Cholesterol exchange and synthesis in the live rat

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Abstract The turnover of plasma cholesterol and de novo cholesterol synthesis were measured simultaneously in the live rat, immediately after administration of [^{3}H]water together with a large volume exchange transfusion of whole blood prelabeled with [^{14}C]cholesterol. It was possible to separate the exchange of unesterified cholesterol from the uptake and secretion of lipoprotein cholesteryl ester, and also to assess the impact of plasma cholesterol exchange on the measurement of in vivo rates of cholesterolgenesis by individual tissues. Cholesterol was measured by an HPLC procedure that effectively separated cholesterol from other structurally similar sterols, and synthesis was determined by the incorporation of [^{3}H]water into cholesterol. Plasma unesterified cholesterol turnover was multiphasic and exceedingly rapid (initial $T_{1/2}$, 4.1 min) in contrast to the near linear and much slower turnover of plasma cholesteryl ester (initial $T_{1/2}$, 59.4 min). Plasma unesterified cholesterol equilibrated with different tissues at different rates, with the liver and adrenal equilibrating most rapidly. Full equilibration of plasma unesterified cholesterol was not achieved with any tissue during the course of this study. For rapidly exchanging tissues like the liver, which was responsible for about 60% of plasma unesterified cholesterol exchange, unesterified cholesterol appeared to be kinetically compartmentalized into rapidly, and much less rapidly, exchangeable pools. After [^{3}H]water administration, the content of newly synthesized cholesterol was greatest in the liver, adrenal, and intestine, and appreciably lower in all other tissues studied. Hepatectomy and intestinal resection resulted in a profound reduction of newly synthesized cholesterol in the plasma and adrenal, but no certain change in the already low amounts at other sites. Thus, while it is clear that appreciable amounts of newly synthesized cholesterol in the adrenal were derived from the plasma by exchange, it was not possible to make this assessment for other selected individual tissues. When, however, newly synthesized cholesterol was determined in the total mass of all extrahepatic and extraintestinal tissues together, exchange could be calculated to account for close to 50% of the new cholesterol recovered in the carcass (in studies of 60 min duration). After correcting for exchange, the liver accounted for 82% of all newly synthesized cholesterol, the intestine for about 10%, and the remaining tissues of the body for just 9%. These results are in marked contrast to recent findings of others and demonstrate that in the live rat cholesterol synthesis is principally confined to the liver. — **Robins, S. J., J. M. Fasulo, M. A. Collins, and G. M. Patton.** Cholesterol exchange and synthesis in the live rat. *J. Lipid Res.* 1985. **26:** 1230–1240.

Supplementary key words plasma cholesterol clearance • in vivo cholesterolgenesis • unesterified cholesterol equilibration • [^{3}H]water • HPLC • hepatectomy • intestinal resection • whole carcass

The turnover of plasma cholesterol in the live animal is customarily traced for prolonged periods and is the result of cholesterol input from diet and new synthesis, and the output of cholesterol from the plasma into tissues (1, for review). Since different tissues have different capacities for both cholesterol synthesis and for cholesterol uptake from the plasma, it is not surprising that the turnover of plasma cholesterol is multiphasic (multi-exponential) (2) and that plasma cholesterol equilibrates at different rates with different tissues (3, 4). Although in long-term studies plasma cholesterol turnover can be viewed as the result of the net movement of cholesterol into and out of the plasma, the short-term plasma cholesterol turnover may also be affected by molecular exchange of cholesterol between the plasma and tissues — a process that is independent of net cholesterol transport.

In the present study we have simultaneously measured the rate of cholesterol exchange from the plasma and rates of de novo cholesterol synthesis in individual tissues of the live rat. This study was undertaken for two reasons: one, to determine rates of cholesterol exchange in vivo and, two, to determine whether exchange would preclude accurate measurements of de novo cholesterol synthesis by individual tissues in vivo.

In the first case, although cholesterol exchange has been the subject of a large number of studies in vitro (5, 6 for reviews), no studies of exchange appear to have been conducted in the live animal. Implicit in this undertaking is the presumption that exchange is much more rapid than net cholesterol uptake and that, therefore, in a relatively brief period, exchange can be measured with reasonable certainty in spite of ongoing net uptake. In the second case, we have sought to determine the feasibility of directly measuring cholesterol synthesis by individual tissues in the live animal. Recently, with the demonstration that absolute rates of cholesterol synthesis can be accurately measured with [^{3}H]water (7, 8), this isotope has been

Abbreviations: UC, unesterified cholesterol; CE, cholesteryl ester; RBC, red blood cell(s); TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

used to assess relative rates of cholesterol synthesis in various tissues in vivo (9-12). These studies have shown that the liver makes a far smaller contribution to total body cholesterol synthesis than previously demonstrated in vitro using ^{14}C-labeled substrates (13, 14), and that synthesis at extrahepatic sites may, in fact, exceed synthesis in the liver itself. Quite clearly these observations might need to be modified if, even during a very brief period in which synthesis is measured, exchange of cholesterol (more specifically, newly synthesized cholesterol) from the plasma into extrahepatic tissues is appreciable.

MATERIALS AND METHODS

Tritiated water (1 Ci/g), [4-^{14}C]cholesterol (60 mCi/mmol), Na$_2$^{51}CrO$_4$, and internal counting standards of [^{3}H] and [^{14}C]toluene were purchased from New England Nuclear (Boston, MA). ^{125}I-Labeled albumin was purchased from Mallinckrodt (St. Louis, MO). Sterols were obtained from the following sources: cholesterol (A grade) from Calbiochem (San Diego, CA), cholestanol and desmosterol from Sedary Research Labs (Ontario, Canada), and 7-Δ-cholestanol as a gift from Dr. E. Caspi (Worcester Foundation, Shrewsbury, MA). Solvents were all reagent or HPLC-grade and were obtained from Fisher Scientific (Medford, MA).

Animals

Male Sprague-Dawley rats were fed Purina Chow ad libitum. They were housed with light cycling at 12-hr intervals for 2-3 weeks prior to study. Experiments were performed when rats weighed 250-300 g and at the midpoint of the dark cycle (i.e., during peak activity of hepatic cholesterol synthesis).

Experimental design

A pair of rats was used for each experiment. Rat no. 1 was injected intravenously with a suspension of [^{14}C]-cholesterol (approximately 20 μCi, 60 mCi/mmol) and bled from the aorta 24 hr later. This blood was heparinized and immediately mixed with [^{3}H]water (from 100 to 200 mCi), ^{51}Cr-labeled rat RBC (1-2 μCi), prepared fresh from an independent donor (15), and ^{125}I-labeled albumin (1-2 μCi). The radiolabeled (donor) blood was then used for injection into a second rat within 5 min. Rat no. 2 was operated upon (using Nembutal anesthesia, 50 mg/kg) to cannulate the common bile duct, a femoral artery, and the femoral vein contralateral to the cannulated artery. This rat was rapidly bled of about 40% of its blood volume (from the arterial cannula) and then immediately injected (through the venous cannula) with an equivalent volume of radiolabeled whole blood, obtained from rat no. 1. Administration of donor blood was completed in 12-15

sec. After the exchange transfusion, arterial blood (about 400 μl), bile, and a variety of tissues were obtained for analysis at sequential times as specified in the figure legends.

In two instances this protocol was modified as follows. In the first instance, to accelerate net uptake and secretion of plasma cholesterol by the liver, rat no. 2 was treated for 4 days with ethinyl estradiol (5 mg/kg per day) prior to surgery and exchange transfusion. In the second instance, to evaluate the contribution of the liver and intestine to cholesterol exchange and synthesis, these organs were eliminated from the circulation. This was accomplished in rat no. 2, just prior to the administration of radiolabeled cholesterol and water, by acutely interrupting the portal venous and arterial blood supply to the liver and by surgically removing the entire small intestine.

Analytical procedures

RBC were separated from plasma and washed two times with saline. Tissues were washed, blotted dry, weighed, and homogenized. Lipids were extracted from RBC as described by Rose and Oklander (16) and from plasma, bile, and tissues by the method of Folch, Lees, and Sloane Stanley (17). In plasma and all excised tissues, cholesteryl esters (CE) were first separated from unesterified cholesterol (UC) by TLC, saponified in 1 N ethanolic KOH (80°C, 3 hr), and extracted into hexane. When cholesterol in the whole animal was determined, the entire animal was initially saponified and total sterols were then extracted.

The mass and radioactivity of cholesterol (in both CE and UC) were determined by the following HPLC procedure. Cholesterol, in amounts from 10-100 μg, was dissolved in hexane and chromatographed in hexane-tetrahydrofuran-acetic acid 500:50:0.1 at 1 ml/min on a silica column (LiChrospher Si-100, 5 μ, EM Laboratories). Cholesterol was detected by absorbance at 205 nm and quantitated by integrating the peak area in comparison with a pure standard to calculate cholesterol mass. The collected cholesterol effluent was dried, solubilized in a toluene-based phosphor (Liquifluor, New England Nuclear), and ^{3}H and ^{14}C radioactivities were determined by liquid scintillation counting (correcting for quenching with internal ^{3}H and ^{14}C standards). Cholesterol recovery by this HPLC procedure averaged 100.8 ± 2.3 (SD) %. This procedure completely separated cholesterol from other closely related 3-β-hydroxysterols with 27 carbons including desmosterol (two double bonds), cholestanol (no double bonds), and 7-Δ-cholestenol (a single double bond in a different position than in cholesterol). In addition, cholesterol isolated by this HPLC procedure (from a radiolabeled sample of liver) also gave a single peak of absorbance at 205 nm and had the same specific activity when rechromatographed in an entirely different system,

using a C18 reverse phase column (Ultrasphere, 5 μ, Altex Scientific) and methanol as a solvent.

^{51}Cr and ^{125}I activities were determined in tissue homogenates and whole blood by gamma counting.

Calculations

The specific activities of [^{3}H] and [^{14}C]cholesterol in tissues were corrected for both RBC and plasma contamination by the amount of ^{51}C and ^{125}I in tissues in relation to the activities of these isotopes in whole blood, which was obtained at the same time.

The rate of cholesterol exchange into the liver, intestine, and selected other individual tissues was determined by dividing the dpm of ^{14}C-labeled UC in a tissue sample by the specific activity of ^{14}C-labeled UC in the plasma, which was obtained at the same time. Cholesterol exchange into the total tissue mass of the whole animal, exclusive of the liver and intestine, was calculated as indicated in the Appendix.

The rate of new cholesterol synthesis was determined as previously described (18) by first dividing the dpm of [^{3}H]cholesterol in a sample by the specific activity of ^{3}H in plasma [^{3}H]water (obtained at that time), and then dividing this value by the number of H atoms incorporated for every 27 C atoms into a molecule of newly synthesized cholesterol (experimentally established as 21 for the liver (7)).

RESULTS

Initial studies were conducted for 2 hr, following the transfusion of whole blood radiolabeled with [^{14}C]cholesterol and [^{3}H]water. During this time, plasma cholesterol concentrations remained unchanged (averaging 40.3 ± 4.8 (SD) mg/dl) and the proportions of UC and CE in the plasma remained constant (UC averaging 30.6 ± 4.3 (SD) % of the total cholesterol).

Plasma cholesterol exchange

[^{14}C]Cholesterol was well equilibrated in the blood of the donor rat just prior to transfusion, as evidenced by the very similar specific activities of donor plasma UC, CE, and RBC cholesterol (**Fig. 1**, insert). After transfusion, 2 min was allowed for mixing of the blood of the donor and recipient rats before the blood of the recipient was first sampled. This time appeared to be adequate for mixing as judged by the linear decline of the specific activities of both plasma CE and RBC cholesterol that ensued after 2 min (Fig. 1). (Assuming that mixing required just about 2 min, it was then possible to estimate, by the extent of dilution of the specific activities of plasma CE and RBC cholesterol of the donor at 2 min, that about 40% of the blood of the recipient rat had been exchanged by transfusion.) In contrast to RBC cholesterol, the specific activity of plasma UC fell nonlinearly and much more
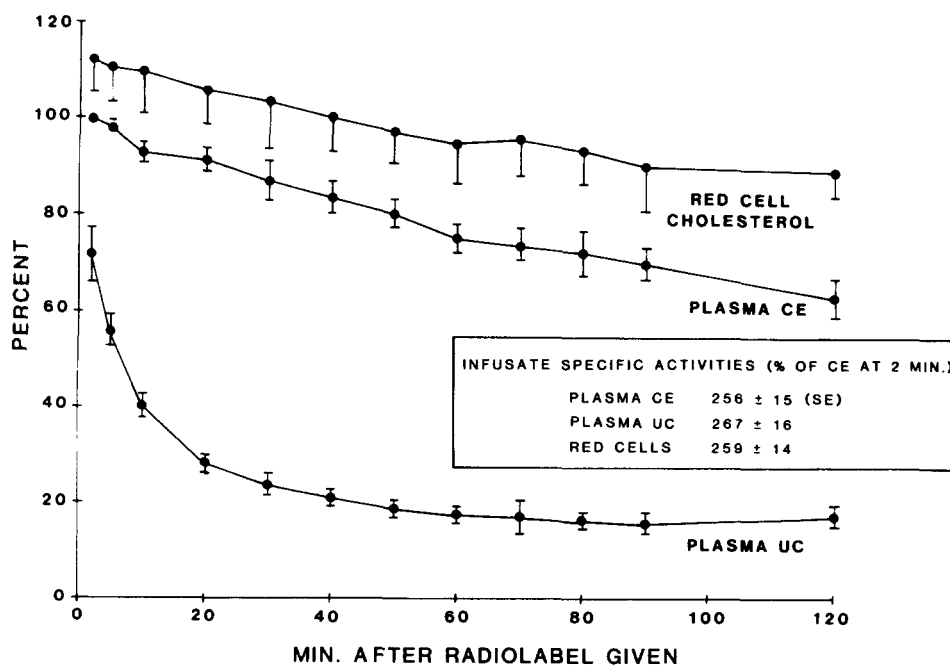

Fig. 1. Change in the specific activities of plasma unesterified cholesterol (UC), esterified cholesterol (CE), and red cell cholesterol after administration of whole blood labeled with [^{14}C]cholesterol. Data are shown as the mean ± SE for three rats. All specific activities have been normalized, assigning the initial plasma CE (which was obtained 2 min after isotope administration) a value of 100%. Values in the insert show the relative specific activities of cholesterol in the injected blood.

precipitously. In fact, at 2 min after transfusion, the specific activity of UC was already about 30% less than that of CE, and by 50–60 min it had reached its nadir—about 70% lower than the specific activity of CE.

The turnover of RBC cholesterol was also traced, and more closely resembled plasma CE than UC. Although infused with the same specific activity as plasma CE, RBC cholesterol was diluted about 10% less than plasma CE in the blood of the recipient. This disparity can probably be attributed to a more restricted space for mixing of RBC (confined to the vascular space) than plasma lipoproteins containing CE (distributed in the interstitial as well as in the vascular space).

To more precisely estimate the initial rate of decline of the specific activity of plasma UC, a second series of rats was similarly transfused but, in contrast to the first group, these rats were bled at more frequent intervals immediately after the transfusion (**Fig. 2**). Again, the completeness of mixing was judged by the onset of linear decay of plasma ^{14}C-labeled CE (at 1.5 min). By extrapolating the linear, terminal portion of the CE decay curve to time zero (the start of transfusion), it was also possible to construct an initial decay curve for ^{14}C-labeled UC—connecting the extrapolated zero time point of CE with the beginning of the first apparent decay curve of UC (at 1.5 min, once mixing of donor and recipient bloods had been completed). As shown, the initial rate of decline in specific activities could be calculated for the first 1.5 min after [^{14}C]cholesterol was administered, and was 14 times more rapid for UC than for CE.

Whereas rapid UC turnover was attributed to molecular exchange of UC from the surface of lipoproteins with tissues, the relatively slow rate of turnover of CE was attributed to the flux out of and into the circulation of whole lipoproteins. This presumption was supported by the following experiment designed to enhance net lipoprotein uptake. A rat was treated with estrogen prior to the administration of [^{14}C]cholesterol in order to increase hepatic lipoprotein receptors and cholesterol uptake from the plasma (19). (A non-estrogen-treated rat was used as a donor.) During the 2-hr period of study, plasma total cholesterol decreased from 23.6 mg/dl (5 min after isotope administration) to 13.2 mg/dl. As shown in **Fig. 3**, estrogen treatment greatly accelerated the turnover of plasma CE, whereas the turnover of UC was only marginally increased and followed the same general pattern of decline as in non-estrogen-treated rats. As anticipated, estrogen did not affect the turnover of RBC cholesterol.

Cholesterol exchange with tissues

To assess the rate of exchange of plasma UC with a variety of tissues, blood and tissues were obtained from rats that were killed at intervals for up to 2 hr after radiolabeled whole blood was transfused. Rates of exchange are
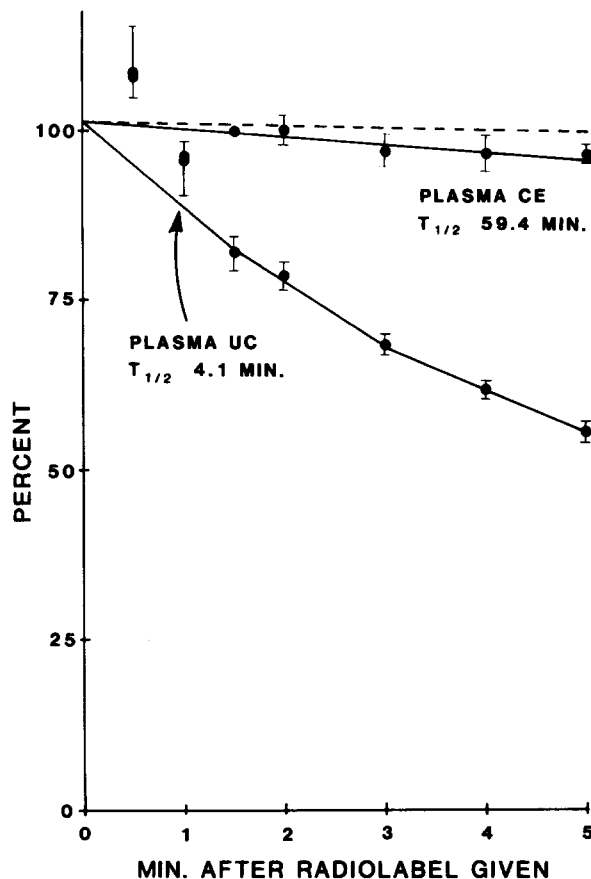

Fig. 2. Change in the specific activities of plasma unesterified cholesterol (UC) and esterified cholesterol (CE) after administration of whole blood labeled with [^{14}C]cholesterol. Data are shown as the mean ± SE for three rats. All specific activities have been normalized, assigning the plasma CE, obtained 1.5 min after isotope administration, a value of 100%. The initial turnover of CE was essentially linear and was calculated by least squares. The initial turnover of UC was calculated from the line connecting the specific activity of CE at time zero with the first point at which linear decay of UC could be recognized, i.e., once mixing had been completed.

shown in **Fig. 4** for eight selected tissues that were excised, washed, homogenized, and corrected for contaminating RBC and plasma (see Methods). Exchange of plasma cholesterol with individual tissues was highly variable with liver, adrenal, and spleen exchanging most rapidly and intestinal mucosa exchanging least. (Data for the spleen were obtained at only one point and may not represent true exchange. RBC are fragmented in the spleen and the ^{51}Cr-labeled hemoglobin, used as a measure of RBC contamination, and RBC membrane [^{14}C]-cholesterol may be separated from each other within the spleen soon after RBC uptake.) For the most actively exchanging tissues, the exchange process appeared to have both fast and slow components, with increases in tissue specific activity reciprocal to the decline in activity of plasma UC.
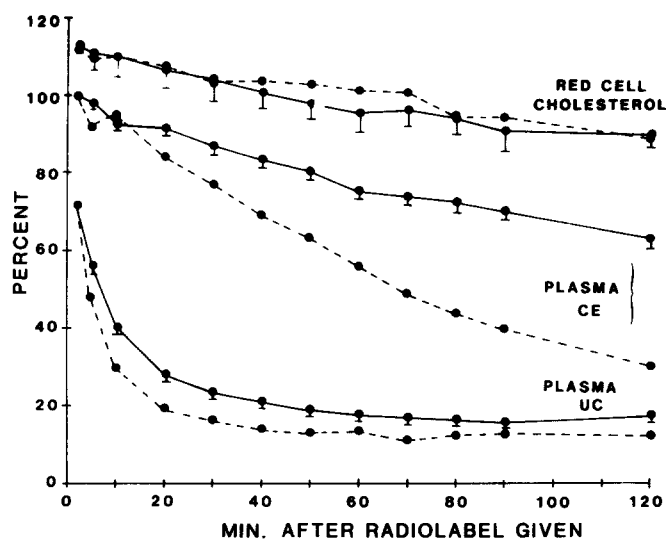
Fig. 3. Effect of estradiol treatment on the turnover of cholesterol. The change in the specific activities of plasma unesterified cholesterol (UC), cholesteryl ester (CE), and red cell cholesterol is shown after administration of blood labeled with [¹⁴C]cholesterol. Values shown by the solid lines are for non-estrogen-treated rats which are shown in Fig. 1, and values shown by the dashed line are for a single estrogen-treated rat. All data have been normalized, assigning the specific activity at 2 min of plasma CE of the non-estrogen group a value of 100%.

(with about 50% equilibration of UC achieved by 1 hr) but then appreciably slowed (with only about 60% equilibration achieved by 2 hr). During this period, the increase of radiolabeled cholesterol in the liver was closely paralleled by the increase of radiolabeled cholesterol in the bile. Indeed, while bile was collected in these studies to avoid contaminating the intestinal mucosa with cholesterol derived from the liver, it is apparent that the pool of UC in the liver is in near-complete equilibrium with cholesterol in the bile and that, therefore, the extent of exchange between plasma and liver can be readily calculated using the bile.

To quantitate the contribution made by the liver to plasma cholesterol exchange, in two rats the blood supply to the liver was acutely interrupted (i.e., a "functional hepatectomy" was performed) just prior to transfusion with [¹⁴C]cholesterol. In addition, since exchange with the intestine was negligible, the intestine was surgically removed to avoid its congestion upon ligation of the portal vein. Compared to results obtained with the circulation intact, eliminating the liver (and intestine) from the circulation resulted in a profound reduction in plasma UC clearance (**Fig. 6**). In addition, with the liver and intestine eliminated, the turnover of plasma CE, reflecting uptake and secretion of lipoproteins, was also markedly reduced.

Cholesterol synthesis in individual tissues

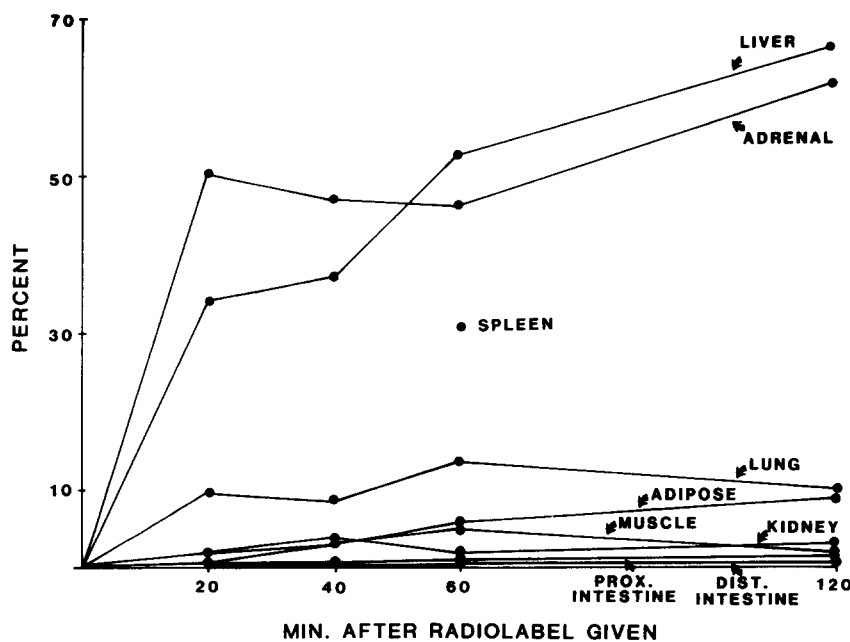At the same times that cholesterol exchange was determined, the amount of newly synthesized cholesterol was

This pattern of exchange was clearly evident in rats in which three liver biopsies were sequentially obtained and bile was continuously collected (**Fig. 5**). The rate of exchange between the plasma and liver was initially rapid

Fig. 4. Relative rates of exchange of plasma unesterified cholesterol with individual tissues of the rat. Values from single animals at each of four time points are shown. All tissue values were corrected for contamination by plasma and red cell cholesterol using ¹²⁵I-labeled albumin and ⁵¹Cr-labeled red cells, respectively. (As indicated in the text, data for the spleen were obtained at only one time point.)
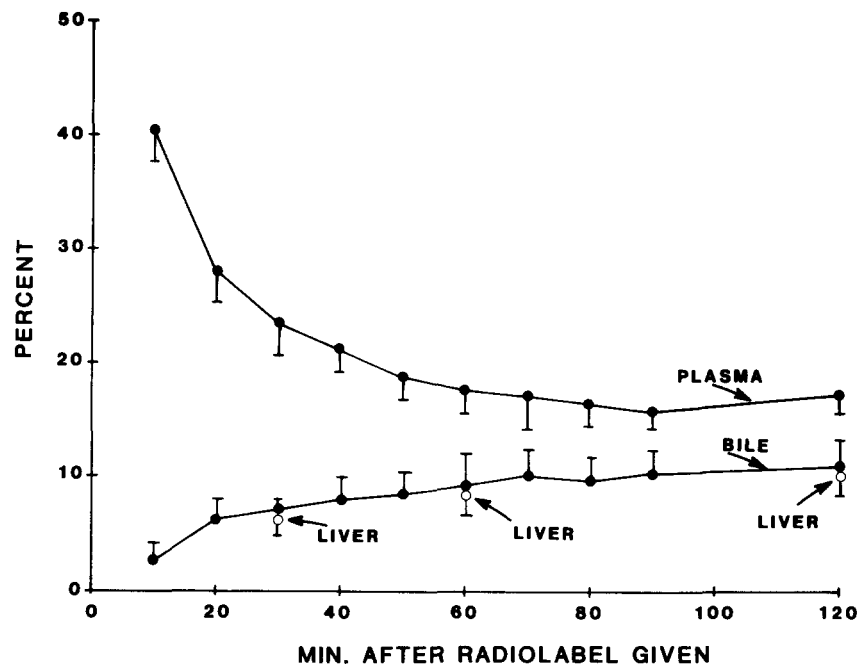
Fig. 5. Change in the specific activities of unesterified cholesterol in plasma, liver, and bile after administration of blood labeled with [14C]cholesterol. Values are from the group of rats shown in Fig. 1 and are normalized as previously indicated. Liver biopsies, obtained at the three times shown, were corrected for contaminating blood (cholesterol) by 51Cr-labeled red cells and 125I-labeled albumin markers (see Methods).

measured in individual tissues, using [3H]water as a precursor. Incorporation of tritium into cholesterol at all times examined was almost exclusively confined to UC. In the liver, for example, UC accounted for 98.4 ± 0.6(SD)% of the total newly synthesized cholesterol at 1 hr and for 96.4 ± 1.8% of the total at 2 hr.

As shown in **Table 1**, in the tissues sampled, amounts of newly synthesized cholesterol per gram of tissue were highest for the liver, followed by the adrenal, and then by the distal and proximal intestinal mucosa. Amounts of newly synthesized cholesterol in the remaining tissues were relatively minimal and, moreover, were in all cases less than in the plasma.

To determine initially whether any of the newly synthesized cholesterol recovered in these extrahepatic and extraintestinal sites might be derived by exchange from the plasma, in a single animal the blood supply to the liver was interrupted and the entire small intestine was resected just prior to the administration of [3H]water. Analysis of individual tissues 1 hr after administration of the tritium demonstrated a profound reduction in the amount of newly synthesized cholesterol in the plasma, a similarly large reduction of newly synthesized cholesterol in the adrenal, but no appreciable change in other tissues (Table 1). Thus, it seemed likely that, at least for the adrenal, appreciable amounts of newly synthesized cholesterol were derived from the plasma. A reliable estimate of exchange could not, however, be performed for other indi-
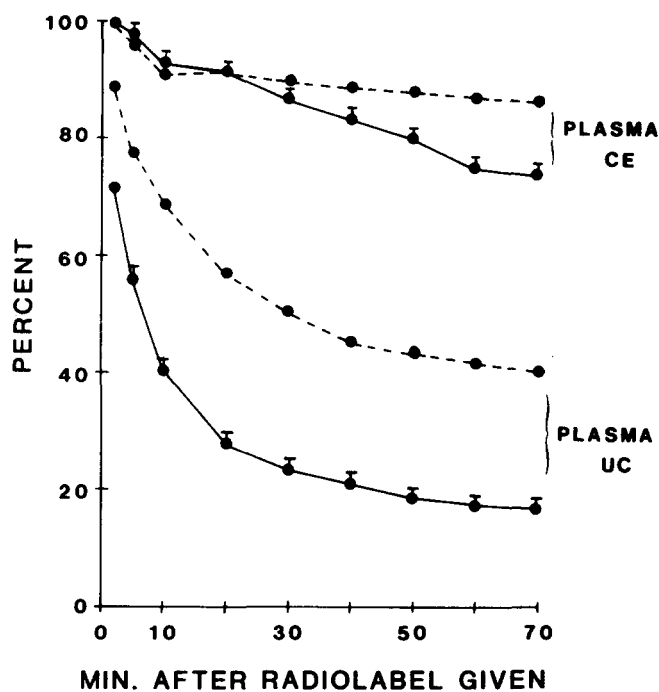

Fig. 6. Effect of functional hepatectomy and small intestinal resection on plasma cholesterol turnover. Values shown by the solid lines are from the group of normal rats shown in Fig. 1 and values shown by the dashed lines are the average from two rats subjected to hepatectomy and intestinal resection. (For these two rats, values at any one single time point did not differ by more than 7%.) All specific activities are normalized as indicated in Fig. 1.

TABLE 1. Rates of cholesterol synthesis in selected individual tissues of the rat

Tissue	Newly Synthesized Cholesterol			
	20 min	40 min	60 min	120 min
	μg/g of tissue (or ml of plasma)			
Liver	15.85	19.35	79.34 (0.44)[a]	184.42
Adrenal	2.04	4.41	15.64 (1.08)	78.79
Distal intestine	2.13	2.63	8.60	40.36
Proximal intestine	2.14	1.32	3.66	23.49
Lung	0.39	0.95	1.21 (1.09)	10.91
Kidney	0.36	0.44	0.83 (0.46)	4.12
Spleen			0.09 (0.18)	
Adipose tissue	0.05	0.05	0.06 (0.11)	0.53
Muscle	0.09	0.14	0.17 (0.11)	0.45
Plasma	0.51	1.68	4.33 (0.06)	19.18

Synthesis was determined by the extent of incorporation of [³H]water into the UC of tissues (see Methods), after correcting for RBC and plasma cholesterol contamination. Values are for a single rat at each of the times shown.

[a] Values shown within parentheses were determined in an additional rat at 60 min, after a functional hepatectomy and intestinal resection.

vidual tissues in which amounts of newly synthesized cholesterol were relatively small, both before and after hepatectomy and intestinal resection. Consequently, newly synthesized cholesterol was next measured and corrected for exchange in the total mass of all body tissues. In these studies, newly synthesized cholesterol was determined in the liver, the bile, the small intestine, the total plasma volume, and the entirety of the remaining carcass 60 min after the administration of [³H]water. The amount of newly synthesized cholesterol in all tissues was corrected for contaminating blood and, as indicated above, for newly synthesized cholesterol that was exchanged from the plasma. The data are shown in **Table 2**. By itself, the liver accounted for 65.4% of whole body synthesis and with the bile included, for 70.1%. However, assuming that virtually all newly synthesized cholesterol in the plasma has originated in the liver [as appears highly probable both from our data (Table 1) and that of others (11)], the liver, in reality, would then account for almost 82% of all cholesterol that was newly synthesized. Of the remaining new cholesterol, 9.7% was contained in the intestine and just 8.9% in all of the remaining tissues of the body. Exchange of newly synthesized cholesterol from the plasma with the carcass was substantial. However, even before correcting for exchange, all extrahepatic–extraintestinal tissues together only accounted for 12.1% of the cholesterol that was newly synthesized (i.e., for 97.4 ± 11.5 μg of new cholesterol, before correcting for exchange).

DISCUSSION

Dietary and newly synthesized cholesterol are sources of plasma cholesterol. Cholesterol moves out of the plasma with the uptake of lipoproteins by the tissues, and both in

and out of plasma simultaneously by a process of molecular exchange. Although exchange does not alter the amount of cholesterol within different body pools, the process of exchange can interfere with the interpretation of cholesterol transport by other mechanisms which do result in a change of net cholesterol distribution. In the present study we have attempted to quantitate plasma cholesterol exchange in the live rat and to assess the impact of exchange on the measurement of cholesterol synthesis in individual tissues in the intact animal.

Cholesterol exchange has been determined by tracing the decline in specific activity of the UC in the plasma immediately after an exchange transfusion of radiolabeled whole blood. The decrease in the specific activity of UC was nonlinear, exceedingly rapid, and virtually complete within 30 min after isotope administration. An initial half-life ($T_{1/2}$) of UC exchange was calculated to be 4.1 min. In sharp contrast to UC, the decline in specific activity of plasma CE was much slower and nearly linear, with an initial $T_{1/2}$ of 59.4 min.

Although there are a relatively large number of studies of plasma cholesterol turnover and cholesterol turnover in specific plasma lipoproteins, no previous study appears to have separately examined the kinetic behavior of plasma UC and CE in the intact animal. It is clear that the extremely different turnover rates of plasma UC and CE could only have been perceived immediately after isotope administration and then only for a very brief time. Almost certainly, the rapid turnover of UC is due to exchange and is a reflection of the location of UC on the surface of lipoproteins and within the surface membranes of the cell. In contrast, the much slower rate of turnover of CE, which is located in the interior of lipoproteins and cells, most probably results from the uptake of whole lipoproteins. This explanation for the different rate of clearance of plasma UC and CE was supported by the effects of estrogen. When an animal was treated with estrogen in order to

TABLE 2. Contribution of liver, intestine, and remaining tissues of the carcass to cholesterol synthesis in the rat

Tissue	Newly Synthesized Cholesterol
	μg in total tissue, bile, or plasma
Liver	525.5 ± 67.8
Bile	37.6 ± 11.5
Small intestine	78.1 ± 16.9
Plasma	91.4 ± 31.0
Remaining carcass	71.1 ± 22.1
Total	803.7 ± 76.5

Cholesterol synthesis was determined in bile duct-cannulated rats at peak hepatic synthesis, 60 min after the administration of [³H]water. Tissue values were corrected for newly synthesized cholesterol contained in contaminating blood (see Methods) and for the exchange of newly synthesized cholesterol from the plasma, reducing the amount of newly synthesized cholesterol in the carcass by 26.3 μg, the extent of exchange of plasma ¹⁴C-labeled UC previously determined after hepatectomy and intestinal resection (Fig. 6 and Appendix). Values are mean ± SE for three rats.

enhance hepatic lipoprotein uptake and secretion, plasma CE turnover was markedly accelerated while the turnover of plasma UC, presumed already near maximum as a result of exchange, was increased to a much smaller extent.

The exchange of plasma UC with individual tissues was highly variable and closely resembled the pattern of equilibration that is described for long-term kinetic studies, in which certain tissues come into equilibration with (total) plasma cholesterol earlier after isotope administration ("pool A" tissues such as the liver) than other tissues ("pool B" tissues, in a two-pool model such as muscle) (4, 20). However, in contrast to long-term studies, in the present study of 2 hr duration, complete equilibration of plasma cholesterol, even of UC, was not achieved with any single tissue. Rather, where exchange was most active (liver and adrenal), after an initial period of rapid labeling of these tissues, an equilibrium was reached in which the specific activity of tissue ^{14}C-labeled UC plateaued at a level that was about 50% less than the specific activity of ^{14}C-labeled UC in the plasma. This particular pattern of UC exchange between plasma and actively exchanging tissues strongly suggests that, even within single tissues, UC is kinetically compartmentalized, and that only a portion of tissue UC is immediately "available" for exchange with plasma UC. Although we have no certain explanation for differences between tissues, it is notable that, in contrast to slowly exchanging tissues, each of the three tissues in which exchange was most rapid has sinusoids that might allow for increased plasma–tissue contact and, therefore, more efficient exchange. We also have no explanation for what appears to be kinetically disparate pools of UC within single tissues. It is possible that this compartmentalization of UC can be attributed to differences in UC exchange in different cell types comprising the same tissue, to the time taken for movement of UC between the plasma membrane and interior membranes in single cells, or even to the time required for the transbilayer movement of UC from the inner to outer portions of single membranes.

The role of the liver in plasma cholesterol exchange was separately evaluated by performing a functional hepatectomy. This procedure was coupled with resection of the entire small intestine with which plasma UC exchange was found to be negligible. As demonstrated by the reduction in plasma ^{14}C-labeled UC turnover with hepatectomy (Fig. 6), the liver by itself accounted for about 60% of the total plasma UC exchange. In addition, this study evidenced the profound impact that the liver and small intestine have on plasma CE clearance, which virtually ceased with removal of these organs from the circulation.

Finally, we also observed that plasma UC that exchanged with the liver was in rapid and complete equilibrium with the cholesterol in the bile, which is all unesterified. Although the precise intrahepatic site of origin of bile cholesterol has not been determined, we (18) and others (21, 22) have previously shown that bile cholesterol predominantly originates from preformed hepatic stores rather than as a result of new synthesis. Since bile and hepatic ^{14}C-labeled UC had similar specific activities in the present study, it is clear that bile cholesterol is derived from that pool of liver UC that is immediately available for exchange with the plasma but is not, however, the sum total of liver cholesterol.

In addition to measuring cholesterol exchange, we also determined relative rates of cholesterol synthesis using [^{3}H]water, first in individual tissues and then in the entire animal. The in vitro use of [^{3}H]water to measure absolute rates of cholesterol synthesis by the liver and a variety of other tissues has been well-validated (7, 8). Extrapolating from these measurements, relative rates of cholesterol synthesis have been reported for a large variety of extra-hepatic and extraintestinal tissues studied in vivo (10–12). Comparative studies have shown that cholesterol synthesis by the liver of the rat is far more active than synthesis by the liver in a variety of other animals (11). However, in the rat, the liver has been found to account for no more than about 50% and 15% of the total body synthesis when synthesis has been measured in vivo 1 hr (9–11) and 6 hr (12), respectively, after administration of [^{3}H]water. In these same studies the intestine accounted for about 24% and 10%, respectively, of the newly synthesized cholesterol. Thus, the contribution by extrahepatic and extraintestinal tissues seemingly could account for 26% or 75% of the total cholesterol synthesized by the whole rat.

We initially focused on the possibility that the measurements by others (9–12) of cholesterol synthesis in tissues other than the liver and intestine might be spuriously high, due to the exchange of newly synthesized cholesterol from the plasma. We found that plasma cholesterol exchange with tissues of the carcass was indeed appreciable, as evidenced by the rapid rate of turnover of plasma ^{14}C-labeled UC in rats in which the liver and intestine had been surgically removed (Fig. 6). Using this rate of exchange of preformed cholesterol in conjunction with the rate of accumulation of newly synthesized cholesterol in the plasma, we could estimate the magnitude of exchange of newly synthesized plasma cholesterol with the carcass (see Appendix). In our studies of 60 min duration, we found that about 27% of the newly synthesized cholesterol that was contained in the carcass was derived by exchange with the plasma. Quite clearly, in studies of synthesis that have been extended to 6 hr, considerably greater amounts of newly synthesized cholesterol will accumulate in the plasma and be exchanged with the carcass than after 1 hr. In no previous study of in vivo cholesterol synthesis has exchange been considered. In a study such as that of Feingold et al. (12) for 6 hr, the failure to correct for exchange will almost certainly result in a substantial overestimation of synthesis by the carcass.

In addition to the issue of exchange, we believe that the large discrepancy between our results and those of others also relates to differences in the manner of correcting tissue samples for cholesterol contained in contaminating blood and to differences in the precision of methods used to isolate cholesterol for analysis. First, we have corrected for the whole of contaminating blood. Because of both large differences in the specific activities of cholesterol in the plasma and RBC and differences in the relative proportion of retained plasma and RBC within different tissues, we found it necessary to correct independently for residual plasma and RBC. Although excised tissues were thoroughly rinsed before homogenization, contamination proved to be extremely variable for single tissues and was often sizable. As one example, the correction for contaminating blood for the kidney resulted in a decrease in specific activity of ^{14}C-labeled UC of 9.9 ± 5.7(SD)% (correcting for plasma) and of 44.0 ± 13.6% (correcting for RBC) and a decrease in specific activity of ^{3}H-labeled UC of 30.3 ± 24.3% (correcting for plasma) and 0.3 ± 0.6% (correcting for RBC). In previous studies, these corrections either were not made (12) or were made only for RBC (10, 11) (which we found to contain no ^{3}H-labeled UC 1 hr after administration of [^{3}H]water), and then apparently extrapolated to correct for plasma contamination.

Second, we have ensured that after [^{3}H]water has been used to measure synthesis, pure [^{3}H]cholesterol has been isolated. The number of ^{3}H atoms from injected [^{3}H]-water that are incorporated into a newly synthesized sterol molecule has only been determined for cholesterol and will likely differ for other sterols. In addition to cholesterol, there is a large variety of structurally similar sterols in animal tissues, especially the skin (23). Quite clearly, if tissues subjected to comparison do not have the same quantitative distribution of sterols, a comparison of [^{3}H]water incorporation in different tissues requires that the comparison either be restricted to cholesterol alone or

separately performed for each of the multiple sterols. Neither condition has seemingly been met in previous studies of in vivo synthesis in which tissue sterols have been isolated by either digitonin precipitation (9–11) or by TLC (12). The use of digitonin to selectively precipitate sterols results in the precipitation of a wide variety of 3-β-hydroxy sterols in addition to cholesterol in amounts that are variable and unpredictable (24, 25), and seem to depend upon the amount of cholesterol also present in the particular sample (25). The use of TLC to isolate cholesterol appears to suffer from an equally serious defect. As shown in **Table 3**, we have compared the analysis of newly synthesized cholesterol by our HPLC procedure with analysis using digitonin precipitation and using TLC (with a mobile phase of benzene–ethyl acetate 5:1). Amounts of newly synthesized cholesterol in the plasma, liver, and intestine were not significantly different, as measured by any one of these three procedures. However, in sharp contrast, amounts of newly synthesized cholesterol in the remaining tissues of the carcass were appreciably greater (and the contribution of the liver to synthesis appreciably less) when cholesterol was measured by either digitonin precipitation or by TLC than when HPLC was used for analysis. (Indeed, the major difference between carcass values determined by HPLC and by either digitonin precipitation or TLC related to the relative precision of these three methods of cholesterol analysis. Using digitonin, newly synthesized cholesterol was 476% greater and using TLC, newly synthesized cholesterol was 354% greater in the carcass than when meassured by HPLC.)

HPLC of samples of the carcass that were initially obtained by digitonin precipitation or by TLC revealed, in addition to a cholesterol peak, a multiplicity of other peaks radiolabeled with tritium (**Fig. 7**). These non-cholesterol components of the carcass did not contain the ^{14}C radiolabel when [^{14}C]cholesterol was transfused into an animal and thus were presumed not to be degradation

TABLE 3. A comparison of methods used to measure newly synthesized cholesterol in the whole rat

	Newly Synthesized Cholesterol		
Tissue	Analysis by HPLC	Analysis after Digitonin Precipitation	Analysis after TLC Isolation
	μg in total tissue or plasma		
Liver	563.1 ± 77.9	656.4 ± 81.9	581.2 ± 72.9
Small intestine	78.1 ± 16.9	124.0 ± 27.6	111.9 ± 23.5
Remaining carcass	71.1 ± 22.1	437.7 ± 52.1[a]	318.3 ± 42.5[a]
Plasma	91.4 ± 31.0	92.6 ± 32.2	92.1 ± 29.6
Total	803.7 ± 76.5	1310.7 ± 70.9[a]	1103.5 ± 64.9[b]

Samples are from the three rats shown in Table 2 and all values have been corrected for contaminating blood and for plasma cholesterol exchange. Amounts of newly synthesized cholesterol in the bile have been added to amounts measured in the liver. Values are shown as mean ± SE.

[a] $P < 0.01$, compared to analysis by HPLC.
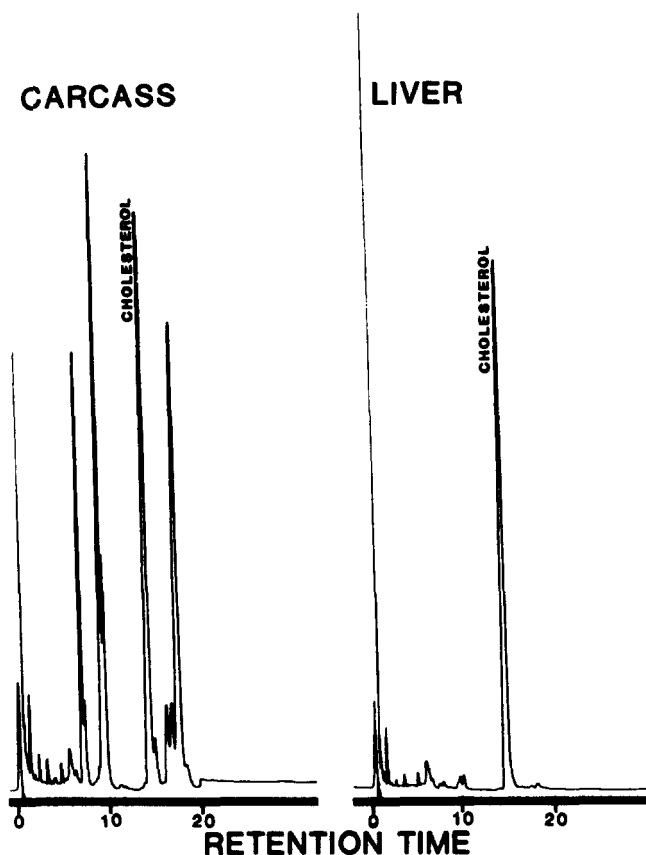[b] $P < 0.05$, compared to analysis by HPLC.

Fig. 7. HPLC tracings of digitonin precipitates of the carcass and the liver of the rat. HPLC was performed as described in Methods to quantitate cholesterol after saponification and digitonin precipitation of the whole animal (identified as "carcass") and the isolated liver. Multiple unidentified peaks were present in addition to cholesterol in the precipitate of the carcass. All peaks, in addition to cholesterol, were radiolabeled with tritium when [³H]water was used to measure cholesterol synthesis in vivo.

products of cholesterol. These other components were also not apparent in chromatograms of the isolated liver (Fig. 7), intestine, or plasma. We made no attempt to identify the non-cholesterol components in samples of the carcass or to determine from which specific tissues they were derived. However, it is evident that when [³H]water is used in studies of cholesterol synthesis in conjunction with isolation of cholesterol by digitonin precipitation or by TLC, tritiated components other than cholesterol in certain tissues will result in erroneously high estimates of cholesterol synthesis.

Finally, we have confined our study to the metabolism of cholesterol in the rat in which the liver has appeared to make a relatively larger contribution to total body cholesterol synthesis than in many other animals. In view of our findings in the rat, however, we think it prudent to reassess comparative rates of cholesterol synthesis in other animals also using [³H]water and methods that permit specific isolation of cholesterol from other highly tritiated sterols. ∎

APPENDIX

The magnitude of transfer of newly synthesized cholesterol from the plasma to the carcass by exchange was calculated as follows.

1. The rate of exchange of plasma cholesterol with tissues of the carcass was estimated from the rate of disappearance of plasma ¹⁴C-labeled UC in animals subjected to hepatectomy and intestinal resection (N = 2). The data were fitted (by iteration, with an error of <2%) to a biexponential disappearance curve as described by

$$Y_t = R_1 C_0 e^{-k_1 t} + R_2 C_0 e^{-k_2 t} \qquad Eq.\ 1)$$

where C_0 is the activity at time zero, R_1 and R_2 are the fractions of C_0 explained by the first and second exponentials, respectively, and k_1 and k_2 are the two disappearance coefficients.

2. The rate of accumulation of newly synthesized cholesterol in the plasma was estimated after intravenous injection of [³H]water in intact animals (N = 3). Newly synthesized cholesterol was first detected in the plasma at an average time of 24 min after the isotope was administered. From its initial time of appearance up to 60 min, the content of newly synthesized cholesterol in the plasma increased linearly at a constant rate. The observed rate of accumulation of newly synthesized cholesterol in the plasma is due to the combination of synthesis, secretion of new cholesterol into the plasma, and exchange of new cholesterol out of the plasma.

3. To estimate the actual amount of newly synthesized cholesterol that would have accumulated, i.e., the amount that would have accumulated if exchange were not taking place, the following model was employed.

At any time t after [³H]cholesterol is detected in the plasma, the C_0 that would have to exist at any zero time can be estimated by the equation:

$$C_0 = C_t/(R_1 e^{-k_1 t} + R_2 e^{-k_2 t}). \qquad Eq.\ 2)$$

The first derivative of equation 1 is:

$$dY/dt = -k_1 R_1 C_0 e^{-k_1 t} - k_2 R_2 C_0 e^{-k_2 t}. \qquad Eq.\ 3)$$

C_0 estimated by equation 2 can then be substituted in equation 3 in order to estimate the exchange rate at any time t. Using equation 3, the rate of exchange of newly synthesized cholesterol was calculated over the entire period of study. The calculation was begun at a near-zero time point on the observed accumulation curve and was continued for 0.1-min intervals thereafter (i.e., from 24 to 60 min), adding the absolute value obtained at each time point to the slope of the observed accumulation curve. The amount of newly synthesized cholesterol exchanged was then obtained by subtracting the area under the observed accumulation curve from the calculated accumulation curve.

4. Assumptions were made that 1) cholesterol exchange between plasma and the carcass is the same in intact animals and in animals subjected to hepatectomy and intestinal resection; and 2) newly synthesized cholesterol in the plasma is derived from the liver (and intestine) and not the remaining tissues of the carcass. This last assumption appears well-substantiated by the study of Spady and Dietschy (11).

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