Input-Output Analysis for Total Input Rate and Total Traced Mass of Body Cholesterol in Man

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ABSTRACT

The Stewart-Hamilton theorems for flow and volume are generalized to yield total input rate and total traced mass in multiple input, steady-state systems with partially labeled input. Application is made to existing decay curves of tracer cholesterol in human serum measured under a control steady state and again under a steady state of neomycin administration which lowered the serum cholesterol level. The effect of neomycin on the total traced mass of body cholesterol was to reduce it by 38, 40, 32, and 24 g, corresponding to 34, 40, 25, and 33%, in four patients studied. The present analysis utilizes only the area and the first time moment of the plasma decay curve. It is applicable to decay curves of more general shape than those that can be fitted by a small number of exponentials. The analysis does not require the assumption of compartments.

ADDITIONAL KEY WORDS
Stewart-Hamilton theorems
first passage indicator-dilution theory
steady-state system
tracer decay curve
neomycin
cholesterol reduction

The method of Stewart (1) and Hamilton (2) was applied to a kinetics problem not usually analyzed from this point of view, namely, the time course of disappearance (decay curve) of tracer cholesterol from the plasma of man (3-11). This approach is made possible by the experimental finding that over most of the decay curve, the specific activity of tracer in the plasma approximates the specific activity of tracer in the fecal end products of cholesterol metabolism (12-21). The decay curve therefore approximates a first passage indicator-dilution curve, for which the Stewart-Hamilton, or input-output, method of analysis was designed. Heretofore, this method has enabled calculation of the total traced mass in systems with only one input channel (22-25) or with multiple input channels only when all input channels are simultaneously labeled with tracer in proportion to local input rate of traced substance (equivalent tracer supply) (26, 27). The present system, however, has multiple input channels of body cholesterol (biosynthesis in various tissues, dietary intake) and tracer cholesterol is injected into none of these but rather into a man-made channel (intravenous injection). The input-output method therefore requires extension to the case of multiple input channels with partially labeled input. This extension is made herein (28).

The present input-output analysis will be applied to the experimental decay curves obtained under a control steady state and also under a steady state of neomycin administration which produced a decrease of serum cholesterol concentration to a lower plateau level (11). The main results are the total input rate and the total traced mass of body cholesterol in each of the two preceding steady states. These parameters are of consid-
erable importance in the study of cholesterol metabolism and of the effect of cholesterol-reducing agents in man. The present method of analysis demonstrates that the preceding two parameters can be derived without postulating the existence of compartments.

Theory

The body is regarded as a biological system into which cholesterol enters via many channels or inlets. These may for convenience be grouped as shown in Figure 1: biosynthesis in rapidly metabolizing cells at rate $I_1$ (g/day), biosynthesis in slowly metabolizing cells at rate $I_2$, dietary intake at rate $I_3$, etc. Each of these rates is itself the sum of many component rates. The total input rate is

$$I_T = I_1 + I_2 + I_3 + \ldots$$  (1)

Cholesterol leaves the body via two main groups of end products, neutral steroids and bile acids. Both groups are excreted almost entirely in the feces (16). For present purposes a cholesterol molecule may be regarded as metabolically transformed into an end product molecule with negligible time delay. Therefore, the two major output rates, $E_1$ (g/day) as neutral steroids and $E_2$ as bile acids, plus minor output rates such as $E_3$ as steroid hormones in urine and $\Delta E_3$ created by blood sampling may be considered combined into one output rate $E$ at a combined outlet $E$, thus

$$E = E_1 + E_2 + E_3 + \Delta E_3 + \ldots$$  (2)

Of the total mass of cholesterol in the system, an amount $M$ is assumed to be accessible to exchange with tracer cholesterol in a time of the order of the maximum time of the experiment. This mass $M$ (g) is denoted the total traced mass. The system is assumed to be in a steady state with respect to body cholesterol metabolism, that is, the total traced mass and all the aforementioned input and output rates are constant in time. The Stewart-Hamilton approach (22-25) is based on a flow theorem and a volume theorem which for the present experimental situation may be extended as follows.

Multiple Inlet, Single Outlet Total Rate Theorem.—In a finite injection experiment the amount $m_0 = \int_0^\infty i(t)dt$ (dpm) of radioactive tracer cholesterol is injected at a time dependent rate $i(t)$ (dpm/day) into the system at a location (mixed venous blood) which is not one of nature's inlets for cholesterol. The steady state of the system is presumed not disturbed by the injection. Starting from the beginning of injection ($t=0$), tracer cholesterol emerges at the combined outlet $E$ at a time-dependent rate $e_0(t)$ (dpm/day). This output rate is the product of the total output rate $E$ (g/day) of body cholesterol and an average specific activity $c_k(t)$ of tracer cholesterol, expressible in terms of the specific activities $c_i(t)$ at the actual individual outlets $i$ by

$$e_0(t) = Ec_k(t) = \Sigma_i c_i(t)c_{Ei}$$  (3)

where $\Sigma_i$ denotes summation (or integration if necessary) over the individual outlets. Conservation of tracer cholesterol requires

$$m_0 = \int_0^\infty i(t)dt = \int_0^\infty e_0(t)dt.$$  (4)
Conservation of body cholesterol in the steady state requires
\[ E = I_T. \] (5)

Equations 3, 4 and 5 yield
\[ I_T = m_o \int_0^\infty c_\phi(t) dt. \] (6)

The specific activity at the combined outlet is assumed sufficiently well approximated by the specific activity in venous plasma at the sampling outlet (12-21, and Discussion), or
\[ c_\phi(t) = c(t). \] (7)

Equations 6 and 7 give
\[ I_T = m_o \int_0^\infty c(t) dt. \] (8)

which is the rate theorem used in the present analysis. Aside from the assumption of equation 7, equation 8 is simply the expression of conservation of mass in the steady state, which is familiar from Stewart-Hamilton theory (24).

**Partially Labeled, Multiple Input Mass Theorem.**—Consider a constant infusion tracer experiment that could be performed on the system, in which the constant rate \( i_w \) (dpm/day) of tracer cholesterol is maintained indefinitely at the same inlet that was used for injection in the finite injection experiment actually performed (Fig. 1). At long (infinite) time in the constant infusion experiment, the tracer would be distributed throughout the system in a steady state, denoted the tracer steady state. In the tracer steady state the constant amount of tracer in the system is \( m_\phi \) (dpm) and the total rate of output of tracer is \( e_x \phi = i_x \phi \). It can be shown (26-28a) that
\[ m_\phi = i_x \phi t_\phi = e_x \phi c_\phi \] (9)

where
\[ t_\phi = \frac{\int c_\phi(t) dt}{\int_0^\infty c_\phi(t) dt} = \frac{\int_i(t) dt}{\int_0^\infty i(t) dt}. \] (10)

is the mean transit time of tracer cholesterol as measured in the finite injection experiment actually performed. The tracer equation 9 can be written in terms of traced substance by multiplying and dividing on the left by \( M \) and on the right by \( E \) to give
\[ \gamma_x M = I_T I_x. \] (11)

where
\[ \gamma_x = (m_x/M)/\left(e_x E/M \right). \] (12)

The average specific activity \( c_{x,E} \) at the combined outlet \( E \) in the tracer steady state is defined in terms of the specific activities \( c_{x,E_i} \) at the individual outlets in the tracer steady state by
\[ c_{x,E} = \frac{c_x E_i}{E_i} = \frac{\Sigma c_{x,E_i}}{\Sigma E_i}. \] (13)

The average specific activity \( c_{x,M} \) in the system in the tracer steady state is defined in terms of the specific activity \( c_x(r) \) at location \( r \) by
\[ c_{x,M} = \frac{m_x/M}{f_x M(r) dM(r)/dM(r)}. \] (14)

where \( f_x \) denotes integration over the elements \( dM(r) \) of traced mass in the system. Equations 12, 13 and 14 give
\[ \gamma_x = c_{x,M}/c_{x,E}. \] (15)

The partially labeled, multiple input mass theorem, equation 11, differs from the single input, Stewart-Hamilton mass theorem (22-25) by the \( \gamma_x \) factor. This factor arises in a multiple input system when tracer is introduced into only part of the input in a finite injection experiment. The factor refers, however, to the specific activity distribution throughout the system in the tracer steady state, that is, at infinite time in a constant infusion experiment. Multiple input systems in which all inputs are labelled in proportion to local input rate of traced substance (equivalent tracer supply) have been shown (26, 27) to be equivalent to single input systems in that in the tracer steady state the specific activity throughout the system is everywhere the same, so that
\[ \gamma_x = 1. \] (16)

For the present application in which only one (man-made) input of the multiple input
system is labeled, the distribution of specific activity in the tracer steady state would not be uniform (the nonlabeled input may be visualized as "diluting" the label in its progress from labeled input to the more-or-less mixed outputs). In general, therefore, \( \gamma_x \neq 1 \). Nevertheless, in the absence of further information, equation 16 will be made an assumption in the present application (see Discussion). Equations 16 and 11 yield

\[
M = I_x t_E. \tag{17}
\]

Substituting the previous assumption, equation 7, into equation 10 and neglecting the input mean time contribution on the right (injection duration negligible relative to mean transit time) gives

\[
I_x = \int_0^\infty c_p(t) dt / \int_0^\infty c_p(t) dt = t_p. \tag{18}
\]

Equations 17 and 18 give

\[
M = I_x t_p, \tag{19}
\]

which was the mass theorem used in the present analysis.

**Methods**

Five patients were studied. The detailed experimental procedure and results are given elsewhere (11). Briefly, the experiment consisted of the intravenous injection of a known amount, \( m_0 \) (dpm), of free cholesterol-7 \( \alpha \)-\(^3\)H into an arm vein of a patient in a clinical steady state and the subsequent measurement (approximately daily for 7 days, thereafter approximately weekly for 23 to 42 weeks) of the specific activity \( c_p(t) \) (dpm/g total cholesterol) in the serum of blood samples drawn from an arm vein. The experiment was repeated after a new steady state had been established under a constant rate of oral administration of neomycin (to four patients) or of a placebo (to one patient). The main criteria of a steady state were considered to be constancy of the serum total cholesterol concentration, the body weight, and the clinical condition of the patients. The tracer experiment under neomycin administration was not begun until the same degree of constancy of serum total cholesterol concentration had been achieved (at a lower level) as in the control experiments. A semilogarithmic plot of each decay curve was fitted by a sum of two exponentials (peeling off process). The experimental data were thus represented as

\[
w(t) = c_p(t)/m_0; \tag{20}
\]

\[
100 \int_0^\infty w(t) dt = a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}, \tag{21}
\]

in which \( a_1 \), \( a_2 \) are the exponential amplitudes and \( \tau_1 \), \( \tau_2 \) are the "e\(^{-1}\)"-lives" (the \( e\(^{-1}\) \) life = 1.443 \times \) half-life is the time for a monoexponential function to decrease to \( 1/e = 0.368 \) of any chosen initial value). Integration of equation 21 yields

\[
100 \int_0^\infty t w(t) dt = a_1 \tau_1 + a_2 \tau_2; \tag{22}
\]

\[
100 \int_0^\infty t^2 w(t) dt = a_1 \tau_1^2 + a_2 \tau_2^2. \tag{23}
\]

The total input rate is given by equations 8, 20 and 22 as

\[
I_x = 1/ \int_0^\infty w(t) dt = 100/(a_1 \tau_1 + a_2 \tau_2). \tag{24}
\]

The mean transit time is given by equations 18, 20, 22 and 23 as

\[
l = (a_1 \tau_1^2 + a_2 \tau_2^2) / (a_1 \tau_1 + a_2 \tau_2). \tag{25}
\]

The total traced mass of body cholesterol is given by equations 19, 24 and 25 as

\[
M = 100( a_1 \tau_1^2 + a_2 \tau_2^2 ) / (a_1 \tau_1 + a_2 \tau_2). \tag{26}
\]

The sum of exponentials curve fit, equation 21, automatically includes an exponential extrapolation from the maximum time of observation to infinite time. This type of extrapolation is customary but represents an assumption (see Discussion). The extrapolated portion of the area under the decay curve is

\[
100 \int_{t_m}^\infty w(t) dt = w_{m} \tau_{2_m}, \tag{27}
\]

where \( w_{m} = a_2 e^{-m/\tau_2} \) is the curve fitted ordinate at the maximum time of observation \( t_m \). The extrapolated portion of the area under the moment curve is

\[
100 \int_{t_m}^\infty t w(t) dt = w_{m} \tau_{2_m}^2 + t_m \tau_{2_m}. \tag{28}
\]

**Results**

The calculated results are given in Table 1. The exponential parameters of equation 21, taken from reference 11, are given in rows 1 to 4 (the reciprocal \( \alpha_1 = 1/\tau_1 \) is given in reference 11). The maximum measurement time, that is, the duration of the experiment, is given in row 5.

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### TABLE 1

**Calculated Results of Neomycin Investigation (in Five Patients)**

<table>
<thead>
<tr>
<th>Row</th>
<th>Parameter</th>
<th>Unit</th>
<th>Control</th>
<th>Neomycin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SS</td>
<td>HP</td>
<td>MR</td>
</tr>
<tr>
<td>1</td>
<td>$a_1$</td>
<td>1/100 g</td>
<td>2.3</td>
<td>2.2</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>$a_2$</td>
<td>1/100 g</td>
<td>0.19</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>3</td>
<td>$r_1$</td>
<td>d</td>
<td>7.52</td>
<td>11.1</td>
<td>11.9</td>
</tr>
<tr>
<td>4</td>
<td>$r_2$</td>
<td>d</td>
<td>69.0</td>
<td>92.6</td>
<td>157.0</td>
</tr>
<tr>
<td>5</td>
<td>$t_m$</td>
<td>d</td>
<td>108.0</td>
<td>161.0</td>
<td>294.0</td>
</tr>
<tr>
<td>6</td>
<td>$a_1r_1$</td>
<td>d/100 g</td>
<td>17.3</td>
<td>24.4</td>
<td>22.6</td>
</tr>
<tr>
<td>7</td>
<td>$a_2r_2$</td>
<td>d/100 g</td>
<td>13.1</td>
<td>37.0</td>
<td>73.8</td>
</tr>
<tr>
<td>8</td>
<td>$100 f w dt$</td>
<td>d/100 g</td>
<td>30.4</td>
<td>61.4</td>
<td>96.4</td>
</tr>
<tr>
<td>9</td>
<td>$w_m r_2$</td>
<td>d/100 g</td>
<td>1.15</td>
<td>6.51</td>
<td>11.3</td>
</tr>
<tr>
<td>10</td>
<td>$a_1 r_1^2$</td>
<td>d^2/100 g</td>
<td>130</td>
<td>271</td>
<td>209</td>
</tr>
<tr>
<td>11</td>
<td>$a_2 r_2^2$</td>
<td>d^2/100 g</td>
<td>905</td>
<td>3340</td>
<td>11585</td>
</tr>
<tr>
<td>12</td>
<td>$100 f w dt$</td>
<td>d^2/100 g</td>
<td>1035</td>
<td>3701</td>
<td>11854</td>
</tr>
<tr>
<td>13</td>
<td>$w_m r_2(r_2 + t_m)$</td>
<td>d^2/100 g</td>
<td>272</td>
<td>1051</td>
<td>5116</td>
</tr>
<tr>
<td>14</td>
<td>$I_T, PR$</td>
<td>g/d</td>
<td>3.29</td>
<td>1.63</td>
<td>1.04</td>
</tr>
<tr>
<td>15</td>
<td>$t_p$</td>
<td>d</td>
<td>34.0</td>
<td>60.3</td>
<td>123.0</td>
</tr>
<tr>
<td>16</td>
<td>$M$</td>
<td>g</td>
<td>112.0</td>
<td>98.3</td>
<td>128.0</td>
</tr>
</tbody>
</table>

Initials identify patients; d = days.
The area integral, equation 22 (row 8), is the sum of contributions from the “fast” exponential (row 6) and the “slow” exponential (row 7). The contribution to the area integral from the exponential extrapolation, equation 27 (row 9), ranges from 3 to 12% of the area integral.

The moment integral, equation 23 (row 12), is the sum of contributions from the “fast” exponential (row 10) and the “slow” exponential (row 11). The contribution to the moment integral from the exponential extrapolation, equation 28 (row 13), ranges from 22 to 45% of the moment integral.

The area and the moment integrals from time zero to the maximum time of observation were also computed by numerical integration of the actual data points. The results did not differ significantly from those resulting from the exponential curve-fitting.

The total input rate, equation 24, is the same as the production rate of compartment analysis (10, 29). The present values (row 14), included for completeness, were previously derived by two-compartment analysis (11).

The mean transit time, equation 25 (row 15), ranged from 34 to 123 days. The effect of neomycin was to reduce it by 9, 44, 29 and 16% in the four patients studied.

The total traced mass, equation 26 (row 16), ranged from 73 to 128 g. The effect of neomycin was to reduce it by 38 g, 40 g, 32 g, and 24 g, that is, by 34, 40, 25 and 33% in the four patients studied.

Discussion

The assumptions of the present analysis are:

1. The system is in a steady state with respect to body cholesterol metabolism and distribution.
2. The system is not appreciably perturbed by, and responds linearly (first order) to, tracer cholesterol.
3. Both body cholesterol and tracer cholesterol are conserved between inlets and outlets.
4. The average specific activity of tracer cholesterol at exit from the system equals the specific activity of tracer cholesterol in the mixed venous plasma, equation 7.
5. At infinite time in a constant infusion experiment, that is, in the tracer steady state, the specific activity of tracer cholesterol is constant throughout the system, equation 16.
6. The exponential extrapolation of the plasma specific activity to infinite time is valid.

Assumption 1, that the system was in a steady state, was considered satisfied in the present experiments by the approximately constant serum cholesterol concentration, body weight, and clinical condition of the patients (11).

Assumption 2, that the system was negligibly perturbed and was linear with respect to tracer, is actually a definition of tracer. The dose levels of radioactive cholesterol in the experiment were considered sufficiently small to satisfy this assumption.

In contrast to assumption 2, it is important to emphasize an assumption that was not made. It was not assumed that the system behaved linearly with respect to body cholesterol (traced substance). Nosslin (30), for example, derived essentially equations 24, 25, and 26 by assuming that the system consisted of \( n \) compartments which were interconnected by first order (linear) exchange rates of traced substance respectively appears and disappears. Linearity assumption for traced substance, would invalidate the application of equations 24, 25, and 26 to many real biological systems. Bergner (26) has emphasized that equations 6 and 19 do not require the assumption of linearity with respect to traced substance, and the same applies to equation 11 (28).

Assumption 3, that tracer and traced substance were conserved, is actually a definition of the inlets and outlets of the system, namely, those locations where the substance respectively appears and disappears. The assumption is “satisfied” by explicitly recognizing where these inlets and outlets are and including all of them in the analysis. In the present application, the difference between the tracer inlet (venous blood) and the traced substance inlets (body cells and gastrointestinal tract) required that equation 11 rather than equation 17 be the starting
point of the analysis for total traced mass.

Assumption 4, that average outlet specific activity equaled plasma specific activity, is the basis of the well-known isotopic balance method for determining the total output rate of body cholesterol (12-21). The specific activity in the fecal end products increases from zero at time zero, crosses the plasma specific activity curve at about 1 week, and thereafter follows the plasma curve in the decay phase except that there may be a relative shift of several days between the two curves. These effects are in opposite directions and are individually of the order of 10 to 20% for the area integral (row 8, Table 1). The net effect on the area integral, hence on the total input rate (row 14) is difficult to estimate at the present time. The mean transit time (row 15) is too low, by approximately the shift of the fecal specific activity curve relative to the plasma specific activity curve in the final decay phase (approximately <5%).

The error in total traced mass (row 16) would be approximately the sum of the error in total input rate and the error in mean transit time. Assumption 4 can be investigated directly experimentally by measuring fecal specific activity simultaneously with plasma specific activity.

Assumption 5, that in the tracer steady state the specific activity was constant throughout the system, is independent of assumption 4 and is also different from the often made assumption (denoted*) that systemic constancy of specific activity occurs in the final monoeponential decay phase of a finite injection experiment. Assumption 5, but not, in general, assumption*, would be fulfilled exactly if the mode of injection is that of "equivalent tracer supply" (see Theory). This mode of injection defines, in the tracer steady state, a uniformly labeled mass of traced substance that is the total traced mass M in equation 11. The lack of fulfillment of assumption 5, that is, the value of \( \gamma_x - 1 \), could in principle be investigated directly experimentally by performing a constant infusion experiment using the same inlets as in the finite injection experiment. In the tracer steady state the specific activity is sampled in a set \( R \) of mass elements \( \Delta M_r \) contained within the system. The average specific activity over this set is

\[
c_{x,R} = \frac{\Sigma c_{x,R} \Delta M_r}{\Sigma \Delta M_r}
\]

where \( c_{x,R} \) is the specific activity in the mass element \( \Delta M_r \) situated at location \( r \). Division of this average specific activity by the average outlet specific activity, equation 13, or

\[
\gamma_x(R) = \frac{c_{x,R}(R)}{c_{x,K}}
\]

gives an approximation to the \( \gamma_x \) factor, equation 15. The difference between \( \gamma_x(R) \) and \( \gamma_x \) disappears if the sampled set \( R \) of mass elements coincides with the entire system. In this case one is measuring what one is trying to deduce (\( m_x \) and \( M \) in equation 12 are in effect being separately measured and equation 11 becomes a trivial identity for \( M \)). In actual cases, however, only a relatively few mass samples \( \Delta M_r \), for example, one in each type of body tissue, would suffice to determine \( \gamma_x \) to satisfactory approximation. The use of equation 11 is then not tautological. Assumption 5 might also be investigated more indirectly by comparing the plasma decay curves obtained with different modes of injection (blood, mouth, precursors). It appears plausible a priori that, because of the rapidity of the blood circulation, intravenous injection would not differ greatly from equivalent tracer supply, so that assumption 5 should not be seriously in error.

Assumption 6, that the final semilogarithmic downslope of the plasma decay curve can be extrapolated linearly indefinitely, can only be checked directly by continuing the experiment indefinitely. The plasma decay curve could conceivably flatten out after any given time in such a way as to produce independent and arbitrary increments to the area and to the mean transit time. After the original calculations were completed (11) it was found that in two of the present patients, observations had been continued in the control period beyond the time originally planned (31, Samuel and Perl, unpublished observations). In patient EA, extension of the time of...
observation from 32 to 50 weeks produced a decrease in total input rate of 9%, an increase in mean transit time of 8% and hence a decrease in total traced mass of 1%. A marked flattening of the plasma decay curve to a smaller down-slope occurred after 20 to 30 weeks. In patient MR, extension of the time of observation from 42 to 54 weeks produced a decrease in total input rate of 5%, an increase in mean transit time of 8% and hence a decrease in total traced mass of 4%. A flattening of the plasma decay curve after 20 to 30 weeks was suggested by the data. These results indicate that the present exponential extrapolation does not introduce serious error. The physical interpretation of a final monoexponential decay phase need not be that a compartment exists. As a counterexample, such a final decay phase also results from a diffusional process in which the specific activity is spatially variable at any given time (32).

In conclusion, we wish to emphasize the importance of distinguishing between deduced information that depends only on the area and the first time moment of the plasma decay curve, and deduced information that depends also on the more detailed shape of the plasma decay curve. The two-exponential curve fit of the present data which in the present paper was merely convenient for calculating the area and the first time moment, originally suggested a two-compartment analysis (10, 11). If all of the present assumptions 1 to 6 are made in the compartment analysis (in compartmental terminology where appropriate) then the present values of total input rate and total traced mass also result from the compartment analysis (the present total input rate is identical with the production rate of reference 10, the present total traced mass is identical with the sum of the two compartment masses in case 1 of reference 11). In addition, the two-compartment analysis yields intercompartmental exchange rates. Of the preceding quantities derived from two-compartment analysis, the exchange rates imply the existence of compartments, but the total input rate and the total traced mass do not imply the existence of compartments.

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References

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