Effect of Neomycin on Exchangeable Pools of Cholesterol in the Steady State

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ABSTRACT Five patients received cholesterol-7α-^{3}H intravenously during control periods. Specific activity of total serum cholesterol was determined serially during the 1st wk and weekly thereafter. 28–59 wk after the injection of the tracer, when no further radioactivity could be detected in serum cholesterol, 2 g of oral neomycin was given daily to four patients for the remainder of the experiment. Average total serum cholesterol concentrations were reduced by 20, 21, 26, and 29%, respectively, in these subjects. The fifth patient, given placebo, had no change in serum cholesterol. After a period of 12–26 wk of medication the intravenous injection of cholesterol-7α-^{3}H was repeated, and while neomycin or placebo administration was continued, serum cholesterol specific activity was again determined serially during the 1st wk and weekly thereafter for 23–42 wk. The data were subjected to a two-compartment analysis. During the administration of neomycin, half-times of the cholesterol radioactivity decay curves were decreased in two patients and remained unchanged in two subjects. The size of the "intermediate" pool of cholesterol decreased in each patient during the administration of neomycin by 33, 36, 40, and 44%, respectively. The absolute decrease was much larger in each case than the concomitant reduction of serum cholesterol. There was no significant change in the data during the administration of placebo in one patient. The size of the "intermediate" pool can be calculated by compartmental analysis from the cholesterol decay curves. For the "slow" pool size and the other kinetic parameters only ranges of values can be deduced from the present experiment.

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INTRODUCTION

Several regimens and a few pharmacologic agents are available to reduce the concentration of serum cholesterol in man. The theoretical justification of lowering the level of serum cholesterol is the assumption that, concomitantly, the amount of cholesterol in the body will be reduced, particularly the amount of cholesterol deposited in atheromas. This, however, is by no means a necessary consequence of serum cholesterol reduction (2, 3), and the decrease or increase of the size of different body pools of cholesterol may depend on the mechanisms of action involved with each agent. The reduction of the concentration of serum cholesterol may occur in any of the following ways: (*a*) decreased biosynthesis; (*b*) decreased intake or absorption; (*c*) increased excretion of neutral

steroids or bile acids; (*d*) shifting of cholesterol from the "serum pool" into "another pool" in the body (red blood cells, liver, other organs, muscles, intima of blood vessels, nervous system tissue, etc.), or any combination of these. It has been reported (4–9) that the daily oral administration of 0.5–2 g of neomycin sulfate significantly reduced the concentration of serum cholesterol in man. This reduction is accompanied by a three to fivefold increase in the fecal excretion of bile acids (*7*, 8) and a twofold increase in the fecal excretion of neutral steroids (8) during the non-steady state, a finding which suggests the involvement of pathway (*c*). In the present study, data are presented on the effect of the administration of neomycin in man on the time course of decay of serum cholesterol specific activity for periods up to 42 wk. The data are interpreted in terms of a two-compartmental model.

METHODS

Experimental. Five patients, two males and three females, were studied. The age range was 45–62 yr. The clinical diagnoses are included in Table I. All patients were ambulatory, their respective diseases under good control, and three of the five were working at their regular jobs during the study. Medications other than experimental were kept constant, and substances known to influence serum cholesterol levels were not given. The diet was uncontrolled, but the patients were instructed to adhere to their customary diets. The patients were seen weekly, with the exception of a few appointments that were missed (vacations, family, job engagements, or other reasons). They were weighed weekly, and physical examination, blood counts, test of urine, blood urea nitrogen, blood sugar, serum bilirubin, serum transaminases, cephalin flocculation, and stool culture for pathogens were carried out periodically.

Cholesterol-7α-^{3}H (New England Nuclear Corp., Boston, Mass.) (225 μc/mmole) was purified by thin-layer chromatography (10). About 20 ml of blood was drawn from each subject 4–6 days before the injection of the tracer, and the serum was separated aseptically and stored at 4°C. An appropriate amount of cholesterol-7α-^{3}H was dissolved in 0.5 ml of ethanol, and 0.5 ml of sterile normal saline solution was added. The serum of each individual patient was mixed aseptically with the solution of the tracer and was incubated at 37°C for 24 hr. After ultrafiltration (size 0.2 μ filter) and radioassay 31–43 μc of cholesterol-7α-^{3}H was given intravenously to each of the five patients. The tube containing the radioactive serum was washed twice with sterile normal saline which was then mixed with the material injected. Simultaneously 100 μc of sodium acetate-1-^{14}C was given intravenously to each subject. There were no reactions to the injection of the tracers. The results of the rate of incorporation of labeled acetate into serum cholesterol will be presented elsewhere.

After the injection of the tracer, blood was drawn serially during the 1st wk (average number of samples, 7.7 during the 1st wk) and weekly thereafter in the fasting state. Total serum cholesterol concentrations were determined in each sample by the method of Abell, Levy, Brodie, and Kendall (11). A separate 3 ml aliquot of the serum was saponified by the addition of 28 ml of ethanol and 2 ml of 33% KOH solution. After the addition of an equal volume of water, neutral steroids were extracted four times with equal volumes of hexane. There was no hexane-extractable radioactive material left in the ethanol-water layer. The combined hexane extracts were washed with water, the hexane was evaporated, and the residue was dissolved in toluene. Parallel determinations of specific radioactivity were identical when this procedure was compared to a procedure involving digitonide precipitation of sterols. Specific activity was measured in a Tri-Carb liquid scintillation spectrometer, with toluene solution containing 4 mg of 2,5-diphenyloxazole and 0.3 mg of 1,4-*bis*-2-(5-phenyloxazolyl)-benzene per ml. All samples were counted in the range from 10^4 to 10^6 counts. The least radioactive samples, of the order of 100 cpm, were counted to 10^4 counts, and the long-term follow-up of radioactivity was terminated at this point. The samples were recounted after the addition of known internal standards for ^{3}H and ^{14}C activity (in 0.1 ml of toluene each). After correction for quenching, specific activity was calculated in disintegrations per minute (12).

28–59 wk after the injection of the tracer, when no further radioactivity (of the order of 100 cpm) could be detected in serum cholesterol, 2 g of neomycin sulfate was given daily by mouth to four patients. The fifth subject was given placebo to control the reproducibility of the tracer methods. After a period of 12–26 wk of neomycin or placebo administration, 31–42 μc of cholesterol-7α-^{3}H (and 100 μc of sodium acetate-1-^{14}C) was injected intravenously for the second time, and while medication was continued, the sequence of procedures outlined above was repeated. Weekly determinations of serum total cholesterol specific activities were carried out for 23–42 wk after the injection of the tracer in both control and medication experiments. The time interval between the two injections of tracer was 44–76 wk. Fig. 1 shows the time sequence of the experiment in one patient.

Theoretical. The experimental data, expressed as specific activity of cholesterol-7α-^{3}H in each serum sample (disintegrations per minute per gram), divided by the injected dose of cholesterol-7α-^{3}H (disintegrations per minute) and multiplied by 100 to give units of % g^{-1} were plotted semilogarithmically against time (Figs. 2–6). Two exponentials, $a_1'e^{-\alpha_1 t}$ and $a_2'e^{-\alpha_2 t}$, were curve-fitted to each plot by the conventional graphical "peeling-off" process, as described in the legend to Fig. 2 a. Each exponential is characterized by an amplitude or coefficient a_i' (% g^{-1}) and a half-life $(t_{\frac{1}{2}})_i$ (day). The half-life is converted to the exponent factor α_i(day^{-1}) by the relation $\alpha_i = 0.693/(t_{\frac{1}{2}})_i$. The subscript $i = 1$ denotes the "intermediate" exponential and the subscript $i = 2$ denotes

the "slow" or smallest exponential. The sum of the two derived exponentials (Appendix, equation 11) fitted the data satisfactorily from approximately 0.5 day to the longest time of measurement.[1] From the earliest measured value (2 hr after injection of tracer) to about 0.5 day, a relatively rapid decrease of serum specific activity occurred which could not be fitted by the sum of two exponentials (Fig. 2b). Since the measurements were scanty in this time interval and since, moreover, the process of exchange of tracer cholesterol between the serum and the various body pools is complicated initially by the interconversion between free and esterified tracer cholesterol, the data in this time interval were not curve-fitted and analyzed.

After Goodman and Noble (13), the two-exponential fit of the data from 0.5 day to the longest measured time was analyzed by a two-compartment model, defined as follows (Fig. 7): compartment a is composed of tissues which contain the amount M_a (grams) of body (non-tracer) cholesterol, from which body cholesterol goes to the tissues of compartment b at the rate r_{ab} (grams per day), which biosynthesize and take up exogenous (dietary) cholesterol at the total rate I_a (grams per day), and which degrade and excrete cholesterol at the total rate E_a (grams per day). The parameters are similarly defined for compartment b. The injected dose of free cholesterol-7α-^{3}H is considered to label compartment a at time zero. Likewise the measured specific activities are considered to be those of compartment a. No distinction between free and esterified forms of cholesterol was made in the analysis (see Discussion).

The compartment model thus formulated was analyzed by conventional first-order, steady-state kinetics (Appendix). It is well known (14–17) that only four combinations of compartment parameters are uniquely derivable from the previously enumerated four exponential parameters representing the present type of experiment. The four combinations are herein taken as:

(a) The pool size M_a (grams) of compartment a, given by Appendix equation 13 as $100/(a_1' + a_2')$.
(b) The total turnover rate $(E_a + r_{ab})/M_a$ (day^{-1}) of compartment a, (Appendix equation 14).
(c) The total turnover rate $(E_b + r_{ba})/M_b$ (day^{-1}) of compartment b, (Appendix equation 15).
(d) The product $(r_{ab}/M_a)(r_{ba}/M_b)$ (day^{-2}) of fractional turnover rates of compartments a and b, (Appendix equation 16).

Although unique values of the individual compartment parameters other than M_a cannot be ascertained from the present experiment, various methods of surveying systematically the ranges of parameter values compatible with the experiment have been developed (17–19). In an effort to extract maximum information from the present experiment such a survey has been made, with a variation of the mathematical method of Skinner, Clark, Baker, and Shipley (19). There are eight compartment parameters (six rates and two pool sizes, Fig. 7). The experiment yields four exponential parameters (two amplitudes and two exponent factors) which by the tracer-kinetic relations (Appendix) yield four conditions on the compartment parameters. The steady-state relations on non-tracer cholesterol flow through each compartment yield two more conditions. Hence the present problem has two degrees of freedom (18), that is, two more conditions must be specified in order to derive the eight compartment parameters. These two conditions will be taken herein as specification of the input-rate ratio $\xi = I_b/I_a$ and the output-rate ratio $\eta = E_b/E_a$. The survey of compartment parameters compatible with the present experiment is then made in terms of ξ and η as survey parameters. The significance of this choice is that the extreme values 0 and ∞ for ξ and also for η turn out to yield maximum or minimum values of the individual compartment parameters; (the extent to which this is true for other choices of survey parameters is not known [18, 19]). Thus the survey of compartment parameters is made simply by solving four so-called limit cases (Appendix), those for which $(\xi, \eta) = (0, 0)$, $(0, \infty)$, $(\infty, 0)$, and (∞, ∞). The true value of any compartment parameter must then be intermediate between the extreme values given by these limit cases.

RESULTS

During the oral administration of neomycin, average total serum cholesterol concentrations fell by 29, 26, 21, and 20%, respectively, in the four patients studied. The differences were statistically significant at the 0.1% level. The reduction of serum cholesterol in these patients was comparable to previous results (4–9). In the fifth patient given placebo, there was no change in the level of serum cholesterol (Table I). Fig. 1 shows the serum cholesterol concentrations of one patient. The administration of the drug was well tolerated. During the first 2 wk of the administration of neomycin two of the four patients experienced temporary loose bowel movements and abdominal cramps. The administration of neomycin was continued, and the symptoms subsided spontaneously by the end of the 2nd wk. These findings are again comparable to previous experience with the use of this drug in this laboratory (4–6, 9). No other side effects were noted, the weight of the patients remained constant within 2 lbs variation, and physical examinations and the monitoring laboratory studies remained unchanged during the ad-

[1] The data, curve-fitting method, and closeness of curve fit were similar to those of Goodman and Noble (13). These authors compared their manual curve-fitting method with a computerized least squares method and concluded that there was little difference in the exponential parameters derived by either method. Their conclusion therefore applies also to the present results.

TABLE I

Average Total Serum Cholesterol Concentrations of Five Patients during Control Periods and during the Daily Oral Administration of 2 g of Neomycin or Placebo

Patient, age, sex	Diagnosis	Medication	Control			Medication			Fall of total serum cholesterol	P
			Duration	n	Average total serum cholesterol	Duration	n	Average total serum cholesterol		
			wk		*mg/100 ml*	*wk*		*mg/100 ml*	%	
S. S., 62, M	Coronary artery disease; diabetes mellitus	Neomycin	34	34	236 ± 17*	46	45	168 ± 9*	29	<0.001
H. F., 47, M	Coronary artery disease; CVA	Neomycin	30	29	313 ± 21	49	47	231 ± 17	26	<0.001
M. R., 53, F	Peripheral vascular insufficiency	Neomycin	42	43	275 ± 21	65	62	216 ± 9	21	<0.001
A. F., 45, F	No clinical disease	Neomycin	28	30	259 ± 15	49	51	206 ± 13	20	<0.001
E. A., 60, F	Pulmonary emphysema	Placebo	41	39	226 ± 17	48	35	231 ± 14	—	>0.5

n, number of cholesterol determinations; CVA, cerebral vascular accident.
* Standard deviation.

ministration of the drug. When the second tracer dose was given, the level of serum cholesterol was at a lower plateau (Fig. 1), in a steady state, and the patients were completely free of side effects.

Figs. 2–6 compare the decay curves of radioactivity during control periods and during the administration of neomycin. The solid and dashed curves in Fig. 2a represent the computed sum of two exponentials and illustrate the closeness of fit to the data for $t > 0.5$ day. Figure 2b shows the initial rapid fall of specific activity for $t < 0.5$ day. This portion of the data is not represented by the sum of two exponentials.

The exponential parameters, namely, the amplitudes a_1', a_2' and the exponential factors α_1, α_2 and equivalent half-lives $(t_{\frac{1}{2}})_1$, $(t_{\frac{1}{2}})_2$ of the two exponentials curve-fitted to each decay curve, are given in Table II. Of the four patients receiving neomycin, the half-lives decreased in two and remained unchanged in two. There was no discernible change in the half-lives of the patient given placebo.

The previously enumerated combinations of compartment parameters which can be derived uniquely from the exponential parameters by compartment analysis are given in Table III. The so-called production rate (13) is also included (see Appendix). The most striking result is that the "intermediate" pool size M_a decreased from 40, 38, 42, and 25 g during control periods, to 27, 23, 24, and 16 g, respectively, during the administration of neomycin in four patients. In the fifth subject M_a was 26.5 g during both control and placebo periods.

The serum total cholesterol contents M_s (grams), calculated from the measured serum total cholesterol concentrations and estimated serum volumes for each subject, are included in Table III. The decreases in M_s during neomycin administration were 2.5, 2.9, 1.3, and 1.1 g. These decreases are much less than the corresponding

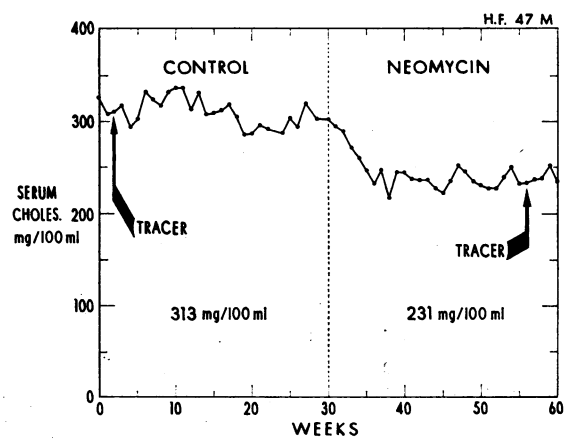

FIGURE 1 Total serum cholesterol concentrations and time sequence of the experiment in patient H.F., 47, M.

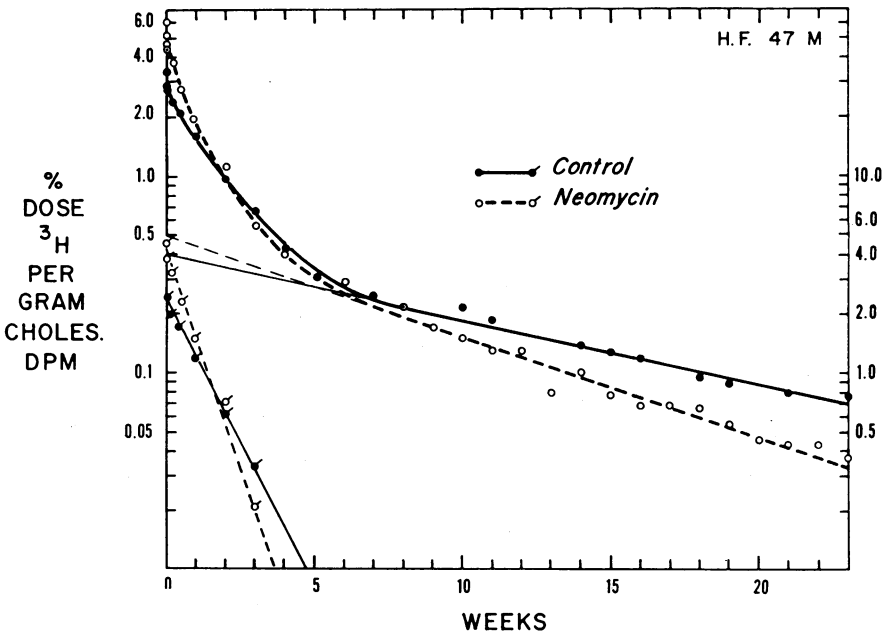

FIGURE 2a Semilogarithmic plot of serum total cholesterol, specific activity decay curves during control periods (closed circles and solid lines) and during neomycin administration (open circles and dashed lines). Exponential peel-off analysis is indicated by the straight lines and flagged points. Ordinate scale at left applies to original data (circles) and to straight lines of the "slow" (later) exponential (wk 7 and later). Ordinate scale at right applies to peeled-off data (flagged circles) and to straight lines of the early ("intermediate") exponential (wk 0–5). The slope of each straight line determines an exponential half-life $(t_{\frac{1}{2}})_i$. The ordinate intercept of each straight line determines an exponential amplitude a_i'. The subscript $i = 1$ corresponds to the "intermediate" exponential (flagged points). The subscript $i = 2$ corresponds to the "slow" exponential.

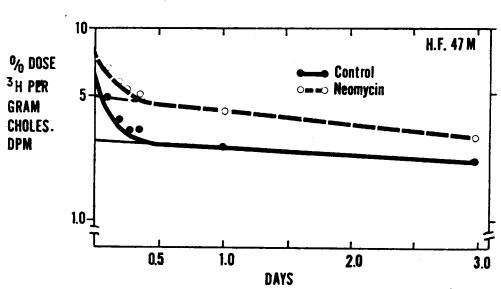

FIGURE 2b Decay of radioactivity during the first 3 days after injection of the tracer in one patient.

decreases of M_a which were 13, 15, 18, and 9 g, respectively.

Compartment parameter survey calculations were made for all subjects. The results for subject H.F. are given in Table IV. These results were obtained from the model solutions for the limit cases (Appendix), listed in nondimensional form in Table V, together with the values for subject H.F. given in Table III. Cases 1–4 are the "limit" cases, in which one input rate and one output rate are set equal to zero. The choice of the vanishing rates is shown in the first four rows of Table IV. Case 5 was chosen to illustrate a possibly plausible situation intermediate between cases 1 and 4, namely, $I_a = E_a$, $I_b = E_b$, and $I_b = 0.25\ I_a$. Several inferences are possible from Table IV: (*a*) upper limits on all compartment parameters (Fig. 7) are established by case 4; (*b*) the large percentage increases in total and fractional turnover rates upon neomycin administration to subject H.F. (Table III) are due, generally speaking, to constancy of the input, output, and exchange rates while both pool sizes decreased. Similar calculations for subject S.S. showed that the various rates changed upon neomycin administration in about the same proportion as the pool sizes, and thus the turnover rates remained approximately unchanged (Table III). The situation for the other two neomycin subjects

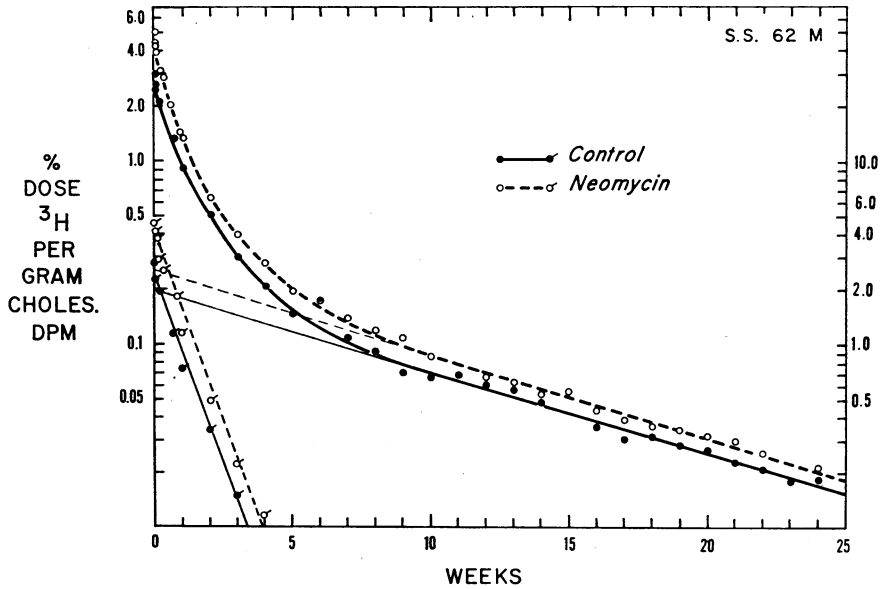

FIGURE 3 See legend to Fig. 2 a.

was intermediate in character to the preceding two; (*c*) the nonvanishing partial turnovers E_a/M_a, E_b/M_b are more constant with respect to case than either the numerator or denominator separately; (*d*) the total output turnover rates $(E_a + E_b)/(M_a + M_b)$ do not vary greatly with case. They are similar in value, but not identical to, the slowest exponential factor α_2 (Table II; the intermediate rate constant α_1 is similar in value, but not identical to, the total turnover rate A of the intermediate compartment a [Table III]); (*e*) the "slow" pool size M_b varies greatly with case. Its value is least reliably deducible from the present experiment. The effect of neomycin was to reduce M_b for corresponding limit cases. This effect was observed for all subjects given neomycin for all limit cases, except for a slight increase for limit case 4 in subject A.F. Although this general trend suggests that M_b, like M_a, is reduced by neomycin administration, this observation must be strongly qualified because of the possibility that neomycin also shifts the case. That

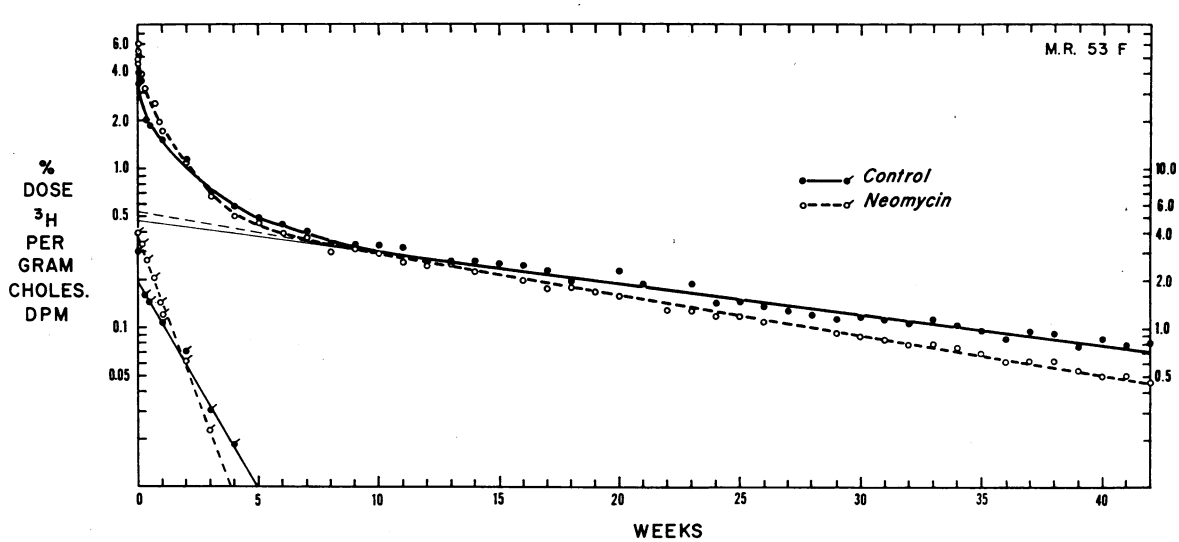

FIGURE 4 See legend to Fig. 2 a.

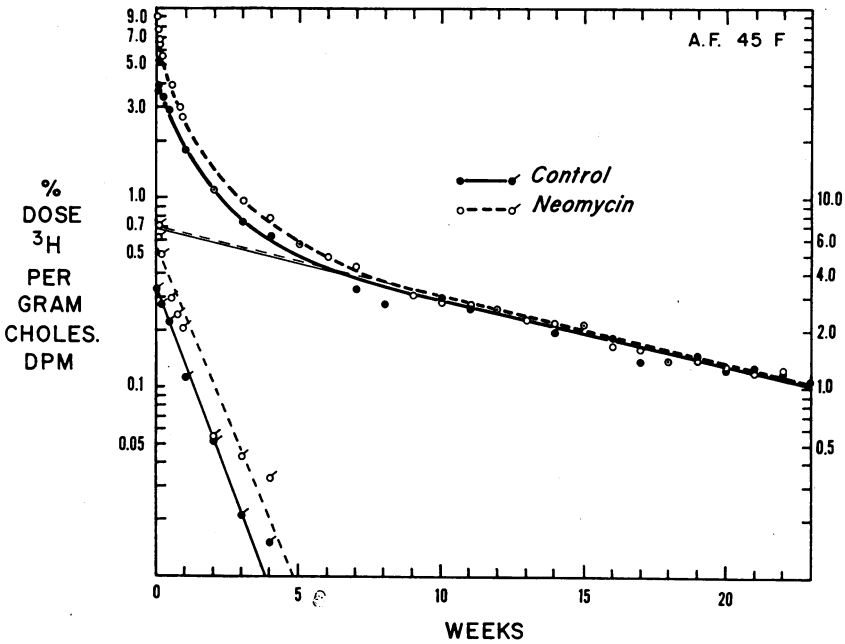

FIGURE 5 See legend to Fig. 2 a.

is, for example, the true case under control conditions might be close to limit case 1, whereas the true case under neomycin administration might be close to limit case 4.

Systematic calculations of all compartment parameters for ξ, η varying between their limiting values of 0 and ∞ were made for subject H.F., control experiment. These calculations showed that no maxima or minima occurred between the limit cases. It may reasonably be inferred that in general the limit cases exhibit the complete range of possible values of compartment parameters compatible with the experimental data.

The intermediate pool size M_a is uniquely given

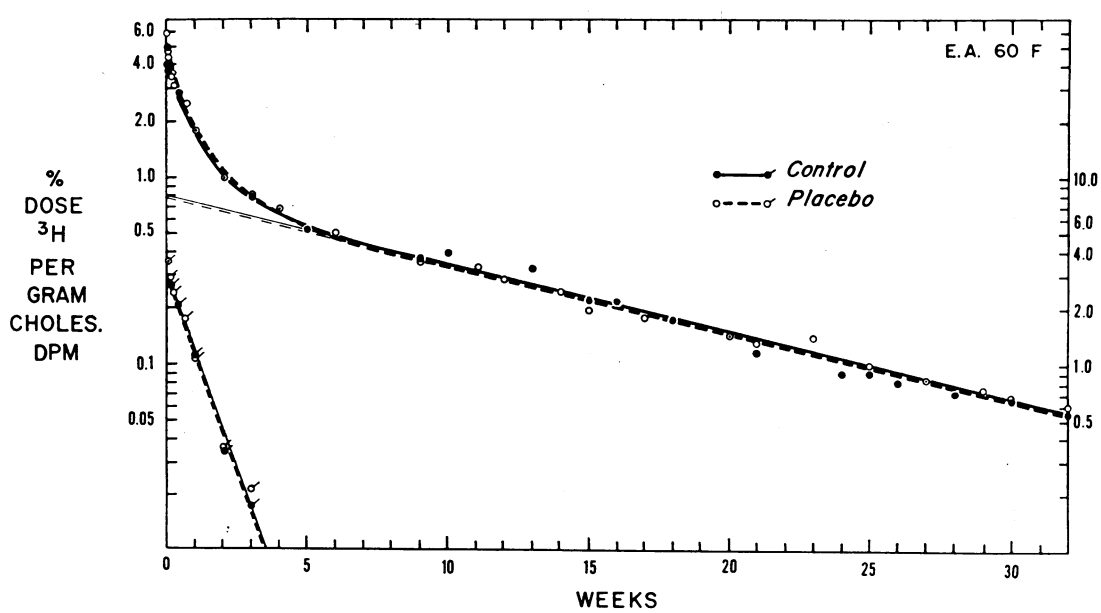

FIGURE 6 See legend to Fig. 2 a, except placebo instead of neomycin.

TABLE II

Amplitudes, Exponent Factors, and Half-Lives from Two Exponential Curve Fit of Data

Exponential parameter	Control					Neomycin				Placebo
	Patients					Patients				Patient
	S. S.	H. E.	M. R.	A. F.	E. A.	S. S.	H. F.	M. R.	A. F.	E. A.
a_1', % g^{-1}	2.3	2.2	1.9	3.3	3.0	3.5	3.85	3.7	5.5	3.0
a_2', % g^{-1}	0.19	0.40	0.47	0.69	0.78	0.25	0.50	0.52	0.70	0.76
100 α_1, day^{-1}	13.3	9.00	8.43	12.9	13.9	13.9	13.6	13.5	11.9	13.9
100 α_2, day^{-1}	1.45	1.08	0.637	1.17	1.19	1.50	1.69	0.822	1.17	1.19
$(t_{\frac{1}{2}})_1$, *day*	5.2	7.7	8.2	5.4	5.0	5.0	5.1	5.1	5.8	5.0
$(t_{\frac{1}{2}})_2$, *day*	48	64	109	59	58	46	41	84	59	58

by the two-compartment analysis of the present experiment only if the initial conditions, that is, the amounts of tracer in compartments *a* and *b* at time zero, are known. The customary assumption (13) was made herein that all the injected tracer is in compartment *a* at time zero. Since, however, the tracer was injected into the blood, all tissues would have some access to tracer in the initial half day during which the tracer exists in blood at specific activities in excess of those given by the two-exponential curve fit. Moreover, tracer would be lost from the system during this initial "high specific activity interval" in excess of that allowed for by extrapolating the two-exponential curve fit from 0.5 day back to time zero. Evidently the customary initial labeling assumption involves some error. This error was investigated by making an approximate fit of a third exponential to the available data from 2 hr after injection to 0.5 day and by making a three-compartment analysis analogous to that previously described, in which the third or "fast" compartment was considered to be the blood serum. Although this analysis was in error because it did not distinguish between the free and esterified forms of cholesterol in the initial interval, the analysis was thought to contain the essential features neglected in the initial labeling assumption of the two-compartment analysis. The results indicated that the present two-compartment values of M_a are overestimated by some

TABLE III

*Parameter Combinations Determined Uniquely from Compartment Analysis**

	Control					Neomycin				Placebo	Percent change: Neomycin				Percent change: Placebo
	S. S.	H. F.	M. R.	A. F.	E. A.	S. S.	H. F.	M. R.	A. F.	E. A.	S. S.	H. F.	M. R.	A. F.	E. A.
M_S*	8.3	11.3	6.4	5.5	4.3	5.9	8.4	5.1	4.4	4.4	−29	−26	−20	−20	2
M_a‡	40.1	38.5	42.1	25.1	26.5	26.7	23.0	23.7	16.1	26.6	−33	−40	−44	−36	0
100 A§	12.4	7.78	6.87	10.9	11.3	13.1	12.2	11.9	10.7	11.3	6	57	73	2	0
100 D‖	2.36	2.30	2.19	3.20	3.81	2.32	3.06	2.38	2.38	3.76	− 2	33	9	26	−1
10^4 BC¶	9.93	8.17	9.70	19.7	26.5	9.58	14.3	17.3	11.4	26.0	− 4	75	78	42	−2
$(I_a)_{I_b=0}$**	3.27	1.63	1.03	1.18	1.14	2.40	1.73	1.10	0.94	1.17	−27	6	7	−8	3

* Serum total cholesterol (grams), not determined by compartment analysis but calculated from measured serum total cholesterol concentration (Table I), hematocrit, body weight, and an assumed blood volume: body weight ratio of 77.7 ml/kg for males and 66.1 ml/kg for females (Handbook of Physiology. American Physiological Society, Washington, D. C., 1962. 1: 52. Table I).

‡ "Intermediate" pool size (grams), $M_a = 100/(a_1' + a_2')$, Appendix equation 13.

§ Total turnover rate of "intermediate" compartment, 100 A = 100 $(E_a + r_{ab})/M_a$, (% day^{-1}), Appendix equation 14.

‖ Total turnover rate of "slow" compartment, 100 D = 100 $(E_b + r_{ba})/M_b$ (% day^{-1}), Appendix equation 15.

¶ Product of fractional turnover rates for both compartments, $10^4\,BC = (100\,r_{ba}/M_b)(100\,r_{ab}/M_a)$ (% $day^{-1})^2$, Appendix equation 16.

** See last paragraph of Appendix.

TABLE IV

Survey of Compartment Parameters Compatible with Experiment for Subject H.F.

Compartment parameter	Control					Neomycin				
	Limit cases				Case	Limit cases				Case
	1	2	3	4	5	1	2	3	4	5
I_a, g day^{-1}	1.6	1.6	0	0	1.3	1.7	1.7	0	0	1.4
I_b, g day^{-1}	0	0	1.6	3.6	0.34	0	0	1.7	4.5	0.36
E_a, g day^{-1}	1.6	0	1.6	0	1.3	1.7	0	1.7	0	1.4
E_b, g day^{-1}	0	1.6	0	3.6	0.34	0	1.7	0	4.5	0.36
r_{ab}, g day^{-1}	1.4	3.0	1.4	3.0	1.6	1.1	2.8	1.1	2.8	1.4
r_{ba}, g day^{-1}	1.4	1.4	3.0	3.0	1.6	1.1	1.1	2.8	2.8	1.4
M_b, g	59	130	130	285	86	35	92	92	239	56
$100\ E_a/M_a$, % day^{-1}	4.2	0	4.2	0	3.5	7.5	0	7.5	0	6.3
$100\ r_{ab}/M_a$, % day^{-1}	3.6	7.8	3.6	7.8	4.3	4.7	12.2	4.7	12.2	5.9
$100\ E_b/M_b$, % day^{-1}	0	1.2	0	1.2	0.39	0	1.9	0	1.9	0.64
$100\ r_{ba}/M_b$, % day^{-1}	2.3	1.1	2.3	1.1	1.9	3.1	1.2	3.1	1.2	2.3
$100\ \frac{(E_a + E_b)}{(M_a + M_b)}$, % day^{-1}	1.7	0.97	0.97	1.1	1.4	3.0	1.5	1.5	1.7	2.3

3–11%. The error is small and moreover similar in any one subject in the control and the neomycin experiment. Hence this effect produced negligible error on the primary result of the present investigation: the percent decrease in M_a under neomycin administration.

DISCUSSION

The rate of disappearance of labeled cholesterol from the serum has been studied extensively. It has been pointed out that in humans the decay of labeled cholesterol in the plasma proceeds at a series of decreasing exponential rates for periods as long as 50 days (20). Similar observations were made in rats (21). However, reports from a number of laboratories demonstrated that in man after about 8–10 wk the decay of specific activity proceeds at a single exponential rate and yields a straight line when plotted semilogarithmically (22–24). The rapid decrease of the initial portion of the decay curves is thought to be due in part to cholesterol transformation or excretion which represents cholesterol turnover, and in part to a molecular interchange between the cholesterol of serum and cholesterol of other tissues (25). The possibility of a significant return into the serum compartment of labeled cholesterol deposited at earlier times in other tissue pools has been suggested (21). The two-compartment model in the present study reflects these considerations.

Exponential half-lives were calculated by a number of authors on cholesterol decay curves in short-term experiments. On the initial portion of the curves, when the decay of radioactivity proceeds at decreasing exponential rates, London and Rittenberg (26) found half-lives of 6 days. Hellman, Rosenfeld, and Gallagher (20) obtained values of 1.6–4 days, and LeRoy (27), values of

TABLE V

Limit Case Solutions for Two Compartment Model

	1	2	3	4
$\xi = I_b/I_a$	0	0	∞	∞
$\eta = E_b/E_a$	0	∞	0	∞
I_a/M_aA	$1 - \beta$	$1 - \beta$	0	0
I_b/M_aA	0	0	$1 - \beta$	$\beta^{-1} - 1$
E_a/M_aA	$1 - \beta$	0	$1 - \beta$	0
E_b/M_aA	0	$1 - \beta$	0	$\beta^{-1} - 1$
r_{ab}/M_aA	β	1	β	1
r_{ba}/M_aA	β	β	1	1
M_bD/M_aA	β	1	1	β^{-1}

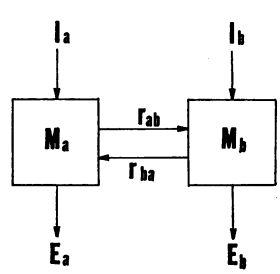

FIGURE 7 The two-compartment model.

4.2–7.2 days. The order of magnitude of these figures is in agreement with that of the intermediate exponential $(t_{\frac{1}{2}})_1$ in the present "peel-off" analysis (Table II). In long-term studies, half-lives of the slowest exponentials were found to be 69–74 days by Kurland, Lucas, and Freedberg (23), and 58–100 days by Chobanian, Burrows, and Hollander (24). Here also, the order of magnitudes are in agreement with those of the slowest exponentials $(t_{\frac{1}{2}})_2$ in the present study (Table II). During the administration of neomycin, corresponding half-lives decreased in two patients and remained unchanged in two subjects (Table II). It should thus be emphasized that the calculated intermediate pool size can decrease during the administration of neomycin (Table III) in the presence of both unchanged or decreased half-lives.

The semilogarithmic plot of the present data did not show any significant deviation from linearity during the duration of this experiment, namely up to 42 wk. This point indicates that up to this time there was no suggestion in the data of a more slowly exchangeable pool of cholesterol.

In scrutinizing for the possible uncertainties of interpretation in the present analysis, two distinct possibilities should be mentioned. First, chemical or biological reproducibility; however, the data of the radioactive decay curves in the patient given placebo were consistently identical during control and placebo periods. The two series of tests (control and placebo) in this patient were carried out with a 66 wk interval from each other, and the analytical procedures covered 32 wk in each instance. The second possible uncertainty concerns the deduction of compartment parameters from the data. The injected tracer cholesterol was in the free form, whereas the measured specific activities were of total cholesterol. It is known that equilibration between free and ester forms of cholesterol in plasma is completed in from 2 to 5 days (20, 28–30). Since the intermediate half-life in the present experiments ranged from 5 to 8 days (Table II), it is evident that some error must be involved in failing to distinguish between free and esterified cholesterol in the first few days after injection. This error is probably not serious, however, since the main time region during which the intermediate exponential decays is approximately four half-lives or 20–30 days (Figs. 2–6). During most of this interval the total tracer cholesterol would be present in all tissues in essentially equilibrium proportions between free and esterified forms so that the compartment parameters are legitimately derived in terms of equivalent total cholesterol. It is also noted that no significant deviation from the fitted sum of two exponentials was apparent down to approximately 0.5 day (Fig. 2a), although substantial interchange between free and esterified forms of serum tracer cholesterol must have been occurring from 0.5 to 2 days. Thus, to the extent that the intermediate exponential in its later time stages characterizes interchange of an equilibrated mixture of free and esterified tracer, the curve fit down to 0.5 day would indicate that the changes due to the initially nonequilibrium distribution are not serious for present purposes.

In addition to the preceding uncertainty, it is well known that from the data presented the individual compartment parameters (Fig. 7) other than intermediate pool size M_a cannot be determined uniquely. However, a range of possible values can be deduced for each parameter (Table IV). It is of interest to note that the input and output rates of all the cases are within the range of values obtained by direct analysis of fecal cholesterol end products (2, 3) (see production rate [13] in Table III).

The control exponential parameters for the clinically "normal" subjects A.F. and E.A., (both free of clinical evidence of atherosclerosis) (Table II) are in good agreement with those obtained by Goodman and Noble (13) for their normal subjects. Since the two-compartment model used by these authors is the same as that adopted here, the present deductions as to control compartment parameters for the clinically normal subjects are in general agreement with those of Goodman and Noble (13). In particular the control intermediate pool size of about 26 g for these subjects (Table III) is in excellent agreement with the previous results. This agreement of course does not imply that $M_a = 26$ g is the true value, only that it is a duplicable value when essentially identical experimental and theoretical methods are used. Goodman and Noble found that the administration of cholestyramine to patients did not significantly alter the intermediate pool size (13). In contrast, the present experiment demonstrated that the admin-

istration of neomycin consistently reduced the intermediate pool size.

In conclusion, the only compartment parameter which can be calculated with acceptable reliability from the present experiment is the size of the intermediate pool M_a. On the basis of the present data it is inferred that the oral administration of neomycin decreased the size of the intermediate pool by 33–44%. The absolute decrease in pool size was, in each case, much greater than the concomitant decrease in serum cholesterol.

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APPENDIX

The compartment kinetic equations (16, 17) are (Fig. 7)

$$dy_a/dt = -Ay_a + By_b \quad [1]$$

$$dy_b/dt = Cy_a - Dy_b \quad [2]$$

in which y_a, y_b (disintegrations per minute) are the amounts of tracer in compartments a and b at time t; the so-called (18) total turnover rates A, D and fractional turnover rates B, C are defined by

$$A = (E_a + r_{ab})/M_a \quad [3]$$

$$D = (E_b + r_{ba})/M_b \quad [4]$$

$$B = r_{ba}/M_b \quad [5]$$

$$C = r_{ab}/M_a. \quad [6]$$

The two independent steady-state conditions for nontracer cholesterol transport may be expressed as

$$I_a + I_b = E_a + E_b \quad [7]$$

$$I_a + r_{ba} = E_a + r_{ab}. \quad [8]$$

The initial conditions correspond to injection at time zero of the amount y_{a0} of tracer into compartment a and no tracer into compartment b, or

$$y_a(0) = y_{a0}, \quad y_b(0) = 0. \quad [9]$$

The solution of equations 1, 2 for compartment a is of the form

$$y_a(t) = a_1e^{-\alpha_1 t} + a_2e^{-\alpha_2 t}. \quad [10]$$

The experimental data are expressed as

$$100\ y_a(t)/M_a y_0 = a_1'e^{-\alpha_1 t} + a_2'e^{-\alpha_2 t} \quad [11]$$

where y_0 is the amount of tracer injected. At time $t = 0$ equation 11 gives

$$M_a = 100\ (y_{a0}/y_0)(a_1' + a_2')^{-1}. \quad [12]$$

Since the injected amount of tracer y_0 is identified in the present analysis as the amount y_{a0} initially in compartment a, equation 12 becomes

$$M_a = 100/(a_1' + a_2'). \quad [13]$$

In addition to M_a, the three other quantities yielded uniquely by the compartment analysis are obtained from equations 1, 2, 10 as (14–17)

$$A = \alpha_1 - \rho\ (\alpha_1 - \alpha_2) \quad [14]$$

$$D = \alpha_2 + \rho\ (\alpha_1 - \alpha_2) \quad [15]$$

$$BC = \rho\ (1 - \rho)(\alpha_1 - \alpha_2)^2 \quad [16]$$

where

$$\rho = a_2'/(a_1' + a_2') \quad [17]$$

and, as check formulas,

$$A + D = \alpha_1 + \alpha_2 \quad [18]$$

$$AD - BC = \alpha_1\alpha_2 \quad [19]$$

Equations 13–19 and Table II were used to calculate the results in Table III.

With M_a, A, D, and BC considered known from the exponential representation of the experiment, equations 3, 4, 7, 8 and the product of equations 5 and 6 constitute five equations for the seven unknown compartment parameters, I_a, I_b, E_a, E_b, r_{ab}, r_{ba}, and M_b. If the two additional equations

$$I_b = \xi I_a \quad [20]$$

$$E_b = \eta E_a \quad [21]$$

are assumed, then each compartment parameter can be solved for as a function of ξ, η, and the known parameters M_a, A, D, BC. This solution can be obtained without difficulty by successive eliminations in the preceding enumerated equations. The results are: define

$$\beta = BC/AD \quad [22]$$

$$b = [(\xi - \eta) - \beta(1 - \xi\eta)]/(1 + \eta) \quad [23]$$

and

$$c = -\beta\xi. \quad [24]$$

Solve the quadratic equation

$$u^2 + bu + c = 0. \quad [25]$$

One root will be positive. Using this root, denoted u, calculate

$$r_{ab}/M_aA = u \quad [26]$$

$$E_a/M_aA = 1 - u \quad [27]$$

$$E_b/M_aA = \eta(1 - u) \quad [28]$$

$$I_a/M_aA = (1 + \xi)^{-1}(1 + \eta)(1 - u) \quad [29]$$

$$I_b/M_aA = \xi(1 + \xi)^{-1}(1 + \eta)(1 - u) \quad [30]$$

$$r_{ba}/M_aA = u + (1 + \xi)^{-1}(\xi - \eta)(1 - u) \quad [31]$$

$$M_bD/M_aA = (1 + \xi)^{-1}[(1 - \xi\eta)u + \xi(1 + \eta)] \quad [32]$$

The limit case solutions are obtained by going to the appropriate limits $\xi, \eta \to 0$ or ∞ in equations 23–25. These solutions are tabulated in Table V. Together with the values for subject H.F. in Table III, they yielded the results in Table IV. Case 5 in Table IV corresponds to $\xi = \eta = 0.25$ in equations 23–32.

The so-called production rate of cholesterol in pool a referred to by Goodman and Noble (PR_A in their equation 5) corresponds in the present notation to I_a under the condition $I_b = 0$. Hence, with $\xi = 0$ by equation 20, the solution of equations 23–25, 29 is

$$(I_a)_{I_b=0} = (E_a + E_b)_{I_b=0} = M_aA\,(1 - \beta). \quad [33]$$

Equation 33 is equivalent to equation 5 of Goodman and Noble, as may be verified by equations 22, 19, 17, and 14. It is noteworthy (13, 15) that the production rate does not depend on $\eta = E_b/E_a$, that is, it is uniquely determined by the present experimental data as expressed in Table III. Another such quantity is $(E_a)_{E_b=0} = (I_a + I_b)_{E_b=0}$ which is also given by equation 33.

PRINCIPAL SYMBOLS

(Symbols in parentheses are the equivalent symbols of Goodman and Noble [13]).

a_1, a_2, dpm, exponential amplitudes of "fast" and "slow" exponentials, respectively, in Appendix equation 10, (M_AC_A, M_AC_B).

a_1', a_2', % g^{-1}, "peeled-off" exponential amplitudes of "fast" and "slow" exponentials, Appendix equation 11, (100 C_A/R_A) (100 C_B/R_A).

α_1, α_2, day^{-1}, "fast" (greater) and "slow" (lesser) exponent factors derived from "peeled-off" exponential half-lives, Appendix equation 11 (α, β).

A, day^{-1}, total turnover rate of compartment a, Appendix equation 3, ($-k_{AA}$).

B, day^{-1}, fractional turnover rate of compartment b, Appendix equation 5, (k_{BA}).

C, day^{-1}, fractional turnover rate of compartment a, Appendix equation 6, (k_{AB}).

D, day^{-1}, total turnover rate of compartment b, Appendix equation 4, ($-k_{BB}$).

E_a, E_b, g day^{-1}, rate of degradation plus excretion of body cholesterol from compartments a, b respectively.

E_a/M_a, day^{-1}, partial or external turnover rate in compartment a, (k_A).

E_b/M_b, day^{-1}, partial or external turnover rate in compartment b, (k_B).

I_a, I_b, g day^{-1}, rate of biosynthesis plus exogenous entry of body cholesterol into compartments a, b respectively, (S_A, S_B); see also last paragraph of Appendix.

M_a, M_b, g, amounts of body cholesterol in compartments a, b respectively, (M_A, M_B).

r_{ab}, g day^{-1}, rate of transfer of body cholesterol from compartment a to compartment b.

r_{ba}, g day^{-1}, rate of transfer of body cholesterol from compartment b to compartment a.

y_a, dpm, amount of tracer in compartment a at time t, (aM_A).

y_0, dpm, amount of tracer initially injected, (R_A).

ρ, Appendix equation 17, ($C_B/[C_A + C_B]$).

t, day, time.

$(t_{\frac{1}{2}})_1$, $(t_{\frac{1}{2}})_2$, day, "fast" and "slow" exponential half-lives "peeled-off" from experimental data, Appendix equation 11, ($t_{\frac{1}{2}}$ first exponential, $t_{\frac{1}{2}}$ second exponential).

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