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Distribution of labeled cholesterol in animal tissues

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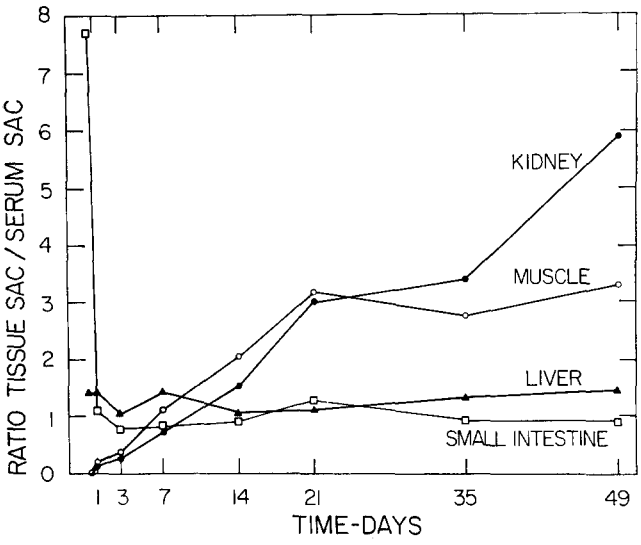
SUMMARY

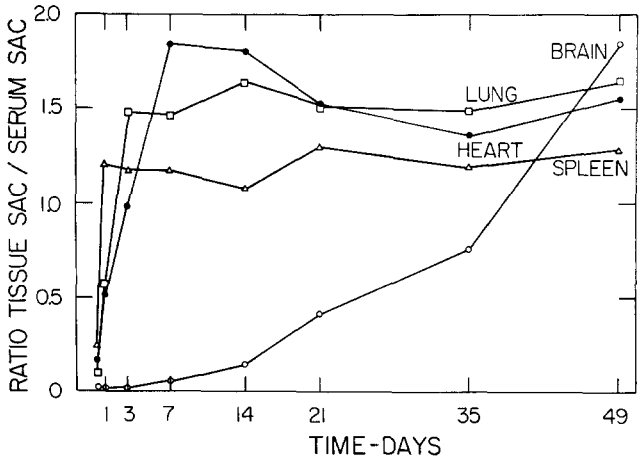
Rats were fed single doses of cholesterol-4-C^{14} and sacrificed at various time intervals. In another experiment, rabbits were injected intravenously with lipoproteins labeled with cholesterol-4-C^{14} obtained from a donor animal and sacrificed at various time intervals. The ratios between specific radioactivities of cholesterol in several tissues and that in the serum were determined and followed as a function of time. For some tissues, this ratio reached values considerably above 1 when a sufficiently long time had elapsed between the administration of label and the time of sacrifice. Time curves for specific radioactivities in various tissues, best fitting the experimental data, were calculated with the aid of a digital computer by assuming that each tissue studied represented a single homogeneous compartment of cholesterol and that there was a flow of radioactive cholesterol from plasma into the tissues. Similar time curves, based on the additional assumption that no significant synthesis of cholesterol took place in the tissues, were also constructed. The results indicate that the treatment of cholesterol in each tissue as a single homogeneous compartment in exchange with plasma cholesterol, with a provision of some cholesterol synthesis in the various organs, is adequate to explain the data in most cases examined.

The rate of disappearance of radioactive cholesterol from the blood has frequently been taken to be a measure of the rate of cholesterol catabolism. One of the assumptions generally made, explicitly or implicitly, is that the turnover of cholesterol in the serum-red cell-liver pool is very fast relative both to the turnover of other body pools of cholesterol and to the rate of isotopic equilibration between the serum pool and other body pools. It has been observed that the cholesterol-disappearance curve becomes progressively flatter when it is followed for longer and longer time intervals after administration of the label (1). This phenomenon obviously suggests, as one possible explanation, that there is significant return into the serum compartment of labeled cholesterol deposited at earlier times in other tissue pools. This, in turn, raises the question of how important the concentration of labeled cholesterol in such pools may be in determining the observed apparent rate constant of disappearance. Some authors have not specifically considered isotopic exchange between various cholesterol pools in evaluating results of experiments in which the distribution of labeled cholesterol was measured (2). That serum cholesterol does exchange with tissue cholesterol has been well demonstrated by Gould (3), Chevallier (4), and Landon and Greenberg (5), but these studies have been of rather short duration. The present studies in rats and rabbits were undertaken to obtain data on the extent of equilibration of serum and tissue cholesterol pools, particularly at longer time intervals after administration of the label. A preliminary report on this investigation has appeared elsewhere (6).

METHODS

Single tracer doses of cholesterol-4-C^{14} (2–20 μc) dissolved in 1 ml of Wesson oil were given by gastric intubation to 7 adult male Sprague-Dawley rats. Individual rats were sacrificed 1, 3, 7, 14, 21, 35, and 49 days after the administration of the label, and cholesterol was isolated from serum and tissues for determination of specific radioactivity. Tissues were homogenized in alcohol—acetone 1:1 in a Waring blendor, the extracted lipids were saponified with KOH, and cholesterol was precipitated as the digitonide. The precipitate was washed and dissolved in methanol. One aliquot was used for determination of cholesterol by the method of Sperry and Webb (7). A second





FIGS. 1, 2. Ratio of specific radioactivity (SAC) of tissue cholesterol to that of serum cholesterol as a function of time after feeding cholesterol-4-C^{14}.

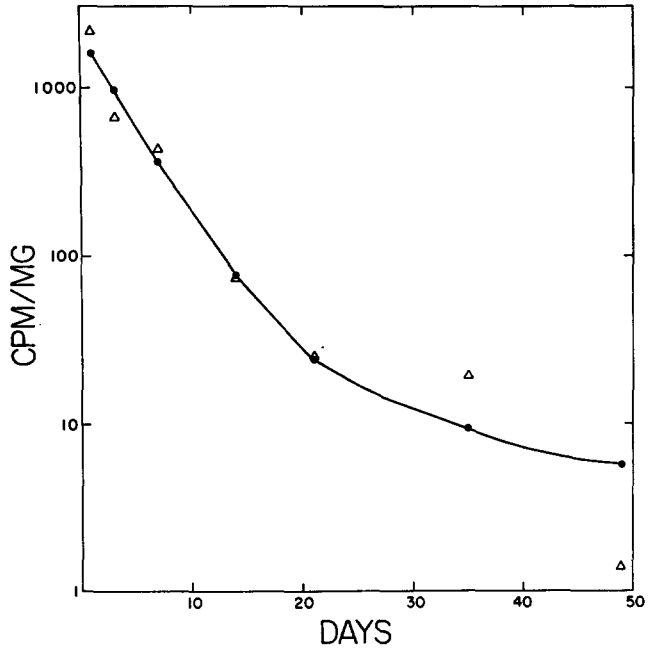


FIG. 3. Specific radioactivity of serum cholesterol in rats sacrificed at different time intervals. Observed specific radioactivities were corrected to take account of different initial doses of C^{14} and are here expressed as cpm/mg/μc of cholesterol-4-C^{14} administered. Triangles represent experimental points; solid circles, fitted values based on equation (2).

aliquot was added to toluene containing diphenyloxazole (4 mg per ml) and counted in a liquid scintillation spectrometer (Packard Instrument Co., LaGrange, Illinois). Larger doses of radioactivity were given to the rats studied over the longer time intervals so that the samples ultimately counted gave not less than 100 counts per minute over background in the case of the tissue samples and not less than 20 counts per minute above background in the case of the serum samples. Samples were counted sufficiently long to reduce the standard error of counting to less than 5%.

Cholesterol-labeled lipoproteins were prepared by feeding a donor rabbit 20 μc of cholesterol-4-C^{14} and sacrificing it 24 hours later. Serum lipoproteins were isolated by adjusting the serum to density 1.21 and centrifuging overnight at 104,000 $\times$ g in a Spinco preparative ultracentrifuge. The isolated lipoproteins were dialyzed overnight against 0.15 M NaCl. Equal aliquots of the labeled lipoproteins prepared in this way were injected intravenously into two recipient rabbits. These were sacrificed 3 and 12 days later, respectively, and the specific activity of cholesterol in serum and tissues was determined as described above.

RESULTS AND DISCUSSION

Results of the studies in rats are given in the form of the *ratio* between the specific activity of the cholesterol in the various tissues examined and that of the serum cholesterol determined at the time of sacrifice (Figs. 1 and 2). In this way the data obtained in the individual animals sacrificed at intervals can be compared independently of the absolute amounts of labeled cholesterol administered or the fraction absorbed. In other words, the form of the curve of disappearance of radioactivity from the serum is assumed to be comparable in the individual animals studied and the tissue levels are then related to the serum levels. The specific activity of the serum cholesterol, of course, fell continuously and markedly over the span of the study (Fig. 3); in order to maintain sufficiently high levels of label in the samples to be analyzed, larger doses of radioactivity were given to

TABLE 1. CHOLESTEROL SPECIFIC RADIOACTIVITIES IN RABBIT TISSUES

Tissue	Ratio: Tissue cholesterol sp. act. / Serum cholesterol sp. act. 3 days	12 days
Liver	1.17	
Kidney	0.47	0.55
Spleen	1.20	0.81
Heart	0.87	1.31
Lung	1.00	1.07
Muscle	0.37	1.31
Brain	0.02	0.08

those animals that were to be sacrificed after longer intervals.

It has been shown that the specific radioactivities of free and of esterified cholesterol in the serum approach a common value soon after administration of labeled cholesterol and then fall in parallel fashion (8). In the present studies, the free and esterified cholesterol, both in serum and in tissue, have been isolated together and treated kinetically as single pools. It is recognized that, at early time intervals, the specific activity of the tissue free and esterified cholesterol may be different. Most of the tissue cholesterol, however, is present in free form. Furthermore, the specific radioactivities of free and esterified cholesterol in the tissues approach each other very closely at longer time intervals. This is shown by the data in Table 2. For these reasons, the treatment of free and esterified cholesterol as a single pool appears to be a justifiable simplification.

Because the radioactivity retained in the tissues after long time intervals is a very small fraction of that administered, it was important to consider the possibility that radioactive impurities in the original preparation or radioactive metabolites other than cholesterol might contribute to the radioactivity found. As shown in Table 2, purification (via the dibromide) of the sterol isolated from brain and kidney in a rat given labeled sterol 5 weeks previously did not significantly alter the observed specific radioactivities.

TABLE 2. SPECIFIC RADIOACTIVITIES OF FREE AND TOTAL CHOLESTEROL IN RAT TISSUES 5 WEEKS AFTER ADMINISTRATION OF LABEL

Tissue	Free Cholesterol	Total Cholesterol
	cpm/mg	*cpm/mg*
Liver	359	355
Brain	132	130 (140)*
Kidney	829 (742)*	727
Spleen	382	349
Small intestine	346	350
Heart	268	290
Muscle	623	661

* After purification as dibromide.

The very high specific activity of intestinal cholesterol at 6 hours (Fig. 1) presumably reflects the persistence of cholesterol-C^{14} *within* the mucosa. (The intestine was slit open and thoroughly washed to remove lumen contents before it was extracted.) This is consonant with the observations of Borgström, Lindhe, and Wlodawer (9) who reported an estimated half-life of 12 hours for newly absorbed cholesterol within the mucosal cells. Throughout the time of the study, from 24 hours to 49 days, the specific activity of the intestinal cholesterol paralleled that of serum cholesterol very closely, indicating rapid and complete equilibration.

Liver cholesterol specific activity also closely paralleled that of the serum cholesterol, although it should be noted that the ratios were somewhat greater than 1.0 at 3 days and at all times thereafter. Similar results have been reported for this tissue by Popják and Beeckmans (10).

The pattern for kidney and skeletal muscle was quite different (Fig. 1). The specific activity of cholesterol in these tissues remained well below that of the serum cholesterol for at least 3 days and reached comparable levels only after about 1 week. Beyond that time, the specific activity of the tissue cholesterol relative to that in the serum rose progressively. By 7 weeks, the specific activity of kidney cholesterol was almost six times as high, and the specific activity of muscle cholesterol a little over three times as high as that of serum cholesterol. To our knowledge, this "crossover" has not been noted previously.

The patterns observed for lung, heart, and spleen were rather similar (Fig. 2). Within the first week, the specific activity of the cholesterol in these tissues rose to values somewhat higher than that of serum cholesterol. Beyond 1 week, the ratio of the two remained at a fairly constant value.

The results for brain were found to be unique among the tissues examined. As expected, the rate at which the label appeared in the brain was very low. Only after 5 weeks was a ratio of unity reached. At the end of the study, however, the ratio had reached about 1.9, and it appeared that it was still rising sharply.

Qualitatively similar results were obtained in the experiment with rabbits (Table 1); in this case, however, the animals were studied at two time intervals only and no curves can be plotted. A comparison of these figures with the rat data suggests that the general pattern is similar but that the specific activity ratios rise more slowly, in keeping with the slower cholesterol turnover rate in the rabbit.

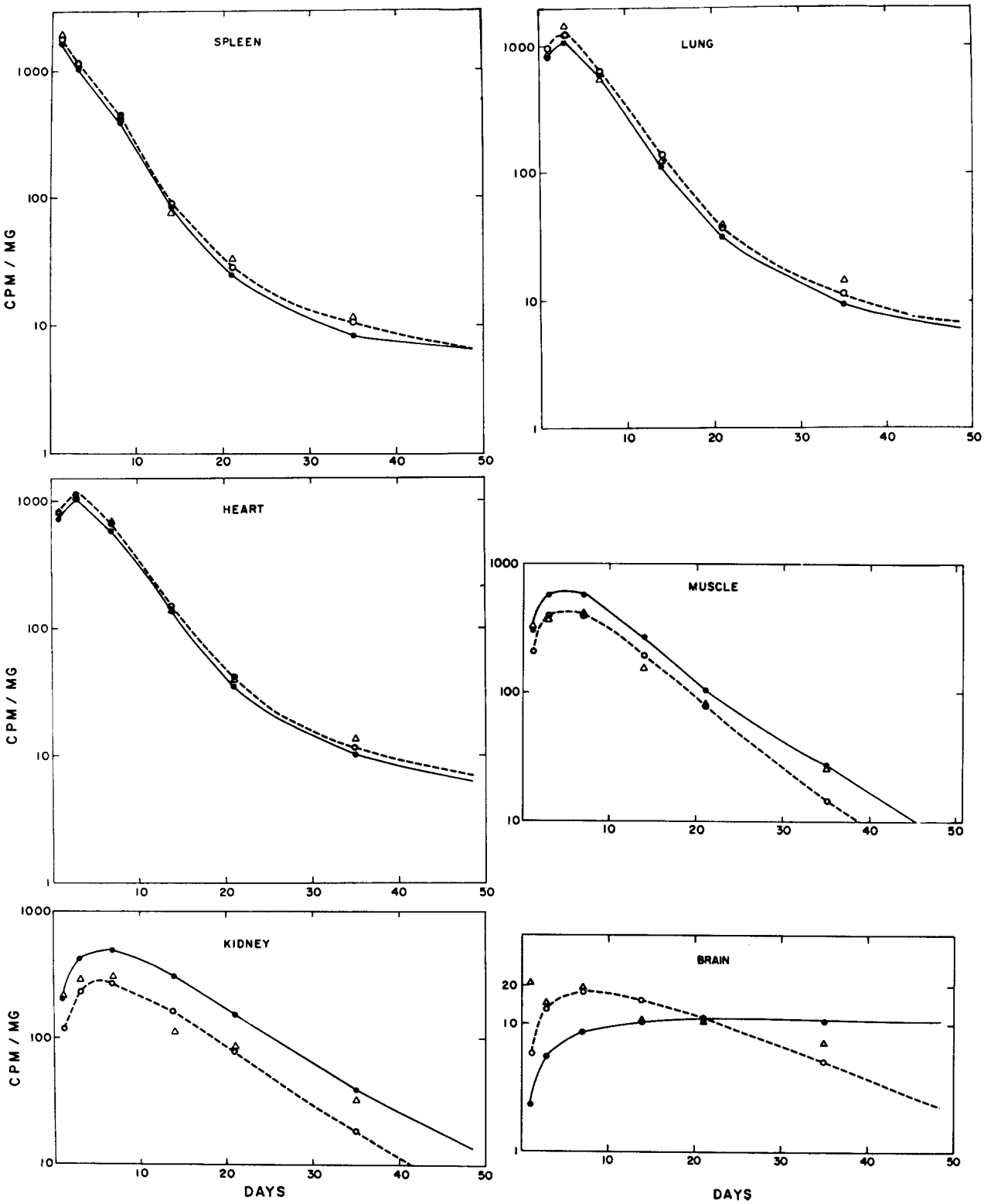


FIG. 4. Time course of cholesterol specific activity per μc of radioactive cholesterol administered in various tissues. Triangles represent experimental points; solid lines (with solid circles), fitted values based on the model, for which cholesterol synthesis is excluded; broken lines (with open circles), fitted values based on the model permitting cholesterol synthesis.

TABLE 3. CALCULATED FRACTIONAL TURNOVER RATES OF CHOLESTEROL IN VARIOUS TISSUES

Tissue	m_s/m_t	Model 1 (Cholesterol Synthesis Permitted) K_2 (day^{-1})	Model 1 K_1* (day^{-1})	Model 2 (Cholesterol Synthesis Ruled out) K_2 (day^{-1})	Model 2 K_1† (day^{-1})
Spleen	3.7	4.0 ± 6.0	1.28	4.0 ± 6.0	1.08
Lung	2.2	0.65 ± 0.23	0.33	0.64 ± 0.33	0.29
Heart	8.9	0.52 ± 0.16	0.065	0.52 ± 0.22	0.058
Muscle	0.22	0.17 ± 0.04	0.55	0.18 ± 0.03	0.82
Kidney	1.6	0.13 ± 0.03	0.043	0.12 ± 0.02	0.075
Brain	0.74	0.059 ± 0.020	0.0046	0.0013 ± 0.0003	0.0017

* Obtained from the computer-calculated K_1 m_s/m_t (equation 2) and the assumed m_s/m_t.
† $K_1 = K_2\ m_t/m_s$.

It has been shown that the sterol nucleus of cholesterol is not degraded to any appreciable extent by the mammalian organism (11). Aside from the almost negligible quantities converted to steroid hormones, the only route for removal of cholesterol is fecal excretion, either as cholesterol *per se* or as bile acids (12). Since our rats were maintained on a diet of constant composition, they were presumably in a steady state with respect to tissue cholesterol concentrations, although the growth over the 7 weeks of the study must have led to some increase in absolute size of the cholesterol pools in each organ. The appearance of cholesterol-4-C^{14} in the tissues most probably reflects an exchange phenomenon, analogous to that demonstrated by Hagerman and Gould in red blood cells (13). In the latter studies, however, a closed system was used and the specific radioactivities of serum cholesterol and red-blood-cell cholesterol tended to approach a common value. In the present studies, the system is an open one with the labeling in the serum decreasing rather rapidly as a function of time.

In an effort to gain some insight into the flow of label in this open system, we have constructed a simple mathematical model. It is recognized that, while the model presented is an over-simplification, it serves to indicate that single pools of cholesterol in each tissue in exchange with the serum pool *may* be sufficient to explain the observed results semi-quantitatively. The assumption is made that the change in the total cholesterol radioactivity in a given tissue at any time (t) is the result of a first-order rate of entrance of label from the serum compartment and a first-order loss of label from the tissue:

$$\frac{dQ_t}{dt} = K_1Q_s - K_2Q_t \qquad (1)$$

where Q_s and Q_t are the total radioactivities in the serum and tissue, respectively, and K_1 and K_2 are rate constants.

If m_s and m_t are defined as the mass of total cholesterol in the serum and tissue, respectively, equation (1) may be rewritten

$$\frac{dC_t}{dt} = \frac{K_1m_s}{m_t}C_s - K_2C_t \qquad (2)$$

where C_s and C_t are the specific radioactivities in the serum and in the tissue.

A digital-computer program (14), developed to treat compartmental systems in general, was employed to obtain least-squares solutions of K_1m_s/m_t and K_2. (Separate solution of K_1, m_s, and m_t is not possible.) For the purposes of the calculations, the serum specific-activity curve was approximated by a sum of two exponentials (Fig. 3):

$$C_s = C_1e^{-\alpha_1t} + C_2e^{-\alpha_2t} \qquad (3)$$

The C_t values were obtained by multiplying the values of C_s from equation (3) by the experimentally determined specific-activity ratios, C_t/C_s (Figs. 1 and 2). Consequently, in fitting the data, the errors derived from possible variations of the theoretical curve for C_s are minimized. Because of experimental uncertainties, zero statistical weight was given to the 49-day data in deriving the fits. The results are shown in Figure 4 (broken lines). The rapid exchange of cholesterol in liver and small intestine with that of serum did not permit a reliable determination of rate constants in these tissues.

If an added assumption is made that synthesis of cholesterol in the peripheral tissues examined is sufficiently slow relative to the rates of exchange that it can be neglected, it follows that

$$K_1m_s = K_2m_t \qquad (4)$$

or

$$\frac{K_1m_s}{m_t} = K_2. \qquad (5)$$

Substitution of this into equation (2) results in

$$\frac{dC_t}{dt} = K_2(C_s - C_t). \qquad (6)$$

This modified model has also been fitted to the experimental data, and the results are shown in Figure 4 for the various tissues (solid lines). Values for K_2 based on both models and their standard errors are given in Table 3. By using published data on the amounts of cholesterol in various tissues of the rat (15, 16, 17), K_1 values have also been calculated from equation (4) (Table 3). The latter, being based on

only approximate values of m_s and m_l, are subject to considerable errors, in contrast to the figures for K_2 which are nearly independent of the cholesterol pool sizes.

In the case of spleen, lung, and heart, the theoretical curves fit the experimental data quite closely, whether or not the contribution of endogenous synthesis was considered (Fig. 4). In the case of muscle and kidney, a better fit was obtained by using the model that allows for endogenous synthesis, but the fit was unsatisfactory at the later time intervals.[1] In the case of the brain, neither model fits the experimental data very closely. These inconsistencies indicate that the experimental situation in some tissues is more complex than assumed and may be due to the presence of two or more kinetically distinguishable compartments that equilibrate with serum cholesterol at widely different rates.

Because of the limited number of animals employed, the quantitative aspects of the present study can only be considered approximate. More work will be needed to establish the degree of variability from animal to animal and to identify factors modifying the distribution of labeled cholesterol. The data do, however, provide a general picture of the fate of labeled cholesterol over long periods of time. In particular, it is clear that a considerable fraction of the radioactive cholesterol disappearing from the serum is entering various tissue compartments rather than undergoing degradation and excretion.

The relative amounts of labeled cholesterol in the tissue compared to the amounts in the blood—liver—intestine pool can be estimated by using the ratios given in Figures 1 and 2 in conjunction with the average tissue weights of the rat (15) and the cholesterol content of these tissues (16). From such a calculation, it is found that at 7 weeks the skeletal muscle pool contained 4.4 times as much label and the kidneys about the same amount as was present in the combined "high turnover pool."

The results obtained here with rats and rabbits are probably qualitatively applicable to other species, including man, except that man's lower rate of cholesterol turnover will influence the height and shape of the time curves for the specific activity ratios.

It is clear that the disappearance of labeled cholesterol from the serum compartment must be determined not only by the rate of degradation and excretion but also, and to a significant degree, by the rates of exchange of labeled sterol into and out of a complex array of tissue pools. Therefore, calculations based exclusively on the rate of disappearance of radioactive cholesterol from the serum do not represent the true rate of cholesterol degradation and excretion.

[1] Since this paper was submitted for publication, Duncan and Best (18) have estimated the half-life of cholesterol in rat kidney by a different method. The absolute value found by them for K_2 is even smaller than that reported here, but their results again show that there is a much slower turnover of cholesterol in the kidney than in most other internal organs.

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