Whole body and tissue cholesterol turnover in the baboon

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Abstract Cholesterol turnover was studied in four baboons by injecting [^{14}C]cholesterol 186 days and [^{3}H]cholesterol 4 days before necropsy, and fitting a two- or three-pool model to the resulting specific activity-time data. At necropsy, cholesterol mass and specific activity were determined for the total body (minus the central nervous system) and for many tissues. A pool model permits the estimation, from the plasma specific activity-time curve alone, of total body cholesterol within a limited range, depending upon the extent of side pool synthesis. The principal aim of this study was to estimate the extent of cholesterol synthesis in the side pools of the model, by computing the amount of side pool synthesis needed to equal the measured total body cholesterol. Central pool synthesis varied from 61 to 89% of the total cholesterol production rate. Thus, approximately 25% (11 to 39%) of the production rate arose from peripheral (pool 3 for the three-pool, and pool 2 for the two-pool model) cholesterol synthesis. Moreover, the finding that the measured total body cholesterol fell within the range obtained from the kinetic analysis by using reasonable assumptions (namely, that zero or that half the production rate occurred in the side pools), provides evidence for the physiological validity of the model. A second aim of this study was to explore cholesterol turnover in various tissues. A pool model predicts that rapidly turning over tissues will have higher specific activities at early times and lower specific activities at later times after injection of tracer relative to slowly turning over tissues, except where significant synthesis occurs. Tissues were ranked 1 to 17 for ^{3}H and 17 to 1 for ^{14}C cholesterol specific activity values. Except for the GI tract and testis, the tissues had similar ranks for both ^{3}H and ^{14}C, further validating model predictions. Results in all four baboons were similar. Turnover rates for the different tissues loosely fell into three groups which were turning over at fast, intermediate, and slow rates. Finally, the magnitude of variation of cholesterol specific activity was moderate for several distributed tissues (fat, muscle, arteries, and the alimentary tract), but was small for liver. Cholesterol turnover in serial biopsies of skin, muscle, and fat could, however, be fitted with a single pool to estimate tissue turnover rates. —**Dell, R. B., G. E. Mott, E. M. Jackson, R. Ramakrishnan, K. D. Carey, H. C. McGill, Jr., and D. S. Goodman.** Whole body and tissue cholesterol turnover in the baboon. *J. Lipid Res.* 1985. **26:** 327-337.

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Kinetic studies of cholesterol turnover have provided a great deal of quantitative information about body cholesterol metabolism in humans (1-6). These studies usually have involved the analysis of plasma decay curves following the injection of radioactively labeled cholesterol. When studies were carried out for 8- to 10-month periods, a three-pool model was found necessary and sufficient to fit the data (2, 5). This model appears to be generally valid for the study of cholesterol metabolism in normal and dyslipoproteinemic humans (5, 6).

The three-pool model provides estimates of the masses of exchangeable cholesterol, and of the rates of flux of cholesterol, in the pools and the body. However, the model has eight unknown parameters, whereas only six model parameters can be determined uniquely from an analysis of the plasma cholesterol turnover curve (2, 7). As a consequence, the size (mass) of the peripheral pools (pools 2 and 3) cannot be determined uniquely. Minimum values for the sizes of the peripheral pools can, however, be calculated by assuming that all of cholesterol synthesis occurs in the central pool (pool 1) (2, 5). Conversely, if all of cholesterol synthesis occurs in the peripheral pools, maximum pool size estimates are obtained.

This model can be used to explore quantitatively the effects of physiological, pharmacological, and nutritional perturbations on body cholesterol metabolism. Such studies have related some of the major model parameters (including production rate, the size of the rapidly exchanging compartment pool 1, and the minimum values of the size of pool 3 and of total body exchangeable cholesterol) to physiological variables (including body size,

Abbreviations: GLC, gas-liquid chromatography; PR, production rate; M, pool size; k, rate constant; R, rate of transfer of cholesterol mass.

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serum lipid levels, and age) (5). The effectiveness of this approach is, however, limited by the uncertainty inherent in the estimation of the sizes of the peripheral side pools. On the basis of both physiological considerations (2, 5), and the findings of Wilson (8) in a study of baboons, we can conclude that the true values for the sizes of the peripheral pools are probably closer to minimum than to maximum values. However, recent studies by Spady and Dietschy (9) have indicated that a substantial proportion of total body cholesterol synthesis may occur in peripheral tissues. If this is the case, then the true values for the sizes of the side pools would be distinctly greater than the minimum estimates, and could only be determined if the extent of side-pool synthesis is known.

The principal aim of the current work was to estimate, in baboons, how much of cholesterol synthesis occurs in the side pools and how much in the central pool of the compartmental model. To obtain this information, we compared cholesterol pool size estimates from kinetic analysis of plasma turnover curves with total body cholesterol content. The measurement of total body cholesterol by carcass analysis permitted the calculation of synthesis occurring in the side pools, and hence the partitioning of total synthesis into central and peripheral components. A secondary aim of this work was to characterize cholesterol turnover in various tissues of the baboon, both by examining the relative rates of turnover in different tissues, and by exploring the variability of turnover rates in region to region for distributed tissues such as muscle and fat.

METHODS

Animals

Four adult male baboons (*Papio cynocephalus* species) were fed a diet containing Purina Special Monkey Chow 25 (Ralston-Purina, St. Louis, MO), lard, egg yolk, vitamins, and crystalline cholesterol (10). The composition expressed as percent of total calories was 41% as fat, 21% as protein, and 38% as carbohydrate. The cholesterol content was 1.7 mg/Kcal and the ratio of polyunsaturated to saturated fatty acids was 0.34. The average daily intake of cholesterol for the four baboons was approximately 2 g. The diet was fed for about 6 months prior to and then during the experiment. Animals C and D were tethered with venous catheters through a backpack approximately 2 months before injection of [4-^{14}C]cholesterol, and remained on tether until day 36 after injection. The catheters permitted frequent sampling of blood without anesthesia.

The mean (± SD) body weights of baboons A, B, C, and D during the experiment were, respectively, 22.4 ± 0.56, 31.1 (mean of two weights, 32.0 and 30.2), 26.6 ± 0.66, and 22.1 ± 0.75 kg. Their serum cholesterol levels during the turnover studies were, respectively, 134 ± 21, 228 ± 29, 176 ± 18, and 148 ± 16 (mean ± SD values in mg/dl, n = 23 to 29).

Turnover studies

Radioactive cholesterol was purified by high performance liquid chromatography on a reverse phase Radial Pak C_{18} column (Waters Associates, Milford, MA) with acetonitrile–isopropanol–methanol 20:3:7, as elution solvent and also on a Silica-Radial Pak column with isooctane–isopropanol 98:2, as elution solvent. [4-^{14}C]Cholesterol was prepared for injection by dissolving about 500 μCi of purified cholesterol (sp act 57 mCi/mmol) in 500 μl of acetone and adding it by syringe to 7 ml of sterile autologous serum. The serum was equilibrated with slow magnetic stirring for 20 hr and then injected intravenously. The precise amount of radioactivity injected was determined by radioassay of an aliquot of the injected serum with a liquid scintillation spectrometer and by subtracting the residual radioactivity in the syringe wash after injection. On day 182 after injection of the [4-^{14}C]cholesterol, [^{3}H]cholesterol was injected intravenously in animals A, C, and D. This injection consisted of purified [1,2-^{3}H]cholesterol dissolved in acetone and resuspended in autologous serum as described above. Each animal received 450 to 500 μCi of [4-^{14}C]cholesterol and 23 to 31 μCi of [1,2-^{3}H]cholesterol. Serum was obtained from blood samples after injection of the [4-^{14}C]cholesterol on days 1, 2, 3, 4, 7, 9, 11, 15, 18, 22, 25, 29, 36, 43, 50, 57, 64, 71, 78, 85, 99, 113, 127, 141, 155, 169, 182, 183, 184, 185, and 186. The animals were sedated with ketamine HCl, 10 mg/kg, before bleedings; no sedation was required during the time animals C and D were tethered.

Assay methods

Serum cholesterol concentrations were determined enzymatically by the methods of Allain et al. (11) and Witte, Barrett, and Wycoff (12) on an ABA-100 Bichromatic Analyzer (Abbott Laboratories, North Chicago, IL). This procedure met the criteria of the Lipid Standardization Program of the Center for Disease Control. The coefficient of variation for duplicate analyses was less than 2%. Serum radioactivity was determined by adding 0.1–0.5 ml of serum to 10 ml Scintisol (Isolab Inc., Akron, OH) and counting by liquid scintillation spectrometry. An external standard counting method was used to calculate dpm.

Serial biopsies (50–200 mg) of skin, of muscle from the posterior lateral aspect of the leg, and of subcutaneous fat from the lateral lower abdominal quadrants were obtained from animals A and B under pentothal anesthesia at 2, 4, 7, 15, 22, 29, 43, 71, and 113 days after injection of the [^{14}C]cholesterol. Tissue specific radioactivity for each biopsy sample was determined as described below.

The animals were killed at 186 days, after ketamine immobilization and under pentothal anesthesia, by exsanguination, and perfused with several liters of cold Krebs-Ringer buffer or isotonic saline. Duplicate samples of approximately 1 g were removed from each tissue, blotted, weighed, and transferred to screw-capped tubes. The samples were saponified with 6 ml of 1.25 N NaOH in absolute ethanol at 15 lbs pressure (121°C) for 1 hr. After the samples cooled, 5 ml of H_2O was added to each and the mixture was extracted with 15 ml of redistilled petroleum ether. Measured portions of the petroleum ether layer were assayed for radioactivity, and cholesterol was determined by a $FeSO_4$-H_2SO_4 colorimetric method (13). During the course of this study we found a large discrepancy between the values for hepatic cholesterol content obtained by the colorimetric method (13) and those obtained by a gas–liquid chromatographic (GLC) method (14). Thus, when we measured the cholesterol content of the liver extracts by GLC on OV-17 with 5 α-cholestane as an internal standard, the values obtained were only 35–60% of those obtained by the colorimetric method. The hepatic cholesterol data derived from the GLC method are reported in this paper. We tested the GLC method on extracts of several other tissues (lung, adrenal, brain, serum, kidney, fat, stomach, intestines, aorta) and found no differences in the results obtained by the GLC and colorimetric methods. Accordingly, for the nonhepatic tissues the cholesterol data presented were derived from the colorimetric method. The specific radioactivity of cholesterol in each sample of serum and tissue was determined by dividing the measured concentration of radioactivity by that of cholesterol mass, and was expressed as dpm/mg of cholesterol.

The remaining tissues and carcass of each animal with the brain and spinal cord removed were pooled and digested in 20–25 liters of ethanolic KOH (final concentration 3.3 N) at room temperature for about 1 month. Total body cholesterol mass was determined by quantitative GLC analysis (14) of samples of the digest with cholestane as internal standard.

Data analysis

[4-^{14}C]Cholesterol specific activity-time data were analyzed by a weighted least-squares technique (15) to determine the parameters of a mammillary model which best describe the data. The curve for each animal was fitted with two-, three-, and four-pool models. There was a significant improvement in fit with a three-pool model, with biologically plausible parameter estimates, in two out of the four baboons; there was no further improvement in fit with a four-pool model in any animal. Accordingly, the data were analyzed according to a three-pool model for baboons A and C, and with a two-pool model for baboons B and D.

The three-pool model has been used extensively in our studies in humans (2, 5, 6). Analysis of the plasma cholesterol specific activity-time curves yields values for six unique model parameters: PR (cholesterol production rate), M_1 (size of the rapidly exchanging central pool 1), and the rate constants k_{12}, k_{13}, k_{21}, and k_{31} (rate constants for transfer between pool 2 or 3 and pool 1). However, it is not possible to estimate the input rates into each of the three pools (i.e., R_{10}, R_{20}, and R_{30}) although the sum of these three mass rates is the production rate, PR. If R_{20} and R_{30} are assumed to be zero (R_{10} equals PR), then minimal estimates of the size of M_2 and M_3 are computed. As R_{20} and R_{30} increase, estimates of the size of M_2 and M_3 increase and the estimate of the amount of total body cholesterol increases. Intermediate values for M_2 and M_3 were computed by assuming that pools 2 and 3 each received one-fourth of the production rate.

Similar considerations apply to the parameters of a two-pool model (no pool 3). In this case the fitting process yields four unique parameters (PR, M_1, k_{12}, and k_{21}), and the size of M_2 depends upon the value of R_{20} (M_2 minimum when $R_{20} = 0$; M_2 intermediate when $R_{20} = 0.5$ PR).

For each animal, the results of compartmental modeling were used together with the directly measured value for total body cholesterol to calculate the mass of the side pools ($M_2 + M_3$ for a three-pool model, M_2 for a two-pool model), and the extent of direct input (synthesis) in the side pools ($R_{20} + R_{30}$ for a three-pool, R_{20} for a two-pool model) in that animal. This was done as follows. First, the mass of the side pools (M_{sp}, which equals $M_2 + M_3$ (three-pool) or M_2 (two-pool)) was computed as:

$$M_{sp} = M_{tot\ (observed)} - M_1 \qquad \text{Eq. } 1)$$

where $M_{tot\ (observed)}$ is the measured total body cholesterol, and M_1 is the mass of pool 1 obtained from the model. If M_{sp} is larger than the minimal estimate of the side pool mass from the model (as computed from the plasma decay curve), this means that direct input into the side pools (R_{sp}, which = $R_{20} + R_{30}$ (three-pool) or R_{20} (two-pool)) is greater than zero. By increasing R_{sp}, the estimated size of M_{sp} from the model can be made to match M_{sp} computed by equation *1*. For the two-pool model, R_{sp} (= R_{20}) was computed as follows:

$$R_{sp} = M_{sp}k_{12} - M_1k_{21}. \qquad \text{Eq. } 2)$$

(Note that $M_{sp}k_{12}$ is R_{12} and that M_1k_{21} is R_{21}, so that R_{sp} is the difference in mass flow out of and into pool 2 from pool 1.) For the three-pool model, increasing R_{20} from zero to PR (i.e., to having all synthesis into pool 2) has a relatively small effect on the model estimates of $M_2 + M_3$; accordingly, R_{20} was left at zero and R_{30} was increased so that the model estimate of $M_2 + M_3$ matched M_{sp} computed from equation *1*. R_{sp} (which

under these conditions equals R_{30}) was then computed as follows:

$$R_{sp} = M_3k_{13} - M_1k_{31} \qquad \text{Eq. } 3)$$

where M_3 equals M_{sp} (from equation *1*) minus the minimum estimate of M_2.

Tissue cholesterol turnover was studied by injecting two isotopes, ^{14}C- and ^{3}H-labeled cholesterol, at two different times before the tissue samples were collected for analysis. By injecting the isotopes at two times and sampling the tissues at a single time, one obtains, in effect, data for two different time points. Thus, [^{14}C]cholesterol was injected 186 days and [^{3}H]cholesterol was injected 4 days before the animals were necropsied and tissue cholesterol specific activity (for both ^{14}C and ^{3}H) was measured. Baboon B received only one isotope. With only two time points, only a single-pool model for tissue cholesterol turnover can be explored. Expressing each tissue as a single pool with the plasma decay curve as input allows computation of a turnover rate for that tissue pool. It is, of course, quite likely that the cholesterol molecules in a tissue turn over with a range of turnover rates; in such a case the estimation of a single rate would represent a weighted average of the separate turnover rates in that tissue. This reasoning leads to a simple, single-pool model: plasma $\rightleftarrows$ tissue. Goodness of fit of this simple model in which a single turnover rate is estimated from two time points, can be judged by computing the residual error of the two points from the fitted curve and expressing the residual error as a coefficient of variation (standard deviation/mean). The coefficient of variation was under 7% (comparable to experimental error) for nearly all tissues. If the coefficient of variation was greater than 15%, we felt that the single pool model was not an adequate description of the data and that more complexity was needed. On physiological grounds, we felt that local synthesis was the most likely explanation for lack of fit; accordingly, in these instances, the single pool model was modified to include unlabeled input (i.e., local synthesis) as well as labeled input from plasma, and this modified model was fitted to the data. Since a model with two parameters exactly fits two points, goodness of fit cannot be assessed.

Serial biopsies of skin, muscle, and fat were obtained from baboons A and B (see above). Analogous to the other tissue data, each set of biopsy data was fitted with a single pool model, without and with synthesis using the plasma curve as input.

Finally, the specific activities of both ^{3}H and ^{14}C in various tissue samples (collected after the animals were killed) were arranged by organ or organ system and ranked 17 (lowest value) to 1 (highest) for ^{3}H and 1 (lowest value) to 17 (highest) for ^{14}C. If a pool model describes the data, then the rank order should invert from early to late time points unless significant local synthesis occurs. The reason for this inversion is illustrated in **Fig. 1**. This figure shows the specific activity-time curves for a rapidly turning over central pool as well as for more slowly turning over pools. The specific activity in the side pools is initially zero and then rises to eventually cross the decay curve for the central pool. Side pool specific activity then remains above central pool specific activity. Thus the specific activity in a side pool is below that in the central pool at early times and above it at later times. Hence, if the early sample is drawn well before the side pool curve crosses the central pool curve, and the later sample drawn well afterwards, then a reversal in rank should be seen. Furthermore, a comparison of the curves for pools 2 and 3 shows that a more slowly turning over pool (pool 3) ranks lower at the early time point and higher at the later time point. Local cholesterol synthesis (in a given tissue) would lower the specific activity-time curve and disturb the ranking for that tissue. Thus, excluding pools with significant local synthesis, a ranking of side pool specific activities should invert between early and later times.

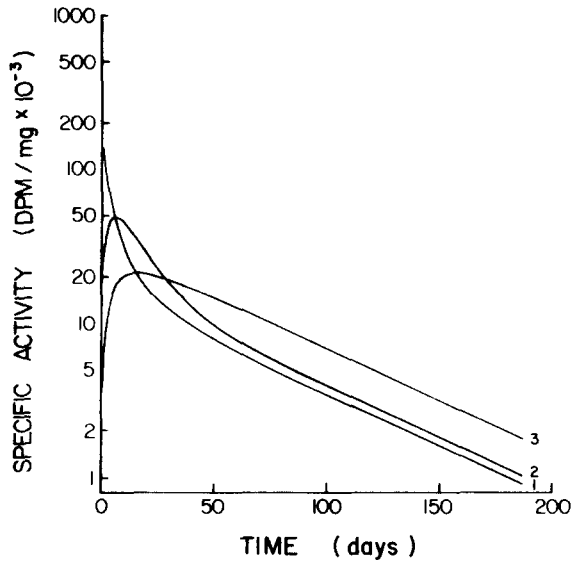

Fig. 1. Specific activity-time curves for cholesterol in plasma (pool 1), and in the side pools of the three-pool model (pools 2 and 3). These particular curves were generated using mean values for parameters from baboons A and C.

RESULTS

Plasma specific radioactivity-time curves

The plasma specific activity-time data together with the fitted curves for all four baboons are shown in **Fig. 2**. The curves represent the best fits obtainable with a three-pool model for baboons A and C, and with a two-pool model for baboons B and D. In each case, the curve fits the data quite well with little residual error. The residual error and the model parameters, both the uniquely determined

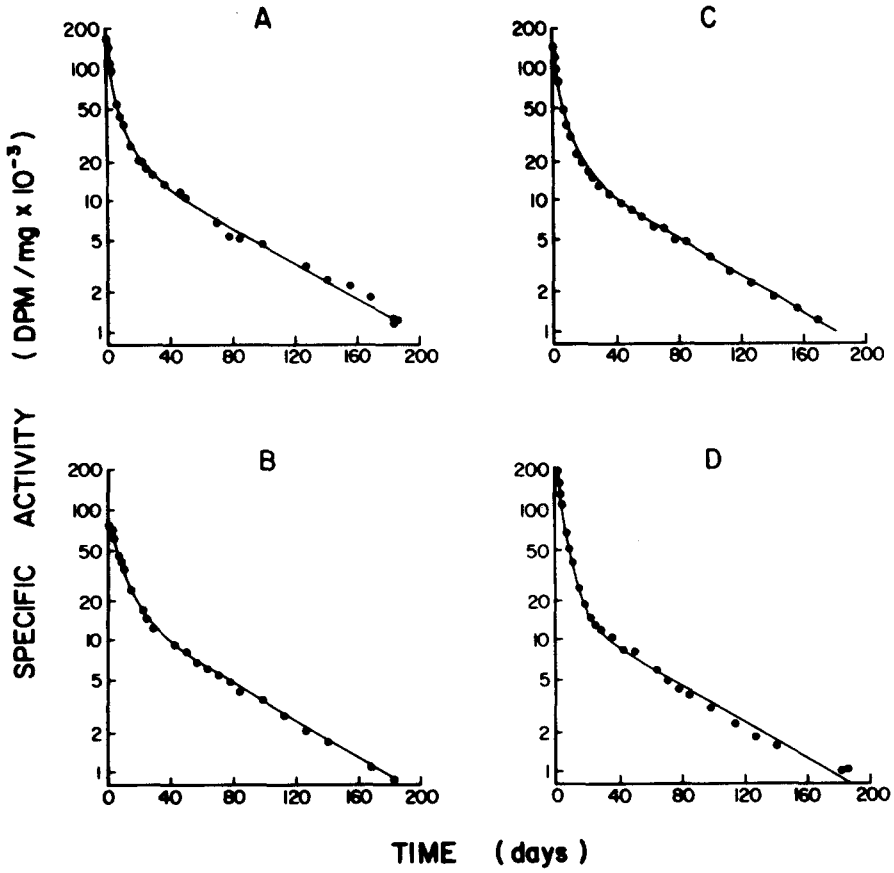

Fig. 2. Turnover of plasma cholesterol in the four baboons studied. The data points indicate the measured specific activity values. The solid curves drawn represent the best fits obtainable with a three-pool model for baboons A and C and with a two-pool model for baboons B and D.

model parameters (PR, M_1, k_{12}, k_{21}, k_{13}, k_{31}) and those for which only an interval estimate can be given (M_2, M_3, and M_{total}), are presented in **Table 1**.

Residual error varied from 2.5 to 7.5%. (Residual error is computed from the sum of squared deviation of the data points from the fitted curve.) For the four baboons, the mean production rate was 565 mg/d, and the mean value for M_1 was 7.0 g. The mean estimates for total exchangeable body cholesterol (M_{tot}) from the kinetic studies were 22.0 g (minimum estimate) and 29.1 g (intermediate estimate).

Side pool synthesis

Estimates of side pool synthesis using the measured total body cholesterol and model parameters, as described in the Methods section, are given in **Table 2**. Side pool cholesterol mass (M_{sp}) was computed by subtracting M_1 (derived from the model) from the directly measured total body cholesterol. Using equations *2* and *3* of the Methods section, the side pool synthesis (input) rate (R_{sp}, column (4) of Table 2) was computed. Subtraction of R_{sp} from the production rate (Table 1) then gave R_{10} (column (5) of Table 2), which represents the rate of entry of cholesterol into the central pool by either synthesis or absorption. R_{10} accounted for 61 to 89% (mean 74%) of the total production rate (column (6) of Table 2). Thus, side pool synthesis accounted for an average of approximately 25% of the total body cholesterol turnover (production) rate.

TABLE 1. Model parameters for all four baboons

Baboon	Residual Error	PR	M_1	k_{12}	k_{21}	k_{13}	k_{31}
	%	*mg/dl*	*g*		*days*$^{-1}$		
A	7.3	478	5.0	0.1022	0.0750	0.0283	0.0642
B	3.6	615	11.3	0.0372	0.0485		
C	2.5	598	6.1	0.1411	0.0515	0.0350	0.0855
D	7.5	570	5.4	0.0298	0.0725		

	Minimum			Intermediate		
	M_2	M_3	M_{total}	M_2	M_3	M_{total}
	g	*g*	*g*	*g*	*g*	*g*
A	3.7	11.5	20.2	4.7	15.7	25.6
B	14.7		25.9	22.9		34.2
C	2.2	15.0	23.4	3.3	19.3	28.7
D	13.0		18.4	22.6		27.9

TABLE 2. Estimates of side pool synthesis using observed total body cholesterol

Baboon	(1) Model M_1	(2) Measured M Total[a]	(3) M_{sp}[b]	(4) R_{sp}[c]	(5) R_{10}[d]	(6) $R_{10}/PR \times 100$[e]
		g		*mg/d*	*mg/d*	%
A	5.0	26.8	21.7	185	293	61
B	11.3	30.7	19.4	177	438	71
C	6.1	25.3	19.1	66	532	89
D	5.4	23.5	18.1	153	417	73

[a]Measured by carcass analysis.
[b]M_{sp}, Mass of cholesterol in the side pools, and = column (2) − column (1) (see Methods section).
[c]R_{sp}, Synthesis (input) rate of cholesterol into the side pools. R_{sp} was computed using equations 2 and 3 in the Methods section.
[d]R_{10}, Input into pool 1, was computed as production rate (Table 1) minus side pool synthesis (R_{sp}), and includes absorbed cholesterol plus cholesterol synthesized de novo in pool 1.
[e]Column 6, Input into pool 1 divided by the production rate.

Tissue specific radioactivity

Over 100 samples of tissue were analyzed for cholesterol specific activity in each baboon. Multiple samples were taken from different regions of the body for fat, muscle, and arteries to permit estimation of spatial heterogeneity (i.e., of the variation of specific activity from region to region in the body for a given type of tissue). Similarly, multiple samples of the alimentary (G.I.) tract and the liver were examined to estimate the variation in specific activity in different parts of these organs. The results of these analyses for all four baboons are shown in **Table 3**. The pooled coefficient of variation of [^{14}C]cholesterol specific activity among fat samples for all four baboons was 20%, for muscle 20%, for arteries 23%, for the alimentary tract 31%, and for the liver 5%. Thus, there was small spatial heterogeneity within the liver and moderate heterogeneity among distributed tissues or organs. In part, this latter heterogeneity appeared to reflect consistent differences between certain anatomic sites. Cholesterol in tongue, for example, appears to turn over more rapidly than cholesterol in the other muscles. The jejunum and ileum also appear to have more rapid cholesterol turnover rates than the rest of the alimentary tract (Table 3).

The tissue specific activity data were pooled to yield weighted means for various organs or tissues. Both the [^{14}C]- and [^{3}H]cholesterol specific activity data were ranked to determined whether the rank order was consistent from animal to animal and to determine whether the ranking of the ^{14}C and ^{3}H data was consistent with a pool model. [^{14}C]Cholesterol specific activity values for 17 organs for all four baboons were ranked 1 to 17 for the lowest to the highest values; the results are shown in **Table 4**. In general, the rank order for the different tissues

TABLE 3. [4-^{14}C]Cholesterol specific activity for selected tissues

Tissue	Baboon A	B	C	D	Pooled C.V.[a]
					%
Fat					
Omentum	3193	1271	3548	3564	
Axillary	2403	1909	2274	3492	
Pericardial	2886	1430	2878	4079	
Perirenal	3313	1714	3918	3490	
Popliteal	3518	1782	4080	4796	
Pelvic	3855	2607	4175	4571	
Mesenteric	3830	1092	3480	3596	
Mean[b]	3285	1686	3479	3941	
SD	520	500	690	550	20
Muscle					
Diaphragm	1667	1116	1241	1240	
Tongue	1434	915	1054	904	
Intercostal	2388	1605	1564	1588	
Biceps	1992	1538	1408	1228	
Deltoid	2086	2080	1268	1383	
Gastrocnemius	1761	—	1442	1293	
Sternocleidomastoid	—	—	1520	1282	
Mean	1888	1451	1357	1274	
SD	338	455	179	205	20
Arteries[c]					
Coronary	2448	3562	3099	2219	
Carotid	3650	2532	3962	3041	
Brachial	3495	1996	3874	2595	
Aortic arch	4431	3318	3051	3473	
Thoracic aorta	4861	3397	4041	3610	
Abd. aorta	3452	2746	4771	4456	
Inf. mesenteric	—	2930	—	—	
Femoral	—	—	3361	3152	
Pulmonary	—	—	1618	—	
Mean	3723	2926	3472	3156	
SD	843	553	940	774	23
G.I. tract					
Esophagus	1604	1088	941	1052	
Stomach	1494	1034	766	1221	
Duodenum	1132	728	752	907	
Jejunum	786	595	500	417	
Ileum	940	383	502	498	
Colon	1128	850	678	795	
Mean	1181	780	690	815	
SD	315	268	170	313	31
Liver					
Left lobe	2409	1830	1939	1839	
Left lobe	1998	1883	2124	1844	
Middle lobe	2365	1979	2116	1750	
Middle lobe	2198	2062	2047	1788	
Right lobe	2076	2008	1931	1781	
Right lobe	—	2031	2013	1781	
Mean	2209	1966	2028	1796	
SD	178	90	84	37	5

[a]Pooled coefficient of variation (C.V.) is the SD for each baboon pooled $\sqrt{(\Sigma(n_i - 1)SD_i^2/\Sigma(n_i - 1))}$ divided by the mean.
[b]Mean is the simple arithmetic mean of the tissue specific activities.
[c]Abd., abdominal; Inf, inferior; the symbol (—) means not analyzed.

TABLE 4. Ranking of specific activity[a] of tissue cholesterol by organs

Tissue/Organ	Baboon A				Baboon B		Baboon C				Baboon D			
	^{14}C		^{3}H		^{14}C		^{14}C		^{3}H		^{14}C		^{3}H	
	dpm/mg	Rank	dpm/mg	Rank	dpm/mg	Rank	dpm/mg	Rank	dpm/mg	Rank	dpm/mg	Rank	dpm/mg	Rank
G.I. tract	1109	1	1143	12	682	1	634	1	1651	11	706	1	1275	12
Testis	1195	2	1264	11	798	2	946	2	1633	12	1007	2	1737	11
Pool 1 (by model)	1242		4132		814		896		4782		1023		5133	
Serum, blood	1295	3	3840	4	861	3	1064	3	5846	2	1042	3	4871	4
Lung	1354	4	3923	3	980	8	1112	6	4776	3	1043	4	4936	3
Gall bladder	1360	5	3664	5	949	5	1086	4	3142	6	1283	12	3006	6
Thyroid	1382	6	1768	8	952	6	1086	5	2736	7	1207	8	2684	7
Spleen	1396	7	4436	1	918	4	1129	8	6439	1	1083	5	5134	2
Adrenal	1429	8	3938	2	961	7	1159	10	4551	4	1251	10	5205	1
Heart	1478	9	3098	6	996	9	1122	7	4287	5	1082	6	4320	5
Pancreas	1491	10	1429	10	1072	10	1153	9	2137	9	1235	9	2188	9
Muscle	1697	11	1680	9	1116	11	1367	11	2110	10	1262	11	1740	10
Prostate	1714	12	891	13	1180	12	1902	13	676	16	1696	13	1091	14
Kidney	1777	13	1863	7	1242	13	1402	12	2334	8	1204	7	2228	8
Urinary bladder	2094	14	643	16	1439	15	1914	14	1011	13	2091	14	1095	13
Tendon, ligament	3107	15	702	14	1391	14	2556	15	654	17	2945	15	787	15
Fat	3368	16	668	15	1630	16	3505	16	758	15	3679	17	625	17
Arteries	4035	17	395	17	2920	17	3697	17	780	14	3433	16	773	16

[a]The tissue specific activities are weighted means of individual values, weighted by the mass of the tissue or organ sampled.

agreed quite well among all four baboons. Also shown in Table 4 is the ranking of tissue [^{3}H]cholesterol specific activity values for the three baboons that received [^{3}H]cholesterol as well as the [^{14}C]cholesterol. The tritium data were ranked from highest to lowest values, i.e., in inverse order from the [^{14}C]cholesterol data. In general, if the data can be explained by a pool model, the early time points (^{3}H data) should be in inverse order from the later time points (^{14}C data). As shown in Table 4, for most tissues this was found to be the case. Notable exceptions were the tissues that showed significant local synthesis (determined as defined in the Methods section), namely the alimentary (G.I.) tract and the testis.

The liver rankings have not been included in Table 4 because the cholesterol specific activity values found in liver (Table 3) were much higher than the values found in serum (Table 4). Other studies in baboons (8) and in humans (16, 17) have indicated that cholesterol in liver turns over rapidly and exchanges rapidly with serum cholesterol. It should be noted (see Methods section) that the liver specific activity values in Table 3 were calculated from the results of GLC mass assays, which (for liver) were quite different from the mass data obtained by the colorimetric method. We cannot explain the discrepancy between the high values seen here in liver (Table 3) and the results of others; studies directed at this issue are currently in progress.

Fig. 3 graphically shows the rank order for each isotope for the 15 tissues that did not show significant local synthesis in these three baboons. The ^{14}C specific activity rank (186 days after injection of isotope) is on the left of each bar, and the tritium rank (4 days after injection of isotope) is on the right side of each bar. A horizontal line connecting the two ranks for a tissue indicates that that tissue has completely inverted its relative ranking and hence is turning over as predicted by a pool model. In general, there is good agreement in rank order between the early and late time points, indicating conformity with a pool model.

Organ turnover rates

The results of using an equation fitted to the plasma cholesterol specific activity-time curve to compute organ turnover rates (see Methods section) are shown in **Table 5**. The tissues are listed in descending order of the turnover rates (in days^{-1}) for baboon A. Again, there is good agreement in ranking for all organs for all four baboons. The turnover rates for the pools of the model fitted to each baboon are also given in Table 5. Inspection of Table 5 indicates that the various tissues can be collected approximately into three groups. One group of tissues had relatively rapid cholesterol turnover rates, comparable to those of blood and serum. Another group of tissues had very slow cholesterol turnover rates, an order of magnitude smaller than those of the first group. A third group of tissues/organs showed intermediate turnover rate values.

The three pools of a compartmental model represent a weighted average of a large number of individual turnover rates and do not have, inherently, a precise anatomical location. Hence, this grouping of tissue turnover rates does not mean that pool 2 or pool 3 is exactly represented by the cholesterol molecules that are present in the tissues with intermediate or slow turnover rates. The data in Table 5 do, however, suggest which tissues are likely to be mainly identified with one or another pool of the model. Thus, it is clear form the table that cholesterol in urinary

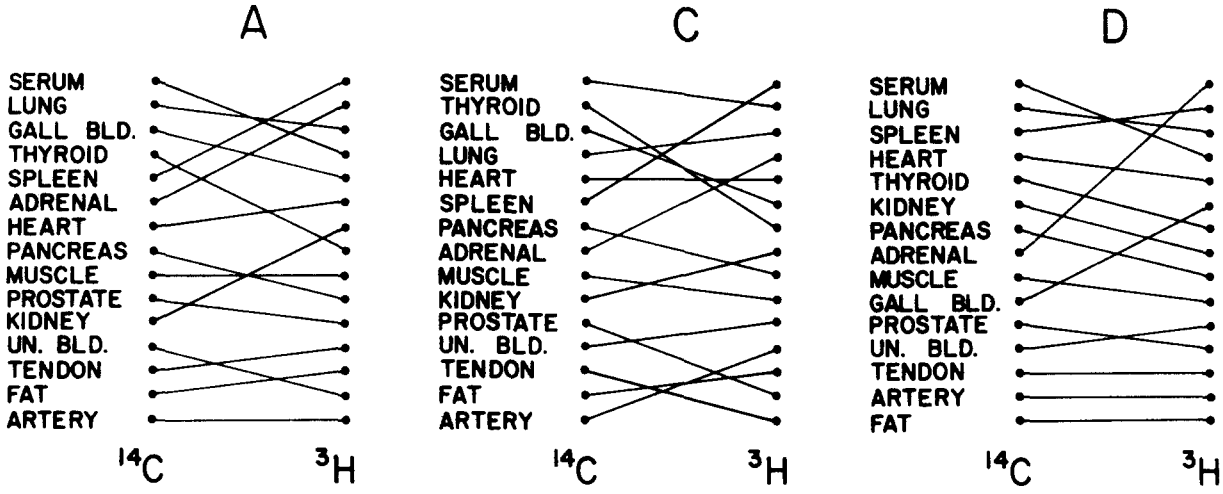

Fig. 3. Ranking of ^{14}C and of ^{3}H data in baboons A, C, and D, for the various tissues and organ systems studied, excluding the tissues (G.I. tract, testis) which showed local synthesis. The ^{14}C ranking has the lowest value on top, whereas the ^{3}H ranking has the highest value on top (inverted). Each line connects the ^{14}C and ^{3}H rankings for a particular organ/tissue.

bladder, tendons, fat, and arteries turned over quite slowly, while cholesterol in blood, spleen, lung, adrenal, gall bladder, and heart turned over quite rapidly; cholesterol in the other tissues was turning over at an intermediate rate. It is probable that the turnover rates for pool 1 of the two-pool model in baboons B and D represent a weighted average of the turnover rates of pools 1 and 2 of the three-pool model, since pool 2 turnover rates in baboons B and D agree with those of pool 3 for baboons A and C.

Biopsy data

The results of the serial biopsies carried out in baboons A and B are shown in **Fig. 4**. Despite considerable scatter in the data points, presumably because of the spatial heterogeneity noted above (Table 3), it was possible to fit each data set with a single pool model. Adding synthesis significantly improved the fit for skin in both baboons but not for muscle or fat in either baboon. The residual errors, expressed as a coefficient of variation, for baboons A and B, respectively, were 17% and 18% for fat, 22% and 34% for muscle, and 17% and 10% for skin with synthesis. These residual errors for fat and muscle are similar to those noted in Table 3. The turnover rates calculated from the biopsy data, for baboons A and B, respectively, were 0.045 and 0.044 $days^{-1}$ for fat, 0.044 and 0.048 $days^{-1}$ for muscle, and 0.050 and 0.039 $days^{-1}$ for skin with synthesis. These values are not significantly different from the muscle and fat turnover values given for baboons A and B in Table 5.

DISCUSSION

The experiments reported here had three major objectives: *1*) to estimate the extent of cholesterol synthesis in the peripheral, side pools of the compartmental model, by computing the amount of side pool synthesis needed in the model to match the measured total body cholesterol; *2*) to estimate the turnover of cholesterol in various tissues, in order to determine whether a pool model provides an adequate description of tissue cholesterol turnover, and to compare the relative turnover rates in the principal organs and tissues of the body; and *3*) to examine the spatial heterogeneity of cholesterol specific activity in distributed tissues and organs and to explore the potential usefulness of small biopsies for the study of cholesterol turnover in various tissues.

TABLE 5. Turnover rates of cholesterol for various organs/tissues and model pools for four baboons

Organ/Tissue	A	B	C	D
			day^{-1}	
Spleen	0.37	0.24	0.69	0.33
Lung	0.27	0.13	0.29	0.30
Adrenal	0.27	0.15	0.26	0.33
Gall bladder	0.24	0.16	0.14	0.13
Heart	0.17	0.12	0.23	0.23
Thyroid	0.086	0.16	0.12	0.11
Kidney	0.086	0.053	0.092	0.088
Muscle	0.077	0.030	0.082	0.065
Testis	0.076[a]		0.079[a]	0.078[a]
G.I. tract	0.074[a]		0.12[a]	0.082[a]
Pancreas	0.067	0.081	0.087	0.086
Urinary bladder	0.031	0.040	0.034	0.032
Tendon	0.026	0.045	0.023	0.022
Fat	0.024	0.034	0.021	0.018
Artery	0.016	0.018	0.021	0.020
Pool 1	0.234	0.103	0.234	0.174
Pool 2	0.102	0.037	0.141	0.028
Pool 3	0.028		0.035	

[a]Local synthesis assumed since without it coefficient of variation of fit was greater than 15%.

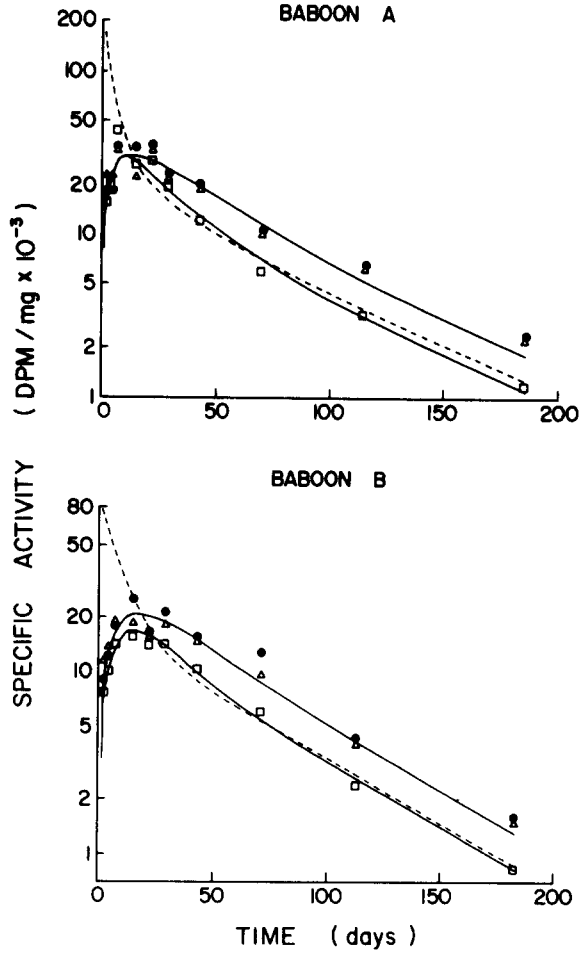

Fig. 4. Specific activity-time data for cholesterol in serial biopsies of skin, muscle, and fat for baboons A and B. The data points are for skin (□), muscle (△), and fat (●). The curves are best fits to the data generated by assuming plasma input to a single pool without or with synthesis (skin) (see Fig. 2). There are three curves: the dashed curve is the plasma decay curve; the upper solid line is the best fit to the fat and muscle data; the lower solid line is the best fit to the skin specific activity data (assumes local synthesis).

Side pool synthesis; model validity

In the three-pool model of body cholesterol turnover in humans, the sizes of the side pools, pools 2 and 3, depend upon the rates of synthesis in these pools. Assuming a steady state and a value for cholesterol absorption, one can compute limiting values for total body cholesterol. If all synthesis is assumed to be in pool 1, then the estimate for total body cholesterol is minimal, while if all synthesis is assumed to be in pool 3 a maximum estimate is obtained. On physiological grounds, neither limiting value should be correct. Thus, since the rapidly exchanging central pool includes the liver and intestines (2, 5), organs that actively synthesize cholesterol (9, 18), the true total body cholesterol mass must be less than the maximum estimate. Conversely, since the carcass and peripheral tissues also contribute to total body cholesterol synthesis (9, 18, 19), the true value for total body cholesterol mass must be greater than the minimum estimate.

Quantitative information about the extent of side-pool synthesis of cholesterol in humans would increase the precision and usefulness of cholesterol turnover studies. Although a theoretical method for this has been described (7), there is no known suitable biosynthetic precursor available for such studies in humans (7). Therefore, in humans, the best approach at present is to determine the ranges of values for side-pool masses, as has been done previously (2, 5).

The validity of kinetic estimates of body cholesterol production rate has been demonstrated by sterol balance studies (4, 20). However, comparable direct confirmation of the physiological validity of kinetic estimates of exchangeable body cholesterol mass is not available in humans. Previous studies in nonhuman primates, including both baboons (8) and squirrel monkeys (21), have indicated that total body cholesterol mass as computed from kinetic studies and two-pool model analysis is not too dissimilar from total body cholesterol mass measured by direct carcass analysis. In the present work, from the kinetic analysis, we computed both the minimum value for total body cholesterol and an "intermediate" value, obtained by assuming that half the production rate occurred in the central pool and half in the side pools. In three of the four baboons, measured total body cholesterol fell within the range of these two computed values, and in the fourth baboon the directly measured value was close to the "intermediate" one. This finding suggests that the modeling approach is a useful and predictive simplification of whole body cholesterol turnover.

The direct measurement of total body cholesterol mass permitted us to calculate the extent of cholesterol synthesis in the peripheral side pools of the model. The brain and spinal cord were excluded from the measurement of total body cholesterol, because studies in both humans (17) and baboons (8) have shown that brain cholesterol is almost entirely nonexchangeable. Approximately 25% of the production rate was found to arise from peripheral cholesterol synthesis. If we assume that whole body cholesterol kinetics are similar in humans and baboons, then the best estimate of total body mass of exchangeable cholesterol in humans is obtained by assuming that 25% of the PR arises in pool 3. This finding is in accord with recent studies that have demonstrated significant cholesterol synthesis in peripheral tissues in intact animals (9, 18, 19).

The studies reported here extend the previous work of Wilson (8), who conducted [^{14}C]cholesterol turnover studies and measured body cholesterol mass in five baboons fed 3 g/day of ^{3}H-labeled cholesterol for 120 days. In this study it was assumed that after 120 days of [^{3}H]cholesterol loading, all exchangeable pools of cholesterol would have the same specific activity. This assump-

tion is true only if there is no side pool synthesis, but significant side pool synthesis is likely to occur. In fact, peripheral synthesis could be estimated from Wilson's data, except that his estimate of total body cholesterol included nervous system cholesterol which is likely to be only slowly exchangeable (we found extremely low ^{14}C counts in the brain after 180 days). Subtracting some 2 g/animal from the observed total in Wilson's study leads to roughly the same side pool synthesis as we have. Similar assumptions were made in the analysis of the ^{14}C turnover data and not enough data are presented in the paper (8) to permit re-analysis. However, it is likely that Wilson's results could be explained more physiologically by side pool synthesis than by nonexchanging pools.

Biopsy measurements

We examined the usefulness of biopsy material to measure cholesterol turnover in a particular tissue in two ways. First, we determined the magnitude of spatial heterogeneity of specific activity for several widely distributed tissues, including fat, muscle, arteries, and the alimentary tract. A moderate degree of heterogeneity was found in all of these tissues, suggesting that a small biopsy may have limited value for the study of tissue cholesterol turnover. Multiple samples of liver showed much lower variability. Second, cholesterol turnover in serial biopsies of skin, muscle, and subcutaneous fat showed considerable scatter, but the data could be fitted with a single pool by using the plasma specific activity-time curve as input. For skin, a single pool with local synthesis was required. The turnover rates so obtained for fat and muscle were not too dissimilar from the values obtained from the necropsy samples. Thus, serial biopsies may provide a useful estimate of a tissue turnover rate if a sufficient number of biopsies spread out over time are taken. The estimate would be improved if more than one site were biopsied at each time.

Turnover rates in individual tissues

The specific activity data of the various tissues examined were quite consistent from animal to animal (see Table 4). Thus, rapidly turning over tissues such as the alimentary tract and testis had low ^{14}C specific activities while slowly turning over tissues such as fat and artery had high ^{14}C specific activities. Also, inversion of ranking from early (4 days) to later (186 days) time points predicted by the compartmental model was observed. Rapidly turning over tissues accumulate radioactivity rapidly and lose it quickly, whereas the opposite is true for slowly turning over tissues. Consistency of inversion in rank order was particularly striking if tissues likely to have significant local synthesis of cholesterol were omitted (Fig. 5).

A turnover rate for each tissue was computed by using the plasma specific activity-time curve as input to a single pool for cholesterol turnover for that tissue. It is likely that each tissue is composed of several pools of cholesterol, each turning over at a certain rate. The single turnover rate computed for a given tissue is, therefore, a weighted average of all of these individual turnover rates. The turnover rates computed in the four baboons for a given tissue were quite similar. It was found (Table 5) that the values for the different tissues fell loosely into three groups that were turning over at fast, intermediate, and slow rates, roughly comparable to the turnover rates calculated for the three pools of the three-pool model. Thus, while each tissue is likely to contain some cholesterol molecules turning over at rates belonging to all three pools, it would seem that in the large majority of the tissues the cholesterol in the tissue turns over at a rate predominantly belonging to a rapid, intermediate, or slowly turning over compartment. Accordingly, the tissue turnover data provide useful information about the anatomic localization of each of the three pools of cholesterol that comprise the three-pool model, and add to our understanding of the physiological meaning of the compartmental model.

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