Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods

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ABSTRACT We have estimated the daily synthesis of cholesterol in man by measuring the excretion of cholesterol and its conversion products during periods of controlled sterol intake (sterol balance method), using isotopic or chromatographic procedures (or a combination of the two). Estimates of daily synthesis by this method are based on the premise that the subject is in the metabolic steady state; i.e., the synthesis of cholesterol is equal to the balance (or difference) between the intake of cholesterol and the excretion of cholesterol and its products. To test this premise, we carried out sterol balances in 11 patients; simultaneously, after administration of isotopic cholesterol, turnover was calculated according to previously described models (one-pool, two-pool, or isotopic steady state models for the distribution of radioactive cholesterol within various pools of the body).

With calculations based on the one-pool model, turnover rates were considerably higher than estimates based on all other models, and reasons are given for considering these to be overestimates. Good agreement was obtained between results calculated from the two-pool model and those based on sterol balance data; neither method is theoretically preferable to the other. However, with the sterol balance method supplemented by isotopic techniques, valid measurements of cholesterol absorption can be obtained; this in turn permits the essential distinction to be made between daily synthesis and daily turnover of cholesterol when the diet contains cholesterol.

In addition, the use of chromatographic isolation procedures provides an accurate measurement of the balance of β-sitosterol. This in turn permits valid corrections to be made for losses (which may be large) of neutral steroids during intestinal transit; this is a unique advantage of the chromatographic method.

SUPPLEMENTARY KEY WORDS one-pool model · two-pool model · isotopic steady state model · sterol degradation

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

PROGRESS in understanding the physiology and pathophysiology of cholesterol metabolism in man depends upon the development of reliable methods for estimation of daily turnover and synthesis rates. Several investigators (1–3) have attempted to estimate daily turnover of cholesterol by analyzing the kinetics of the specific activity of plasma cholesterol after pulse labeling with isotopic cholesterol. Data derived from such studies can be interpreted variously, depending upon the assumptions made about the distribution and exchange of cholesterol in the several body pools; thus, a wide range of values has been reported for cholesterol turnover in man.

Another approach to an estimation of daily cholesterol synthesis has been designated the method of sterol balance (4–7). In most balance studies synthesis is estimated by measuring excretion of all steroids derived from cholesterol under steady state conditions. The metabolic steady state is assumed to be present when the following conditions are met: constant plasma cholesterol concentrations, unchanging fecal excretion of steroids, and constant body weight all coexist during long periods of study of patients who are clinically stable and free from complicating metabolic abnormalities. In the metabolic steady state the input (synthesis plus dietary intake) is balanced by outflow (excretion): in other words, synthesis equals excretion minus intake.

For the measurement of the fecal excretion products of cholesterol we have utilized the two most reliable procedures currently available: the isotope balance technique (5) and the chromatographic balance method (6, 7). It is the purpose of this paper to present estimates of daily synthesis and (or) turnover in 11 patients, by using various combinations of these two balance methods, and to compare them with estimates based on analyses of

isotope kinetics derived from specific activity–time curves of plasma cholesterol determined for the same patients over the same period of time. These comparisons permit a rigorous assessment of all underlying assumptions.

METHODS

Patients

Cholesterol balance studies were carried out on 11 patients during hospitalization on a metabolic ward at The Rockefeller University Hospital. The age, sex, body weight, and clinical diagnosis of each patient are listed in Table 1. There were 5 males and 6 females, aged 21–68 yr, with a rather narrow range of adiposity [only 1 had a body weight greater than 120% of normal, according to life insurance tables (8)]. All but 1 patient had abnormal lipoprotein patterns, determined according to Fredrickson, Levy, and Lees (9), and 8 of 11 patients had some manifestation of vascular disease. Patient 3 was studied twice under different conditions: during the second period (3B) she received 500 mg of sodium taurocholate twice daily by mouth in a capsule. Patient 5 also was studied under two regimens: in the first period (5A) she ingested 37 mg of cholesterol per day, and in the second (5B), 1600 mg/day.

Diets

During all study periods, all patients were fed orally and exclusively by the technique of liquid formula feeding (10). In 10 patients dietary fat contributed 40%, protein 15%, and glucose 45% of total caloric intake; patient 2 received a fat-free diet in which glucose provided 85% and protein 15% of calories. Total caloric intake was adjusted in every case to hold body weight constant throughout the balance study.

Table 2 lists the dietary fats used in these studies, and their sterol contents. The 11 patients have been grouped according to daily intake of cholesterol: for patients 1–5, the formula diets were essentially free of cholesterol whereas for patients 6–11, diets contained cholesterol as an inherent component of butter or egg yolk, with intakes ranging from 295 to 577 mg/day. Plant sterols were inherent components of corn and cottonseed oils; sitosterols were added to butter oil formulas by dissolving crystalline compounds in the oil before its addition to the other ingredients during formula manufacture. Crystalline plant sterols were obtained as "β-sitosterol" from Mann Research Laboratories, Inc., New York, and were further purified and prepared in microcrystalline form by Dr. Erold R. Diller, Eli Lilly & Co., Indianapolis, Ind.; the resultant preparation contained 90% β-sitosterol and 10% campesterol. Patient 2, who was on a fat-free diet, received plant sterols twice daily in equal doses in capsule form. Cholesterol and plant sterol contents of each formula were determined by gas–liquid chromatographic methods (6).

TABLE 1 CLINICAL DATA

Patient	Age	Sex	Weight and % of Ideal Wt.*	Diet	Total Plasma Cholesterol	Total Plasma Glycerides	Diagnosis
	yr		*kg (%)*		*mg/100 ml*		
Group I (Low-Cholesterol Diets)							
1 (H.Sp.)	50	F	78 (137)	A	200 ± 10 (16)†	155 ± 19 (16)†	IHD‡, hypercholesteremia (type II §) essential hypertension
2 (R.T.)	48	M	65 (108)	B	377 ± 40 (6)	2048 ± 74 (6)	IHD, hyperglyceridemia (Type V)
3A (L.M.)	58	F	50 (97)	C	403 ± 34 (7)	192 ± 26 (7)	Hypercholesteremia (Type II), xanthomatosis
3B (L.M.)¶	58	F	50 (97)	C	401 ± 19 (6)	200 ± 22 (6)	Hypercholesteremia (Type II), xanthomatosis
4 (R.G.)	58	F	61 (120)	C	407 ± 13 (9)	256 ± 46 (9)	IHD, xanthomatosis, hypercholesteremia (Type II)
5A (J.Sh.)	68	F	63 (117)	A	180 ± 12 (11)	115 ± 16 (11)	IHD, normocholesteremia
5B (J.Sh.)	68	F	63 (117)	D	199 ± 16 (13)	96 ± 18 (13)	IHD, normocholesteremia
Group II (Moderate-Cholesterol Diets)							
6 (H.Sa.)	54	M	78 (117)	E	416 ± 19 (7)	1529 ± 140 (7)	Hyperglyceridemia (Type V)
7 (N.S.)	21	F	52 (90)	F	549 ± 11 (11)	191 ± 20 (11)	IHD, hypercholesteremia (Type II)
8 (N.A.)	30	M	67 (102)	G	257 ± 6 (5)	179 ± 9 (5)	IHD, hypercholesteremia (Type II)
9 (J.H.)	39	M	74 (104)	G	447 ± 13 (7)	293 ± 33 (7)	Hypercholesteremia (Type II)
10 (J.R.)	36	F	53 (98)	G	464 ± 24 (12)	188 ± 31 (12)	IHD, PVD‡, xanthomatosis, hypercholesteremia (Type II)
11 (D.A.)	55	M	64 (103)	H	403 ± 18 (8)	2089 ± 266 (8)	IHD, hyperglyceridemia (Type V)

For diets, see Table 2.
* According to life insurance tables (8).
† Means are given ± SD (No. in parentheses = biweekly determinations throughout the period of study).
‡ IHD = ischemic heart disease; PVD = peripheral vascular disease.
§ Type of hyperlipoproteinemia according to Fredrickson et al. (9).
¶ In the second period (3B) the patient received 500 mg of sodium taurocholate twice daily by mouth.

TABLE 2 DIETARY FATS AND THEIR STEROL CONTENTS

Diet	Dietary Fat	Cholesterol	Total Plant Sterols	β-Sitosterol	Source of Plant Sterols
		*mg/500 cal**			
A	Corn oil	6.2	200	148	Inherent in dietary fat
B	Fat-free	6.2	—	—	—
C	Cottonseed oil	10	80	71	Inherent
D	Corn oil	368	200	148	Inherent
E	Butter	80	—	—	—
F	Butter	147	52	33.5	Corn sterols (Staley)
G	Butter	80	40	35	β-Sitosterol (Mann)
H	Butter	66	51	45	β-Sitosterol (Mann)

* Except for diet B, all diets were orally-fed liquid formulas containing 40% of calories as dietary fat. Thus, a 500 calorie unit contained 22.2 g of fat; hence, the sterol contents of the fats themselves can be calculated by multiplying the figures shown above by 4.5 to give "mg of sterol per 100 g of fat."

Isotopes

One of the following isotopically labeled forms of cholesterol was administered intravenously to each patient at the beginning of the study: -4-^{14}C, -26-^{14}C, -1,2-^{3}H, or -7-^{3}H. These labeled compounds, obtained from New England Nuclear Corp., Boston, Mass., were checked for radiopurity by thin-layer chromatography before use. For intravenous administration, 1 ml of ethanol containing the radioactive tracer was dispersed in 150 ml of physiologic saline; immediately thereafter the mixture was administered. The concentration and specific radioactivity of plasma cholesterol were then determined biweekly. The concentration of plasma cholesterol was measured by the method of Abell, Levy, Brodie, and Kendall (11); its radioactivity was measured on another aliquot of that extract in a Packard Tri-Carb scintillation counter (model 3003), as previously described (6).

In certain patients constant amounts of radioactive cholesterol were administered daily by mouth for 11–16 wk. To achieve a constant input of isotope, we dissolved radioactive cholesterol in 10 ml of ethanol and added it to 40-kg batches of formula during their preparation; patients were maintained solely on these formulas for the duration of the study. Specific activities of dietary cholesterol were determined by counting an aliquot of the sterol fraction used for analysis of sterol content by GLC (6). Specific radioactivity was always found to be uniform throughout the entire batch and to remain unchanged throughout freezing, storage, thawing, and serving the formulas.

Cholesterol-7-^{3}H was the first tritiated isotope of cholesterol that was available for these studies and was used in patient 5B; the isotope, obtained from New England Nuclear Corp., was reported to be a mixture in which 90% of the tritium was located at C-7, with undetermined proportions of tritium at 7α- and 7β-. When cholesterol-7α-^{3}H is converted into bile acids, tritium is lost from the steroid molecule (12); therefore, measurements of fecal bile acid radioactivity are meaningless when this labeled compound is administered. Likewise, when cholesterol-26-^{14}C (which was used in patients 8–10) is converted to bile acids, isotopic carbon is lost and the resulting bile acids are unlabeled. In studies carried out with these two isotopic cholesterol preparations radioactivity is not lost from *neutral* steroids and measurement of radioactivity in this fraction is not affected by the position of ^{14}C in the cholesterol used.

Analysis of Fecal Steroids

Fecal neutral and acidic steroids[1] were isolated separately, and their mass and specific activities were measured by the methods developed recently in this laboratory (6, 7). These procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit. *In this paper the term "fecal neutral steroids" is restricted to the sum of cholesterol and its bacterial conversion products, coprostanol and coprostanone; plant sterols and their conversion products (which are measured separately from products of cholesterol) are not included under this term.*

Corrections for Sterol Losses and Fecal Flow

In a recent report (13) we presented evidence that neutral steroids are lost during intestinal transit and we have stated our reasons for believing that these losses are due to degradation of the sterol ring structure. The losses may be very large, as much as 60%; whether the magnitude of these losses is abnormally large in formula-fed patients we do not know, since we have made no comparable studies in patients fed solid foods. However, we have demonstrated that the amount of neutral steroid lost is roughly proportional to the length of time that feces are retained in the colon (14). The losses in any one patient tend to remain quite constant from one collection period to another and from one type of formula to another, but from patient to patient the extent of neutral sterol loss is highly variable.

However, losses of cholesterol can be accurately corrected *in each stool collection period* according to the fractional recovery of β-sitosterol (and of fecal steroids de-

[1] The term *steroid* is used in preference to *sterol* because of the significant amounts of ketonic metabolites of cholesterol that are usually present in neutral and acidic fractions.

rived from it). Daily excretion of fecal neutral steriods derived from cholesterol (mg), corrected for losses = daily excretion of neutral steroids derived from cholesterol (mg) × [daily intake of β-sitosterol (mg) ÷ daily excretion of β-sitosterol (mg)]. When this correction is applied, variations in fecal flow are also automatically corrected (13).

On the other hand, acidic steroids are not lost during intestinal transit in man (13). Thus the data for daily excretion of acidic steroids need be corrected only for variations in fecal flow by reference to the excretion of the inert marker chromic oxide (Cr_2O_3) administered orally in a constant daily amount as an internal standard. Mass measurements of Cr_2O_3 were carried out according to the method of Bolin, King, and Klosterman (15) with some modifications described by Davignon, Simmonds, and Ahrens (14). Daily excretion of acidic fecal steroids (mg), corrected for fecal flow = [daily intake of Cr_2O_3 (mg)/mg of Cr_2O_3 per g of fecal homogenate] × (mg of fecal acidic steroids per g of fecal homogenate).

For all patients in the present investigation, the Cr_2O_3 excretion data indicated fecal flow patterns that were satisfactory for sterol balance studies, according to the criteria established by Davignon et al. (14).

THEORETICAL CONSIDERATIONS AND CALCULATIONS

In this study the method of sterol balance has been compared with other methods that have been developed in recent years for estimating daily turnover, synthesis, and absorption of cholesterol in man. The theoretical basis of each of these methods will be presented in the present section, and the findings of these comparisons will be given in the Results section.

Two of these methods for measuring turnover and synthesis utilize models for interpretation of specific activity–time curves of plasma cholesterol obtained after administration of a single intravenous dose of radioactive cholesterol: the one-pool model proposed by Chobanian, Burrows, and Hollander (1, 2) in 1962 and the two-pool model suggested by Goodman and Noble (3) in 1968.

The method of sterol balance for estimation of cholesterol turnover[2] and synthesis is based on the assumption that the daily rate of cholesterol turnover in the steady state equals the daily excretion of fecal steroids derived from body cholesterol. For such studies of sterol balance, fecal steroids can be measured by the isotopic balance technique developed by Hellman, Rosenfeld, Insull, and Ahrens (5), or by the chromatographic methods developed in this laboratory and reported in 1965 (6, 7). The theoretical basis of these two methods and the necessary calculations are discussed in this section. In view of the complicated terminology and frequent overlaps between the various approaches to the determination of turnover and synthesis of cholesterol, Table 3 is presented as a simplified guide to the presentation that follows.

One-Pool Model

Chobanian et al. (1, 2) proposed a method for calculation of the rate of turnover of total cholesterol in man which makes use of the specific activity–time curve of plasma cholesterol (Fig. 1, left). The calculation is based on the assumption that, during the linear phase of decay in specific activity which begins 4–6 wk after intravenous administration of the isotope, the exponential decline reflects the turnover of a single pool of readily miscible cholesterol.

The rate of turnover of this cholesterol pool, which was considered to exclude cholesterol in the nervous system, was calculated as follows.

Eq. 1. Daily cholesterol turnover (mg/day) = $\beta \cdot M_{chol} = 0.693/t_{1/2} \times R/B$, where $t_{1/2}$ = half-life (days) of radioactivity in the body, obtained graphically from linear portion (β') of curve; β = a function of the slope of the linear portion of the decay curve, namely $(\ln 2)/t_{1/2} = 0.693/t_{1/2}$; R = injected dose of radioactivity (dpm); B = specific activity of plasma cholesterol at zero time extrapolated from linear portion (β') of curve (dpm/mg); and M_{chol} = miscible cholesterol pool (mg) = R/B.

Modified One-Pool Model

Besides estimating the size of the cholesterol pool graphically by extrapolation, as above, Chobanian et al. (2) also employed a second method for measuring pool size (R/B). In this model they set out to correct R (the total radioactivity injected) for losses of isotope occurring prior to attainment of the linear portion of the specific activity–time curve. Feces were collected during the phase of nonlinear decay in specific activity, and the total amount of radioactivity excreted in feces was determined. Radioactive cholesterol retained in the body equalled the difference between the amount injected and that excreted up to that point. The total miscible pool was then calculated by dividing the retained dose of radioactivity at the beginning of the linear phase of the curve by the specific activity of plasma cholesterol at that time. Theoretically, the modified one-pool model seemed to represent an improvement, since in the unmodified method no account was taken of radioactivity lost from

[2] In this report the term *turnover* is used to denote the total amount of new cholesterol that enters body pools (excluding the intestinal lumen) each day, by absorption of dietary cholesterol through the intestine and by synthesis, or that is excreted daily from these pools. Unabsorbed dietary cholesterol is excluded from our use of the term "body cholesterol"; it does not contribute to the value of turnover. This definition of turnover is applicable to all of the methods examined in this study.

TABLE 3 GUIDE TO THE VARIOUS APPROACHES NOW AVAILABLE FOR MEASUREMENT OF DAILY SYNTHESIS, TURNOVER, AND ABSORPTION OF CHOLESTEROL IN INTACT ORGANISMS

Method	Analysis Required	Information Obtained	Equations
I. *Specific activity–time curves*			
A. One-pool model	Plasma cholesterol SA*	Cholesterol turnover	1
B. Modified one-pool model	Plasma cholesterol SA; fecal radioactivity	Cholesterol turnover	1, 2
C. Two-pool model	Plasma cholesterol SA	Cholesterol turnover	3
II. *Sterol balance methods*			
A. Isotope balance method	Plasma cholesterol SA; fecal neutral and acidic steroid radioactivity	(1) Fecal neutral and acidic steroids	4, 5
		(2) Cholesterol synthesis (cholesterol-free diet)	6
		(3) Cholesterol turnover (cholesterol-containing diet)	7
B. GLC balance method	Dietary sterols (chromatographic); fecal neutral and acidic steroids (chromatographic)	(1) Fecal neutral and acidic steroids	
		(2) Cholesterol synthesis (cholesterol-free diet)	8
		(3) Cholesterol synthesis (cholesterol-containing diet)	9
C. Combined isotopic and GLC balance methods			
1. Pulse labeling (intravenous)	Same as in II A and II B	(1) Cholesterol absorption (Method I)	10, 11
		(2) Cholesterol turnover and synthesis (cholesterol-containing diet)	12, 13
2. Constant daily labeling (oral)	Same as in II A and II B, plus dietary cholesterol SA	(1) % of cholesterol in plasma derived from absorbed cholesterol	14
		(2) Cholesterol absorption (Method II)	11, 15, 16
		(3) Cholesterol turnover	17
		(4) Cholesterol absorption (Method III, isotopic steady state)	18

* SA = specific activity.

the body during the many weeks required for equilibration of isotopic cholesterol between plasma and different tissues.

In the modified one-pool method, turnover of plasma cholesterol was calculated as in equation 1, but with M_{chol} obtained as follows:

Eq. 2. M_{chol} (mg) = radioactivity (dpm) retained in body (at beginning of linear phase of decay) ÷ specific activity (dpm/mg) of plasma cholesterol (at the same time).

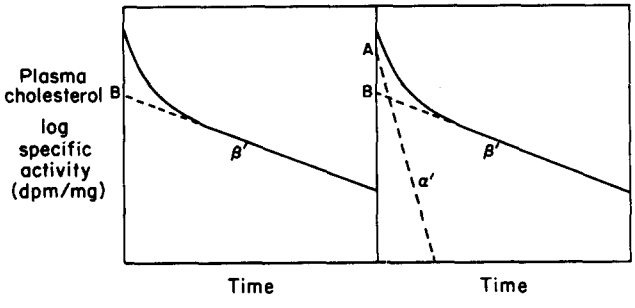

FIG. 1. Schematic specific activity–time curves for plasma cholesterol after administration of a single intravenous dose of radioactive cholesterol. Left = one-pool model. With extrapolation of the linear portion of the curve to zero time to provide the intercept B, daily cholesterol turnover is obtained by the expression $\beta \cdot (R/B)$ (equation 1). Right = two-pool model. With subtraction of line β' from the experimental curve to provide another line (α') and intercept A at zero time, daily turnover of cholesterol is calculated by equation 3, $R\alpha\beta/(\alpha B + \beta A)$.

Two-Pool Model

Theoretical equations derived from various two-pool models have been presented by Tait, Tait, Little, and Laumas (16) and by Gurpide, Mann, and Sandberg (17) for use in their studies of the metabolism of steroid hormones. These equations have recently been adapted by Goodman and Noble (3) for estimating the daily turnover of cholesterol in man. As in the one-pool model, this calculation is derived from specific activity–time curves of plasma cholesterol, but with a different interpretation of the data. Goodman and Noble proposed that radioactive cholesterol given intravenously does not exchange uniformly with a single pool; rather, it exchanges with a pool of readily miscible cholesterol (pool A) which at the same time exchanges more slowly with a second pool of cholesterol (pool B) (Fig. 2). They have presented an equation for calculation of the rate of entry of nonlabeled cholesterol from any source (synthesis, absorption, or pool B) into pool A; this rate was referred to as the "production rate" in pool A. In the special case in which cholesterol is not removed from pool B by excretion or degradation, as they suggest to be the case in man, the rate of entry of cholesterol into pool A should be equal to the turnover rate of total body cholesterol. With these assumptions they calculated the rate of cholesterol turnover from specific activity–time curves obtained in 10 patients.

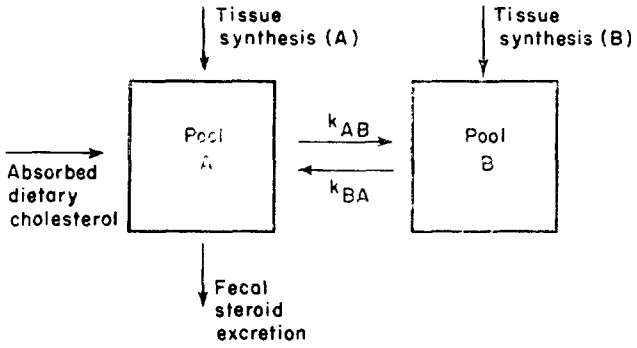

FIG. 2. General two-pool model. Rate constants are denoted by k values [after Goodman and Noble, (3)]. This model appears to describe the circumstances in man. Unlabeled cholesterol enters pool A from three sources: exchange with pool B, new synthesis, and absorbed dietary cholesterol. Unlabeled cholesterol enters pool B only from exchange with pool A, or by new synthesis. Cholesterol is excreted only from pool A.

According to the two-pool model the following equation gave the daily turnover of body cholesterol (Fig. 1, right):

Eq. 3. Daily cholesterol turnover (mg/day) = $R\alpha\beta/(\alpha B + \beta A)$ where R, β, and B are the same as in the one-pool model, α = a function ($\ln 2/t_{1/2}$) of the slope of the straight line obtained by subtracting line β' from the original curve, and A = specific activity of plasma cholesterol at zero time, extrapolated from line α' (dpm/mg).

Sterol Balance, Measured Isotopically

Hellman et al. (5) described a sterol balance method, hereafter called the isotopic balance method, which can be used for measurement of turnover of cholesterol. With this technique the excretion of fecal steroids was derived from specific activity–time curves of plasma cholesterol after labeling with a single pulse of isotopic cholesterol, in conjunction with measurements of fecal radioactivity. This approach is based on the supposition that, in the metabolic steady state, the daily turnover of cholesterol equals the daily excretion of steroids derived from body pools of cholesterol (endogenous cholesterol). In species in which the intestine is the major route of excretion of these steroids it is necessary to carry out quantitative measurement of radioactivity in acidic and neutral steroid fractions in feces. Moreover, it has been shown to be advantageous to correct excretions of fecal steroids for variations in fecal flow with the marker, Cr_2O_3 (14).

A single dose of radioactive cholesterol is administered intravenously; thereafter, the radioactivity in the fecal acidic and neutral steroid fractions is measured and compared to the specific activity of plasma cholesterol. Sufficient time should be allowed for cholesterol and bile acids in the bile to attain specific activities equal to those of plasma cholesterol (as much as 3 wk in some cases). Excretions of neutral and acidic steroids of endogenous origin are calculated as follows:

Eq. 4. Daily excretion of fecal neutral steroids (endogenous) (mg/day) = total radioactivity in neutral steroid fraction (corrected for variations in fecal flow) (dpm/day) ÷ specific activity of plasma cholesterol (dpm/mg) 1–2 days previously.

Eq. 5. Daily excretion of fecal acidic steroids (endogenous) (mg/day) = total radioactivity in acidic steroid fraction (corrected for variations in fecal flow) (dpm/day) ÷ specific activity of plasma cholesterol (dpm/mg) 3–6 days previously.

For the rationale for choosing 1–2 and 3–6 days respectively in equations 4 and 5, see below.

When the patient is maintained in the metabolic steady state on a diet free of cholesterol, fecal neutral and acidic steroids are derived entirely from cholesterol newly synthesized in the body.Under these conditions, daily synthesis of cholesterol equals daily excretion of products derived from cholesterol.

Eq. 6. Daily cholesterol synthesis (*cholesterol-free diet*) (mg/day) = daily fecal neutral steroids (corrected for variations in fecal flow) (mg/day) + daily fecal acidic steroids (corrected for variations in fecal flow) (mg/day).

When the diet contains cholesterol, the fecal neutral and acidic steroids are derived only in part from newly synthesized cholesterol; the remainder is derived from absorbed dietary cholesterol which has become mixed with plasma and other body pools of cholesterol, then reexcreted into the intestinal tract. But this absorbed and reexcreted cholesterol is now indistinguishable from newly synthesized cholesterol; therefore, both are called "endogenous." *Thus, when the diet contains cholesterol, "fecal steroids of endogenous origin" reflect daily turnover and not daily synthesis.*

Eq. 7. Daily cholesterol turnover (*cholesterol-containing diet*) (mg/day) = daily fecal neutral steroids of endogenous origin (corrected for variations in fecal flow) (mg/day) + daily fecal acidic steroids of endogenous origin (corrected for variations in fecal flow) (mg/day).

The isotopic balance method has been adopted in several laboratories and its validity has been examined in detail by Grundy and Ahrens (18). There are two major difficulties with this method as it has been used in the past: one relating to certain assumptions which must be made in regard to variations in fecal flow; the second, the inability to correct for losses (which may be large) of neutral steroids that occur during intestinal transit.

Regarding the first issue, Lindstedt and Ahrens (19) have shown that the specific activity of the cholesterol in the bile is the same as that of cholesterol in plasma; but after the intestinal contents have passed entirely through the intestinal tract a finite time will have elapsed, and the specific activity of fecal neutral steroids of en-

dogenous origin will be higher than that of plasma at the time the feces are collected. To correct for this time lag, the actual transit time may be measured with markers, so that the calculation of fecal steroid mass can be related more accurately to the plasma cholesterol specific activity at the time the biliary sterols were passed into the intestinal lumen. Alternatively, the plasma cholesterol specific activity 1–2 days prior to the stool collection period may be arbitrarily selected for this calculation. Although these assumptions do not introduce large errors in calculations of fecal neutral steroids by the isotopic balance method, the calculation of acidic steroids poses a more difficult problem: the specific activity of plasma cholesterol *several* days earlier must be utilized in equation 5, since newly formed bile acids recirculate extensively before being excreted in the feces. Unfortunately, the half-life of the pool of bile acids varies from patient to patient, and from time to time in any one patient; thus, an arbitrary choice of time interval between bile acid synthesis and excretion necessarily introduces errors.

Regarding the second difficulty, that of neutral steroid losses, no correction can be applied if the isotopic balance method is used without the concurrent measurement of the percentage recovery of dietary β-sitosterol. The extent of the losses, from patient to patient, may range from zero to 60%.

Sterol Balance, Measured by Chromatographic Methods

In this laboratory methods have been developed for the quantitative analysis of fecal neutral and acidic steroids, using a combination of thin-layer and gas–liquid chromatography (TLC and GLC) (6, 7). The sterol balance method based on these procedures, alone and *not* in combination with isotopic labeling, will hereafter be called the chromatographic (or GLC) balance method.

The GLC balance method eliminates dependence on the administration of radioisotopes to patients. It affords a direct measurement of mass in the two steroid fractions, yet provides a qualitative and quantitative pattern of steroid excretion. Finally, it permits quantitative analysis of plant sterols and their conversion products in feces, independently of cholesterol. This is a cardinal advantage of the GLC method: when the diet contains plant sterols, a measurement of plant sterol balance provides an accurate correction for losses of neutral steroids in the course of transit through the intestine as well as for variations in fecal flow (see Methods). Acidic steroids are apparently not lost during intestinal transit (13), and corrections for variations in fecal flow of bile acids are best carried out with the inert marker Cr_2O_3.

Fecal neutral steroids measured by the GLC method include unabsorbed dietary cholesterol as well as cholesterol of endogenous origin, and thus the GLC method used alone does not allow the relative contributions of these two subfractions of the total fecal neutral steroids to be estimated. On the other hand, the GLC method provides a more precise measurement of fecal acidic steroids than the isotopic balance technique (see above, discussion of equation 5).

Synthesis of cholesterol in the metabolic steady state is estimated by the chromatographic method as follows:

Eq. 8. Daily cholesterol synthesis (*cholesterol-free diet*) (mg/day) = daily fecal neutral steroids (corrected for neutral steroid losses and for variations in fecal flow) (mg/day) + acidic steroids (corrected for variations in fecal flow) (mg/day).

Eq. 9. Daily cholesterol synthesis (*cholesterol-containing diet*) (mg/day) = daily total fecal steroids [neutral + acidic] (corrected for neutral steroid losses and for variations in fecal flow) (mg/day) − daily cholesterol intake (mg/day).

Combined Sterol Balance Method (Isotopic and Chromatographic)

When the isotopic balance technique and the chromatographic method are combined, an estimation of cholesterol absorption can be made that neither method provides when used alone. As noted above, fecal neutral steroids measured by the isotopic balance method represent neutral steroids of endogenous origin, whereas neutral steroids determined by the chromatographic method include this fraction plus unabsorbed dietary cholesterol. Thus, the difference between the results obtained by the two methods represents that portion of exogenous cholesterol that has not been absorbed during its passage through the intestine.

Eq. 10. Daily unabsorbed dietary cholesterol (mg/day) = daily fecal neutral steroids (chromatographic method) (mg/day) − daily fecal neutral steroids (isotopic balance method) (mg/day)

Cholesterol absorption, then, is the difference between the intake and the amount of unabsorbed dietary cholesterol in the feces, as follows:

Eq. 11. Exogenous cholesterol absorbed daily (mg/day) = daily cholesterol intake (mg/day) − daily unabsorbed dietary cholesterol (mg/day).

Use of equations 10 and 11 for the calculation of the daily absorption of cholesterol will be referred to subsequently as Method I (absorption from decay curve data after intravenous labeling).

Turnover of cholesterol can be calculated by combining results obtained by the isotopic and chromatographic methods:

Eq. 12. Daily cholesterol turnover (*cholesterol-containing diet*) (mg/day) = daily fecal neutral steroids (isotopic)) mg/day) + fecal acidic steroids (chromatographic) (mg/day.)

This estimate of turnover is preferred to that of equation 7 because the excretion of acidic steroids is determined

with greater accuracy by the chromatographic method than by the isotopic balance technique.

By definition, the daily turnover of endogenous cholesterol equals the sum (cholesterol absorbed + cholesterol synthesized); therefore,

Eq. 13. Daily cholesterol synthesis (*cholesterol-containing diet*) (mg/day) = daily cholesterol turnover (mg/day) − daily absorption of exogenous cholesterol (mg/day).

Synthesis calculated by equation 13 should be equal to that calculated by equation 9.

Isotopic Steady State Method

The concept of the isotopic steady state was introduced by Morris, Chaikoff, Felts, Abraham, and Fansah (20) in 1957; these workers fed radioactive cholesterol in the diets of rats until the specific activities of plasma cholesterol became constant, and from the level of the plateau of specific activity in plasma as compared to that of the dietary cholesterol they calculated the relative contributions of dietary and newly synthesized cholesterol to the plasma cholesterol. Their findings for rats fed diets containing large amounts of cholesterol are represented schematically in Fig. 3A; the plateau of plasma cholesterol specific activity approached 90% of the dietary cholesterol radioactivity. They interpreted this finding—a small contribution from synthesis relative to that from absorption—as indicating that feedback inhibition of cholesterol synthesis had been demonstrated; but, since an absolute value for absorption could not be obtained, they were unable to make quantitative estimations of synthesis.

This technique has subsequently been applied to studies of cholesterol synthesis in rats by Chevallier (21) and in man by Taylor, Patton, Yogi, and Cox (22). More recently, Wilson and Lindsey (4) have measured sterol balance during the isotopic steady state in human beings and succeeded in making quantitative estimates of cholesterol synthesis and absorption.

To produce the isotopic steady state, the investigator gives radioactive cholesterol by mouth in constant amounts daily. When, after some weeks, the specific activity of plasma cholesterol no longer increases despite the continued feeding of radioactive cholesterol, it is postulated (but has not yet been proven) that all readily miscible pools of cholesterol are in isotopic equilibrium with the plasma pool. Thus, by the reasoning of Morris et al. (20):

Eq. 14. Cholesterol in plasma derived from absorbed cholesterol (%) = plasma cholesterol specific activity (dpm/mg) × 100 ÷ dietary cholesterol specific activity (dpm/mg).

Fig. 3B shows the situation which has been consistently found to pertain in man, in whom the greater fraction of

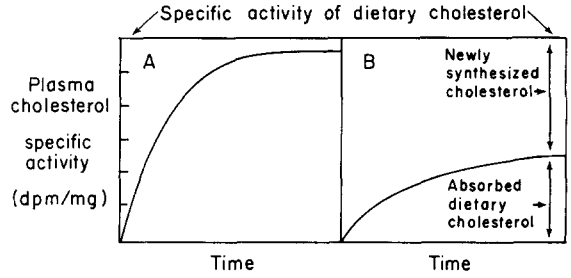

FIG. 3. Schematic specific activity–time curves for plasma cholesterol. These curves are typical of those produced by continuous feeding of radioactive cholesterol in high cholesterol diets to rats (A) and to man (B). The specific activity of dietary cholesterol is shown at the top by a horizontal line. A. In rats, specific activity of plasma cholesterol increases until a plateau is reached at approximately 90% of specific activity of dietary cholesterol. This value represents the relative contribution of absorbed dietary cholesterol to the plasma pool; the remaining 10% is presumably derived from new synthesis. B. The plateau of specific activity is reached much more slowly in man, and the relative contribution of absorbed dietary cholesterol to the plasma pool is much smaller than in rats (usually less than 50%).

the plasma cholesterol is derived from newly synthesized cholesterol, and a much smaller fraction from absorbed exogenous cholesterol. Taylor and his associates have shown repeatedly (22) that in man cholesterol synthesis always contributes more to the plasma cholesterol than does the diet even when large amounts of cholesterol are ingested; these findings have been confirmed by Wilson and Lindsey (4), by Frantz (personal communication), and in this laboratory.

Cholesterol absorption can be calculated during the oral administration of radioactive cholesterol from the following simultaneous equations (these equations are valid at any time after 4 days of isotope feeding; the isotope steady state need not be attained):

Eq. 15. $Z = X + Y$, where: Z = total fecal neutral steroids (mg/day); X = fecal neutral steroids of endogenous origin (mg/day); and Y = unabsorbed dietary cholesterol (mg/day).

Eq. 16. $Z \cdot SA_{(z)} = X \cdot SA_{(x)} + Y \cdot SA_{(y)}$, where: $SA_{(z)}$ = specific activity of total fecal neutral steroids; $SA_{(x)}$ = specific activity of plasma cholesterol; and $SA_{(y)}$ = specific activity of dietary cholesterol.

Equation 15 states merely that the fecal neutral steroids are made up of two components: endogenously derived neutral steroids and unabsorbed dietary cholesterol. Equation 16 is a restatement of this fact, expressing the radioactivity of each component as its mass times its specific activity.

After solving for Y (unabsorbed dietary cholesterol), absorbed cholesterol is calculated as the difference between dietary intake and unabsorbed dietary cholesterol in the fecal neutral steroid fraction, as in equation 11.

This approach to cholesterol absorption will be referred to as Method II (absorption from specific activity data after daily oral labeling).

Reasoning from equation 13, we can calculate turnover of cholesterol from data in the isotopic steady state, since we know how much cholesterol has been absorbed in absolute terms and also the proportion of the plasma cholesterol derived from absorbed cholesterol (equation 14):

Eq. 17. Cholesterol turnover (*cholesterol-containing diet*) (mg/day) = daily absorption of dietary cholesterol (mg/day) ÷ fraction of plasma cholesterol derived from absorbed dietary cholesterol.

In patients receiving cholesterol in the diet, daily synthesis of cholesterol can be calculated in the isotopic steady state by either equation 9 or equation 13; according to concepts used in developing these equations, they should give identical results.

Wilson and Lindsey (4) have proposed another method for estimating cholesterol absorption which can be carried out only in the isotopic steady state. The validity of this method depends on the assumption that in the isotopic steady state the percentage of plasma cholesterol derived from dietary cholesterol reflects the same percentage in all miscible pools. If such is the case, cholesterol absorption could be determined as follows:

Eq. 18. Exogenous cholesterol absorbed daily (mg/day) = daily cholesterol turnover (mg/day) (Eq. 3 or 12) × fraction of plasma cholesterol derived from absorbed dietary cholesterol.

Wilson and Lindsey (4) actually calculated absorption according to equations 12 and 18. However, turnover measured by the two-pool model (equation 3) can be used as an alternative in equation 18. This method will be designated Method III (absorption from data in the isotopic steady state). It is valid *only* in the isotopic steady state because prior to the time of attaining that state, isotopic cholesterol from the diet is going into tissues faster than it is returning into plasma, and so the total contribution of dietary cholesterol to the plasma compartment is underestimated. In the period prior to attainment of the isotopic steady state, values calculated for cholesterol absorption would be erroneously low.

In our experience with formula-fed patients given isotopic cholesterol daily, a plateau in specific activity of plasma cholesterol may be reached only after an exceedingly long period (for instance, in two patients not described in this report, the plateau was not reached even after 17 wk). We have found recently that this plateau can be more rapidly attained by giving a large priming dose of radioactivity at the beginning of the study, either orally or intravenously (Fig. 4).

RESULTS

Sterol balance data were obtained in two groups of patients (see Table 1): five patients on a low intake of cholesterol (less than 40 mg/day) and six patients on a moderate intake (295–577 mg/day). These patients also received isotopic cholesterol, either by pulse or continuous labeling as described in the previous sections. The resulting time curves of specific activities of plasma cholesterol were used for various calculations of cholesterol turnover. Values for rates of turnover and of synthesis of body cholesterol obtained by the sterol balance method are presented first; these results are then compared to values obtained from plasma specific activity data through application of the one- and two-pool models.

Sterol Balance Methods

Group I ("Cholesterol-Free" Diets). Table 4 lists the mean daily fecal excretions of neutral and acidic steroids determined by the GLC balance method in five patients ingesting less than 40 mg/day of cholesterol.The excretion data are presented in two forms: (*a*) analyses of stool pools of 2–4 day duration without correction, and (*b*) the above results corrected for cholesterol losses and for variations in fecal flow. Correction of neutral steroid losses with β-sitosterol as internal standard produced higher values for daily excretion in all except patient 2 (see footnote, Table 4); at the same time, day-to-day variations were reduced. We have previously shown that the standard deviations of the percentage losses of β-sitosterol from successive stool collections are small, reflecting the constancy of the percentage loss in any one patient (13). Day-to-day variations in excretion of acidic

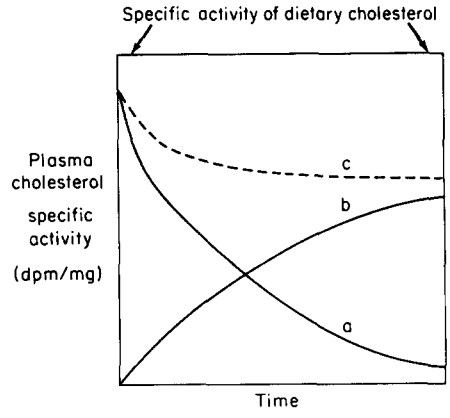

FIG. 4. Use of a priming dose to attain isotopic steady state. Two specific activity–time curves of plasma cholesterol, each in dpm/mg, are shown (*a*) after a single intravenous injection of cholesterol-^{14}C and (*b*) during repeated daily oral administration of cholesterol-^{3}H. The summed curve *c* shows that a plateau in specific activity is reached more rapidly than in curve *b*. In practice, only one isotopic form of cholesterol would be used for primary and daily doses.

TABLE 4 STEROL BALANCE DATA IN FIVE PATIENTS ON "CHOLESTEROL-FREE" DIETS (CHROMATOGRAPHIC BALANCE METHOD)

Patient No.	1	2	3A	3B	4	5A
Days: No. of determinations*	32:8	38:9	55:14	14:7	44:11	68:17
Cholesterol intake (mg/day)	36	34	27	28	34	37
Isotope given intravenously (pulse labeling)	1,2-^{3}H	4-^{14}C	4-^{14}C	1,2-^{3}H	1,2-^{3}H	4-^{14}C
			mg/day ± SD			
Fecal steroids (uncorrected)†						
a. Neutral	367 ± 160	400 ± 105	318 ± 105	458 ± 84	426 ± 150	428 ± 234
b. Acidic	188 ± 86	394 ± 74	245 ± 95	319 ± 30‡	326 ± 121	191 ± 81
c. Total	555 ± 241	794 ± 161	563 ± 188	777 ± 112	752 ± 211	619 ± 291
Fecal steroids (corrected)§						
a. Neutral	608 ± 37	342 ± 65¶	471 ± 46	813 ± 74‡	675 ± 59	522 ± 36
% loss	39.6	None	32.5	43.7	36.9	18.0
b. Acidic	202 ± 25	431 ± 62	218 ± 45	267 ± 57‖	302 ± 99	189 ± 41
c. Total	810 ± 49	773 ± 89	689 ± 63	1080 ± 94	977 ± 124	711 ± 38

* Duration of balance study (days) and number of successive stool pools analyzed. All stools were collected and analyzed; the ratio of the two figures in this column gives the average stool collection period in days.

† Uncorrected for neutral steroid losses and for variations in fecal flow.

‡ During this period patient 3 received sodium taurocholate (1 g/day) by mouth. This medication caused increased frequency of stools and a larger output of fecal neutral steroids than in 3A, which we attribute to a more rapid intestinal transit time. The finding of increased neutral steroid excretion in this patient during feeding of sodium taurocholate is exceptional; in five other patients fed this drug essentially no change was found in neutral steroid excretion as compared to a control period (unpublished data).

§ Corrected for neutral steroid losses and for variations in fecal flow.

¶ In patient 2 recovery of dietary plant sterols during the 38 days of this study corresponded to the amount fed during 42.9 days; thus, the raw excretion data for neutral steroids were higher than the corrected data.

‖ The results for acidic steroids include only endogenous acidic steroids calculated according to equation 5. Exogenous acidic steroids are excluded from this value.

steroids also were reduced in most cases after correction with Cr_2O_3 as internal standard.

In these patients fed diets essentially free of cholesterol, fecal steroids were derived exclusively from endogenous sources. Thus, the values for corrected total steroids in Table 4 (bottom line) represent daily turnover as well as synthesis of cholesterol (equation 6). In these five patients daily synthesis ranged from 689 to 1080 mg/day; in 7 to 14 successive stool collection periods in each patient the coefficients of variation ranged from 5.3 to 12.8%.

Group II (Moderate Cholesterol Diets). Table 5 presents results of sterol balance in six patients who ingested formula diets containing 295–577 mg cholesterol per day. Uncorrected values of fecal neutral and acidic steroid excretion obtained by the chromatographic method are shown at the top of Table 5. Corrected data, including endogenous and exogenous components of the neutral steroid fraction obtained by the combined sterol balance method, are presented in the middle of Table 5. Calculations of turnover and synthesis are shown at the bottom of Table 5.

In five of six patients, losses of neutral steroids during intestinal transit ranged from 32 to 52% (average 40%). The formula feeding of patient 6 contained no β-sitosterol, and in this case neutral steroids were corrected only for irregularities in fecal flow with chromic oxide as internal standard. We justify his inclusion in this study on the basis that, in a subsequent balance period in which corn oil constituted the sole dietary fat, we obtained complete recovery of β-sitosterol. Results of the balance study in patient 6 have already been reported in detail (18).

Absorption of dietary cholesterol, calculated by Method I (equation 11), ranged from 34 to 63% of daily intake, and mean daily turnover rates (equation 12) ranged from 610 to 1514 mg/day. Daily synthesis of cholesterol calculated by equations 9 or 13 gave results which ranged, from patient to patient, from 367 to 1407 mg. In these six patients on a moderate cholesterol intake the mean synthesis rate (706 mg/day) was somewhat lower from that of the five patients on a cholesterol-free diet (840 mg/day), but the range of values in the two groups was so great that any real difference in synthesis rates, if there was one, was hidden. Furthermore, we could find no meaningful relationship between daily synthesis rates and degree of hypercholesteremia in our 11 patients, nor with the types of hyperlipidemias manifested by them.

Isotopic Steady State Method

Attainment of the Isotopic Steady State. Previous investigators (4, 22) have shown that feeding radioactive cholesterol to patients daily leads to a plateau in specific activity of plasma cholesterol after only a few weeks. However, in many of our studies we have failed to attain constant specific activity even after several months. In patient 5B (Fig. 5) it is doubtful that the specific activity–

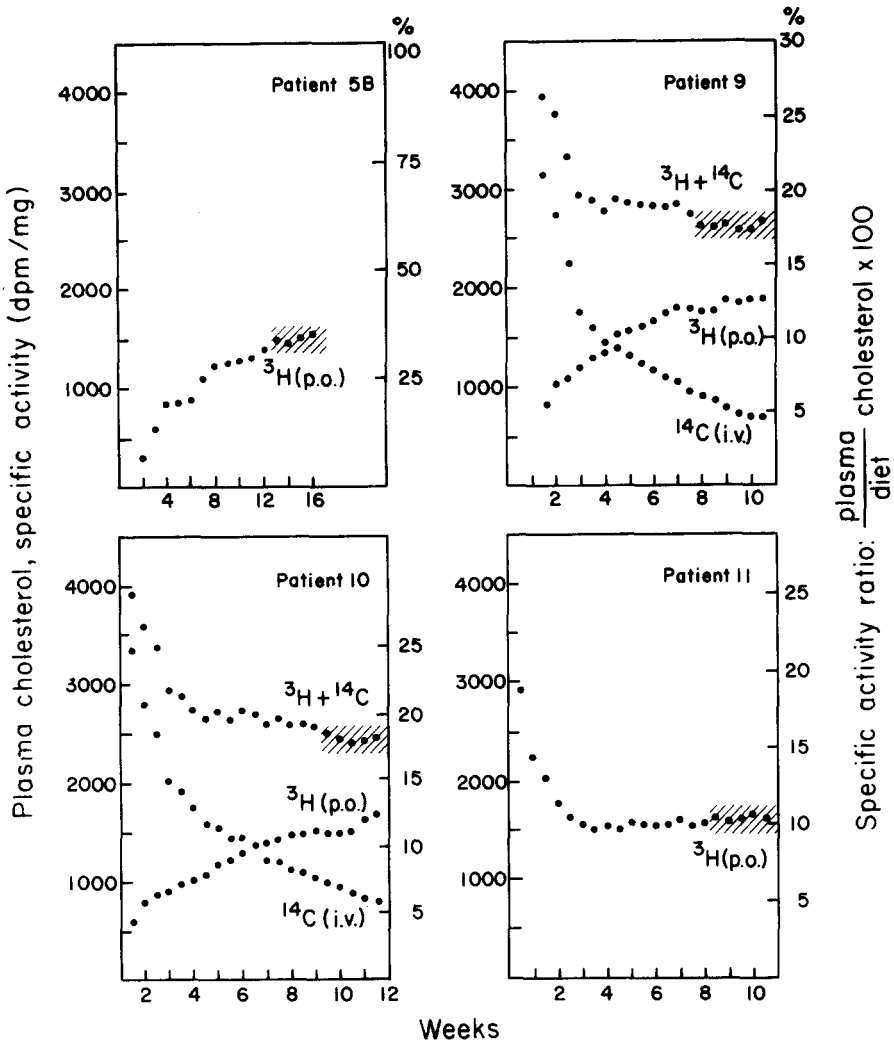

FIG. 5. Application of principles shown in Figs. 3 and 4. Four patients received radioactive cholesterol intravenously, orally, or both. Shaded areas represent isotopic steady state periods in which calculations of cholesterol turnover and synthesis were made (for details see Table 6). Patient 5B received cholesterol-7-^3H orally for 16 wk (this curve was reported in a different context in reference 13). Patients 9 and 10 received cholesterol-26-^{14}C intravenously at the beginning of the study and cholesterol-1,2-^3H orally throughout. The curve obtained by adding the two curves is shown. Patient 11 received cholesterol-1,2-^3H intravenously at the beginning of the study and orally thereafter.

time curve had reached a plateau even after 16 wk, at which time the study had to be ended. However, when a priming dose of cholesterol-1,2-^3H (50 μc) was fed to patient 11 and small doses (2 μc) of the same isotope were fed daily, a plateau of specific activity of plasma cholesterol was reached in 3 wk (Fig. 5). The usefulness of a priming dose of radiocholesterol was tested in a different manner in patients 9 and 10: cholesterol-1,2-^3H was fed daily, and cholesterol-26-^{14}C was given as a single dose intravenously. Fig. 5 shows that at 11 wk a plateau for the orally administered isotope had still not been reached, but when the specific activity–time curves for the two isotopes (expressed as dpm/mg) were added, the additive curve showed a plateau in both patients in 8–9 wk. This additive curve is assumed to represent the single curve that would have been seen had a single isotope been given in the same program. The validity of this treatment rests on the assumption that with small daily doses of isotopic cholesterol a plateau will be reached if the feeding is continued long enough: "priming" labels the tissues more rapidly, thus accelerating the attainment of isotopic equilibrium.

Cholesterol in Plasma Derived from Absorbed Cholesterol. At the top of Table 6 is shown that fraction of plasma cholesterol derived from dietary cholesterol, calculated as the ratio of the specific activities of plasma cholesterol and dietary cholesterol (equation 14). The specific activities used in this calculation represent an average of the values during the isotopic steady state (shown in the shaded areas in Fig. 5); in patients 9 and 10 these values

TABLE 5 STEROL BALANCE DATA IN SIX PATIENTS ON MODERATE CHOLESTEROL INTAKES (COMBINED STEROL BALANCE METHOD)

Patient No.	6	7	8	9	10
Days: No. of determinations*	40:10	30:6	72:18	36:9	76:19
Cholesterol intake (mg/day)	300	577	378	452	295
Isotope given intravenously (pulse labeling)	4-^{14}C	1,2-^{3}H	26-^{14}C	26-^{14}C	26-^{14}C
			mg/day ± SD		
Fecal steroids (uncorrected)					
a. Neutral	852 ± 274	405 ± 119	433 ± 65	641 ± 117	442 ± 305
b. Acidic	754 ± 269	141 ± 52	238 ± 92	110 ± 20	107 ± 89
c. Total	1606 ± 523	546 ± 170	671 ± 131	751 ± 132	549 ± 392
Fecal steroids (corrected)					
a. Neutral steroids					
(1) Total†	908 ± 226	782 ± 54	647 ± 45	1169 ± 194	645 ± 72
% loss	—‡	52.0	33.1	45.2	31.5
(2) Endogenous	715 ± 161	457 ± 35	510 ± 42	998 ± 242	527 ± 55
(3) Unabsorbed dietary	192 ± 81	326 ± 47	138 ± 23	171 ± 72	118 ± 54
(4) Absorbed dietary cholesterol (Method I, Eq. 11)	108 ± 80	252 ± 47	240 ± 23	281 ± 72	176 ± 58
b. Acidic steroids	799 ± 212	162 ± 26	220 ± 73	110 ± 11	93 ± 19
c. Total steroids (total neutral + acidic)	1707 ± 418	944 ± 65	867 ± 87	1279 ± 198	738 ± 77
Cholesterol turnover (endogenous neutral + acidic steroids, Eq. 12)	1514 ± 352	619 ± 56	730 ± 76	1108 ± 247	620 ± 58
Cholesterol synthesis (Eq. 9 or 13)	1407	367	489	827	443

* See same footnote in Table 4.
† The term "total neutral steroids" includes all neutral steroids derived from cholesterol, and excludes steroids derived from plant sterols.
‡ Neutral steroid excretion corrected only for fecal flow with Cr_2O_3 in this patient, since the diet contained no β-sitosterol.

TABLE 6 COMPARISON OF CHOLESTEROL TURNOVER AND SYNTHESIS DETERMINED BY STEROL BALANCE METHODS AND ISOTOPIC STEADY STATE MODEL

Patient No.	5B	9	10	11
Diet	D	G	G	H
Days: No. of determinations*	20:5	20:5	21:15	11:11
Cholesterol intake (mg/day)	1,600	452	295	290
Isotope fed daily	7-^{3}H	1,2-^{3}H	1,2-^{3}H	1,2-^{3}H
Dietary cholesterol SA (*dpm/mg*)	4,159	15,000	13,500	15,553
Plasma cholesterol SA (*dpm/mg*)	1,500	2,600	2,450	1,600
Proportion of plasma cholesterol derived from absorbed cholesterol (Eq. 14)	0.36	0.17	0.18	0.10
		mg/day ± SD		
Cholesterol absorption				
Method I (Eq. 11)	417 ± 172	258 ± 18	108 ± 23	—
Method II (Eq. 15, 16, 11)	514 ± 143	237 ± 18	132 ± 9	147 ± 31
Method III (Eq. 12 and 18)	414	161	108	126
Method III (Eq. 3 and 18)		222	133	
Cholesterol turnover				
Isotopic steady state model				
I. By Eq. 17, with absorption by Method I	1,156	1,489	595	—
II. By Eq. 17, with absorption by Method II	1,425	1,368	728	1,511
Combined Sterol Balance Method (Eq. 12)	1,050 ± 78	948 ± 58†	598 ± 54†	1,256 ± 98
Cholesterol synthesis				
Isotopic steady state model				
I. By Eq. 17 and 13, with absorption by Method I	739	1,231	487	—
II. By Eq. 17 and 13, with absorption by Method II	911	1,131	596	1,364
GLC balance method (Eq. 9)	633	700†	490†	1,114

SA = specific activity.
* See same footnote in Table 4.
† These values do not agree with those in Tables 5 or 8, because of differences in durations of study periods. The data in this table relate only to isotopic steady state periods.

were derived from the sum of ^{3}H and ^{14}C curves. The proportion of plasma cholesterol derived from dietary cholesterol ranged from 10 to 36%.

Absorption of Cholesterol by Three Methods. Table 6 presents results for cholesterol absorption in four patients calculated by Methods I (intravenous label), II (oral label), and III (isotopic steady state data). These calculations showed good agreement between results

obtained by all methods, with poorest agreement in patient 9. Calculations according to Method III are valid only during the isotopic steady state (equation 18); since the values obtained by Method III generally agreed well with those derived by Methods I and II, the data suggest that the isotopic steady state had been approximated in all patients.

However, the reasonable correspondences between values obtained by Method III in patients 9 and 10 resulted only when Method III was applied to the sum of the ^{3}H and ^{14}C curves; had absorption been calculated solely from the specific activities derived from the orally administered isotope (^{3}H) (Fig. 4), values for absorption calculated by equations 12 and 18 would have been considerably lower (104 and 63 mg/day, respectively) than those derived from the summed specific activity curves (161 and 108 mg/day).

Fig. 6 presents a comparison of absorption data obtained by Methods I and II in four patients. In every case cholesterol-^{14}C was administered intravenously as a pulse label and cholesterol-^{3}H was incorporated into the formula feeding before the beginning of the absorption measurements; the number of days after first administration of each label prior to the initial analysis is shown.

In general, Method II seems to be superior to Method I for measuring cholesterol absorption. In most cases the results by Method II were more constant than those by Method I, and constant absorption data were attainable almost immediately after initiating oral administration of isotope. Certain factors appear to contribute to the greater constancy obtained by Method II. (*a*) Isotopic calculation of unabsorbed dietary cholesterol (which usually constitutes a much smaller fraction of the fecal neutral steroids than do the endogenous neutral steroids)

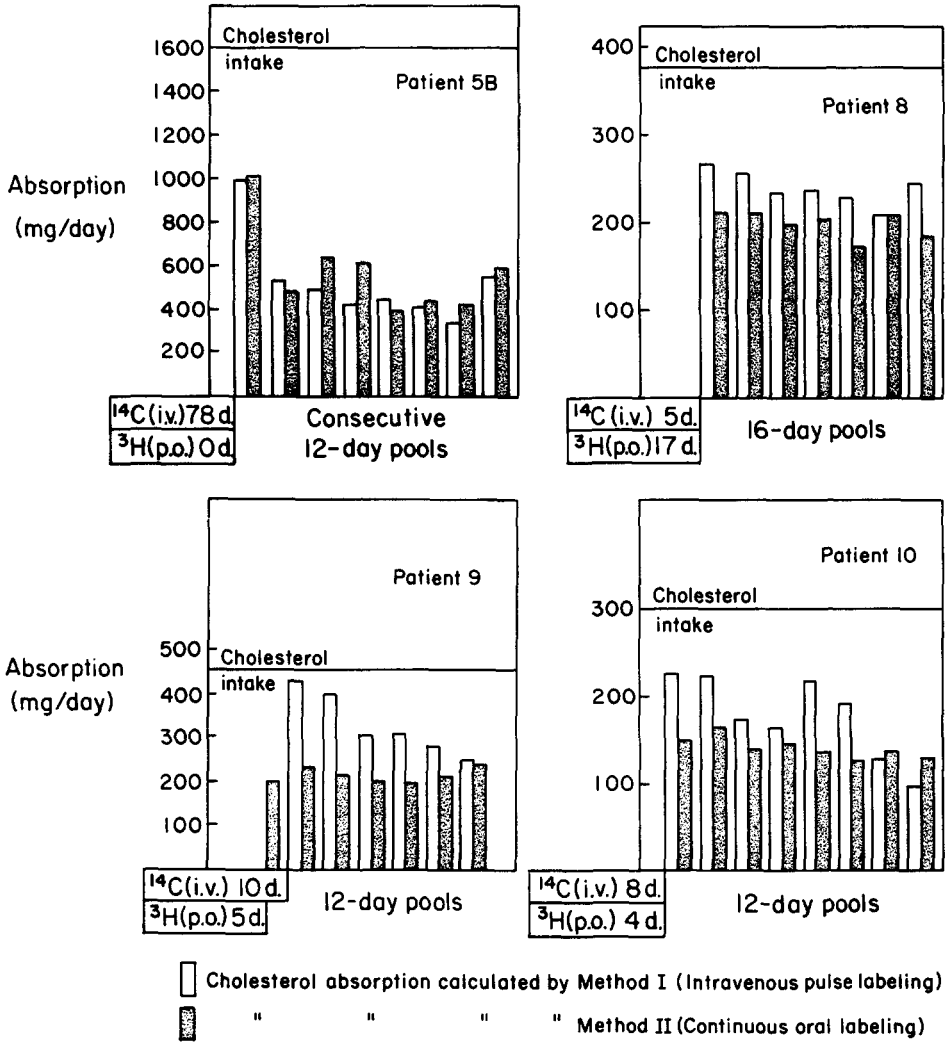

FIG. 6. Cholesterol absorption calculated by Method I (intravenous labeling) and by Method II (continuous oral labeling). Box below each graph shows the number of days (d.) after administration of cholesterol-^{14}C intravenously and institution of daily cholesterol-^{3}H by mouth before absorption measurements were begun. Daily cholesterol absorption by each method is shown relative to daily cholesterol intake.

is a direct measurement facilitated by the high specific activity of dietary cholesterol. On the other hand, in Method I, unabsorbed dietary cholesterol is measured indirectly as the difference between two larger values obtained independently, total fecal steroids and endogenous neutral steroids. (*b*) In Method II the amount of isotope excreted in the form of endogenous neutral steroids is small as compared to that of unabsorbed dietary cholesterol, and correction of specific activity of neutral steroids for delay in colonic emptying time is not critical by this method. In Method I the estimation of this delay is important, but it cannot always be made with precision. (*c*) Method II does not depend upon complete equilibration of isotope between plasma and intestinal pools of cholesterol as does Method I. If the specific activity of fecal endogenous neutral steroids is lower than that of plasma cholesterol, unabsorbed dietary cholesterol would be overestimated and cholesterol absorption underestimated by Method I. This error does not appear to occur in these four patients, but in certain patients in whom cholesterol metabolism has been altered radically we have observed such errors to occur (unpublished data of the authors). Whatever the factors which cause an erroneous measurement of absorption by Method I, they seem to be predominant in the period shortly after the intravenous administration of labeled cholesterol and during the phase of rapid nonlinear decay in the specific activity of plasma cholesterol. Gradually, as the measurements are extended in time, the two methods give very similar results.

Turnover and Synthesis of Cholesterol. The lower half of Table 6 shows values for daily turnover and synthesis of cholesterol obtained by analysis of data obtained by three methods: the isotopic steady state model, the GLC balance method, and the combined sterol balance method. Methods I and II were both applied for measurement of absorption, since patients 5B, 9, and 10 were labeled in two ways: by pulse labeling (to give absorption by Method I) and by daily doses to attain the isotopic steady state (absorption by Method II). Therefore two estimates of turnover and synthesis, based on different methods for measuring absorption, could be calculated during the isotopic steady state.

Since values for synthesis and turnover derived from isotopic data depend so critically upon the accuracy of absorption measurements, it is pertinent to note, first, that daily absorption calculated by Methods I and II agreed so well, in the three patients in whom this comparison could be made, that as a result the estimates of synthesis and turnover using these two sets of absorption values also compared favorably.

Small absolute differences in estimation of cholesterol absorption result in larger absolute differences in estimates of cholesterol synthesis based on data obtained during the isotopic steady state. For example in patient 5, values obtained for cholesterol absorption by Methods I and II were 417 and 514 mg/day, respectively. While the absolute difference in absorption by the two methods was only 97 mg/day, synthesis calculated from Method I was 739 and from Method II was 911 mg/day, an absolute difference of 172 mg/day. The percentage differences in absorption, in turnover, and in synthesis were all the same (12.3%) using Methods I and II.

Estimates of cholesterol turnover and synthesis obtained by the isotopic steady state method were invariably higher than those obtained by the two sterol balance methods. In the four patients in whom absorption data by Method II were available, turnover estimates were 20 to 43% higher by the isotopic steady state method; for synthesis, 22 to 61% higher. The estimates for turnover, in absolute terms, ranged 130–420 mg/day higher, and for synthesis, 106–431 mg/day higher by the isotopic steady state calculations.

One- and Two-Pool Models vs. Sterol Balance Methods

Table 7 presents data obtained from specific activity–time curves of plasma cholesterol after intravenous administration of radioactive cholesterol. In addition, it shows rates of turnover of body cholesterol derived from these data according to the one-pool model (equation 1) and the two-pool model (equation 3). Values obtained according to the one-pool model were uniformly higher than those calculated by the two-pool model; the absolute differences ranged from 71 to 1756 mg/day higher, percentage differences from 11 to 175% higher.

In Table 8 these results for turnover of cholesterol obtained by isotopic labeling are compared with those obtained by sterol balance methods (equations 6 and 12). Rates of turnover of cholesterol calculated by the two-pool model correlated more closely with those obtained by the methods of sterol balance ($r = 0.90$) than those calculated by either the one-pool model ($r = 0.74$) or its modification ($r = 0.36$). The difference in rate of cholesterol turnover between the sterol balance methods and the two-pool model was on the average only 136 mg/day, in contrast to a mean difference of 573 mg/day between results obtained by the sterol balance methods and the one-pool model. The mean percentage differences were, respectively, 15 and 68%.

Table 8 also presents calculations of turnover in eight patients according to the modified one-pool model (equations 1 and 2), in which the pool size was estimated from the radioactivity retained in the body at the start of log-linear decay. Despite the conceptual differences between the one-pool model (equation 1) and its modification (equations 1 and 2), these two methods showed results for turnover that were often remarkably similar.

TABLE 7 CHOLESTEROL TURNOVER RATES DERIVED FROM ONE-POOL AND TWO-POOL MODELS AFTER INTRAVENOUS ADMINISTRATION OF RADIOCHOLESTEROL

Patient	Isotope	Dose of Isotope	A	B	$t_{1/2}$ of First Exponential*	$t_{1/2}$ of Second Exponential†	Cholesterol Turnover		Difference	
							One-Pool Model (Eq. 1)	Two-Pool Model (Eq. 3)	Absolute	Percentage
		dpm × 10^8	*dpm/mg*	*dpm/mg*	*days*	*days*	*mg/day*	*mg/day*	*mg/day*	%
1	4-^{14}C	1.89	10000	2600	4	40	1260	908	+352	39
2	4-^{14}C	1.89	6400	2600	5.6	35	1440	1026	+414	40
3A	4-^{14}C	1.95	5000	2600	5.6	50	1040	853	+187	22
3B	1,2-^{3}H	1.78	4350	3270	4.5	31	1210	1017	+193	19
4	1,2-^{3}H	1.83	5500	2000	4.8	23	2760	1004	+1756	175
5A	4-^{14}C	1.89	6000	1700	5.6	51	1500	1084	+416	38
6	4-^{14}C	1.56	3400	1530	5.5	29	2440	1710	+730	43
7	1,2-^{3}H	2.22	4200	2610	8.4	95	620	549	+71	11
8	26-^{14}C	1.84	5000	1800	5.6	60	1180	939	+241	26
9	26-^{14}C	2.44	3825	2320	6.5	45	1610	1309	+301	23
10	26-^{14}C	1.71	3950	2075	7	64	880	738	+142	19
						Mean	1449	1012	437	40
						± SD	± 638	± 302	± 473	

* $t_{1/2}$ of line α' in Fig. 1, right.
† $t_{1/2}$ of line β' in Fig. 1, right.

TABLE 8 CHOLESTEROL TURNOVER RATES OBTAINED BY THE COMBINED STEROL BALANCE METHOD, COMPARED TO RATES OBTAINED BY ISOTOPIC LABELING

Patient	Sterol Balance Method	Two-Pool Model			One-Pool Model			One-Pool Model (Modified)		
	Turnover (Eq. 6, 12)*	Turnover (Eq. 3)	Difference†		Turnover (Eq. 1)	Difference†		Turnover (Eq. 1, 2)	Difference†	
	mg/day	*mg/day*	*mg/day*	%	*mg/day*	*mg/day*	%	*mg/day*	*mg/day*	%
1	810	908	+98	12	1260	+450	55	898	+88	11
2	773	1026	+253	33	1440	+667	86	—	—	—
3A	689	853	+164	24	1040	+351	51	980	+291	42
3B	1080	1017	−63	−6	1210	+130	12	1149	+69	6
4	977	1004	+27	3	2760	+1783	182	3420	+2443	250
5A	711	1084	+373	52	1500	+789	111	1665	+954	134
6	1514	1710	+196	13	2440	+926	61	—	—	—
7	619	549	−70	−11	620	+1	0.0	—	—	—
8	730	939	+209	29	1180	+450	62	1250	+520	71
9	1108	1309	+201	18	1610	+502	45	1470	+362	33
10	620	738	+118	19	880	+260	42	935	+315	51
Mean ± SD	876 ± 304	1017 ± 348	+136	15	1449 ± 598	+573	65	1470 ± 696	+594	68
		r = 0.90‡			r = 0.74‡			r = 0.36‡		

* For patients 1–5 turnover was calculated by Eq. 6; for patients 6–10, by Eq. 12.
† Difference from value in sterol balance method.
‡ Correlation coefficient compared to sterol balance method.

DISCUSSION

Daily Turnover of Cholesterol Estimated from Specific Activity–Time Curves

In our nine hyperlipidemic patients, calculations of the daily rate of cholesterol turnover by the one-pool model gave results (average 1444 ± 538 mg/day) similar to those reported (2) in seven hypercholesterolemic patients by Chobanian et al. (1830 ± 551 mg/day).

However, our values for daily turnover based on the two-pool model were considerably smaller, and they agreed well with results obtained in eight hyperlipidemic patients by Goodman and Noble (3). In addition, we found a high degree of correlation (r = 0.90) in turnover data obtained for our 10 patients by the two-pool model and by sterol balance methods; the mean difference between results obtained by the two methods was only 15%. The fact that concordant results were obtained by the latter two methods, which are so different in approach, leads us to believe that these results probably approximate the true rate of turnover.

According to Goodman and Noble (3), it is not possible to calculate the size of pool B, and hence of the total body pool of cholesterol, using the two-pool model under the conditions of cholesterol metabolism that pertain in man. They state that the size of pool B can be determined only if entry and removal of cholesterol from pool B, except by isotopic exchange with pool A, is negligible. Since the quantitative aspects of synthesis of cholesterol within pool B cannot be evaluated at this time, any calculations of the size of this pool, based on the two-pool model, should be viewed with caution. Indeed, it must be emphasized that pool B is a conceptual rather than a defined anatomical entity at this time and that there are no data available on the morphologic counterparts of pools A and B in any species.

The use of specific activity–time curves for the calculation of cholesterol turnover after pulse labeling obviously requires less effort than does any method of sterol balance. Results are obtained no sooner, however, since 4–6 wk are needed for log-linearity to be reached in the decay curve. It is a *dis*advantage of specific activity–time curve analyses that daily synthesis of cholesterol (in contrast to turnover) cannot be calculated from decay curves when the diet contains appreciable amounts of cholesterol: newly synthesized and exogenous cholesterol (both of which are unlabeled) contribute to the rate of decay of specific activity, yet their relative contributions to the rate of decay cannot be differentiated by the isotopic technique alone. An exception to this latter statement exists if the patient is also given isotopic cholesterol by mouth and has attained the isotopic steady state; at this time it is possible to determine the relative contributions of dietary and of newly synthesized cholesterol and in this way to estimate synthesis.

All methods based on analyses of curves of specific activity depend upon two basic assumptions: that the size of the total miscible pool of cholesterol does not change during the period of calculation, and that the rate of entry of new cholesterol into body pools (from synthetic or exogenous sources) is constant. In a previous study (18) we have discussed the effects of a change in the pool size or in the rate of synthesis that is induced during the phase of log-linear decay: (*a*) an increase in the size of body pools or a decrease in the rate of synthesis will cause the slope for decay of specific activity of plasma cholesterol to decrease; or (*b*) if either factor (pool size or synthesis) changes in the other direction, the decay of specific activity will be accelerated. While the magnitude of changes in either of these counterbalancing factors cannot be determined, qualitative alterations in the slopes of the specific activity curve can at times offer valuable insights into the effects of drug therapy or various manipulations of diet upon the metabolism of cholesterol.

Estimation of Synthesis of Cholesterol by Sterol Balance Methods

Sterol balance methods depend upon the precise measurement and careful administration of dietary constituents as well as upon total collection of feces and accurate analysis of fecal neutral and acidic steroids. Compensating for these efforts, the balance approach appears to provide more information than analysis of isotopic decay curves alone. There are three advantages inherent in the chromatographic method of sterol balance. First, estimations of cholesterol synthesis are made possible by the GLC balance method in patients in whom the administration of isotopes would not be permissible—normal volunteers, young adults, and children. Second, the GLC method is the only method available which permits valid correction of neutral steroid losses during intestinal transit. Third, with the sterol balance method valid measurements of absorption can be obtained; this in turn permits the essential distinction to be made between daily synthesis and daily turnover of cholesterol when the diet contains cholesterol.

The model of the isotopic steady state first applied in man by Taylor et al. (22) and used in association with sterol balance data by Wilson and Lindsey (4) represents another variation of the combined sterol balance method in which chromatographic and isotopic techniques are employed simultaneously in patients fed cholesterol-containing diets. Although this approach to the measurement of daily synthesis of cholesterol appears to be sound theoretically, its application to patients poses two problems. (*a*) In our clinical experience, a period of many weeks of daily feeding of radioactive cholesterol is required before the specific activity of plasma cholesterol appears to reach a plateau. In four patients the administration of a priming dose of radioactive cholesterol at the beginning of the study period shortened the apparent equilibration time, but even this procedure may not resolve the following consideration. (*b*) It is a premise of the concept of the isotopic steady state that, at the time of the apparent plateau in specific activity of plasma cholesterol, all miscible pools have specific activities identical with that in the plasma. This premise has not yet been tested. Although the plasma cholesterol may appear to have reached constant specific activity, it is possible that a slow exchange of isotope may continue to occur between cholesterol in the plasma compartment and that of slightly lower specific activity in tissues. In this case the method would give results for synthesis that would be erroneously high, because of the movement of less-labeled cholesterol into the plasma compartment. Indeed, we found that results for daily synthesis by this method were uniformly higher than those obtained by the chromatographic sterol

balance method; these differences may be due to falsely high values obtained with the isotopic steady state model, owing to incomplete attainment of isotopic equilibrium.

If the *isotopic* steady state method is handicapped by tardy attainment of isotopic equilibrium, the major problem in calculating cholesterol synthesis by the combined or GLC sterol balance methods is that of determining whether a patient has reached a *metabolic* steady state with respect to constancy of total body cholesterol. It is evident that during periods when concentrations of cholesterol in plasma and tissue pools are rapidly changing, the measurement of synthesis rates by the sterol balance method will give erroneous results. But it is conceivable that tissue concentrations of cholesterol may be changing even when the concentration in the plasma is not changing. In that case rates of synthesis and excretion will not be equal; to the extent of the difference the sterol balance method will be in error in estimating daily synthesis. At the present time the metabolic steady state is arbitrarily defined: constant plasma cholesterol concentrations, unchanging fecal excretion of steroids, and constant body weight must all apply during long periods of study of patients in stable clinical states. A less arbitrary definition of this state clearly is needed.

In view of the theoretical handicaps which beset these available methods for measuring cholesterol synthesis rates in man, it is highly encouraging to find such close agreement between the results obtained by them. Whether the two-pool model *over*estimates and the sterol balance method *under*estimates daily synthesis (or both) during the metabolic steady state remains uncertain. Indeed, a recent report by Samuel, Holtzman, Meilman, and Perl (23) indicated that the two-pool model may overestimate turnover by 3–11% because of the fact that in the first few hours after intravenous pulse labeling the decay curve fails to conform to that required by a perfect two-pool system. This may explain a considerable part of the 15% discrepancy in our analyses of turnover (Table 8) by the cholesterol balance and two-pool models.

During any *un*steady state caused by diet or drug intervention, neither method, nor any other known today, can be used to measure daily synthesis; only when the body has reestablished a new steady state is it possible to test the effects of such interventions upon cholesterol synthesis.

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