A model for cholesterol absorption: isotope vs. mass; single dose vs. constant infusion

Donald B. Zilversmit[1]

Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853[2]

Abstract A study is presented to evaluate the relative merits of isotope and cholesterol mass measurements for cholesterol absorption. In this study, cholesterol absorption is simulated as a sequence of 10 two-pool segments in which a concentration gradient of cholesterol mass and/or label exists between the site of exogenous cholesterol entry and that of fecal loss. The model is governed by first order rate constants both for label and for mass. The appearance of labeled cholesterol in lymph and feces provides a reliable measure of the cholesterol mass increment in lymph due to exogenous cholesterol absorption. Net cholesterol absorption, calculated from constant infusion experiments, differs numerically from this mass increment. A dual isotope fecal ratio method agrees with other labeling techniques, but gives reliable information only when feces are collected for a sufficiently long time.—**Zilversmit, D. B.** A model for cholesterol absorption: isotope vs. mass; single dose vs. constant infusion. *J. Lipid Res.* 1983. **24:** 297–302.

Various methods for determining the absorption of cholesterol from the intestinal tract have been proposed: measuring cholesterol mass, isotopically labeled cholesterol, or both. Measurement of cholesterol absorption is complicated by the fact that cholesterol in the intestinal tract comes from two sources: endogenous as well as dietary. In humans this endogenous component is derived mainly from bile and, to a lesser extent, from secretion of the intestinal tract or from remains of sloughed cells. Measurement is further complicated by the fact that cholesterol, once absorbed from the small intestine, may re-enter the intestinal lumen by way of an active entero-hepatic circulation and/or by exchange of cholesterol between intestinal mucosa and lumen. Thus, the same cholesterol molecule may enter the intestinal lumen several times, presenting itself for absorption more than once.

The following model of cholesterol absorption contains an array of many elements, each element representing a small segment of intestinal lumen and mucosa. This model was devised to evaluate the relative merits of isotopic and mass measurement procedures for cholesterol absorption, as well as to obtain a clearer picture of the movements of cholesterol newly synthesized by the intestinal mucosa, the cholesterol secreted from the intestinal wall into the lumen, and the cholesterol exchanged between lumen and intestinal mucosa. The model is also likely to portray the actual cholesterol absorption processes more realistically than previous models, because it assumes that cholesterol mass and isotope concentration gradients exist between the duodenum and the lower jejunum where, it is assumed, there is almost no cholesterol absorption.

Model

The model consists of 10 or more segments, each segment consisting of two compartments. Flux through the model is governed by first order rate constants for both label and mass. **Fig. 1** shows the first two duodenal segments, each segment consisting of a lumen and a mucosal pool. In the following discussion we shall compare some isotopic procedures, in which feces are collected or in which lymph is sampled, with the net cholesterol mass absorption procedure of Grundy and Mok (1), in which a triple lumen constant infusion technique is employed. In the latter model, the first segment of Fig. 1 represents the place at which the cholesterol mass or label, infused upstream of this location, is sampled by aspiration. Subsequent segments represent segments of small intestine, with the last segment sampled in the same manner as the first.

The rate constants were chosen to give results that correspond to data obtained in human studies. For example, Grundy and Mok (Table 5, ref. 1) report mean net cholesterol absorption rates of 43 mg/hr when cholesterol inflow into the intestinal lumen averages 99 mg/hr. Samuel et al. (2), in a study with the isotope ratio method, report cholesterol absorption of 46 ± 11%. In our model we aimed at a cholesterol absorption of 50%. No data are available for the cholesterol synthesis or pool size of that portion of the intestinal mucosa that participates in cholesterol absorption,

[1] Career Investigator of the American Heart Association.

[2] Reprint requests should be addressed to: Division of Nutritional Sciences, 202 Savage Hall, Cornell University, Ithaca, NY 14853.

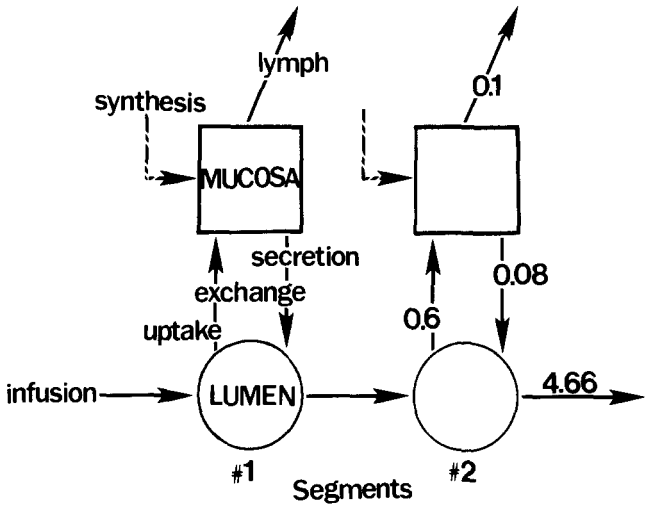

Fig. 1. Multi-segmental cholesterol absorption model. Segment #2 shows first order rate constants employed in the calculated values of Tables 1 and 2. The descriptions in segment #1 show the transfer processes described by these constants.

nor is it known how much cholesterol is transferred by mucosal secretion and sloughed cells and the amount of cholesterol exchanged between the intestinal lumen and mucosa. We have chosen values that appear to be reasonable for the absorptive process in humans and we have observed that, in results not shown here, changes in the model parameters do not alter the conclusions drawn from this study to a significant extent.

The segments in Fig. 1 are composed of a circle, which represents the lumen contents, and a square, which represents the intestinal mucosa. The relation between cholesterol contents of the lumen and intestinal mucosa is such that for each segment the transfer of cholesterol from lumen into mucosa is 0.6 times the lumen contents per hr and the hourly transfer of cholesterol from mucosa to lumen is 0.08 times the mucosal cholesterol pool that participates in cholesterol absorption. In addition, an arrow with a transfer rate constant of 0.1 hr^{-1} represents the transfer of cholesterol from the active mucosal cholesterol pool to lymph. Each pool of mucosal cholesterol also has an input arrow, which represents the cholesterol synthesis in that particular pool. As for the intestinal lumen, each section is connected to the next one by an arrow representing the transfer of cholesterol from one section of intestinal lumen to the next. The value for the rate constant between two lumen sections is 4.66 hr^{-1} for a 10-segment model. This represents a 10-segment transit time of approximately 2 hr. This means that in approximately 2 hr, the unabsorbed portion of a bolus of cholesterol present at the first segment will have been propelled by convective flow or peristalsis to emerge from the last intestinal segment of the model. For the studies of Grundy and Mok (1), who used a 100-cm length of intestine, such a transit time appears reasonable.

In segment #1 of the model, one additional arrow is present as an input into the lumen. This input could represent a constant infusion or a single dose of cholesterol label or mass. In the discussion of the present model, I wish to draw particular attention to the quantitative behavior of the isotope and to the fate of exogenous cholesterol mass in the Grundy and Mok model (1).

The constants assigned to the 10-segment model, shown partially in Fig. 1, were derived to mimic the experimental findings in the Grundy and Mok study. A constant infusion of 100 units into the proximal segment of the intestinal lumen might represent 100 mg/hr of cholesterol which compares, for example, to an input of 500 mg of dietary cholesterol plus 1000 mg of endogenous cholesterol during an assumed 15-hr absorptive period for an average person. The same number could, however, also reflect an input of 100 dpm of labeled cholesterol per hr in infusion experiments in which both mass and isotopic cholesterol are employed. Finally, the arrow labeled "synthesis" of cholesterol (in the mucosal pool that participates in cholesterol absorption) is assumed to be 3 mg/hr per segment or a total of 30 mg/hr over the entire length of intestine that is being studied.

Table 1 presents the calculated results from the infusion of 100 mg of cholesterol/hr. It shows that the

TABLE 1. Cholesterol mass and label in ten segments of the intestinal model[a]

	Cholesterol[b]			
	Mass		Label	
Segment	Lumen	Mucosa	Lumen	Mucosa
	mg		*dpm*	
1	20.3	84.3	20.0	66.8
2	19.2	80.7	18.7	62.3
3	18.2	77.3	17.4	58.1
4	17.2	74.1	16.3	54.3
5	16.4	71.2	15.2	50.6
6	15.5	68.4	14.2	47.3
7	14.8	65.9	13.2	44.1
8	14.1	63.5	12.3	41.2
9	13.4	61.3	11.5	38.4
10	12.8	59.2	10.8	35.9
Total	161.9	705.9	149.6	499.0
	Lymph	70.6 mg/hr	Lymph	49.9 dpm/hr
	Feces	59.4 mg/hr	Feces	50.1 dpm/hr

[a] Infusion rates: 100 mg/hr and 100 dpm/hr. Rate constants as shown in Fig. 1. Cholesterol synthesis: 3 mg/segment per hr.

[b] Tabulated values in Table 1 were calculated by mass or label balance equations. For example, in segment #1, if the lumen pool size = L mg, mucosal pool = M mg, and mucosal cholesterol synthesis = 3.0 mg/hr, 100 + 0.08 M = (4.66 + 0.6)L and 0.6 L + 3.0 = (0.1 + 0.08)M, which gives L = 20.3 and M = 84.3 mg.

TABLE 2. Cholesterol absorption as calculated from lymph cholesterol mass and label increments and from intestinal lumen cholesterol content

Infusion into Segment #1	Lymph Cholesterol	Absorption	Intestinal Cholesterol Segment #1	Intestinal Cholesterol Segment #10	Net Absorption
		%	mg		%
Mass					
100 mg/hr	70.6 mg/hr	49.9[a]	20.3	12.8	36.9[b]
50 mg/hr	45.6 mg/hr	49.8	10.3	7.4	28.2
25 mg/hr	33.2 mg/hr	50.0	5.3	4.7	11.3
10 mg/hr	25.7 mg/hr	50.0	2.3	3.1	<0
0 mg/hr	20.7 mg/hr		0.3	2.0	<0
Label					
100 dpm/hr	49.9 dpm/hr	49.9			

[a] Absorption from lymph cholesterol increment: e.g., 100 (70.6–20.7)/100, and from labeled cholesterol in lymph: 100(49.9/100).

[b] Absorption from intestinal lumen contents according to the method of Grundy and Mok (1), e.g.: 100[1 − (12.8/20.3)].

lumen cholesterol concentration decreases from a value of 20.3 in the first segment to 12.8 in segment #10. It shows a parallel decrease in the pool size of that portion of the mucosa that participates in cholesterol absorption. Although this decrease is not meant to signify that the actual amount of mucosal tissue per segment of intestine decreases from the proximal to the distal end, the decrease in mucosal cholesterol pool size parallels the decrease of the lumen cholesterol pools from the proximal to the distal portions of the intestinal segment. This parallelism reflects the assumption that the same rate constants prevail in different segments of the intestinal model. The amount of cholesterol leaving the system in lymph is calculated to be 70.6 mg/hr and in feces as 59.4 mg/hr, which adds up to 130 mg/hr. This equals the sum of an infusion rate of 100 mg/hr and 30 mg/hr from cholesterol synthesis by intestinal mucosa. Table 1 also shows the steady state isotopic concentrations and outputs in the intestinal system if 100 dpm of labeled cholesterol are infused per hr. Again, one sees an exponentially falling concentration of isotope in the lumen and in the mucosa from the proximal towards the distal end. The output in lymph and feces is 49.9 and 50.1 dpm/hr, respectively, which adds up to 100 dpm/hr; this equals the input of label into the system.

Absorption calculations

The data in Table 1 show the steady state solutions of the 10-segment system presented in Fig. 1. The same calculations were also made for a 100-segment system, but the results differ less than 1% from those for the 10-segment system. We can now compare the calculation of cholesterol absorption based on mass with that based on the measurement of isotope. It seems reasonable to use the output of lymph cholesterol mass as a reference point for comparison of absorption obtained by other means. Thus, if one takes the amount of cholesterol transported into lymph in the absence of cholesterol infusion into the upper intestine as a baseline, one can calculate that upon infusion of 10 to 100 mg/hr, the increment in total lymph cholesterol mass transported per hr represents 49.8–50% of the infused dose. Therefore, the absorption of cholesterol in this system may be said to equal 50%. When one compares this figure to the absorption calculated from the amount of label in lymph, or from the amount of label in feces subtracted from the infused label, one finds that in both cases the absorption of cholesterol label equals 49.9%, showing an excellent agreement with the increment in cholesterol mass in lymph. A comparison of these calculations with those for "net absorption" proposed by Grundy and Mok is shown in **Table 2.** Apparently, in this method the calculated net percent absorption varies greatly with the infused cholesterol load and may even be negative. The latter case results from the appearance of synthesized cholesterol in the lumen when little or no cholesterol is infused.

It is of some interest to consider the significance of the cholesterol transfers between intestinal lumen and mucosa in more detail. Fig. 1 shows a unidirectional flux labeled "uptake", another labeled "secretion", and a bidirectional flux labeled "exchange". The latter represents an equimolar transfer that does not contribute to net mass transfer but does allow labeled cholesterol to reach the mucosal and lymph compartments.

In **Fig. 2** the transfer of cholesterol mass between lumen and mucosa in the first segment of intestine is shown for two examples: A) 100 mg of cholesterol enters the first lumen section, and B) no cholesterol enters

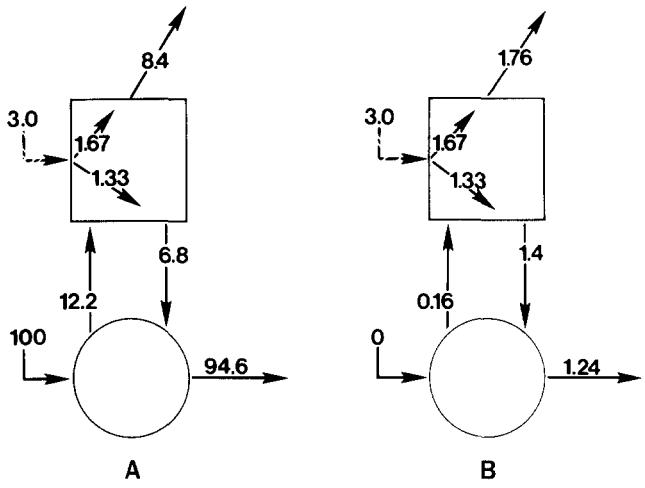

Fig. 2. Cholesterol flux in segment #1. A, 100 mg per hr cholesterol infusion into lumen compartment. B, No infusion into lumen compartment. Numbers indicate transfers in mg/hr, and are rounded to about 1%.

this section. Analysis of the cholesterol transfers show that, of the 3.0 mg/hr synthesized in mucosa, 1.67 mg/hr is secreted into lymph and 1.33 mg/hr is secreted back into the intestinal lumen. If the total return to the intestine is 6.8 mg/hr, then the difference 6.8 − 1.33 = 5.47 mg/hr is due to exchange. Of the 12.2 mg/hr entering the mucosa, the exchange component is 5.47 mg/hr (to equal that going in the opposite direction) and the remaining 12.2 − 5.47 = 6.73 mg/hr is a net uptake component.

A similar analysis in Fig. 2B shows an exchange of 0.07 mg/hr and a net uptake of 0.16 − 0.07 = 0.09 mg/hr. In this case the net uptake is not exogenous cholesterol, which is 0, but is composed of cholesterol, originally synthesized by mucosa, returning to that compartment. If we compare the lymph outflows in Fig. 2A and B we see a difference of 8.431 − 1.756 = 6.68 mg/hr, which represents an absorption by the first segment of 6.68% of the 100 mg/hr exogenous cholesterol infusion. This percentage absorption by the first segment corresponds to the outflow of labeled cholesterol in lymph. According to the data in Table 1, when 100 dpm is infused per hr, the mucosa of the first segment contains 66.8 dpm at the isotopic steady state. One-tenth of this pool or 6.68 dpm (i.e., 6.68% of the infused label) appears in lymph per hr. This amount of label would appear in lymph even if no exogenous cholesterol mass were supplied to the lumen. The agreement between label and mass absorption is not a fluke. If the mucosal synthesis is changed from 3.0 to 100 mg/hr, the mass increment in lymph when 100 mg/hr is infused is still 6.68% of the infused amount and the percent of label appearing in lymph is also unchanged.

The apparent contradiction of finding 6.68% of the infused labeled cholesterol in lymph from segment #1 under conditions in which no exogenous cholesterol is supplied and thus no net absorption occurs is frequently attributed to the transfer of label by an exchange reaction which has little or no physiological significance. Far from being meaningless, however, the presence of label in lymph shows that *if* cholesterol mass were entering the upper intestinal lumen, *then* the first segment would absorb 6.68% of the infused cholesterol mass.

Now let us see how the Grundy and Mok net cholesterol absorption method (1) interprets the data in Fig. 2. According to Table 1, which represents the same model, the lumen cholesterol in segment #1 equals 20.3 mg and that in segment #2 equals 19.2 mg. The percent cholesterol absorption in segment #1 would have been calculated as 100[1 − (19.2/20.3)] = 5.4%. The same number of results from the difference between the cholesterol entering and leaving segment #1, i.e., 100 − 20.3 × 4.66 = 5.4 mg/hr or 5.4% of the infused amount. This percentage is a sizable underestimate of the 6.7% obtained from the lymph cholesterol mass or label increment.

Single dose of labeled cholesterol

The 10-segment model of Fig. 1 can also be used to test the validity of a commonly used single dose, dual isotope procedure (3, 4). If the arrow representing the "infusion" is made to represent a single dose of 100 units of [^{3}H]cholesterol and 100 units of [^{14}C]beta-sitosterol introduced into segment #1, the concentration of isotopically labeled sterols in segments 1 to 10 can be calculated by integration of the 20 differential equations describing the model. This was done on a Hewlett Packard 9825 with a fourth-fifth order Runge-Kutta routine with variable step size (5). **Fig. 3** shows the cumulative outflow of labeled sterols from segment #10 to "feces". Curve A represents labeled cholesterol; curves B to D represent labeled sitosterol fecal outputs in models that make different assumptions about the behavior of this sterol. Curve B results when the rate constants for beta-sitosterol are assumed to be the same as for labeled cholesterol, except that output into lymph = 0. Curve C, which reaches the asymptotic ratio much faster, results from an increase in the mucosa → lumen rate constant from 0.08 to 0.26. This adjustment equalizes the mucosal pool sizes of the two labeled sterols. Data on the relative mucosal pool sizes of the two sterols in humans are lacking, but in rats the amount of labeled sitosterol in the mucosa was found to be much smaller than that of labeled cholesterol (6, 7). Thus the fourth curve (D) is based on the assumption that labeled sitosterol does not enter the mucosa at all

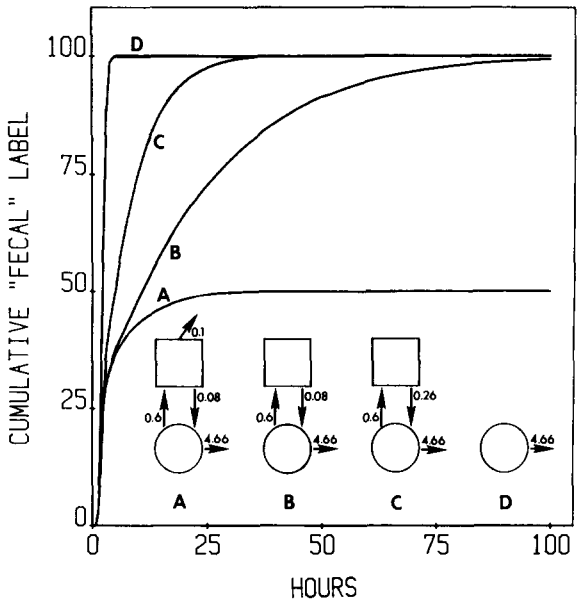

Fig. 3. Accumulation of labeled cholesterol and sitosterol in "fecal" outflow from segment #10. Y axis is percent of single dose introduced into segment #1. Curves correspond to models shown in figure: A is cholesterol of which 50% is absorbed; B–D are sitosterol models. Rate constants in B are the same as in A except for no outflow in lymph. Constants in C are adjusted so as to equalize the presence of labeled cholesterol and sitosterol in mucosa under constant infusion conditions. Model D shows no sitosterol uptake by mucosa. At steady state, model B would contain very large amounts of sitosterol in the mucosa, model C would contain an intermediate amount, and model D none at all. These models probably cover the extremes of the actual states under physiological conditions.

and is, therefore, confined to the intestinal lumen. In this instance the labeled sitosterol is removed from the intestinal tract at a much faster rate.

If the fecal outflows from the 10-segment models A and C in Fig. 3 are converted to isotope (cholesterol/sitosterol) ratios, a curious effect becomes apparent. The earliest "fecal" samples (curve E, **Fig. 4**) have a ratio of 1.00 which is the same as that of the dose. The fecal isotope ratio reaches a minimum of 0.27 at about 12 hr and then rises rapidly. At 5 hr about 50% of the administered sitosterol and 35% of the cholesterol dose have been excreted from segment #10, giving rise to a cumulative fecal isotope ratio of 0.7 in curve F, Fig. 4. The correct ratio for overall absorption is 0.5, which is not achieved in the pooled feces until about 25 hr after the dual isotope dose (curve F, Fig. 4). At this time 97.5% of the sitosterol and 98.5% of the labeled cholesterol that are ultimately excreted are present in feces.

Of the three sitosterol models described in Fig. 3, model C, used in the previous paragraph, gives accurate isotope ratios at an earlier time than the other two models. With the one-pool model for sitosterol (curve D, Fig. 3) the cumulative cholesterol/sitosterol isotope ratio (not shown) goes through a minimum of 0.35 at 3 hr and does not reach the correct value of 0.5 until 40 hr after dosage. If model B is chosen for sitosterol, the isotope ratio converges upon the asymptotic value even more slowly.

There is one additional property of the isotope ratio that to my knowledge has not been described previously. If one uses the 10-segment models A and D to generate the instantaneous fecal isotope ratios, these ratios decrease in a log-linear fashion during the first 2 hr, during which nearly one-half of the sitosterol dose is excreted. During this initial phase one can show mathematically that the isotope ratio $R = e^{-0.6t}$ in which t = time in hours. The exponent 0.6 is equal to the first order rate constant of cholesterol uptake by mucosa (Fig. 3, model A). This value can therefore, in principle, be obtained experimentally from the initial "slope" of the isotope ratio vs. time curve.

From the foregoing considerations it is apparent that the fecal isotope ratio may be a less than reliable index of cholesterol absorption if only small portions of feces are collected. The instantaneous ratio at early times is probably too high and that of the middle period may be too low. This U-shaped ratio profile has been observed in timed collections of feces from some rabbits fed labeled cholesterol and sitosterol (8). The late rise in isotope ratio is usually ascribed to the return to the lumen of a portion of the absorbed isotopic cholesterol that has reached the bloodstream. Although this may

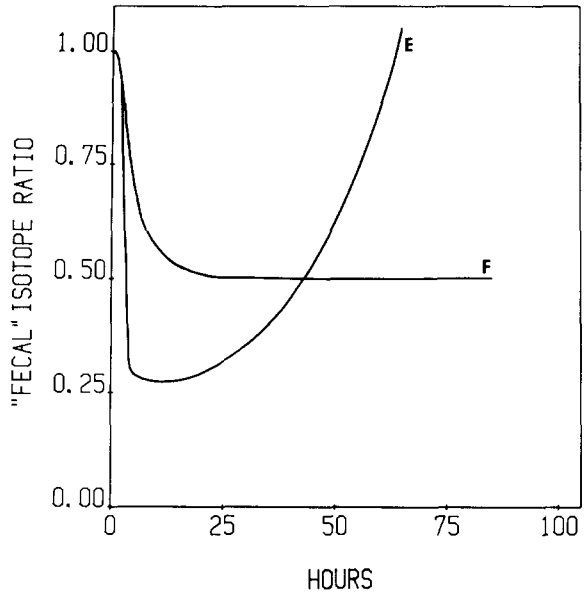

Fig. 4. Cholesterol/sitosterol isotope ratios of "fecal" outflow from segment #10 for models A and C in Fig. 3. E, Instantaneous isotope ratios in fecal outflow. F, Isotope ratios of feces collected up to the time on abscissa.

well be the case under some conditions, the models described in the present paper do not contain such a "resecretion" pathway. Instead, the late rise in isotope ratios in the multisegmental models results from the unequal transit times of the two sterols. It should be noted, however, that the sharp rise in fecal isotope ratio shown in Fig. 4 pertains only to the final 2.5% of labeled fecal sterol. Other models may show differing tendencies to produce a U-shaped ratio profile.

The results show that when absorption is calculated from the amount of lymph or fecal isotopically labeled cholesterol after constant infusion or a single dose, one obtains a measure of exgenous cholesterol absorption that is in very good agreement with that derived from the increment in lymph cholesterol mass. Since the amount of cholesterol label appearing in lymph also appears to be a good measure of the increment in lymph cholesterol mass, it would appear that methods based on the measurement of labeled cholesterol in plasma may give equally valid measures of exogenous cholesterol absorption. In rats (9, 10) and in humans (2, 11, 12), a dual isotope plasma ratio method has been found to correspond closely to values obtained from fecal analysis.

The author wishes to thank P. L. Fox and L. Staib for writing computer programs used in the calculations, and J. Dougherty for art work and manuscript preparation. Several discussions with Dr. S. M. Grundy have contributed significantly to the development of the model. This investigation was supported by NIH Research Grant HL 10940 from the National Heart, Lung, and Blood Institute of the U.S. Public Health Service.

Manuscript received 3 May 1982 and in revised form 25 October 1982.

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