# Improved estimation of body masses and turnover of cholesterol by computerized input–output analysis

**Paul Samuel and Sidney Lieberman**

The Rockefeller University, New York 10021, The Arterioscler osis Research Laboratory, Queens Hospital Center (Long Island Jewish-Hillside Medical Center Affiliation), Jamaica, New York 11432, and The Department of Mathematics, Queens College of The City University of New York, Flushing, New York 11367

**Abstract** In 23 patients, the decay curves of serum cholesterol specific activity after a single intravenous dose of radioactive cholesterol were measured for 16–66 wk and were subjected to computerized input–output analysis. Of 17 patients with decay curves followed for longer than 50 wk, a three-exponential curve fit was better in 12, and a two-exponential curve fit in 5, according to computerized $F$ tests. Of six patients with decay curves followed for less than 50 wk, a two-exponential curve fit was better in five and a three-exponential curve fit in one.

In the 13 patients who exhibited three-exponential curve fits, the third exponential appeared after 13–43 wk of observation (average, 25 wk). In 12 patients of this group who were followed for 50 wk or more, turnover rates and exchangeable masses of cholesterol were measured at maximum lengths of the curves (50–66 wk), and these parameters were then compared with measurements made with curves successively shortened down to 10–12 wk. The average differences between analyses of the minimum vs. the maximum lengths of the curves were: $I_T$ (input rate: absorbed dietary plus biosynthesized cholesterol), 14% larger (1.24 vs. 1.09 g/day); $M_a$ (rapidly exchangeable mass of cholesterol), no change (34 vs. 33 g); $M$ (total exchangeable mass), 26% smaller (67 vs. 91 g); $M - M_a$ (remaining exchangeable mass), 39% smaller (40 vs. 65 g).

Significant differences in $I_T$, $M$, and $M - M_a$ (minimum vs. maximum curve lengths) were found in both normolipidemic and hypercholesterolemic patients, and the differences were of similar magnitude in the two groups. Since only 12 of 17 patients followed for 50 wk or longer demonstrated three-exponential curve fits, various means were sought by which it might be predicted at the outset whether a given patient must be studied for so long a time; none was found. However, in the group with two exponentials the value of $M_a$ was significantly larger than those with three-exponential curve fits, and this difference was apparent at as early as 10–12 wk.

**Supplementary key words** computerized $F$ test · multi-exponential decay · curve fitting · hypercholesterolemia · coronary artery disease

In recent years, tracer methods have become available for the estimation of body masses and turnover of exchangeable cholesterol in man (1, 2). After the intravenous injection of a single dose of labeled cholesterol, the decay of specific activity in the blood was followed for various periods. The data points were plotted semilogarithmically, and the decay curves were analyzed by input–output (1) or compartmental analysis (2).

To verify the validity of the data obtained through these procedures, Grundy and Ahrens (3) compared the results simultaneously derived from compartmental analysis and a complete sterol balance method in the same patients. The parameter which could thus be verified was the magnitude of production or input rates of cholesterol, equivalent to the sum of the daily absorbed dietary and biosynthesized cholesterol. They found that the results obtained by direct chemical measurements were an average of 15% smaller than the data derived from tracer analysis. Although the difference was small between the two methods, the possible causes of this discrepancy were discussed by Grundy and Ahrens (3). They raised the question whether the two-pool model *over*estimated or the sterol balance method *under*-estimated daily turnover. The former case could obtain if in fact isotopic equilibrium[1] had not been attained between plasma and tissue cholesterol, whereas the

[1] The term "isotopic equilibrium" is used here to define a state when the specific activities in serum and specific tissues reach a constant ratio, as illustrated for intestinal mucosa and bile by Grundy, Ahrens, and Salen (4). For some compartments the time needed to attain this state may be very long (i.e., xanthoma cholesterol, Ref. 5).

TABLE 1. Clinical data, serum lipid levels, and results of computerized curve fitting in 23 patients

| Patient Number, Age, and Sex | | | | Clinical Diagnosis | Total Serum Cholesterol | Serum Triglyceride | Lipoprotein Pattern[a] | Length of Study | Number of Specific Activity Data | Best Curve Fit: Number of Exponentials | Best Curve Fit: $F$[b] | Best Curve Fit: $P$ | Point of Change[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mg/100 ml ± SD | | | wk | | | | | wk |
| 1 | B.R. | 53 | M | Multiple sclerosis | 232 ± 17 | 168 ± 14 | | 66 | 50 | 3 | 3.45 | <0.001 | 19 |
| 2 | A.B. | 52 | M | Coronary artery disease | 220 ± 18 | 125 ± 24 | | 66 | 56 | 3 | 5.99 | <0.001 | 27 |
| 3 | B.P. | 64 | F | Coronary artery disease | 307 ± 18 | 110 ± 17 | II | 66 | 41 | 3 | 1.53 | NS | 23 |
| 4 | J.L. | 49 | M | Coronary artery disease | 318 ± 19 | 99 ± 20 | II | 63 | 48 | 3 | 2.85 | <0.001 | 35 |
| 5 | N.A. | 50 | F | Coronary artery disease Xanthomatosis | 542 ± 26 | 121 ± 28 | II | 63 | 58 | 3 | 2.45 | <0.001 | 30 |
| 6 | A.W. | 46 | M | Coronary artery disease Xanthomatosis | 631 ± 14 | 148 ± 14 | II | 63 | 32 | 2 | 0.93 | | |
| 7 | S.K. | 67 | F | No clinical disease | 234 ± 12 | 122 ± 14 | | 60 | 44 | 3 | 4.88 | <0.001 | 13 |
| 8 | H.H. | 59 | M | Multiple sclerosis | 224 ± 20 | 90 ± 33 | | 60 | 46 | 3 | 2.93 | <0.001 | 18 |
| 9 | N.S. | 72 | M | Coronary artery disease | 197 ± 23 | 100 ± 17 | | 58 | 42 | 2 | 0.95 | | |
| 10 | S.P. | 49 | F | Essential hypertension | 272 ± 32 | 84 ± 12 | II | 57 | 51 | 3 | 4.01 | <0.001 | 13 |
| 11 | G.K. | 63 | M | Coronary artery disease | 407 ± 31 | 113 ± 21 | II | 57 | 37 | 3 | 3.47 | <0.001 | 30 |
| 12 | T.B. | 45 | M | Coronary artery disease Xanthomatosis | 580 ± 33 | 128 ± 16 | II | 57 | 49 | 2 | 0.96 | | |
| 13 | D.B. | 43 | F | Essential hypertension | 224 ± 15 | 94 ± 19 | | 55 | 39 | 3 | 1.35 | NS | 29 |
| 14 | M.R. | 53 | F | Peripheral vascular insufficiency | 289 ± 24 | 104 ± 19 | II | 54 | 45 | 3 | 1.64 | <0.05 | 43 |
| 15 | A.K. | 18 | M | Coronary artery disease Xanthomatosis | 699 ± 45 | 78 ± 15 | II | 53 | 44 | 2 | 0.95 | | |
| 16 | C.A. | 41 | F | Multiple sclerosis | 228 ± 11 | 44 ± 10 | | 50 | 35 | 3 | 8.33 | <0.001 | 18 |
| 17 | E.A. | 60 | F | Pulmonary emphysema | 224 ± 17 | 86 ± 11 | | 50 | 25 | 2 | 0.81 | | |
| 18 | E.D. | 52 | M | Coronary artery disease | 240 ± 18 | 110 ± 8 | | 41 | 29 | 2 | 0.92 | | |
| 19 | S.S. | 62 | M | Coronary artery disease Diabetes mellitus | 236 ± 17 | 227 ± 63 | IV | 29 | 25 | 3 | 1.03 | NS | 24 |
| 20 | A.F. | 45 | F | No clinical disease | 249 ± 15 | 79 ± 14 | | 28 | 20 | 2 | 0.88 | | |
| 21 | H.F. | 47 | M | Coronary artery disease | 313 ± 21 | 133 ± 14 | II | 26 | 15 | 2 | 0.82 | | |
| 22 | N.M. | 40 | F | Multiple sclerosis | 271 ± 18 | 102 ± 16 | | 23 | 15 | 2 | 0.82 | | |
| 23 | H.Z. | 43 | F | Coronary artery disease Xanthomatosis | 551 ± 28 | 103 ± 19 | II | 16 | 14 | 2 | 0.60 | | |

[a] Ref. 7.
[b] The three-exponential curve fit was better when the value of $F$ was larger than 1.
[c] Number of weeks of observation at which a two-exponential curve fit could be shown to be better than a three-exponential fit.

latter could occur if a metabolic steady state was not in fact achieved (3).

During a long-term study, we have noted that the slow slope of the decay curves showed a further flattening after 20–30 wk of follow-up in some patients (6). When serum cholesterol specific activity was observed for as long as 63 wk after the injection of tracer, in five of seven patients the data could not be fitted with two exponentials. A three-exponential curve fit was clearly exhibited by three subjects when curves were fitted by eye, and in two, the curve fitting of the data was equivocal. The average input rate in the patients with three-exponential curve fits was 15% smaller than that of the group with two exponentials. It was thought that the discrepancy described by Grundy and Ahrens (3) could be explained by the appearance of a third exponential in long-term studies (6).

In the present report, a simple computerized procedure for input–output analysis of the decay curves in 23 patients is described. The program includes curve fitting by two-, three-, and four-exponentials, and the computer statistically determines the best fit. The parameters on turnover and exchangeable body masses of cholesterol using the best exponential fit are then calculated and printed.

## METHODS

### Patients

23 patients were studied; age, sex, and clinical diagnosis of each are included in Table 1. 11 patients had serum lipid levels regarded as normal, 11 subjects had hypercholesterolemia (type II lipoprotein pattern), and 1 had hypertriglyceridemia (type IV lipoprotein pattern) and diabetes mellitus. All subjects were ambulatory outpatients, and their respective diseases, if any, were kept under good control. Medications, if any, were kept constant, and substances known to influence serum lipid levels were not given. The diet was uncontrolled, but the

patients were instructed to adhere to a low fat, low cholesterol diet that contained approximately 250 mg of cholesterol daily. In the suggested diet, carbohydrate constituted 54% of the total calories, protein 20%, and fat 26%. The diabetic patient was instructed to adhere to a 2000 kcal diabetic diet.

Physical examinations, complete blood counts, urinalysis, blood urea nitrogen, blood sugar, serum bilirubin, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, and cephalin flocculation were carried out periodically and remained practically unchanged during the study. The weights of the patients did not fluctuate more than 4 pounds. Some of the data in 16 of 23 patients have been reported previously in other connections (1, 5, 6, 8).

### Radioactive sterols

Radioactive sterols were processed and administered as previously described (8). [7α-$^{3}$H]Cholesterol (225 μCi/mmole) or [4-$^{14}$C]cholesterol (50 μCi/mmole) (New England Nuclear Corp., Boston, Mass.) was further purified by thin-layer chromatography (toluene–ethyl acetate 9:1), and only that material that moved with the same $R_F$ as a cholesterol standard was administered to the patients. The radioactive cholesterol was incubated with the serum of each individual, and 31–83 μCi was given intravenously to each patient at the beginning of the experiment.

### Procedures and length of follow-up

After the injection of labeled cholesterol, blood was drawn at each visit weekly or biweekly (except for a few appointments missed by the patients for personal reasons). Serum cholesterol specific activity was determined as described previously (8). The results were expressed as 100 times specific activity (dpm/gram total serum cholesterol), divided by the injected dose (dpm) of radioactive cholesterol (percentage of dose of radioactivity given per 1 g of total serum cholesterol). The first blood sample was taken 1 wk after the injection of the tracer.

Total serum cholesterol concentrations were determined in each sample by the method of Abell et al. (9). Serum triglyceride levels were determined by the method of Van Handel and Zilversmit (10). The values in Table 1 are averages (± SD) of all samples obtained during the study.

17 patients were followed for 50–66 wk (average, 59 wk) after the injection of the tracer. In the six remaining patients the study was of 16–41 wk duration (average, 27 wk). The length of the study and the number of data points available for each patient are included in Table 1.

### Computer analysis

All computations were done on an XDS Sigma 7 computer. The time required for the entire computation (including the calculation of parameters on shortened curves) varied from 0.5 to 3 min/patient, depending on the number of points initially available. A copy of the program (in Fortran language) is available on request.

*Curve fitting and F tests.* The data for each patient were subjected to the following analysis (two-, three-, and four-exponential curves):

$$100\,w = a_1e^{-b_1t} + a_2e^{-b_2t} + a_3e^{-b_3t} + a_4e^{-b_4t}$$

(four-exponential curve)

where $w$ denotes percentage of injected dose/gram; $a$ denotes the exponential amplitude; and $b = 0.693/t_{1/2}$ in days. The best two-exponential curve fit, the best three-exponential curve fit, and the best four-exponential curve fit were obtained by performing a standard nonlinear least squares fit, using the reciprocal of each value as the weighting factor. The initial guess in this iterative least squares procedure was obtained by the conventional peeling-off method. The weighted variances were computed and compared with the $F$ test.

*Computation of parameters.* The theoretical input–output analysis and the method of calculation of the kinetic data are described elsewhere (1). The computer calculates and prints the following parameters:

Daily input rates, $I_T$ (grams/day), representing the sum of absorbed dietary and biosynthesized cholesterol obtained as

$$I_T = 1\Big/\int_0^\infty w(t)dt \qquad \text{Eq. 1}$$

$$= 69.3/\Sigma_j a_j(t_{1/2})_j \qquad \text{Eq. 2}$$

where $w(t)$ denotes percentage of injected dose/gram at time $t$ (days), $a_j$ is the exponential amplitude (1/100 g), and $(t_{1/2})_j = (0.693\ bj)$ the half-lives (days). $j = 1, 2, 3$ for a three-exponential curve fit and $j = 1, 2$ for a two-exponential curve fit.

The mean transit time, $\bar{l}_p$ (days), of tracer cholesterol:

$$\bar{l}_p = \int_0^\infty tw(t)dt\Big/\int_0^\infty w(t)dt \qquad \text{Eq. 3}$$

$$= \Sigma_j a_j(t_{1/2})_j^2/0.693\Sigma_j a_j(t_{1/2})_j \qquad \text{Eq. 4}$$

The total exchangeable mass, $M$ (grams), of body cholesterol

$$M = I_T\bar{l}_p \qquad \text{Eq. 5}$$

The rapidly exchangeable mass, $M_a$ (grams), of

TABLE 2. Values of $I_T$ (sum of absorbed dietary and biosynthesized cholesterol) in 12 patients with three-exponential curve fits and in 5 patients with two-exponential curve fits[a]

| Patient | Length of Analysis (wk) 50–66[b] | 40 | 30 | 20 | 10–12 |
|---|---|---|---|---|---|
| | | | *g/day* | | |
| Three-exponential curves | | | | | |
| 1 | 1.84 | 1.84 | 1.82 | 1.80 | 2.08 |
| 2 | 1.14 | 1.14 | 1.09 | 1.22 | 1.23 |
| 3 | 0.78 | 0.78 | 0.78 | 0.82 | 0.88 |
| 4 | 1.02 | 1.00 | 1.10 | 1.10 | 1.14 |
| 5 | 1.14 | 1.22 | 1.22 | 1.20 | 1.25 |
| 7 | 0.65 | 0.64 | 0.66 | 0.69 | 0.77 |
| 8 | 1.17 | 1.17 | 1.17 | 1.16 | 1.24 |
| 10 | 1.17 | 1.17 | 1.17 | 1.19 | 1.47 |
| 11 | 1.27 | 1.27 | 1.34 | 1.35 | 1.38[c] |
| 13 | 1.06 | 1.10 | 1.11 | 1.08 | 1.19 |
| 14 | 0.87 | 0.97 | 0.97 | 0.97 | 1.05 |
| 16 | 1.07 | 1.07 | 1.06 | 1.06 | 1.20 |
| Average | 1.09 | 1.11 | 1.12 | 1.14 | 1.24 |
| Difference[d] | | | | 4.6% | 13.8% |
| $F$[d] | | NS | NS | <0.05 | <0.001 |
| Two-exponential curves | | | | | |
| 6 | 1.51 | 1.53 | 1.53 | 1.52 | 1.52 |
| 9 | 0.92 | 0.92 | 0.94 | 0.96 | 0.99 |
| 12 | 1.31 | 1.32 | 1.34 | 1.32 | 1.27 |
| 15 | 1.28 | 1.28 | 1.28 | 1.26 | 1.25[c] |
| 17 | 1.14 | 1.14 | 1.14 | 1.12 | 1.09[c] |
| Average | 1.23 | 1.24 | 1.25 | 1.24 | 1.22 |

[a] The data for each patient are presented in terms of the entire observational period and also at successively shortened intervals.
[b] Full length of experiment.
[c] 16 wk.
[d] Compared with data for 50–66 wk.

TABLE 3. Values of $M_a$ (rapidly exchangeable mass of cholesterol) in 12 patients with three-exponential curve fits and in 5 patients with two-exponential curve fits[a]

| Patient | Length of Analysis (wk) 50–66[b] | 40 | 30 | 20 | 10–12 |
|---|---|---|---|---|---|
| | | | *g* | | |
| Three-exponential curves | | | | | |
| 1 | 39.0 | 38.9 | 39.1 | 39.2 | 39.6 |
| 2 | 24.7 | 24.7 | 25.0 | 26.1 | 25.6 |
| 3 | 17.4 | 17.6 | 17.3 | 19.7 | 16.0 |
| 4 | 25.5 | 26.3 | 31.4 | 29.1 | 25.7 |
| 5 | 37.0 | 37.9 | 37.4 | 38.0 | 37.8 |
| 7 | 15.5 | 16.2 | 15.8 | 13.1 | 16.9 |
| 8 | 25.2 | 23.0 | 22.0 | 23.0 | 24.8 |
| 10 | 20.5 | 21.2 | 21.6 | 21.2 | 25.2 |
| 11 | 44.3 | 45.3 | 53.9 | 53.4 | 52.5[c] |
| 13 | 28.2 | 28.9 | 28.5 | 29.4 | 27.0 |
| 14 | 18.8 | 28.2 | 24.5 | 24.5 | 20.7 |
| 16 | 17.1 | 16.6 | 16.8 | 16.9 | 19.2 |
| Average[d] | 26.1[e] | 27.1 | 27.8 | 27.8 | 27.6 |
| Two-exponential curves | | | | | |
| 6 | 60.4 | 59.1 | 58.8 | 59.0 | 59.0 |
| 9 | 40.2 | 40.1 | 39.6 | 39.1 | 38.4 |
| 12 | 66.7 | 65.8 | 64.9 | 64.9 | 64.9 |
| 15 | 60.3 | 59.1 | 58.1 | 60.0 | 60.5[c] |
| 17 | 27.5 | 27.0 | 25.2 | 26.6 | 28.0[c] |
| Average[d] | 51.0[e] | 50.2 | 49.3 | 49.9 | 50.2 |

[a] The data for each patient are presented in terms of the entire observational period, and also at successively shortened intervals.
[b] Full length of experiment.
[c] 16 wk.
[d] No significant differences among the averages calculated at the various time intervals.
[e] Differences between the means obtained at every time interval (three- vs. two-exponential curves) were significant ($P < 0.01$).

body cholesterol

$$M_a = 100/w(0) \quad \text{Eq. 6}$$

$$= 100/\Sigma_j a_j \quad \text{Eq. 7}$$

The remaining exchangeable mass of body cholesterol is defined as the difference: $M - M_a$ (grams). The computer program included Eq. 1, 3, 5, and 6, using the trapezoidal rule for area integration.

(The initial and the final slopes of the curves were determined by exponential curve fitting for the purpose of extrapolation back to time zero and forward to infinity.)

*Computation of differences between short- and long-term studies due to recognition of a third exponential.* We have developed a method for the determination of the possible degree of error incurred in short-term studies. Such error occurs when a third, slowest exponential, not revealed by the early points of a shorter curve, is exhibited by later data points.

After the analysis was completed using the full lengths of the curves, the last point was removed by the computer and the best two-, three-, and four-exponential fits, as well as the parameters $I_T$, $\bar{l}_p$, $M_a$, $M$, and $M - M_a$, were again computed on this shorter curve. This procedure of removing points one at a time (beginning with the last one) and recomputing the curve fits, as well as the parameters, was repeated on progressively shorter curves until only 10–12 points were left. The difference in the value of each parameter obtained on the full lengths and on the shortened curves was calculated. The average differences for each parameter were then determined, using the data obtained in all patients in this study.

## RESULTS

### Curve fitting and *F* tests

Of 17 patients with decay curves followed for longer than 50 wk, a three-exponential curve-fit was better in 12, according to computerized $F$ tests (Table 1). In 10 of the 12 patients with three-exponential fits, the value of $F$ was statistically significant (see $P$ values in Table 1). In five patients studied for 50 wk or longer, two-exponential fits were better (Table 1).

In the remaining six patients, the decay curves were followed for less than 50 wk (15–41 wk). In five of these subjects, two-exponential curve fits were better, whereas in one patient the best fit was three-exponential (Table 1); however, the value of $F$ in this subject was not statistically significant. The reasons for this seemingly arbitrary division into two groups (more than and less than 50 wk of data) will become apparent below.

The program included an attempt at curve fitting with four exponentials in each patient. In none of the 23 subjects did the data comply with four-exponential curve fits. Either the computer failed to iterate the data or the value of $F$ demonstrated nonvalidity of the four-exponential fits.

The computer analysis indicated that by shortening the curves the third (slowest) exponential disappeared at 13–43 wk (average, 25 wk) after the injection of the tracer. In two patients, data points of 13-wk length exhibited three-exponential curve fits, in seven subjects the point of change was at 18–29 wk, and in four it was at 30–43 wk (Table 1).

### Input rates and exchangeable masses of cholesterol

Average parameters of input–output analysis were calculated in 17 patients studied for more than 50 wk. Only 12 of these 17 exhibited a three-exponential curve fit (Tables 2–5); in the other 5 the best fit was obtained with two exponentials.

In the 12 patients in whom a three-exponential curve fit was obtained, the average value of $I_T$ (input rate: sum of absorbed dietary and biosynthesized cholesterol) derived from the maximum lengths of the curves (50–66 wk) was 1.09 g/day (Table 2). As the length of each decay curve was shortened by the computer, the value of $I_T$ gradually increased. At the shortest analyzed length (10–12 wk), the value of $I_T$ was 1.24 g/day, or 13.8% larger than at full length. In the other patients, however, with two-exponential curve fits, the value of $I_T$ showed no perceptible change (Table 2) with curve shortening.

The average value of $M_a$ (rapidly exchangeable mass of cholesterol) did not change when the length of the curves was decreased by the computer in any of the 17 patients exhibiting three-exponential curve fits (Table 3). The average $M_a$ was 26.1 g, using 50–66 wk data, and 27.6 g, using 10–12 wk data. However, the average value of $M_a$ in the five patients who exhibited two-exponential curve fits was 51.0 g at the maximum length of the study. This figure was nearly twice as large ($P < 0.01$) as the average value exhibited by the 12 patients with three-exponential curve fits (Table 3).

The values of $M$ (total exchangeable body mass of cholesterol) showed marked decreases as the curves were shortened only in the 12 patients with three-exponential curve fits (Table 4). The average $M$ calculated at 50–66 wk was 90.8 g vs. 67.3 g at 10–12 wk, a difference of 25.9%. Again, there was little or no change with curve shortening in patients with two-exponential curve fits.

The largest variations were exhibited by the value of $M - M_a$ (remaining exchangeable mass of cholesterol) (Table 5). Calculated at 50–66 wk, the average value of $(M - M_a)$ was 64.8 g, but at 10–12 wk it was 39.8 g, a difference of 38.6%. Again, the patients with two-exponential curve fits showed little variation with curve shortening.

Of the 12 patients included in the above analysis, 6 had serum lipid levels regarded as normal and 6 had hypercholesterolemia (type II lipoprotein pattern). To ascertain whether there were differences in the possible error incurred in the analysis between these two groups, the average parameters were calculated separately. In these two groups of patients, differences in calculations of $I_T$, $M$, and $M - M_a$ using maximum and minimum length of study were equally large.

## DISCUSSION

The computerized procedure for input–output analysis of serum cholesterol specific activity decay curves described in this report represents a considerable improvement over previously used manual procedures. First, the time required for the analysis is a few seconds or minutes, instead of many hours. Second, curve fitting is carried out by an unbiased machine. Third, the present method conclusively proves that many decay curves must be fitted by three rather than by two exponentials.

When the parameters of the present study were calculated by exponential curve fitting and by area integration, the results by the two methods were practically identical. In 16 of the 23 patients in the present study, the results had previously been calculated by visual curve fitting: the average values in these subjects obtained by the "manual" procedure as compared with the present computerized method were, respectively, $I_T$, 1.31 and 1.32 g/day; $M_a$, 41.8 and 37.9 g; and $M$, 105.2 and 106.3 g.

Application of the present procedure clearly demonstrated that long-term experiments yielded different results from those obtained with short-term curves. Although the data obtained here by long-term input–output analysis were not validated by carrying out simultaneous sterol balance studies, indirect evidence suggesting an improvement in estimates of $I_T$ were obtained by comparing the figures of $I_T$ derived from curves of different lengths with the data of Grundy and Ahrens (3). In those patients in whom a third exponential was demonstrated, the average value of input rates ($I_T$) decreased by 14% (Table 2). This figure is in good agree-

TABLE 4. Values of $M$ (total exchangeable body mass of cholesterol) in 12 patients with three-exponential curve fits and in 5 patients with two-exponential curve fits[a]

| Patient | Length of Analysis (wk) 50–66[b] | 40 | 30 | 20 | 10–12 |
|---|---|---|---|---|---|
| | | | *g* | | |
| Three-exponential curves | | | | | |
| 1 | 141.1 | 140.0 | 145.6 | 152.2 | 99.0 |
| 2 | 75.8 | 75.9 | 95.1 | 62.3 | 59.7 |
| 3 | 54.5 | 54.7 | 54.1 | 50.8 | 36.9 |
| 4 | 82.4 | 93.8 | 77.3 | 72.3 | 62.1 |
| 5 | 129.9 | 87.6 | 85.4 | 89.8 | 83.6 |
| 7 | 57.6 | 63.2 | 55.3 | 45.0 | 39.8 |
| 8 | 72.1 | 68.5 | 67.0 | 70.1 | 61.4 |
| 10 | 109.4 | 112.3 | 114.1 | 108.1 | 76.7 |
| 11 | 99.1 | 100.5 | 94.9 | 92.6 | 87.8[c] |
| 13 | 103.8 | 86.5 | 85.4 | 89.2 | 76.8 |
| 14 | 102.8 | 103.7 | 93.5 | 93.7 | 78.0 |
| 16 | 61.6 | 60.1 | 61.0 | 61.9 | 45.9 |
| Average | 90.8[d] | 87.2 | 85.8 | 82.3 | 67.3[e] |
| Difference[f] | | | | 9.4% | 25.9% |
| $P$[f] | | NS | NS | <0.05 | <0.001 |
| Two-exponential curves | | | | | |
| 6 | 118.2 | 113.0 | 111.4 | 113.2 | 113.2 |
| 9 | 87.8 | 87.4 | 84.8 | 82.0 | 77.9 |
| 12 | 147.3 | 143.1 | 138.4 | 140.0 | 146.5 |
| 15 | 98.4 | 96.9 | 95.8 | 100.9 | 102.5[c] |
| 17 | 75.9 | 75.0 | 72.1 | 75.5 | 80.6[c] |
| Average | 105.5[d] | 103.1 | 100.5 | 102.3 | 104.1[e] |

[a] The data for each patient are presented in terms of the entire observational period and also at successively shortened intervals.
[b] Full length of experiment.
[c] 16 wk.
[d] Differences between the means obtained (three- or two-exponential curves) at 50–66, 40, 30, and 20 wk were not statistically significant.
[e] Difference between the means obtained (three- or two-exponential curves) at 10–12 wk was significant ($P < 0.01$).
[f] Compared with data for 50–66 wk.

TABLE 5. Values of $M - M_a$ (remaining exchangeable mass of cholesterol) in 12 patients with three-exponential curve fits and in 5 patients with two-exponential curve fits[a]

| Patient | Length of Analysis (wk) 50–66[b] | 40 | 30 | 20 | 10–12 |
|---|---|---|---|---|---|
| | | | *g* | | |
| Three-exponential curves | | | | | |
| 1 | 102.2 | 101.0 | 107.4 | 113.0 | 59.4 |
| 2 | 51.2 | 51.2 | 70.1 | 36.2 | 34.1 |
| 3 | 37.1 | 37.1 | 36.8 | 31.1 | 20.9 |
| 4 | 56.9 | 67.5 | 45.9 | 43.2 | 36.4 |
| 5 | 93.0 | 49.7 | 48.0 | 51.8 | 45.8 |
| 7 | 42.1 | 47.0 | 39.5 | 31.8 | 22.8 |
| 8 | 46.8 | 45.4 | 45.0 | 47.1 | 36.6 |
| 10 | 88.9 | 91.1 | 92.5 | 86.9 | 51.5 |
| 11 | 54.9 | 55.2 | 41.1 | 39.3 | 35.3[c] |
| 13 | 75.6 | 57.6 | 56.9 | 59.8 | 49.8 |
| 14 | 84.0 | 75.5 | 69.0 | 69.2 | 58.4 |
| 16 | 44.5 | 43.5 | 44.3 | 44.9 | 26.8 |
| Average | 64.8[d] | 60.2 | 58.0 | 54.5 | 39.8[e] |
| Difference[f] | | | | 15.9% | 38.6% |
| $P$[f] | | NS | NS | <0.05 | <0.001 |
| Two-exponential curves | | | | | |
| 6 | 57.9 | 53.9 | 52.6 | 54.2 | 54.2 |
| 9 | 47.6 | 47.3 | 45.2 | 43.0 | 39.5 |
| 12 | 80.6 | 77.3 | 73.5 | 75.1 | 81.6 |
| 15 | 38.1 | 37.9 | 37.7 | 40.9 | 42.1[c] |
| 17 | 48.3 | 48.0 | 46.8 | 48.9 | 52.6[c] |
| Average | 54.5[d] | 52.9 | 51.2 | 52.4 | 54.0[e] |

[a] The data for each patient are presented in terms of the entire observational period and also at successively shortened intervals.
[b] Full length of experiment.
[c] 16 wk.
[d] Differences between the means obtained (three- or two-exponential curves) at 50–66, 40, 30, and 20 wk were not statistically significant.
[e] Difference between the means obtained (three- or two-exponential curves) at 10–12 wk was significant ($P < 0.05$).
[f] Compared with data for 50–66 wk.

ment with the data of Grundy and Ahrens (3), who demonstrated a 15% difference between chemical and tracer analysis.

Of 17 patients studied for 50 wk or longer, in 12 (71%) a three-exponential curve fit was better than a two-exponential curve fit. It is interesting to note that in the report of Grundy and Ahrens (3), 8 of 11 studies (73%) demonstrated larger production rates using the two compartmental model compared with chemical balance measurements of turnover. Whether these data and their remarkable agreement with our own delineate two different populations with regard to cholesterol turnover is not clear at present. As we had pointed out previously (6), the two categories of patients failed to show any clear differentiation on the basis of age, sex, clinical diagnoses, or serum lipid levels. The only difference between the two groups was in the average size of $M_a$: this was twice as large in patients with two-exponential fits as in those with three exponentials. Whether the size of $M_a$ obtained in a short-term experiment will correctly predict the existence or absence of a third exponential later on in the study is not clear at present, but with larger numbers of data obtainable in future experiments, measurement of $M_a$ after about 12 wk of study may prove to be useful in determining whether to terminate or to extend the observations.

The appearance of a third exponential markedly increased the values of $M$ (total exchangeable mass) and $M - M_a$ (remaining exchangeable mass of cholesterol) (Tables 4 and 5). A physiological explanation of the flattening of decay curves in compartmental terms was proposed in a previous paper (5), i.e., with the progress of time the exchange process between serum and different tissue cholesterol radioactivities becomes slower and slower. With longer periods of observation and with the appearance of a third exponential, the exchange of larger masses of slowly turning over tissue cholesterol is revealed and thus measured by the present analysis. It is, of course, not known whether the third exponential is a final one or whether still longer periods of observation

would have revealed further exponentials corresponding to still more slowly exchanging masses of cholesterol. It is known, for instance, that cholesterol in the brain exchanges very slowly with serum cholesterol, and it may take a lifetime to attain total isotopic equilibration with serum (5).

The calculation of $M$ (total exchangeable mass of cholesterol) by Eq. 5 assumes that the input of cholesterol (dietary and biosynthesized) occurs uniquely into the rapidly exchangeable mass ($M_a$) (1). The results so obtained represent a minimum value (limit case 1, Ref. 8) for the size of $M$. A maximum value (limit case 4, Ref. 8) can be calculated by assuming that input occurs uniquely into the slowly turning over mass. Since the exact input rates (i.e., cholesterol biosynthesis) in different body masses of cholesterol are unknown, neither of these two limit cases is the true case. However, it is felt that the minimum limit case used in the present computer program (and in previous studies) is far more supportable on physiological grounds (1, 5, 8).

In contrast to the marked effects of curve shortening on $I_T$, $M$, and $M - M_a$, the value of the rapidly exchangeable mass of cholesterol ($M_a$) was not affected. This indicated that the value of $M_a$ is independent of the demonstration of a third exponential.

Since the first blood sample was taken 1 wk after the injection of labeled cholesterol, it is possible that a very rapid, initial exponential was missed in the present analysis. To detect this missed exponential, several more data points in the first week would be required; had these data been available to us, the curves we have described as three-exponential might have become four-exponential, and the curves described as two-exponential would have become three-exponential. The implications of this consideration are that, in the present analysis, the size of $M_a$ may have been slightly overestimated, whereas the value of $I_T$ and $M$ would not be appreciably affected.

One of the fundamental assumptions of input–output analysis is that the specific activity of tracer cholesterol at exit from the system (fecal neutral and acidic steroids) equals the specific activity of tracer cholesterol in plasma (determined no more than 5 days earlier). This assumption has repeatedly been corroborated in the sterol balance studies carried out by Grundy and Ahrens (3), though in certain exceptional patients this rule does not hold true, in which cases kinetic analysis gives incorrect values (4).

When the data of 50–66 wk duration in 17 patients were analyzed so that only every second, third, fourth, fifth, or sixth data point was used by the computer ("thinning procedure"), the value of $I_T$ remained essentially unchanged as compared with the values obtained using all available data. This was done by retaining data points of the first 2 and the last 6 wk, and thinning the points remaining in between. Thus, it seems likely that the most accurate analysis of $I_T$ will be obtained if studies are carried out for at least 45 wk, but the inconvenience and labor involved in such a long study can perhaps be greatly reduced by measuring serum cholesterol specific activity as infrequently as once a month after injection of radioactive cholesterol. A more detailed publication of these data will follow.

The idea of the application of input–output analysis of the decay curves of serum cholesterol specific activity (1), in lieu of compartmental analysis, occurred when we first noted that some of these decay curves exhibited more than two exponentials (1, 6, 8). Contrary to compartmental analysis in which the number of pools should correspond to the number of exponentials revealed, input–output analysis is independent of the number of exponentials. Thus, the main difference between compartmental and input–output analysis is that the latter does not require the interpretation of a multiexponential curve in terms of discrete body pools of cholesterol (1). Indeed, the equations required for solution of $I_T$ (or $PR$), $M$ (or $M_A + M_{Bmin}$), and $M_a$ (or $M_A$) are essentially interchangeable: given a set of experimental data describing a typical decay curve, the two calculations (input–output analysis and compartmental analysis) must necessarily give exactly the same results for the above parameters.

Input–output analysis utilizes only the area and the first time-moment (meant transit time of tracer cholesterol, or the "center of gravity" of the area under the curve) of the plasma decay curves. It does not yield intercompartmental exchange rates, and indeed these calculated rates have never been validated by direct measurement, but it is applicable to decay curves of more general shape than those that can be fitted by a small number of exponentials. For example: should increased observation time demonstrate more than three exponentials, the same assumptions and Eq. 1, 3, 5, and 6 herewith could be applied, without invoking the concept of additional discrete pools, which are admittedly mathematical constructs rather than anatomical sites (11, 12). Because of this, input–output analysis has the potential of application with any curve shape or duration of experiment, and it is simpler in concept than compartmental analysis.

We are grateful to Dr. Edward H. Ahrens, Jr., for his interest and advice throughout many discussions of this study, and for his help in the preparation of the manuscript.

The cooperation of Dr. William Perl and Dr. Edward Meilman is gratefully acknowledged. We thank Mrs. Tea E. Siil, Lois R. Markowski, Mary Steiger, and Estelle F. Fisher for excellent technical assistance.

This work was supported in part by grants from the Nassau Heart Association, the Alan Kahn Memorial Fund, and by

grants HE-07188 and FR-5496 from the National Institutes of Health (USPHS).

*Manuscript received 21 July 1972; accepted 3 November 1972.*

## REFERENCES

1. Perl, W., and P. Samuel. 1969. Input–output analysis for total input rate and total traced mass of body cholesterol in man. *Cir. Res.* **25:** 191–199.
2. Goodman, DeW. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. *J. Clin. Invest.* **47:** 231–241.
3. 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.
4. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
5. Samuel, P., W. Perl, C. M. Holtzman, N. D. Rochman, and S. Lieberman. 1972. Long-term kinetics of serum and xanthoma cholesterol radioactivity in patients with hypercholesterolemia. *J. Clin. Invest.* **51:** 266–278.
6. 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.
7. Fredrickson, D. S., and R. S. Lees. 1966. Familial hyperlipoproteinemia. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, New York. 429–485.
8. 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.
9. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.
10. Van Handel, E., and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50:** 152–157.
11. Wilson, J. D. 1970. The measurement of the exchangeable pools of cholesterol in the baboon. *J. Clin. Invest.* **49:** 655–665.
12. Wilson, J. D. 1972. The relation between cholesterol absorption and cholesterol synthesis in the baboon. *J. Clin. Invest.* **51:** 1450–1458.