Pool	3-Pool	% Difference	P^a
M_1 (g)	24.2 ± 0.8	23.4 ± 1.1	−3.8 ± 1.8	<0.1
Total M,[b] minimum (g)	58.2 ± 2.3	70.4 ± 2.5	21.2 ± 1.2	<0.001
Total M,[b] maximum (g)	79.9 ± 4.2	106.8 ± 5.7	33.7 ± 1.9	<0.001
Half-time of final exponential (days)	43.3 ± 1.2	62.1 ± 2.4	43.2 ± 2.5	<0.001

Values are means ± SEM for the six men studied.
[a] Parameters were compared statistically by the paired t test for the individual subjects for each parameter.
[b] Total $M = M_1 + M_2$ for the two-pool model and $M_1 + M_2 + M_3$ for the three-pool model.

of virtually nonexchangeable cholesterol to the two-pool model was proposed and discussed by Wilson (7), who indicated that, in the baboon, this pool consisted not only of the cholesterol in the central nervous system but also of much of the cholesterol in bone, and apparently also of some of the cholesterol in skeletal muscle, skin, and other tissues rich in fibrous connective tissue. In the baboon this pool contained approximately 0.5 g of cholesterol/kg body weight and comprised about 38% of the total body cholesterol (7). It should be noted, moreover, that a much longer-term study than the ones reported here might possibly require a four-compartment model for optimal analysis of the data, reflecting the effects of the exceedingly slowly exchangeable body cholesterol on the plasma turnover curve. Nonexchangeable cholesterol would then represent a fifth compartment.

From our current knowledge of cholesterol metabolism, it is likely that almost all of cholesterol catabolism and excretion occurs via the tissues which comprise the rapidly turning over compartment, pool 1 (see Ref. 1, 3, 4, 7, 20, and 21 for discussions of this point). In the proposed three-pool model, therefore, there is no independent exit of cholesterol from the body via pools 2 and 3, and exit of cholesterol from the body is assumed to occur only by way of pool 1, at a mass flow rate of R_{01}. Under these circumstances, and assuming steady state conditions, the production rate in pool 1 is equivalent to the total body turnover rate. Evidence for the validity of this interpretation has been obtained by studies in which the total body cholesterol turnover rate calculated (as the PR) from plasma turnover curves (with the two-pool model) was compared with the turnover rate measured directly by sterol balance methods (4, 5). The turnover rates obtained by the two methods agreed quite closely in all cases, with the sterol balance method generally providing a slightly lower value than the isotopic kinetic method. In 11 studies carried out in 10 patients, there was a mean discrepancy of 15% in the results obtained by the two methods (4), and in 5 additional studies carried out in 4 patients the mean discrepancy in the results was only 8% (5). Given this single constraint on the model (loss of cholesterol only via pool 1), and assuming that there is no direct transfer of cholesterol between pools 2 and 3 (i.e., the tissue pools in pools 2 and 3 interchange cholesterol only via pool 1, as shown in Fig. 3), it is possible to calculate all of the model parameters listed in Table 3.

Although the assumption that all exit from the body occurs via pool 1 appears to be generally valid, it is clear that it is not absolutely so. Recent studies from other laboratories (22)[3] have demonstrated that a small amount of excretion of cholesterol appears to occur directly via the skin, and hence via slowly equilibrating tissue pools. The amount of cholesterol so excreted (estimated at about 50–100 mg/day [22][3]) is apparently sufficiently small so as not to seriously affect the overall quantitative validity of the results obtained with the model as formulated.

A major parameter of cholesterol metabolism, which we would like to be able to estimate accurately, is the total amount of exchangeable cholesterol in the body. Unfortunately, neither the size of pool 2 (M_2) nor that of pool 3 (M_3) can be estimated with any precision with the three-pool model shown in Fig. 3. It is, however, possible to calculate upper and lower limiting values for both M_2 and M_3 by making assumptions about the relative extent of synthesis of cholesterol in the tissues which comprise pools 1, 2, and 3. Thus, if independent entry of cholesterol into pool 2, or pool 3, is negligibly small (i.e., if R_{20}, or R_{30}, = 0), then the lower limiting value for M_2, or M_3, is obtained. On the other hand, the upper limiting value for either M_2 or M_3 is obtained by assuming that cholesterol synthesis occurs entirely in that particular pool (i.e., is represented by R_{20} or by R_{30}, respectively), with R_{10} representing only cholesterol absorbed from the diet. The true values for M_2 and M_3 are probably much closer to the lower than to the upper limiting values, since the tissues which comprise pools 2 and 3 undoubtedly are much less active in the synthesis of cholesterol than are the liver and the gastrointestinal

[3] Nikkari, T., and E. H. Ahrens, Jr. Personal communication.

tract (which are presumbly part of pool 1) (23). Wilson (7) and Dietschy and Wilson (21) have suggested that the assumption that the entry of new cholesterol into the system occurs almost entirely via the rapidly exchangeable compartment (pool 1) may be reliable. This approach (i.e., assuming that R_{20} and $R_{30} = 0$) to the estimation of M_2 and M_3 may prove useful for lean human subjects. It is likely, however, that this approach would not be adequate for obese subjects, since there is evidence that large adipose stores contribute significantly to the production rate (3). This approach may also not be valid for certain kinds of hyperlipidemic or other subjects.

How would the results obtained by data analysis by the method of "input–output analysis" (8, 9) compare with those obtained by multicompartmental analysis as reported here? Input–output analysis of the present data would provide values for "total input rate" equivalent to, and presumably identical with, the values obtained for the production rate with the three-pool model. The values for total exchangeable mass of body cholesterol calculated by input–output analysis would be equivalent to the lower limiting values for total exchangeable cholesterol (minimum values for $M_1 + M_2 + M_3$) reported here, since the input–output method assumes that all entry of cholesterol into the system occurs in the same location where the label is introduced (hence into pool 1), and that exit from the system also occurs from a single location. As discussed above, this assumption may be valid for estimation of body pools in some circumstances, but might well be inadequate in others. The calculation of the "mean transit time" of cholesterol by input–output analysis involves the same assumption of single sites of entry into and exit from the system. Finally, it should be noted that input–output analysis, while potentially useful if a satisfactory multicompartmental model cannot be formulated, considerably limits the amount of information which can be derived from the data, as compared with that obtainable by multicompartmental analysis.

The question of the duration of a turnover study required for satisfactory delineation of the three-pool model warrants consideration. In the six studies reported here, fairly satisfactory definition of the parameters of the three-pool model could have been achieved in each case with the data from about the first 22–25 wk of study. The critical question in deciding whether a given study has been conducted for a long enough period, and can be terminated, is the question of whether the time of study was long enough to clearly define the final exponential in the curve (i.e., the final term in Eq. 4) and whether the number of data points was sufficient to define all parameters. Since the question of a possible change in slope of the turnover curve near or beyond 20 wk in some patients remains to be resolved, it would probably be desirable to conduct future long-term studies for at least 30–35 wk (or more) whenever this is feasible. A better definition of some of the model parameters can also be obtained by collecting more frequent data points during the first weeks of study.

In contrast to the long-term studies, the results of medium-term studies (10–12 wk duration) are satisfactorily described by the two-pool model. In the studies reported here, when only the first 12 wk of data were analyzed the three-pool model did not provide a better description of the data than did the two-pool model. The important question therefore arises as to how the parameters of cholesterol metabolism calculated (by the two-pool model) from medium-term data compare with the parameters calculated from long-term data (by analysis according to the three-pool model). In the present studies, the results obtained from the long-term studies compared with those obtained from 12 wk of data as follows. (*a*) Similar values were obtained by both analyses for M_1, the size of the rapidly exchangeable pool. This finding was expected, since M_1 is calculated from the values of the injected dose of ^{14}C and of the *y* intercept of the turnover curve extrapolated back to zero time (which is minimally affected by carrying out a longer-term study). (*b*) Considerably larger values were obtained for both the upper and lower limiting values of total body exchangeable cholesterol, and for the sizes of the slowly exchanging pools of cholesterol, with the long-term data (analyzed by the three-pool model) than with the medium-term (12 wk) data. (*c*) The production rate calculated from the long-term data was slightly (mean of 8.4%) lower than that calculated from 12 wk of data, a statistically highly significant difference. (*d*) The half-life of the final exponential obtained with long-term data was considerably larger than that obtained with medium-term (12 wk) data.

These findings indicate that some of the conclusions previously derived from medium-term turnover studies are probably valid, whereas others are not. Thus, previous estimates of the size of the rapidly exchangeable compartment of cholesterol (of M_1) can be considered as valid in the patients studied, and the previous inferences regarding the identity of the tissue pools which make up pool 1 remain unchanged. In contrast, previous estimates of the size of the slowly exchangeable compartment of cholesterol, obtained from medium-term studies, can no longer be considered as quantitatively valid. It is, of course, possible that previous conclusions regarding the effects of certain factors (e.g., body weight [3]) on the size of the slowly exchangeable compartment remain relatively correct. Further data will be required in order to examine adequately this possibility. It should be emphasized that even with long-term data there will be significant uncertainty in the estimates of the sizes of the

pools of slowly exchangeable cholesterol in any group of patients, since the upper limiting values for both M_2 and M_3 differed considerably from the corresponding lower limiting values in every subject studied here. Although we would like to use kinetic studies and compartmental analysis to examine the effects of therapeutic intervention (e.g., with hypolipidemic drugs) on body pool sizes of cholesterol, this may prove to be unfeasible because of the degree of uncertainty inherent in the estimations of M_2 and M_3.

In view of the findings summarized in Table 5, previous estimates of the production rate obtained from medium-term studies can be considered as quantitatively valid if corrected by a reduction of 8–9%. When this correction factor is applied to published results, the daily turnover calculated from turnover studies (as the production rate) agrees almost precisely with that measured directly, in the same patients and at the same time, by sterol balance methods (4, 5).

APPENDIX

Calculation of Model Parameters

The number of terms in a multiexponential equation, when applied to cholesterol data, denotes the number of compartments in a multicompartmental system. Furthermore, the rate constants of the system can be calculated from the estimated values of the parameters of the multiexponential equation. The assumptions and calculations for a two-pool model have already been described (1, 3). If the three-compartment model shown in Fig. 3 is assumed, then the rate constants and pool sizes are calculated as follows (adapted from Skinner et al. [24]):

$$M_1 = \text{amount injected}/(A_1 + A_2 + A_3)$$

$$\text{let } d_1 = k_{22} + k_{33} = \alpha_2 + \alpha_3 + (\alpha_1 - \alpha_2)(A_2M_1) + (\alpha_1 - \alpha_3)A_3M_1$$

$$d_2 = k_{22}k_{33} = (\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)A_2M_1 - \alpha_2^2 + d_1\alpha_2$$

$$d_3 = k_{11} = \alpha_1 + \alpha_2 + \alpha_3 - d_1$$

$$d_4 = k_{13}k_{31} + k_{12}k_{21} = d_2 + d_3d_1 - \alpha_1\alpha_2 - \alpha_2\alpha_3 - \alpha_3\alpha_1$$

$$d_5 = k_{13}k_{22}k_{31} + k_{12}k_{33}k_{21} = -\alpha_1\alpha_2\alpha_3 + d_3d_2$$

$$\text{then } k_{11} = d_3$$

$$k_{22} = (-d_1 + \sqrt{d_1^2 - 4d_2})/2$$

$$k_{33} = (-d_1 - \sqrt{d_1^2 - 4d_2})/2$$

$$k_{12} = k_{22}$$

$$k_{13} = k_{33}$$

$$k_{21} = (d_5 - d_4k_{22})/[(k_{33} - k_{22})k_{22}]$$

$$k_{31} = (d_4k_{33} - d_5)/[(k_{33} - k_{22})k_{33}]$$

$$M_2 = (R_{20} + M_1k_{21})/k_{22}$$

$$M_3 = (R_{30} + M_1k_{31})/k_{33}$$

Minimum M_2 or M_3 occurs when R_{20} or $R_{30} = 0$.
Maximum M_2 or M_3 occurs when R_{20} or $R_{30} = PR - 0.2$.

We are grateful for the expert assistance of Mr. R. Sciacca, Ms. G. Stanton, and Ms. A. Campbell. This work was supported by grants HL-14236 (SCR), AM-05968, and HD-03993 from the National Institutes of Health, Bethesda, Md., and by grants from the Sharon Research Institute and the Upjohn Company. Drs. Goodman and Dell are Career Scientists of the Health Research Council of the City of New York.

Manuscript received 3 July 1972; accepted 3 November 1972.

REFERENCES

1. Goodman, DeW. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. *J. Clin. Invest.* **47:** 231–241.
2. Samuel, P., C. M. Holtzman, E. Meilman, and W. Perl. 1968. Effect of neomycin on exchangeable pools of cholesterol in the steady state. *J. Clin. Invest.* **47:** 1806–1818.
3. Nestel, P. J., H. M. Whyte, and DeW. S. Goodman. 1969. Distribution and turnover of cholesterol in humans. *J. Clin. Invest.* **48:** 982–991.
4. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
5. Salen, G., E. H. Ahrens, Jr., and S. M. Grundy. 1970. Metabolism of β-sitosterol in man. *J. Clin. Invest.* **49:** 952–967.
6. Moore, R. B., I. D. Frantz, Jr., and H. Buchwald. 1969. Changes in cholesterol pool size, turnover rate, and fecal bile acid and sterol excretion after partial ileal bypass in hypercholesteremic patients. *Surgery.* **65:** 98–108.
7. Wilson, J. D. 1970. The measurement of the exchangeable pools of cholesterol in the baboon. *J. Clin. Invest.* **49:** 655–665.
8. Samuel, P., and W. Perl. 1970. Long-term decay of serum cholesterol radioactivity: body cholesterol metabolism in normals and in patients with hyperlipoproteinemia and atherosclerosis *J. Clin. Invest.* **49:** 346–357.
9. Perl, W., and P. Samuel. 1969. Input–output analysis for total input rate and total traced mass of body cholesterol in man. *Circ. Res.* **25:** 191–199.
10. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276:** 34–44, 94–103, 148–156, 215–225, 273–281.
11. Block, W. D., K. J. Jarret, and J. B. Levine. 1965. Use of a single color reagent to improve the automated determination of serum total cholesterol. *In* Automation in Analytical Chemistry. L. T. Skeggs, Jr., editor. Mediad, New York. 345–347.
12. Noble, R. P., and F. M. Campbell. 1970. Improved accuracy in automated fluorometric determination of plasma triglycerides. *Clin. Chem.* **16:** 166–170.

13. Kessler, G., and H. Lederer. 1965. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. L. T. Skeggs, Jr., editor. Mediad, New York. 341–344.
14. Marquardt, D. W. 1963. An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Indust. Appl. Math.* **11:** 431–441.
15. Cramer, H. 1963. Mathematical Methods of Statistics. Princeton Univ. Press, Princeton, N.J.
16. Mann, H. B. 1949. Analysis and Design of Experiments. Analysis of Variance and Analysis of Variance Design. Dover, New York.
17. Brownell, G. L., M. Berman, and J. S. Robertson. 1968. Nomenclature for tracer kinetics. *Int. J. Appl. Radiat. Isotop.* **19:** 249–262.
18. Field, H., Jr., L. Swell, P. E. Schools, Jr., and C. R. Treadwell. 1960. Dynamic aspects of cholesterol metabolism in different areas of the aorta and other tissues in man and their relationship to atherosclerosis. *Circulation.* **22:** 547–558.
19. Chobanian, A. V., and W. Hollander. 1962. Body cholesterol metabolism in man. I. The equilibration of serum and tissue cholesterol. *J. Clin. Invest.* **41:** 1732–1737.
20. Goodman, DeW. S. 1970. The measurement of cholesterol pools and turnover in man. *In* Atherosclerosis: Proceedings of the Second International Symposium. R. J. Jones, editor. Springer-Verlag, New York. 242–248.
21. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. *N. Engl. J. Med.* **282:** 1128–1138, 1179–1183, 1241–1249.
22. Bhattacharyya, A. K., W. E. Connor, and A. A. Spector. 1970. Excretion of sterols from the skin of man: implications for sterol balance studies. *Clin. Res.* **18:** 621. (Abstr.)
23. 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.
24. Skinner, S. M., R. E. Clark, N. Baker, and R. A. Shipley. 1959. Complete solution of the three-compartment model in steady state after single injection of radioactive tracer. *Amer. J. Physiol.* **196:** 238–244.