# Measurement of cholesterol synthesis in kinetically defined pools using fecal steroid analysis and double labeling technique in man[1]

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**Abstract** The purpose of the study was to develop a kinetic method for measurement of different parameters of cholesterol metabolism in man using labeled cholesterol precursors that could initially be incorporated even into the slowly exchangeable cholesterol pool. For this purpose, tritiated water and [2-$^{14}$C]mevalonate were given to five normocholesterolemic subjects and the activities for serum cholesterol and body water were measured serially for up to eight weeks. Elimination of cholesterol was measured by fecal analysis of neutral and acidic steroids. For comparison, two subjects received a mixture of [4-$^{14}$C]cholesterol and [2-$^{3}$H]mevalonate.

The data were subjected to multicompartmental analysis by computer, with the assumption that synthesis occurred in two compartments. The rapidly exchangeable cholesterol (pool 1) and the fractional hydrogen transport constant from body water to cholesterol could not be measured directly; therefore, the influence of two different mass transport values was tested. The best fit was obtained with the smaller mass of cholesterol in pool 1 associated with a hydrogen transport constant of 0.700 (32 out of 46 hydrogens originate from water).

Kinetic analysis of the data allows estimates of the exchangeable cholesterol mass, flux rates of cholesterol between pools 1 and 2, and synthesis of cholesterol separately in the two pools. The results of computer analysis suggested that, in contrast to what has been assumed earlier on the basis of studies with radiolabeled cholesterol, 22–53% of endogenous cholesterol synthesis took place in pool 2 from body water and that this synthesis tended to correlate with the total body fat mass.

The study with [2-$^{3}$H]mevalonate and [4-$^{14}$C]cholesterol indicated synthesis in pool 2 to be 20–22% of the total. Up to 50% of the DL[2-$^{14}$C]mevalonate dose was incorporated into cholesterol. The fractional incorporation of DL-mevalonate into pool 2 was correlated with that of tritiated water, indicating that both precursors studied yielded essentially the same kinetic result.

**Supplementary key words** water as cholesterol precursor · mevalonate · multicompartmental analysis

Cholesterol synthesis in man has been measured either from the labeled plasma cholesterol disappearance curve after an intravenous administration of radioactive cholesterol (2–8) or from sterol balance studies (9–14). A close agreement has been observed between these two methods (9). The labeled plasma cholesterol disappearance curve has been subjected to either multicompartmental analysis (2–4, 8) or input–output analysis (5–7). The kinetic limitations of both methods when using prelabeled cholesterol as a marker substance have been repeatedly discussed. Owing to uncertainty concerning the sites of endogenous cholesterol input into the system, only minimum and maximum values of exchangeable cholesterol mass in the body can be calculated (2–4, 8). Input–output analysis is subject to the same limitations; in addition, no fractional transport rates between compartments are calculable when input–output analysis is applied (5–7). The prerequisite for use of prelabeled cholesterol in the above-mentioned kinetic approach is that all tracer cholesterol is initially found in a rapidly mixing pool comprising primarily the blood, liver, and gastrointestinal tract cholesterol. Erroneous estimates of the cholesterol mass in this pool would otherwise be incurred. So far, no validation has been presented for this prerequisite, and no detailed analysis of cholesterol kinetics has been performed in man after administration of small molecular precursors that could be incorporated into cholesterol even in a slowly exchangeable pool.

In the present study the problems of the site of endogenous cholesterol synthesis and cholesterol

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

[1] This work was presented in part at the Symposium on Regulation and Control in Physiological Systems sponsored by the International Federation of Automatic Control, the American Physiological Society, and the International Union of Physiological Science, August 1973, and published in the conference proceedings (1).

TABLE 1. Data of experimental subjects

| Subject | Sex | Age | Weight | Height | RBW[a] | Serum TG | Serum Cholesterol | Fat Tissue Mass | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | acc. to Moore et al. (23) | acc. to Francois (24) |
| | | | *kg* | *cm* | % | *mg/dl*[c] | *mg/dl*[c] | *kg* | |
| R.S. | F | 29 | 56 | 157 | 110 | 40 | 137 | 16.4 | 12.7 |
| P.Ö. | M | 54 | 69 | 170 | 100 | 131 | 174 | 17.1 | 12.9 |
| T.H. | F | 54 | 69 | 162 | 128 | 167 | 188 | 24.0 | 19.9 |
| T.H.[b] | M | 60 | 55 | 169 | 93 | 207 | 234 | 12.7 | 5.9 |
| V.W.[b] | M | 59 | 75 | 173 | 114 | 146 | 221 | 20.4 | 16.7 |
| V.N. | M | 68 | 78 | 176 | 114 | 122 | 170 | | |
| A.S. | M | 67 | 80 | 178 | 115 | 133 | 180 | | |

[a] RBW is the relative body weight or percent of ideal body weight.
[b] Subjects with coronary heart disease.
[c] Mean values during the experiment, 19 determinations in subjects R.S.–V.W., 15 in subject V.N., and 17 in subject A.S.

kinetics were investigated, using the collated information from sterol balance and labeled plasma cholesterol data. The latter were obtained by employing a double labeling technique with tritiated water and [2-$^{14}$C]-mevalonate as cholesterol precursors. As a kinetic check of the results, additional experiments were carried out using prelabeled [4-$^{14}$C]cholesterol and [2-$^{3}$H]mevalonate. The results reveal that a significant portion of endogenous cholesterol synthesis occurs outside the rapidly mixing cholesterol pool.

## METHODS

### Patients

Seven adult subjects were studied (**Table 1**). Five of them were ambulatory during the experiment and received a tracer mixture of tritiated water and [2-$^{14}$C]-mevalonate for labeling their cholesterol pools. Two of them, T.H. and V.W., had 3 years earlier experienced a myocardial infarction. They had no angina or heart failure and were in good clinical condition. After the myocardial infarction both had altered their diet, which was low in cholesterol and animal fat during the study. Three other subjects were cardiovascularly healthy and were on their regular diet during the study. During the experimental period of 8 weeks, no significant change was found in body weight, serum cholesterol, or triglycerides of these five subjects, indicating that they were in a steady state in regard to cholesterol metabolism.

The remaining two subjects (V.N. and A.S.) were hospitalized because of cerebral infarction and were in the hospital during the study. [4-$^{14}$C]Cholesterol and [2-$^{3}$H]mevalonate were injected intravenously four weeks after the initial attack, when the patients had recovered from the stroke and were clinically in a steady state. They were on a regular ad libitum hospital diet moderately low in cholesterol (ca. 300 mg/2400 kcal). Though serum cholesterol and triglycerides were stable the patients tended to lose weight; the loss was 1 kg for V.N. during the experimental period of 38 days and almost 2 kg for A.S. during the experimental period of 64 days. Patient V.N. died suddenly from a recurrence of infarction on the 42nd day, and the study could not be completed for this reason. The purpose and design of the study were explained in detail to all subjects and they all volunteered for it.

### Experimental procedures

The study was started by giving to the five ambulatory patients 8 mCi of tritiated water orally and 50 $\mu$Ci of [2-$^{14}$C]mevalonate intravenously (both from the Radiochemical Centre, Amersham, England). Since a fairly large amount of tritiated water was expected to be used in order to have sufficient counts in cholesterol, only volunteers were studied and the number of subjects was limited to five.[2] Blood samples were drawn serially during the experimental period of 8 weeks for lipid and radioactivity measurements. The latter indicated that the tritium dose could have been reduced to 3 mCi. A small sample of urine was obtained simultaneously with the blood to measure the specific activity of tritium in the body water pool. One 3-day stool collection was performed on each of the five subjects 6 weeks after the administration of the isotopic precursors in order to measure elimination of radioactive cholesterol and cholesterol mass by the fecal route. Fecal flow (15) and possible degradation of cholesterol during intestinal passage (16) were checked with the aid of $Cr_2O_3$ and $\beta$-sitosterol,

[2] Calculations revealed that the dose of tritiated water corresponded to 8–14 rads.

respectively. For this purpose 600 mg of both $Cr_2O_3$ and β-sitosterol (both obtained from Orion Oy, Helsinki, Finland) were given daily to the subjects, starting one week before the collection. In our unpublished studies from seven healthy subjects on a solid food diet, double determinations of fecal steroids yielded a mean error of 4.2%, indicating that the actual error from a single collection was small.

To demonstrate possible differences between the kinetics of cholesterol synthesized endogeneously from mevalonate and of that injected intravenously as cholesterol, 50 μCi of [4-$^{14}$C]cholesterol (The Radiochemical Centre, Amersham, England) and 100 μCi of [2-$^{3}$H]mevalonate were intravenously administered to the two remaining patients. For this purpose [4-$^{14}$C]cholesterol was purified by thin-layer chromatography and dissolved in sterile pyrogen-free ethanol, which was diluted before intravenous injection with sterile saline to give a 20% ethanolic solution. Blood samples were obtained serially from V.N. up to the 38th day and from A.S. up to the 64th day after administration of the precursors. A 3-day stool collection was performed from A.S. after the seventh experimental week using $Cr_2O_3$ and β-sitosterol as internal unabsorbable markers.

### Chemical analysis

Serum cholesterol was measured by the method of Pearson, Stern, and McGawack (17) from a petroleum ether extract prepared according to Abell et al. (18). Specific activity of serum cholesterol was measured, however, after the cholesterol fraction of the petroleum ether extract had been purified by TLC. For this purpose silica gel G plates (0.5 mm thick) were used and the plates were developed in ethyl ether–heptane 55:45. TLC purification was necessary in the studies in which $^{3}H_2O$ was used, because the measurement of the specific activity of [$^{3}$H]cholesterol directly from the petroleum ether extract gave slightly but significantly higher values than after the TLC purification. Further purification appeared unnecessary because digitonin precipitation had no additional effect on the specific activity. Serum triglycerides were measured according to Carlson (19).

Fecal acidic and neutral steroids were measured by TLC gas–liquid chromatographic methods (20, 21) and $Cr_2O_3$ according to Bolin, King, and Klosterman (22). Gas–liquid chromatographic determination of cholesterol gave identical results with the method of Pearson et al. (17), indicating that mass measurements and specific activities of fecal and plasma sterols were comparable. Since the recovery of administered β-sitosterol from the stools was complete, no degradation of neutral sterols had apparently occurred and therefore the results of fecal steroids were expressed in relation to $Cr_2O_3$.

In the studies in which $^{3}H_2O$ had been used as the precursor of the cholesterol, digitonin precipitation insignificantly reduced the specific activity of $^{3}$H-labeled sterols in the fecal steroid method. In the case of bile acids, however, the specific activity of total bile acids isolated in bulk by TLC for GLC analysis tended to be higher than those of deoxycholic acid and lithocholic acid (isolated separately by TLC). This difference was not seen with $^{14}$C-labeled bile acids synthesized from [4-$^{14}$C]cholesterol after administration of [2-$^{14}$C]mevalonate, suggesting that the total bile acid fraction contained some tritiated impurities. These probably emerged in peaks occurring before 5α-cholestane in the GLC run. The isolated deoxycholic acid and lithocholic acid (containing isolithocholic acid) fractions, which comprised about 95% of the total bile acids, had the same specific activities and gave single peaks in GLC. In addition, the specific activities of the two bile acids were identical with the serum cholesterol in the beginning of the 3-day stool collection. Thus, the fractions were considered to be reasonably pure. Since deoxycholate was the major fraction, the total radioactivity of $^{3}$H in the acidic steroid fraction was obtained by multiplying the specific activity of deoxycholate by the amount of total fecal bile acids quantitated in bulk by GLC.

The specific activity of $^{3}$H in the body water was measured from the urine samples. For this purpose urine was distilled and a small portion was used for radioactivity measurements. Radioactivities of the water, serum cholesterol, and fecal steroids were counted with a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL) with an external standard for the correction of quenching.

### Definition and determination of different parameters

For the analysis it was necessary to define and determine a relatively large number of different parameters in each subject. They were obtained from primary data determined in the present investigation or calculated according to information found in the literature, alone or in combination with the present data. The multicompartment model that was used as a base for the kinetic analysis of cholesterol metabolism is presented in **Fig. 1.** The following parameters were required.

TBW Total body water (kg) was measured by extrapolating the specific activity of the body $^{3}$H-labeled water curve to the zero point. The intercept was

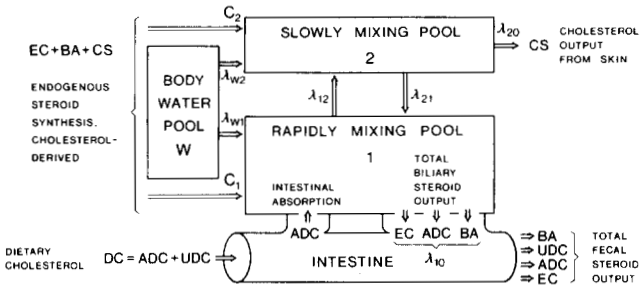


**Fig. 1.** Three-compartment model used in computer simulations. Flux, cholesterol mass, and bile acid mass symbols are defined in Methods.

considered to represent the specific activity of $^3H_2O$ at zero time (equals TBW) in which the administered dose of 8 mCi $^3H_2O$ water was distributed. (Subscript $w$ is used to define TBW-pool in λ- and ρ-notations.)

TBWH Total body water hydrogen = 0.111 × TBW (kg).

TBF Total body fat mass (kg). Two methods were used to calculate the total fat mass:

I. Using formulas given by Moore et al. (23): Fat-free body mass (FFB) = TBW/0.732, TBF = body weight − FFB.

II. Using an empirical formula, derived for the best fit of the data by François (24). When the plot of body weight vs. TBF of his data was analyzed, a suitable general equation was found to be:

$$y = k(x - a)^2$$

(This equation gave a significantly better fit with the data than, for instance, a simple square law.) The constants for a series of women (14 subjects with body weights of 40–99 kg) were $k = 0.0047$ and $a = 5.04$, with the abscissas (body weight) and ordinates (TBF) plotted in kg; $r = 0.963$. The corresponding constants for males (16 subjects with body weights 40–99 kg) were $k = 0.00677$, $a = 25.37$; $r = 0.921$. When analyzing the possible effect of the height of François' test subjects on the deviations from the derived curve, no significant correlation was found between deviation from mean height and deviation of actual calculated TBF from the value read from the curve ($r = 0.24$). The regression line deviated little from the horizontal (indicating that an approximate 10 cm increment from the mean height would cause overestimation of TBF by 1 kg) and therefore the effect of height in the calculation was ignored.

$M_1$ Cholesterol mass (g) in rapidly exchangeable pool 1. Two methods were used to calculate the pool 1 cholesterol mass.

I. Cholesterol of the blood, liver, and intestine is considered to form pool 1 (Moore et al. (25)). In this method hepatic and intestinal cholesterol are estimated according to necropsy data from the literature (72.9 and 54.3 mg/kg, respectively (26)). Serum and red cell cholesterol were directly determined from serum and packed red cells. Knowing the plasma volume (4.5% of body weight) and hematocrit, the cholesterol content of the blood compartment could be calculated.[3]

II. To correct the above-mentioned pool 1 mass values to be used with the mean pool 1 mass values reported by Goodman et al. (**Fig. 2**), both sets of pool 1 mass values were converted to body weight units (kg). The mean values of the control subjects of Goodman et al. were compared to values obtained according to Moore et al. (25) by using the line derived from our values (Fig. 2). The latter were on an average 11.5% lower than those of Goodman, Noble, and Dell (8).

[3] It should be borne in mind that pools 1 and 2 in cholesterol metabolism are kinetic parameters and can not be defined exactly anatomically since, for instance, the liver and intestine can include small pool 2 compartments, and rapidly exchangeable cholesterol (pool 1) is found in many extravisceral tissues.

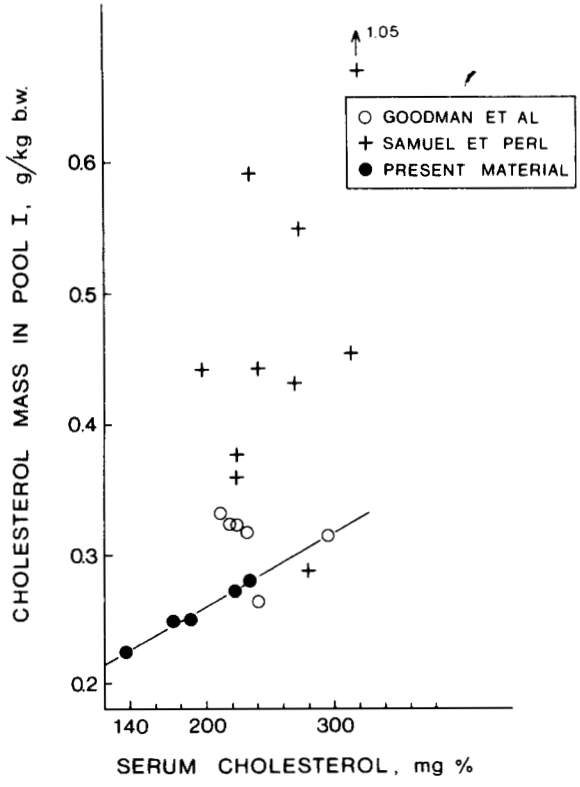


**Fig. 2.** Plot of cholesterol mass in pool 1 against serum concentration. Present material and data from studies of Samuel and Perl (6) and Goodman et al. (8).

Cholesterol of the blood compartment was measured in our patients. Therefore, the tissue compartment of $M_1$ in the present series was 22.5% smaller than in that of Goodman et al. To have the two series comparable this correction was made for the pool 1 mass of patients, giving the second estimate of $M_1$. In cases A.S. and V.N., $M_1$ was calculated from the dilution of the [4-$^{14}$C]cholesterol administered in plasma at zero point according to Goodman and Noble (2).

$M_1H$ Hydrogen (g) of the pool 1 cholesterol = $0.1198 \times M_1$.

TS Fecal total steroids (mg/day) determined in each case (except for subject V.N.) by the procedure presented in the Methods sections of Ref. 20 and 21. It includes, in terms of mg/day, fecal bile acids plus fecal neutral steroids of cholesterol origin.

NS Fecal neutral steroids (mg/day) of cholesterol origin. This fraction includes cholesterol synthesized within the body, dietary unabsorbed cholesterol and dietary absorbed cholesterol re-excreted as fecal neutral steroids.

BA Fecal bile acids (mg/day) determined in each case with the TLC–GLC method. They originate from cholesterol synthesized within the body and from dietary absorbed cholesterol.

$\rho_{20}$ Removal of cholesterol via the skin. This was assumed to be constant in every case and the value of 83 mg/day obtained from the study by Bhattacharyya, Conner, and Spector (27) was used for this purpose. As illustrated in Fig. 1, this removal was assumed to take place from the slowly mixing pool 2 and therefore it has been denoted by $\rho_{20}$ in the kinetic analysis.

ENS Fecal neutral steroids of endogenous origin (mg/day). They were determined (9) in each subject by dividing the daily radioactivity of the fecal neutral steroid fraction in the three-day stool collection by the specific activity of serum cholesterol at the beginning of the stool collection period. This fraction includes endogenously synthesized cholesterol and absorbed dietary cholesterol re-excreted as fecal neutral steroids.

UDC Unabsorbed dietary cholesterol (mg/day) = NS − ENS.

ADC Absorbed dietary cholesterol (mg/day). This was calculated assuming, on the basis of the results in the literature (14, 28–30), that the absorption was 35%. Then 35/65 × UDC equals the absorbed cholesterol in terms of mg/day.

EC Fecal steroids derived from cholesterol synthesis (mg/day) = ENS + BA − ADC.

$\rho_{10}$ Fecal total steroids of endogenous origin (mg/day), which is the sum of BA and ENS. In subject V.N., from whom no fecal sample was available, $\rho_{10}$ was assumed to be identical with that in subject A.S., especially since the body weights, obesity, and serum cholesterol concentrations of these patients were identical.

PR Cholesterol turnover (mg/day) is the sum of $\rho_{20}$ and $\rho_{10}$.

PRE Endogenous cholesterol production (mg/day) is the difference between PR and ADC.

HT Hydrogen transport (mg/day) to endogenously synthesized cholesterol = $0.1198 \times$ PRE.

HTW Hydrogen transport (mg/day) from the body water to PRE. The determination of this parameter implies that the number of water-derived hydrogens among the hydrogen molecules in endogenously synthesized cholesterol molecules is known. Two different numbers were used as follows.

I. The constant 0.543 was derived from the work by Loud (31) and Loud and Bucher (32) in which it was shown that at least 25 out of 46 hydrogen atoms of the cholesterol molecule originate from water. Accordingly HTW would be $0.543 \times$ HT.

II. Re-analysis of human data on incorporation of heavy water deuterium into serum cholesterol by Taylor et al. (33) in two patients constitutes the basis for the second constant. Their experimental design (daily oral intake of heavy water) was actually one of constant infusion into a multicompartment system. Therefore an analysis was undertaken to resolve their data into exponential components using the two pool model of cholesterol kinetics as the basis of the analysis. In this procedure any values clearly deviating from the curve were disregarded (3 out of 21 points were removed for both subjects), as otherwise no analysis with a reasonable degree of accuracy would have been possible. The remaining data points were clearly resolvable into the exponential components according to the two pool model ($r$ = 0.99 for Subject 1 and 0.97 for Subject 2). At the end of the experiments the curves did not yet approach their asymptotes. Two different analyses were therefore performed: *1*) a straightforward best-fit analysis of the data; and *2*) using for preassigned $\lambda_{10}$ the mean of our healthy subjects, 0.043 (Table 6). The results of both analyses agreed completely with Subject 2 of Taylor et al. (33), but less well with their Subject 1. However, the means of both were close to 0.700, indicating that 32 out of 46 hydrogens of cholesterol originated from body water. To find which one of the two constants

would give the better fit in our kinetic analysis, two HTW values were tested: 0.543 × HT and 0.700 × HT.

$M_2$ Mass of slowly exchangeable cholesterol pool (g). This could be calculated applying the mass flow identity formula

$$\rho_{12} = \rho_{21} + \rho_{20} - \rho_{w2}$$

$M_t$ Mass of total exchangeable cholesterol pool of the body (g), equaling $M_1 + M_2$.

$C_1$ Fraction of [2-$^{14}$C]mevalonate (% of dose) incorporated into cholesterol in pool 1.

$C_2$ Fraction of [2-$^{14}$C]mevalonate (% of dose) incorporated into cholesterol in pool 2. $C_1$ and $C_2$ were obtained from the hybrid computer solution, and the incorporation in pool 1 as well as in pool 2 was assumed to take place almost instantaneously, with no recirculation of label.

$C_{1\%}$ Proportion of [2-$^{14}$C]mevalonate incorporated into cholesterol in pool 1, in percent of total [2-$^{14}$C]mevalonate incorporation in cholesterol in pools 1 and 2, calculated from the formula

$$100 \times C_1/(C_1 + C_2)$$

$C_{2\%}$ Proportion of [2-$^{14}$C]mevalonate incorporated into cholesterol in pool 2, in percent of total [2-$^{14}$C]mevalonate incorporation in cholesterol in pools 1 and 2, calculated from the formula

$$100 \times C_2/(C_1 + C_2)$$

$\lambda_{21}$ Fractional daily transport of cholesterol from pool 2 to pool 1.

$\lambda_{12}$ Fractional daily transport of cholesterol from pool 1 to pool 2. Both $\lambda_{21}$ and $\lambda_{12}$ are obtained from the computer solution and they were assumed to be identical in the synthesis both from [2-$^{14}$C]mevalonate and from $^3H_2O$.

$\lambda_{10}$ Fractional daily rate of cholesterol removal from pool 1 out from the body. This value was calculated to the computer solution by dividing $\rho_{10}$ by $M_1$.

$\lambda_{20}$ Fractional daily rate of cholesterol removal from pool 2 directly out from the body. This parameter was bound to the fixed $\rho_{20}$ value (elimination of blood cholesterol through the skin) in the computer program.

$\lambda_{w1}$ Fractional daily incorporation of body water hydrogen into cholesterol in pool 1.

$\lambda_{w2}$ Fractional daily incorporation of body water hydrogen into cholesterol in pool 2. $\lambda_{w1}$ and $\lambda_{w2}$ were obtained from the computer solution after the sum of $\lambda_{w1} + \lambda_{w2}$ had been precalculated for each subject by dividing HTW by TBWH.

$\lambda_{w1\%}$ Proportion of $^3$H incorporated into cholesterol in pool 1, in percent of total hydrogen incorporation from $^3H_2O$ into cholesterol in pools 1 and 2. This was obtained from the formula

$$(\lambda_{w1} \times 100)/(\lambda_{w1} + \lambda_{w2})$$

$\lambda_{w2\%}$ Proportion of $^3$H incorporated into cholesterol in pool 2, in percent of total hydrogen incorporation from $^3H_2O$ into cholesterol in pools 1 and 2. This was obtained from the formula

$$(\lambda_{w2} \times 100)/(\lambda_{w1} + \lambda_{w2})$$

$\lambda_{w0}$ Fractional daily removal of water from the body (the pathway is not illustrated in Fig. 1). This was obtained from the decay of $^3H_2O$ in the body water.

$PRE_1$ Endogenous cholesterol production in pool 1 (mg/day). This was separately calculated for mevalonate and for BWH using the respective formulas $C_1/(C_1 + C_2) \times PRE$, and $\lambda_{w1}/(\lambda_{w1} + \lambda_{w2}) \times PRE$.

$PRE_2$ Endogenous cholesterol production in pool 2 (mg/day). This was separately calculated for mevalonate and BWH using the respective formulas $C_2/(C_1 + C_2) \times PRE$, and $\lambda_{w2}/(\lambda_{w1} + \lambda_{w2}) \times PRE$.

Four series of computer resolutions were performed using the following mass and fractional $^3$H-labeled water incorporation constants.

| | | |
|---|---|---|
| Series I. | $M_1$ acc. Moore et al. (25) | HTW = 0.543 × HT |
| Series II. | $M_1$ acc. Moore et al. (25) | HTW = 0.700 × HT |
| Series III. | $M_1$ corr. acc. Goodman et al. (8) | HTW = 0.543 × HT |
| Series IV. | $M_1$ corr. acc. Goodman et al. (8) | HTW = 0.700 × HT |

In experiments with [4-$^{14}$C]cholesterol and DL[2-$^3$H]mevalonate as sources of labeled plasma cholesterol, $M_1$ was calculated from the dilution of plasma [4-$^{14}$C]cholesterol at zero point and $\lambda_{10}$ in test subject A.S. as described earlier; in subject V.N. $\lambda_{10}$ was considered to be identical to that in subject A.S. $C_1$, $C_2$, $\lambda_{20}$, $\lambda_{21}$ and $\lambda_{12}$ were handled in a similar manner as described above.

## Computer solution

As indicated earlier and also shown in Fig. 1, metabolic and kinetic steady states were assumed to

exist for cholesterol in the patients studied. Then the flow is related to mass and decay rates by

$$\rho_{ji} = \lambda_{ji} m_j \qquad \text{Eq. 1}$$

The steady state assumption gives the following equilibrium equations:

$$\rho_{w1} + \rho_{12} + \text{ADC} = \rho_{21} + \rho_{10} \qquad \text{Eq. 2}$$

$$\rho_{w2} + \rho_{12} = \rho_{21} + \rho_{20} \qquad \text{Eq. 3}$$

The metabolism could be described with the state variables

$x_1$, normalized $^3$H activity of cholesterol in pool I
$x_2$, normalized $^3$H activity of cholesterol in pool II
$x_3$, normalized $^3$H activity of body water
$x_4$, normalized $^{14}$C activity of cholesterol in pool I
$x_5$, normalized $^{14}$C activity of cholesterol in pool II.

The equation for the components is then of the form

$$\dot{x}_i = -x_i \sum_{k \neq i} \lambda_{ki} + \sum_{k \neq i} \lambda_{ik} x_k \qquad \text{Eq. 4}$$

and the state equation could be written

$$\dot{x} = \mathbf{A}(\lambda) x \qquad \text{Eq. 5}$$

where $\mathbf{A}$ is a matrix function of the decay rates $\lambda$.

$$\mathbf{A}(\lambda) = \begin{Bmatrix} -(\lambda_{12} + \lambda_{10}) & \lambda_{21} & \lambda_{w1} & 0 & 0 \\ \lambda_{12} & -(\lambda_{21} + \lambda_{20}) & \lambda_{w2} & 0 & 0 \\ 0 & 0 & -\lambda_{w0} & 0 & 0 \\ 0 & 0 & 0 & -(\lambda_{12} + \lambda_{10}) & \lambda_{21} \\ 0 & 0 & 0 & \lambda_{12} & -(\lambda_{21} + \lambda_{20}) \end{Bmatrix}$$

The solution of this system is

$$x(t) = \exp(\mathbf{A}(\lambda) t)\, x(0) \qquad \text{Eq. 6}$$

$$\exp(\mathbf{A}t) = \mathrm{I} + t\mathbf{A} + t^2\mathbf{A}^2/2! + t^3\mathbf{A}^3/3! + \ldots \qquad \text{Eq. 7}$$

The initial state $x(0) = (0,0,1,C_1,C_2)$ is obtained from the arrangement of the experiment. Because some amount of the $^{14}$C intake immediately leaves the organism, the relation $C_1 + C_2 < 1$ is valid.

Denote the measured quantities

$z_1$ measured $^3$H activity of blood cholesterol
$z_2$ measured $^{14}$C activity of blood cholesterol
$z_3$ measured $^3$H activity of urine

The measurements are then related to the system by

$$z = y + n \qquad \text{Eq. 8}$$

where $n \in \mathbb{R}^3$ is the noise corrupting the measurements. The noise is coming from measurement errors. $\mathbb{R}$ is the set of real numbers. $y$ is defined through the output relation

$$y = \mathbf{C}x. \qquad \text{Eq. 9}$$

$$\mathbf{C} = \begin{Bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{Bmatrix}$$

It can be shown that the system represented by Equations 5 and 9 is observable.

The measurement could be written as a vector valued N-tuple

$$\mathcal{M} = (z(s(1)\tau), \ldots, z(s(\mathrm{N})\tau)) \qquad \text{Eq. 10}$$

and a simulation run with known decay rates and known initial state as

$$\zeta = (y(s(1)\tau), \ldots, y(s(\mathrm{N})\tau)) \qquad \text{Eq. 11}$$

where $s$: $\mathbb{N} \rightarrow \mathbb{N}$ is the sampling function ($\mathbb{N}$ is the set of natural numbers). The $i$th measured point was at the time instant $s(i)\tau$ where $\tau$ is the unit sampling interval. Eq. 12

The problem could now be stated: determine the decay rates and the initial state so that $\zeta$ is as close to $\mathcal{M}$ as possible. This problem could be solved with standard methods for nonlinear programming, when a suitable objective function is defined. In this case the sum of squared relative errors that was used, defined the functions $f_1, f_2$ and $f_3$ as

$$f_k(\mathcal{M},\zeta) = \sum_{i=1}^{\mathrm{N}} \left[ \frac{z_k(s(i)\tau) - y_k(s(i)\tau)}{y_k(s(i)\tau)} \right]^2. \qquad \text{Eq. 13}$$

The minimization of $f_3$ can be separated from the minimization of $f_1$ and $f_2$. The best fit can then be obtained by first minimizing $f_3$ and then the sum $f_1 + f_2$.

A hybrid computer solution of the problem is economically advantageous. The nonlinear programming method used was an accelerated random creep. Because the value of the objective function varies randomly, a sequential decision test was used to decide whether a new point was better than the old one.

The decay-rate $\lambda_{20}$ corresponding to the known flow $\rho_{20}$ (27) can be calculated from the equilibrium equation (Eq. 3). To get the sum $\lambda_{w1} + \lambda_{w2}$ within the calculated limits, a penalty function technique was used. The penalty function $p$ : $\mathbb{R}^2 \rightarrow \mathbb{R}$ was defined as

$$p(\lambda_{w1},\lambda_{w2}) = \text{abs}\left(\lambda_{w1} + \lambda_{w2} - \frac{\text{HTW}}{\text{TBWH}}\right)$$

TABLE 2. Different parameters of cholesterol metabolism calculated and/or measured before computer analysis

| | Subject | | | | | |
|---|---|---|---|---|---|---|
| | R.S. | P.Ö. | T.H. female | T.H. male | V.W. | Mean ± SEM |
| $M_1$, g (Series I + II)[a] | 12.54 | 17.18 | 17.31 | 15.40 | 20.42 | 16.57 ± 1.29 |
| $M_1$, g (Series III + IV) | 13.92 | 19.07 | 19.21 | 17.09 | 22.67 | 18.39 ± 1.43 |
| NS, mg/day | 504 | 848 | 1142 | 389 | 631 | 703 ± 134 |
| BA, mg/day | 150 | 198 | 90 | 78 | 133 | 130 ± 22 |
| ADC, mg/day | 69 | 205 | 177 | 62 | 87 | 120 ± 30 |
| UDC, mg/day | 129 | 380 | 328 | 115 | 162 | 223 ± 55 |
| ENS, mg/day | 375 | 468 | 814 | 274 | 469 | 480 ± 91 |
| PR, mg/day | 608 | 749 | 987 | 435 | 685 | 693 ± 90 |
| PRE, mg/day | 539 | 544 | 810 | 373 | 598 | 573 ± 70 |
| $\lambda_{10}$, $day^{-1}$ (Series I + II) | 0.0419 | 0.0388 | 0.0522 | 0.0229 | 0.0295 | 0.0371 ± 0.0051 |
| $\lambda_{10}$, $day^{-1}$ (Series III + IV) | 0.0362 | 0.0349 | 0.0471 | 0.0206 | 0.0266 | 0.0331 ± 0.0045 |
| TBWH, kg | 3.36 | 4.74 | 3.97 | 3.95 | 5.02 | 4.21 ± 0.30 |
| $(\lambda_{w1} + \lambda_{w2}) \times 10^5$, $day^{-1}$ (Series I + III) | 1.04 | 0.75 | 1.33 | 0.62 | 0.77 | 0.90 ± 0.13 |
| $(\lambda_{w1} + \lambda_{w2}) \times 10^5$, $day^{-1}$ (Series II + IV) | 1.34 | 0.97 | 1.86 | 0.80 | 0.99 | 1.19 ± 0.19 |

[a] Series I: $M_1$ acc. Moore et al. (25) HTW = 0.543 × HT
Series II: $M_1$ acc. Moore et al. (25) HTW = 0.700 × HT
Series III: $M_1$ corr. acc. Goodman et al. (8) HTW = 0.543 × HT
Series IV: $M_1$ corr. acc. Goodman et al. (8) HTW = 0.700 × HT

Abbreviations: $M_1$, cholesterol mass in pool 1; NS, fecal neutral steroids of cholesterol origin; BA, fecal bile acids; ADC, absorbed dietary cholesterol; UDC, unabsorbed dietary cholesterol; ENS, fecal neutral steroids of endogenous origin; PR, cholesterol turnover; PRE, endogenous cholesterol production; $\lambda_{10}$, fractional rate of cholesterol removal from pool 1; TBWH, total body water hydrogen; $\lambda_{w1} + \lambda_{w2}$, fractional incorporation of body water hydrogen into cholesterol in pool 1 and 2. See section on definition and determination of parameters in text.

and was incorporated in the function to be optimized with

$$f = f_1 + f_2 + \alpha p$$

where $\alpha$ is a real number that was decreased to zero at the end of the optimization.

To utilize the information contained in the experiment, not only optimum but also second order behavior should be calculated. This was done by estimating the hessian matrix of second order partial derivatives. Large eigenvalues of the hessian then characterize sensitive directions in the parameter space and small eigenvalues insensitive ones. These directions are given by the eigenvectors corresponding to the eigenvalues. For references for the system theoretical approach and the solution method see Ref. 1, 34–36.

## RESULTS

### Sterol balance data

The data based on sterol balance and body water determinations are presented in **Table 2**. The average mass of cholesterol in pool 1 was 16.57 g by the method of Moore et al. (25) and 18.39 g after correction to the data of Goodman et al. (8). The mean cholesterol turnover (including fecal and skin output) was 693 mg/day and the endogenous cholesterol synthesis 573 mg/day. Cholesterol absorption was calculated to be 120 mg/day, indicating that at 35% absorption the dietary intake had been 343 mg/day, of which 223 mg/day was unabsorbed and excreted in the stools as neutral sterols. The latter amounted to 703 mg/day. The cholesterol transformation into bile acids accounted for 130 mg/day. Fecal total steroids of endogenous origin were 610 mg/day, indicating that the fractional daily turnover of cholesterol in pool 1 was 0.0371 or 0.0329 with the smaller (16.57 g) or larger (18.39 g) size of the pool, respectively.

### Hydrogen transport

The mean of body water hydrogen transported to cholesterol synthesis was 37.2 mg/day when the smaller constant 0.543 (25 out of 46 hydrogens derived from water) was used, and 49.2 mg/day with the constant 0.700.

### Validation of computer analyses

The computer resolutions made it possible to evaluate the validity of the assumptions made in the calculations concerning pool 1 masses and hydrogen transport constants. The "best fit" with the experimental data could be assessed from the lowest single objective function value in each set of runs (not less

TABLE 3. Computer solution values obtained with different $M_1$ and hydrogen transport constants

| Subject | Exp. Code[a] | $C_1$ | $C_2$ | $\frac{100 \times C_2}{C_1 + C_2}$ | $\lambda_{w1} \times 10^5$ day$^{-1}$ | $\lambda_{w2} \times 10^5$ day$^{-1}$ | $\frac{100 \times \lambda_{w2}}{\lambda_{w1} + \lambda_{w2}}$ | $\lambda_{12}$ day$^{-1}$ | $\lambda_{21}$ day$^{-1}$ | Objective Function |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *% of dose* | | | | | | | | |
| R.S. | I | 8.5 | 3.0 | 25.8 | 0.958[a] | 0.205[a] | 17.6 | 0.0747 | 0.0230 | 2.47 |
| | II[b] | 8.5 | 4.9 | 36.5 | 0.955 | 0.390 | 29.0 | 0.0759 | 0.0172 | 2.33 |
| | III | 9.7 | 3.3 | 25.4 | 1.048 | 0.033 | 3.0 | 0.0798 | 0.0209 | 2.68 |
| | IV | 9.7 | 1.3 | 12.0 | 1.140 | 0.301 | 20.9 | 0.0760 | 0.0281 | 2.09 |
| P.Ö. | I | 5.6 | 2.3 | 29.1 | 0.762 | 0.091 | 10.7 | 0.0795 | 0.0220 | 7.27 |
| | II[b] | 5.6 | 3.2 | 36.6 | 0.753 | 0.208 | 21.6 | 0.0793 | 0.0177 | 7.15 |
| | III | 6.2 | 3.1 | 33.3 | 0.844 | 0.166 | 16.5 | 0.0835 | 0.0187 | 8.02 |
| | IV | 6.2 | 2.4 | 28.3 | 0.821 | 0.120 | 12.5 | 0.0820 | 0.0204 | 7.45 |
| T.H. female | I | 7.7 | 7.4 | 49.1 | 0.866 | 0.460 | 34.7 | 0.0538 | 0.0153 | 1.61 |
| | II[b] | 7.7 | 9.7 | 55.7 | 0.851 | 0.883 | 50.9 | 0.0508 | 0.0110 | 1.65 |
| | III | 8.6 | 5.6 | 39.4 | 0.940 | 0.398 | 29.7 | 0.0559 | 0.0170 | 1.70 |
| | IV | 8.6 | 15.1 | 63.8 | 0.947 | 0.761 | 44.6 | 0.0599 | 0.0085 | 2.05 |
| T.H. male | I | 6.8 | 1.0 | 13.3 | 0.642 | 0.083 | 11.4 | 0.0520 | 0.0200 | 4.75 |
| | II[b] | 6.8 | 3.5 | 34.2 | 0.638 | 0.193 | 23.2 | 0.0563 | 0.0137 | 4.64 |
| | III | 7.5 | 1.5 | 16.9 | 0.755 | 0.190 | 20.1 | 0.0873 | 0.0216 | 6.29 |
| | IV | 7.5 | 0.8 | 10.0 | 0.741 | 0.064 | 7.9 | 0.0876 | 0.0238 | 5.64 |
| V.W. | I | 10.6 | 9.5 | 47.4 | 0.457 | 0.320 | 41.2 | 0.0332 | 0.0084 | 3.72 |
| | II[b] | 10.6 | 11.4 | 52.0 | 0.469 | 0.530 | 53.1 | 0.0328 | 0.0070 | 3.44 |
| | III | 11.6 | 7.1 | 38.1 | 0.506 | 0.254 | 33.4 | 0.0217 | 0.0095 | 4.32 |
| | IV | 11.6 | 13.1 | 53.1 | 0.506 | 0.494 | 49.4 | 0.0325 | 0.0061 | 4.15 |

[a] See footnote *a* to Table 2.

[b] Data of Series II were obtained with the best computer fit and are considered as final results.

Abbreviations: $C_1$ and $C_2$, fraction of [2-$^{14}$C]mevalonate incorporated into cholesterol in pools 1 and 2; $(100 \times C_2)/(C_1 + C_2)$, proportion of [2-$^{14}$C]mevalonate incorporated into cholesterol in pool 2; $\lambda_{w1}$ and $\lambda_{w2}$, fractional incorporation of body water hydrogen into cholesterol in pools 1 and 2; $(100 \times \lambda_{w2})/(\lambda_{w1} + \lambda_{w2})$, proportion of $^3$H incorporated into cholesterol in pool 2; $\lambda_{12}$ and $\lambda_{21}$, fractional transport of cholesterol from pool 1 to pool 2 and from pool 2 to pool 1, respectively.

than five). The differences were small on the whole (**Table 3**). The decreasing order of best fit II–I–IV–III was apparent because none of the interseries differences was equivalent to less than a ratio of 4:1 in level (lower: higher objective function). The consistency with a preassigned $\lambda_{w1} + \lambda_{w2}$ value, and its fluctuations around the mean in individual runs, is a test for closest fit with net flux assumptions and of best overall convergence of the computer resolutions. The results (**Fig. 3**) showed that series II and IV fit the expected $\lambda_{w1} + \lambda_{w2}$ very closely, while series I and III tend to display too high values. This implies that the fraction of water-derived hydrogen molecules in the cholesterol molecule is above 0.543 in man. The fact that the variations of individual runs were smallest in series II is obviously an indication of the tendency to yield an unambiguous resolution. With these parameters the computer resolution was within 2% limits of the precalculated $\lambda_{10}$ and $\lambda_{w1} + \lambda_{w2}$. That means that the model approximates the actual behavior of the system and conclusions about the actual system could be drawn from the model. Thus, the hydrogen transport constant 0.700 (32 out of 46 hydrogens of cholesterol originated from the water) and the smaller $M_1$, calculated according to Moore et al. (25), gave the best fit with the experimental data and were regarded as the final results.

### Sensitivity analysis

The parameters of the model fit were tested in each case for their sensitivity to changes. It was observed that the fit is sensitive to changes in $\lambda_{w1}$ and $\lambda_{12}$ but not very sensitive to those in $\lambda_{w2}$ and $\lambda_{21}$. The model fit was not sensitive to changes in $\lambda_{10}$ although the solution was. The fit was more sensitive to changes in $C_1$ and $C_2$ than to those in $\lambda_{w2}$, $\lambda_{21}$, and $\lambda_{10}$, though not as sensitive as with regard to $\lambda_{w1}$ and $\lambda_{12}$.

### Cholesterol kinetics after administration of labeled mevalonate and water

The parameters of the hybrid computer solution are given in Table 3, the calculated mass and transport values in **Tables 4** and **5**, and an example of the resolution and experimental values in **Fig. 4.** From 17.6 to 44.0% of the biologically active L-form of [2-$^{14}$C]mevalonate was incorporated into cholesterol. The computer analysis determines accurately $\lambda_{w1}$ in $PRE_1$; because $\lambda_{w1} + \lambda_{w2}$ was known from PRE, $PRE_2$ is the difference between PRE and $PRE_1$. The successful computer analysis of the experimental data

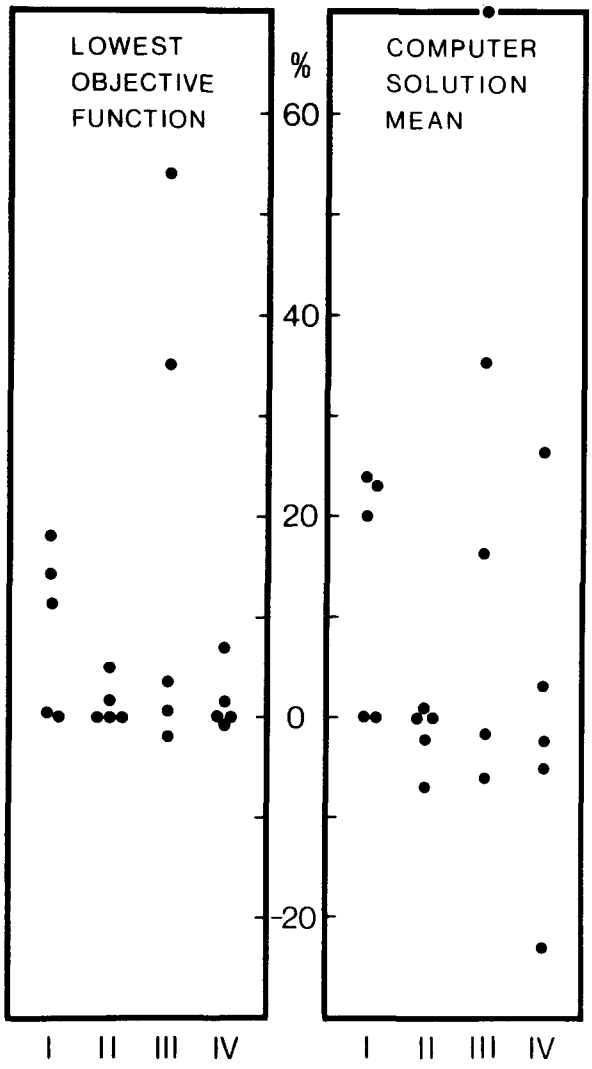


**Fig. 3.** Deviation of computer solutions from expected $\lambda_{w1} + \lambda_{w2}$ values. Left, lowest objective function resolutions from Series I–IV (used in further calculations of cholesterol metabolism). Right, means of all runs (each point refers to one individual) in Series I–IV.

provided that a significant amount of cholesterol is synthesized in pool 2 both from [2-$^{14}$C]mevalonate and from [$^{3}$H]water. In experimental animals mevalonate is known to be unequally incorporated into tissue sterols, the synthesis of precursors being high in the kidneys in particular (37, 38). This kind of unequal distribution may explain the finding in the present study that the relative amount of cholesterol produced in pool 2 was higher in the analysis of [2-$^{14}$C]mevalonate than of $^{3}H_2O$ data (Table 3). According to the latter analysis, from 21.6 to 53.1% (87 to 412 mg/day) of the overall cholesterol synthesis took place in pool 2. The total exchangeable cholesterol ranged from 72 to 149 g and the daily cholesterol transport from pool 1 to pool 2 from 0.67 to 1.36 g.

Since the role of fat tissue in the cholesterol metabolism has received great attention (4, 39, 40) cholesterol synthesis in pool 2 ($PRE_2$) was related to TBF as shown in **Fig. 5.** The subjects with higher TBF tended to have higher $PRE_2$ values, indicating a greater synthesis in pool 2 of obese than of lean subjects.

### Studies with [2-$^{3}$H]mevalonate and [4-$^{14}$C]cholesterol

**Table 6** and **Fig. 6** show the kinetic results and resolution graphs pertaining to two experiments in which [2-$^{3}$H]mevalonate and [4-$^{14}$C]cholesterol were injected intravenously. All of the [4-$^{14}$C]cholesterol was assumed to enter primarily pool 1. Trials in which the [4-$^{14}$C]cholesterol dose was distributed between pool 1 and 2 produced no better fit of the model with the experimental data. According to the computer results, 20–22% of [2-$^{3}$H]mevalonate was initially incorporated into cholesterol in pool 2. This is reflected also in the decay curves of plasma cholesterol in **Fig. 7.** The percent decreases of [$^{3}$H]cholesterol and [4-$^{14}$C]cholesterol in the plasma cholesterol of subject V.N. are presented as percentages of the 4th day value. The [4-$^{14}$C]cholesterol disappeared more rapidly than the [$^{3}$H]cholesterol, to a ratio of about 0.70 by the end of the experiment. Similar results were obtained from A.S.

Thus the successful computer analysis of the data obtained after administration of labeled water and mevalonate and the deviation of the decay curves according to Fig. 7 are the findings of the present study indicating that human cholesterol is also synthesized in pool 2.

## DISCUSSION

When the kinetic approach was first adopted in the measurement of cholesterol turnover in man (2) the sites of cholesterol synthesis could not be pointed out, and this unfortunate situation prevails even today. This causes difficulties in the calculation of the exchangeable cholesterol mass in the body (2–4, 8). It is mainly on the basis of in vitro studies with various organs that the greater part of cholesterol synthesis has been assumed to take place in kinetic pool 1 [the "rapidly exchangeable cholesterol pool", e.g., pool "A" of Goodman et al. (2), of which the blood, hepatic, and gastrointestinal cholesterols constitute the most important part (41)], while no production would take place in pool 2 (pool B, slowly exchangeable pool).

The present data are the first estimates concerning the relative proportions and amounts of cholesterol synthesis in kinetically defined pools. By treating

TABLE 4. Endogenous cholesterol synthesis, exchangeable cholesterol mass and rate of transport

| Subject | Exp. Code[a] | Endogenous Cholesterol Synthesis in Pool I according to [$^3$H]water | Endogenous Cholesterol Synthesis in Pool I according to [$^{14}$C]mevalonate | Endogenous Cholesterol Synthesis in Pool II according to [$^3$H]water | Endogenous Cholesterol Synthesis in Pool II according to [$^{14}$C]mevalonate | Exchangeable Cholesterol Mass in Pool II | Exchangeable Cholesterol Mass Total | Daily Cholesterol Transport from Pool I to Pool II |
|---|---|---|---|---|---|---|---|---|
| | | *mg/day* | | | | *g* | | *g* |
| R.S. | I | 444 | 400 | 95 | 139 | 41.2 | 53.7 | 0.94 |
| | II[b] | 383 | 342 | 156 | 197 | 59.5 | 72.1 | 0.95 |
| | III | 523 | 402 | 16 | 137 | 50.0 | 63.9 | 1.11 |
| | IV | 426 | 474 | 113 | 65 | 38.8 | 52.7 | 1.06 |
| P.Ö. | I | 486 | 386 | 58 | 158 | 67.8 | 85.0 | 1.52 |
| | II[b] | 426 | 345 | 118 | 199 | 79.0 | 96.2 | 1.36 |
| | III | 454 | 363 | 90 | 181 | 85.4 | 104.5 | 1.59 |
| | IV | 476 | 390 | 68 | 154 | 75.9 | 95.0 | 1.56 |
| T.H. female | I | 529 | 412 | 281 | 398 | 74.1 | 91.4 | 0.93 |
| | II[b] | 398 | 359 | 412 | 451 | 110.1 | 127.4 | 0.88 |
| | III | 569 | 491 | 241 | 319 | 72.6 | 91.8 | 1.08 |
| | IV | 449 | 293 | 361 | 517 | 168.9 | 188.1 | 1.15 |
| T.H. male | I | 330 | 323 | 43 | 50 | 38.1 | 53.5 | 0.80 |
| | II[b] | 286 | 245 | 87 | 128 | 63.6 | 79.0 | 0.87 |
| | III | 298 | 310 | 75 | 63 | 68.7 | 85.8 | 1.49 |
| | IV | 344 | 336 | 29 | 37 | 60.8 | 77.9 | 1.50 |
| V.W. | I | 352 | 315 | 246 | 283 | 100.0 | 120.4 | 0.68 |
| | II[b] | 280 | 287 | 318 | 311 | 129.0 | 149.4 | 0.67 |
| | III | 398 | 370 | 200 | 228 | 88.2 | 110.9 | 0.72 |
| | IV | 303 | 280 | 295 | 318 | 155.6 | 178.2 | 0.74 |

[a] See footnote *a* to Table 2.
[b] See footnote *b* to Table 3.

sterol balance data as well as tracer kinetic data after administration of labeled water and mevalonate (used as cholesterol precursors), and prelabeled cholesterol with the hybrid computer, the best fit solutions revealed that pool 2 contributed 20–50% to body cholesterol synthesis in the subjects studied. The kinetic behavior of [4-$^{14}$C]cholesterol as compared to [$^3$H]cholesterol in Fig. 7 indicates that a deviation of the specific activities is to be expected if a part of the [$^3$H]cholesterol primarily synthesized from [2-$^3$H]-mevalonate in pool 2 flows to pool 1 during the mixing phase (up to about 3 weeks from the start of the experiment), enriching its tracer content.

The source of pool 2 cholesterol synthesis has become increasingly interesting after the observations that an increase in TBF produces a closely linear increase in PR (4, 12, 39, 41) and cholesterol mass in pool 2 (40). In the present study an increase of $PRE_2$ with that of TBF was apparent. However, this type of kinetic analysis cannot point to any site of synthesis for $PRE_2$ in terms of specific tissues. The question therefore remains open whether the present estimates

TABLE 5. Pertinent cholesterol mass and mass transport results from series II, based on [$^3$H]water

| Parameter | Test Subject R.S. | P.Ö. | T.H., f. | T.H., m. | V.W. | Mean ± SE |
|---|---|---|---|---|---|---|
| $M_1$, g | 12.5 | 17.2 | 17.3 | 15.4 | 20.4 | 16.6 ± 1.3 |
| $M_t$, g | 72.1 | 96.2 | 127.4 | 79.0 | 149.4 | 104.8 ± 14.7 |
| PR, mg/day | 608 | 749 | 987 | 435 | 685 | 693 ± 90 |
| PRE, mg/day | 539 | 544 | 810 | 373 | 598 | 573 ± 70 |
| $PRE_1$, mg/day | 383 | 426 | 398 | 286 | 280 | 355 ± 30 |
| $PRE_2$, mg/day | 156 | 118 | 412 | 87 | 318 | 218 ± 63 |
| $\lambda_{w2\%}$ | 29.0 | 21.6 | 50.9 | 23.2 | 53.1 | 35.6 ± 6.8 |
| $\rho_{12}$ g/day | 0.95 | 1.36 | 0.88 | 0.87 | 0.67 | 0.95 ± 0.11 |
| $\lambda_{10}$ day$^{-1}$ | 0.0419 | 0.0388 | 0.0522 | 0.0229 | 0.0295 | 0.0371 ± 0.0051 |

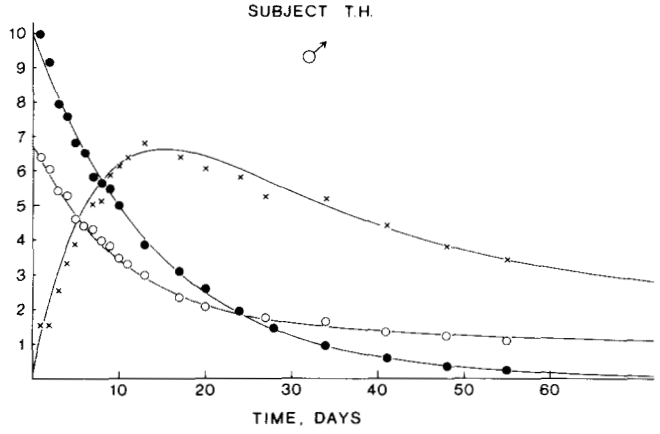


**Fig. 4.** Plasma cholesterol radioactivity curves (crosses, [$^3$H]-cholesterol; circles, [$^{14}$C]cholesterol) and body tritiated water curves (black dots) from one test subject. Ordinate, specific activity of [$^3$H]cholesterol, [$^{14}$C]cholesterol and [$^3$H]water, arbitrary units.

of $PRE_2$ are related to the actual cholesterol synthesis in the fat tissue of the subjects examined. The remarkable similarities between cholesterogenesis by adipose tissue in monkeys (42) and increase in PR related to excess body weight in man has been pointed out (9), however. More recent in vitro studies suggested that cholesterol synthesis in adipose tissue from many precursors, including tritiated water, is negligible (43).

The experimental design of the present study is characterized by 19 blood samples spread over a period of 8 weeks, a quantitative fecal steroid analysis about 40 days after the start of the experiment, and the use of double labeling on one hand and sensitivity analysis practiced on hybrid computer resolutions on the other hand. The two principal methods used at present in measuring cholesterol turnover in man—kinetic isotopic methods and sterol balance method—both serve equally the basic functions in this paper. The cholesterol turnover was measured from fecal steroid excretion added with a constant cutaneous cholesterol output, while the cholesterol transport characteristics were estimated by applying kinetic methods.

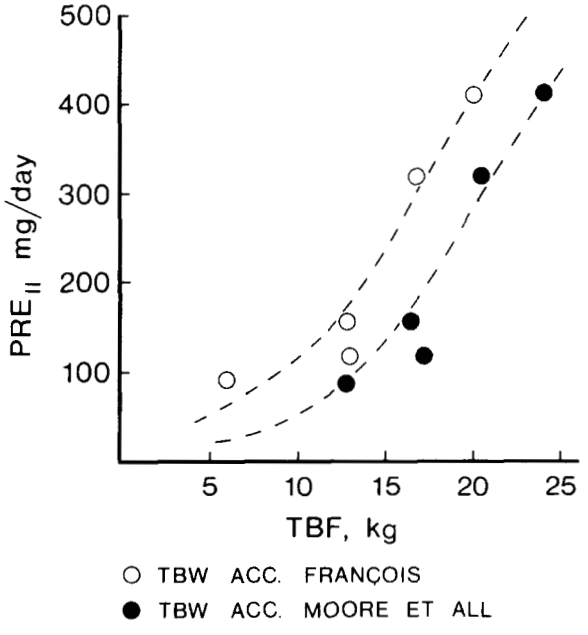


**Fig. 5.** Plot of total body fat mass (TBF) (calculated according to Moore et al. (23) or to François (24)) vs. cholesterol synthesis in pool 2 ($PRE_2$) in subjects in experiments with tritiated water and DL[$^{14}$C]mevalonate.

The experimental design allowed the determination of $\lambda_{w1}$ and $\lambda_{12}$ from the tracer data, while $\lambda_{10}$ and $\lambda_{w2}$ are parameters essentially unmeasurable from the tracer curves. An 8-week period would most evidently have been too short for kinetic analysis alone, owing to the comparatively slow exchange of cholesterol between the different pools. A kinetic deficiency of this kind would decrease $M_t$ and $M_{t-1}$ (8, 44) and, in multicompartment analysis, increase PR (8, 44). However, the drawback of the short follow-up period was successfully overcome by predetermining $\lambda_{10}$ with the aid of fecal steroid determinations. This is evidenced by a close agreement of the values of the total exchangeable cholesterol and removal characteristics in the present study with those of the control subjects in the two studies with long-term kinetic data subjected to a critical kinetic and computer scrutiny (Table 7). All three series present parameters on the same level. The third cholesterol pool (7, 8) implied by a third exponential component in the long-term studies has been ignored in our model. It has probably merged with pool 2 in the computer resolution of the present data. As regards $\lambda_{w2}$, it should be noted that the sum $\lambda_{w1} + \lambda_{w2}$ is fixed in advance (see section on calculation), and because $\lambda_{w1}$ is measurable, $\lambda_{w2}$ will be fixed.

Several parameters had to be taken from the

TABLE 6. Cholesterol mass and turnover data of two subjects in experiment with DL [$^3$H]mevalonate and [$^{14}$C]cholesterol

| Subject | $\lambda_{10}$ | $M_1$ | $M_t$ | $\lambda_{12}$ | $\lambda_{21}$ | $C_{2\%}$ |
|---|---|---|---|---|---|---|
| | *day*$^{-1}$ | *g* | *g* | *day*$^{-1}$ | *day*$^{-1}$ | |
| V.N. (male) | 0.023[a] | 29.4 | 178[a] | 0.1142 | 0.0186 | 21.8 |
| A.S. (male) | 0.023 | 29.4 | 95 | 0.0622 | 0.0184 | 20.0 |

[a] Study ended at 39 days due to reinfarction and death of the subject before fecal collections could be made; therefore value of $\lambda_{10}$ was assumed to be identical to that of subject A.S. It probably has been underestimated and the computer solution reflects how the solution can deviate when incorrect $\lambda_{10}$ is used.

literature for the development of the present method and the implication of this will be discussed briefly. The estimates used for $\rho_{20}$ and ADC in the calculations are not thought to be critical in the experiments. The excretion of cholesterol through the skin (27) was incorporated in our program as a constant (83 mg/day) because this parameter is not affected by the serum cholesterol concentration, and the range of variation of the values is very narrow (27, 45). The percentage of cholesterol absorption from the gut varies within fairly narrow limits according to recent investigations (14, 28–30); the mean figure of 35% was used in this paper. This constant is partly reflected in ADC, $\rho_{10}$, PRE, and HT. It finally enters the procedure when the computer is supplied with a constant value of $\lambda_{w1} + \lambda_{w2}$ and thus influences the estimations of parameters by the computer. However, the amount of dietary cholesterol was fairly low so that variation in the absorption percentage would change the ADC fairly little. Assuming that the mean absorption percentages were 25% and 45% instead of 35%, the mean PRE in our test subjects could have been 619 mg/day and 511 mg/day, respectively, provided the negative feedback mechanism was active. Since the computer solution measured $PRE_1$, the change in PRE from 619 to 511 could have taken place in $PRE_2$, decreasing its percentage from 40% to 27% of PRE. At the higher mean cholesterol absorption, the computer fit became poor, allowing no further calculation to be performed. If the real experimental isotopic data had been available at two different levels of absorbed dietary cholesterol, the negative feedback mechanism had apparently changed hepatic (pool 1) synthesis and subsequently the initial radioactivity curves. The change in the latter ones had been revealed by the computer solution as diminished $PRE_1$.

Two other parameters, $M_1$ (pool 1 cholesterol mass) and the constants 0.543 or 0.700 (the relative contribution of body water hydrogen to the total hydrogen transport to the cholesterol molecule), were derived from the literature. Since the amount of labeled cholesterol formed from mevalonate was not known, it was not possible in our experimental design to estimate $M_1$ by the dilution principle. $M_1$ was therefore determined according to Moore et al. (25) in the present study. It includes the individually measured blood cholesterol measure plus the mean cholesterol concentrations of certain organs (liver, spleen, and intestine) derived from the literature.

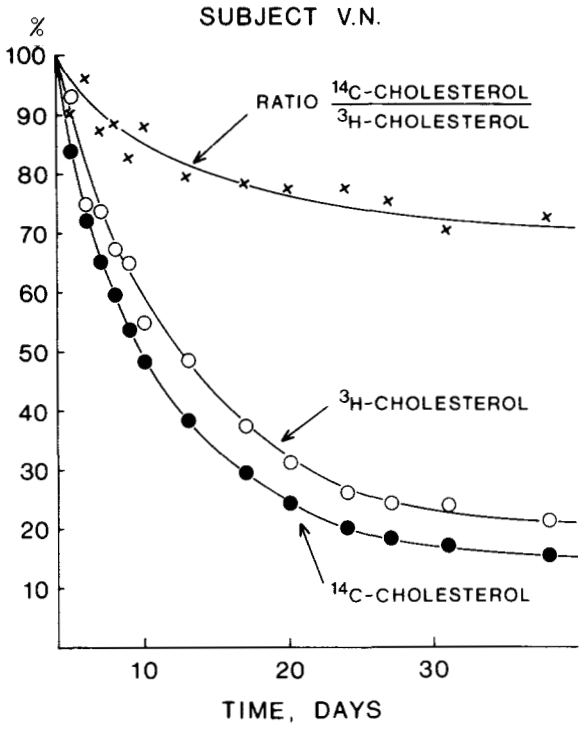


**Fig. 7.** Plasma [$^{14}$C]cholesterol and [$^{3}$H]cholesterol curves of subject V.N., percentages of 4th day value, and their ratio.

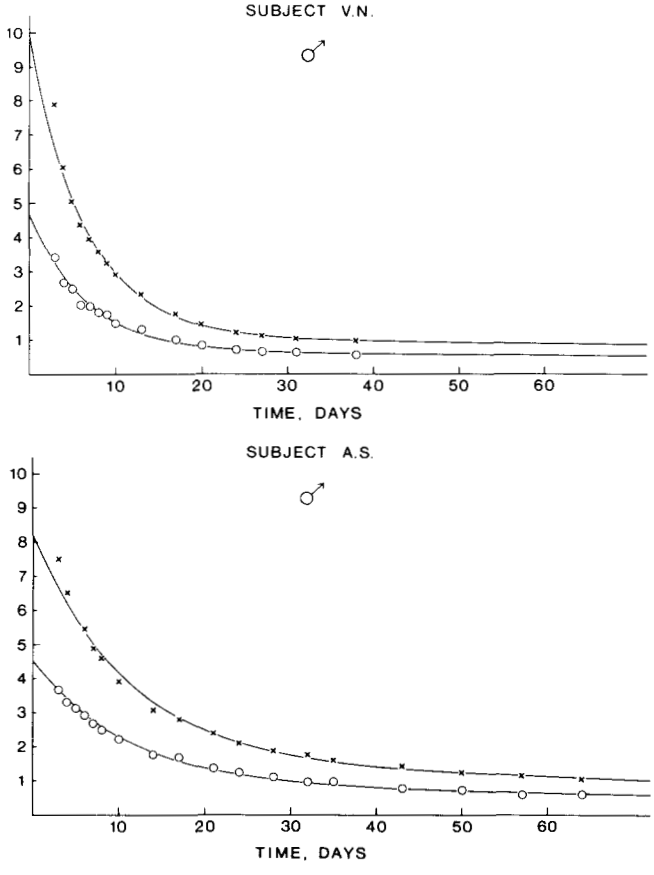


**Fig. 6.** Individual plasma radioactivity curves (crosses, [$^{14}$C]-cholesterol; circles, [$^{3}$H]cholesterol) in subjects in experiments with DL[$^{3}$H]mevalonate and [$^{14}$C]cholesterol. Ordinate, specific activity of [$^{14}$C]cholesterol and [$^{3}$H]cholesterol, arbitrary units.

TABLE 7. Findings of long-term kinetic studies concerning healthy subjects reported in the literature, compared with the present results

| | $\lambda_{10}$ | $M_t$ | PR | B. Wt |
|---|---|---|---|---|
| | $day^{-1}$ | $g$ | $g.\ day^{-1}$ | $kg$ |
| Goodman et al.[a] Ref. (8) | 0.044 | 72.3[d] | 1.12 | 77 |
| | 0.044 | 119.2[e] | 1.12 | 77 |
| Samuel and Perl[b] Ref. (6) | 0.042 | 71.9 | 1.00 | 60 |
| Present material[c] | | | | |
| Series I | 0.044 | 76.7 | 0.78 | 65 |
| Series II | 0.044 | 98.6 | 0.78 | 65 |

[a] Subjects F.C., R.M. and R.N.
[b] Subjects A.F., S.K., E.A., and D.B.
[c] Subjects R.S., Ö.P., and T.H. (female).
[d] Minimum estimate.
[e] Maximum estimate.

The estimation of the pool 1 mass and the cholesterol turnover by the use of a single tracer cholesterol provides that all the tracer cholesterol primarily enters pool 1. This has not been validated so far, though fairly good agreement with the sterol balance method (9, 40) has been taken as an indirect validation. The cholesterol dilution principle (2–8) gives a maximum estimate of the pool 1 cholesterol mass. This estimate is reduced by a possible direct cholesterol incorporation into pool 2 and by the initial mixing phenomena (3). The results concerning the measurement of $M_1$ by the dilution principle seem to vary. Thus Fig. 2 shows that $M_1$ reported by Samuel and Perl (6) is significantly greater than that found by Goodman et al. (8). Our series III and IV with greater $M_1$ values, which conformed to those of Goodman et al. (8) (Fig. 2), gave clearly inferior computer resolutions compared to series II and I with smaller $M_1$ values, suggesting that $M_1$ determined according to Moore et al. (25) is highly applicable to the present kinetic analysis.

The fractional hydrogen transport from body water to the cholesterol molecule plays a central analytical role when water is used as a precursor. Loud (31, 32) made a thorough analysis of this question and concluded that at least 25 out of the 46 hydrogen atoms in the cholesterol molecule originate from body water in the rat. This value (25:46 = 0.543) was considered to be the minimum estimate, suggesting that the correct figure would be between 0.543 and one. To find the correct constant for human subjects, the data presented by Taylor et al. (33) were reanalyzed (see Methods). These were the only data in the literature applicable to the determination of this constant (33, 46). Only two of the experiments (Subjects 1 and 2) by Taylor et al. (33) seemed to be suitable for the analysis that was based on a multicompartment model involving the same number of pools as in the present paper. On the basis of these analyses the constant 0.700 (32:46) was obtained, indicating that, in contrast to what was suggested by Moore et al. (25), the fraction of hydrogen incorporation from water to cholesterol hydrogen is greater than 0.5 but less than 1.00.

Studies on the possibility of measuring $M_1$ and HTW individually with the hybrid computer from the experimental data are in progress.

The difficulties concerning $M_1$ and HTW hamper especially the estimation of the endogenous cholesterol synthesis in pools 1 and 2 when tritiated water is used as a precursor. The $C_1$ and $C_2$ determined by the computer in connection with mevalonate also constitute elements of the total tracer data resolution. However, in this resolution $C_1$ and $C_2$ are not too firmly linked with the mass and the hydrogen transport characteristics. Thus, cholesterol synthesis characterized by $C_1$ and $C_2$ in pools 1 and 2, respectively, is obtained largely independently from that obtained from tritium data. Mevalonate has a fast turnover in the rat, displaying a rapid and a slow exponential component (38). Its bulk appears in the kidneys, where it is converted to cholesterol precursors, which are then converted to cholesterol in other tissues (37, 38).

According to the present findings, 18–44% of the biologically active mevalonate was actually converted into cholesterol in man. A systematic incorporation of [2-$^{14}$C]mevalonate in pool 2 cholesterol was noted; it was at a relatively higher percent level than that of tritiated water and was independent of the series or of the subject. It is impossible at present to say anything about the possible site of this synthesized extra 10%.

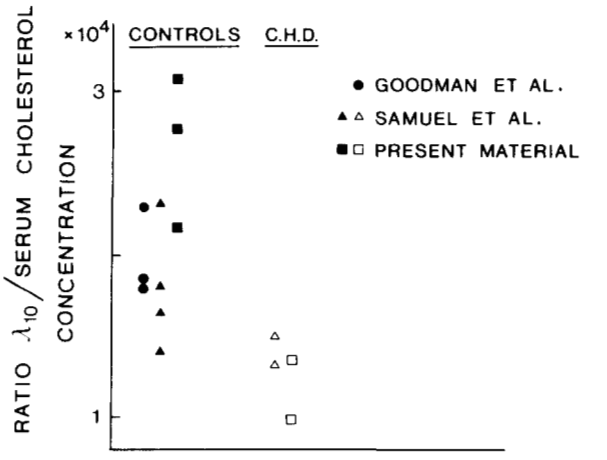


**Fig. 8.** Ratio of $\lambda_{10}$/serum cholesterol concentration as indicator of efficiency of removal in subjects T.H. (male) and V.W. in the present material, and in CHD patients studied by Samuel and Perl (6) and Goodman et al. (8).

This may depend primarily on the uneven distribution of mevalonate between different tissues (37, 38). Accordingly it may be more appropriate to use body water tracer data for the final calculations to obtain estimates of the cholesterol synthesis in different pools since, in contrast to mevalonate, the distribution of the body water apparently shows no disparity in its distribution between the sites of cholesterol synthesis in the body. A question still left open is the theoretical possibility of unequal fractional hydrogen transport to the cholesterol molecule in different tissues.

Some preliminary comparisons were performed between the kinetic behavior of cholesterol in our subjects with coronary heart disease (CHD) and that found by other authors. Our subjects T.H. (male) and V.W. differed from the other subjects by a slow $\lambda_{10}$ and low synthesis rate in pool 1 and a small mass transport between pools 1 and 2. In combination, these values are generally connected with what is called a "removal defect" as the principal cause of an elevated cholesterol concentration in man (47). Our CHD subjects and those of Samuel and Perl (6) (Subjects N.S. and E.D.) behaved rather similarly (**Fig. 8**) with a low $\lambda_{10}$ serum cholesterol concentration ratio compared with the controls.

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