Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglyceride in man

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SUMMARY Pool sizes and turnover rates of hepatic triglycerides and of lipids of three plasma lipoprotein fractions of human subjects were defined from studies of the rates of incorporation of labeled free glycerol and of labeled palmitate into these lipids, and of distribution and clearance from plasma of intact, endogenously labeled lipoproteins.

Incorporation of labeled glycerol and labeled palmitate into plasma lipoproteins was prompt. Triglycerides of the lowest density lipoprotein class ($S_f > 20$) were most highly labeled of all plasma lipoproteins, and turnover was most rapid in this class.

The kinetic behavior of radioactive plasma triglyceride was more complex when palmitate was used than when glycerol served as a labeled precursor, probably because glycerol recycled less than did palmitate.

An incompletely coupled, two-compartment, nonrecycling catenary (linked in series) model of hepatic triglyceride and of plasma $S_f > 20$ triglyceride was validated. The $S_f > 20$ triglycerides turned over in a volume approximating that of plasma. Apparently unique to man of various species previously reported is the relatively slow turnover and consequent rate-determining role of $S_f > 20$ triglyceride in this two-pool system.

The hepatic pool greatly exceeded that in plasma and turned over approximately three times more rapidly.

KEY WORDS model · liver · plasma · triglyceride · turnover · man · glycerol as tracer · rate-determining compartment

AHRENS AND CO-WORKERS (1) have recently suggested that patients with turbid hypertriglyceridemic plasma previously designated as having "essential hyperlipemia" (2). may be classified in two groups according to their response to dietary manipulation. The syndromes of these two groups were termed "carbohydrate-induced" or "fat-induced" hypertriglyceridemia.

Previous studies have suggested that the principal cause of fat-induced lipema is grossly delayed removal from plasma of newly absorbed dietary triglyceride (chylomicrons), probably due to a deficiency of tissue lipase enzyme(s) assayed in vitro as plasma post-heparin lipase activity (1, 3). In contrast, the observation that plasma post-heparin lipolytic activity is normal in carbohydrate-induced hypertriglyceridemia (1, 3) suggests that the triglyceride-rich lipoproteins present in plasma in this disorder are removed normally, and that the lipemia may be initiated primarily by an accelerated input of newly synthesized triglyceride into plasma.

In order to assess directly the input and removal rates of plasma triglyceride in man, an accurate method of measurement of liver and plasma triglyceride turnover rates was required. In this paper we describe such a method, which employs radioactive glycerol as a precursor of newly synthesized triglyceride. Using this method, we present evidence for a hepatic triglyceride synthesis rate many times that required to account for plasma triglyceride turnover.[1] From our data a two-compartment, nonrecycling, catenary (linked in series) model for hepatic and plasma triglyceride turnover has been derived. Over 90% of the hepatic precursor triglyceride was calculated to enter a blind intrahepatic "sink," wherein hydrolysis to free glycerol and fatty acid is presumed to occur. Therefore the single hepatic precursor pool is *incompletely* coupled with the product pool

[1] Preliminary reports have been given (4, 5).

in plasma. This model seems unique to man, of the animals so far studied.

MATERIALS

Glycerol-2-H^3, either 229 or 455 μc/μmole, glycerol-1,3-C^{14}, 15 μc/μmole, and palmitate-1-C^{14}, 22 μc/μmole, were from New England Nuclear Corp., Boston, Mass. The purity of glycerol was preserved after storage for 10 weeks at 4°, as shown by finding a single peak of radioactivity on Whatman No. 3MM paper in an area with correct R_F for glycerol after ascending chromatography in butanol–acetic acid–water 4:1:5, v/v/v. The paper strips were counted in a strip counter[2] at full scale deflection for the glycerol peak.

METHODS

Details of experimental procedures for other than the liver biopsy studies are described in the Results section. Selected human subjects were fasted for 10 hr overnight, and unless otherwise specified, fasting was continued for a total of 20 hr. Subjects were at rest for the first 12 hr; they then commenced habitual activities. Labeled compounds dissolved in 10 ml of 0.15 M NaCl were injected into an antecubital vein. Following the injection, venous blood samples were obtained at various intervals up to 168 hr. Clotting of blood was prevented either by addition of 1 mg EDTA per ml or, if lipoproteins were to be recovered and later reinfused, by withdrawal of blood into a syringe wetted with heparin (Liquaemin Sodium, Upjohn, 1,000 units/ml). Plasma was removed after prompt centrifugation at 4° and stored at that temperature until processed further.

Liver Biopsies

Five studies were done on four subjects. Study 1 was done on a 71 yr old male (subject F. C.) with inoperable carcinoma of the bronchus and radiologic evidence of bony metastases. He had lost 12 kg shortly before the study, but body weight was stable during the week prior to biopsy. Various liver function tests were normal. He was given 150 g of glucose intravenously over a 4 hr period the evening before biopsy to insure an adequate caloric intake. Glycerol-2-H^3 (300 μc) was infused intravenously, and percutaneous liver biopsy was performed 65 min later. The biopsy segment was maintained in 0.15 M NaCl at 4° for 1 hr. The tissue was weighed, lipids were extracted, and the triglycerides isolated and counted.

Study 2 was done on a 33 yr old male (subject C. H.) with a diagnosis of schizophrenia and of subsiding obstructive jaundice secondary to oral chlorpromazine medication. There was a slight elevation of plasma direct bilirubin. Many other liver function tests were normal. Liver biopsy was performed 110 min after the infusion of 300 μc of glycerol-2-H^3.

Study 3 was done on a 21 yr old male (subject G. S.) with a tentative diagnosis of sarcoidosis. (Later diagnoses were bronchiectasis and membranous glomerulonephritis). Selected liver function tests and routine laboratory studies were normal except for moderate proteinuria. A solid food diet of 38 cal/kg body weight, containing fat, carbohydrate, and protein in proportions approximating 25:60:15 (per cent of calories) was consumed by the patient for 4 days prior to the biopsy. A weight gain of 0.6 kg occurred during this period. Liver biopsy was performed 100 min after infusion of 300 μc of glycerol-2-H^3.

Study 4 was done on a 52 yr old male (subject G. B.) following 3 weeks of a liquid formula diet containing fat, carbohydrate, and protein in proportions of 5:80:15 (per cent of calories). This individual, who had evidence of arteriosclerotic heart disease, was under study for response of plasma and hepatic lipid and carbohydrate metabolism to high and low fat diets. This subject received a simultaneous infusion of 300 μc of glycerol-2-H^3 and 35 μc of palmitate-1-C^{14} (albumin-bound) and the liver biopsy was performed 120 min later.

Isolation of Plasma Lipoproteins

The technique of Havel, Eder, and Bragdon (6) was used with modifications as follows. The cellulose centrifuge tubes[3] were dried after a rinse in a solution of EDTA (5.0 mg/100 ml). The salt solutions used to adjust plasma density contained the same concentration of EDTA in order to chelate polyvalent cations and thereby inhibit auto-oxidative reactions (7). A No. 50 rotor[3] was used at 50,000 rpm (150,925 × g at the center of the tube) in place of the No. 40 rotor to shorten centrifuge times to 16, 16, and 24 hr for the $S_f > 20$ S_f 0–20, and high density (d 1.063–1.21) lipoproteins, respectively. Lipoproteins were removed by aspiration rather than by means of a tube slicer. For reinfusion experiments, polyethylene tubes were used instead of cellulose, and these and their caps were sterilized by boiling and subsequently exposing them to ultraviolet irradiation for 18 hr.

Extraction and Analysis of Lipids

All samples were processed in a manner designed to decrease the likelihood of oxidative and hydrolytic changes in lipids (8). Lipids were extracted from lipoprotein

[2] Automatic Windowless Paper Chromatogram Strip Counter, RSC-160, Atomic Accessories, Inc., Bellrose 26, N.Y.

[3] Purchased from the Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif.

fractions or from whole plasma with mixtures of chloroform, methanol, and water by the method of Bligh and Dyer (9). The chloroform extract was washed twice with equal volumes of a blank upper phase, methanol–0.58% NaCl–chloroform, 47:48:3. The interface was rinsed twice with small amounts of blank upper phase. The washed lower phase solvents were removed in a partial vacuum in a rotary evaporator at 37°, using generous amounts of ethanol to prevent foaming and to remove traces of water as a lower boiling azeotrope. Lipids were dissolved in warm petroleum ether (bp 60–70°) and filtered through glass wool.

Cholesterol esters, triglycerides, free cholesterol, and phospholipids of selected lipid extracts were separated on columns of silicic acid by the method of Hirsch and Ahrens (10), modified by performing the chromatography at 8° instead of 24°. In experiments where total recovery of small amounts (<5 mg) of triglyceride was the sole object, they were separated by thin-layer chromatography (8) using petroleum ether (bp 60–70°)–ethyl ether 72:28. Neutral lipids were extracted from Silica Gel G (Brinkmann Instruments, Inc., Great Neck, N.Y.), or from dry residues of column eluates by three extractions with the solvent system of Dole (11), omitting acid from the water used to form the two phases. Phospholipids were extracted by the method of Bligh and Dyer (9).

Lipid extracts were analyzed for weight of total lipids, and for lipid phosphorus (12), cholesterol (13), and triglycerides (14). A molecular weight of 850 was assumed for triglycerides. Recovery of 50 μmoles of pure triolein from silicic acid columns was 97 and 99% on two trials, and percentage recovery of 1–4 μmoles of triolein from single 20 × 20 cm silica gel plates was 96 ± 1.5 (mean ± SD) in 10 trials.

Hydrolysis of Glycerol and Fatty Acid Moieties

Selected triglyceride samples were hydrolyzed in alcoholic NaOH and the fatty acids recovered by extraction after acidification (15). The aqueous infranate was neutralized, and evaporated down at room temperature (15). Methanol was added at intervals during evaporation to maintain a volume not less than one-third of the original. Fatty acids were methylated in anhydrous HCl–methanol (16). The radioactivity in methyl esters and in 0.2 ml aliquots of the methanolic extract was determined.

Isotope Counting

Lipids were dissolved in 10 ml of a liquid scintillation fluid,[4] and counted with an accuracy of ±2% in a model 314EX-2 or Model 3000 Packard Tri-Carb Liquid Scintillation Spectrometer. The activity of H^3 and C^{14} in mixtures was calculated by the discriminator ratio method (17). Aliquots of 0.2 ml of the methanolic aqueous hydrolysate of selected triglyceride samples were counted in a similar fashion.

[4] Toluene–1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene–2,5-diphenyloxazole, 1000 ml/0.3 g/5.0 g.

Definitions

TG = triglyceride. Pool = an assumed anatomical space which contains the substance under study. SA = specific activity. $SA_{(x)}$ = SA of a precursor pool in an open 2-pool system. $SA_{(y)}$ = SA of a product pool. SA_{max} = maximum SA. t_{max} = time of maximum SA. t_0 = time of injection of a labeled compound into a subject. $t_{1/2}$ = half time. t_r = turnover rate = amount of material entering or leaving a pool or compartment per unit time. k_1, k_2 = rate constant or fractional turnover rate of material in precursor and product pools respectively = fraction of a pool turning over per unit time. Precursor classes: absolute, the product is derived only from the precursor, and all the precursor enters only the product pool; unique, the same as absolute except the precursor enters pools in addition to the product pool.

RESULTS

Comparison of H^3- and C^{14}-Labeled Glycerol in Turnover of Plasma $S_f > 20$ Triglycerides

A 48 yr old white male (subject J. P.) was given a mixture of 100 μc of glycerol-2-H^3 and 10 μc of glycerol-1,3-C^{14}. Very low density ($S_f > 20$) plasma lipoproteins were isolated from blood obtained at various intervals up to 48 hr and the specific activity of the triglycerides was determined.

Figure 1 demonstrates that the shapes of the two specific activity–time curves of $S_f > 20$ triglyceride labeled with either 2-H^3 or 1,3-C^{14} forms of glycerol are indistinguishable. Since β-particle energies of H^3 are about 8-fold lower than of C^{14}, but the spectrometer efficiency of H^3 is one-half that of C^{14}, we chose glycerol-H^3 for most of our studies because this allowed us to expose the subjects to less radiation.

Incorporation of Glycerol-2-H^3 and Palmitate-1-C^{14} into the Three Major Lipoprotein Fractions

A 41 yr old white male (subject E. C.) received a mixture of 300 μc of glycerol-2-H^3 and 35 μc of palmitate-1-C^{14}. Palmitate was complexed to human serum albumin by the method of Felts and Masoro (18). Blood was withdrawn at various intervals, the three major lipoprotein classes of plasma were isolated ($S_f > 20$, S_f 0–20, and high density), and specific activities of H^3 and C^{14} in the triglycerides, phospholipids, and cholesterol esters deter-

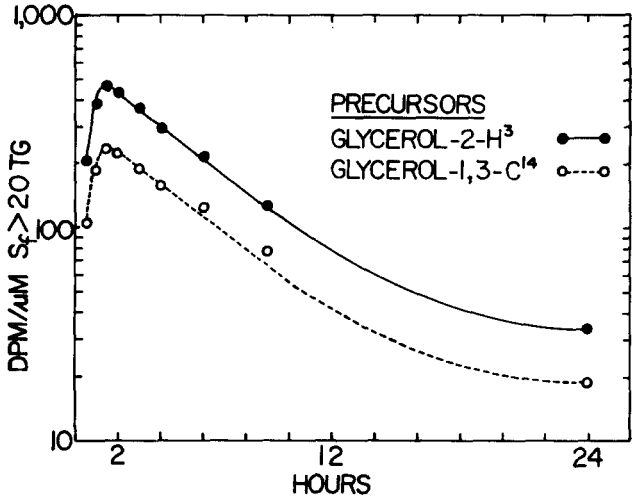

FIG. 1. Specific activity (SA)–time curves of $S_f > 20$ lipoprotein triglycerides following simultaneous rapid intravenous infusion of glycerol-1,3-C^{14} and glycerol-2-H^3 (subject J. P.). SA–time curves of H^3 and C^{14} adjusted to values based on an assumed administered dose of 1.94 μc and 0.67 μc/kg body weight, respectively.

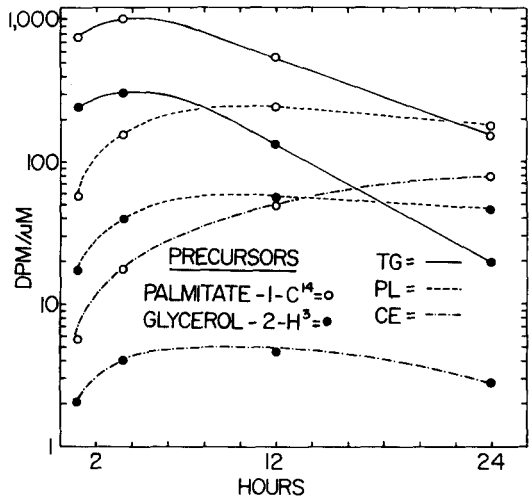

FIG. 2. Comparison of SA–time curves of triglycerides (TG), phospholipids (PL), and cholesterol esters (CE) of $S_f > 20$ lipoproteins (subject E. C.) after simultaneous infusion of glycerol-2-H^3 and of palmitate-1-C^{14}. SA–time curves adjusted to values based on an assumed administered dose of 3.34 μc and 1.1 μc/kg body weight, respectively.

mined. In addition, recoveries of H^3 and C^{14} in glycerol and fatty acid moieties were determined from aliquots of $S_f > 20$ triglyceride from selected samples.

A complete analysis of the relative proportion of each label present in the separate lipid classes was carried out in the $S_f > 20$ lipoproteins (of subject E. C.) at various times after infusion of labeled compounds (Table 1 and Fig. 2). The most striking result, albeit predictable, was the virtual absence of H^3 as compared to C^{14} in cholesterol esters (Fig. 2). Also, palmitate-1-C^{14} appeared somewhat more rapidly in phospholipid and disappeared slightly more slowly than did glycerol-2-H^3 (Fig. 2, Table 1). However, the rate of phospholipid labeling from either isotope was delayed as compared with triglyceride, and maximum C^{14} incorporation into cholesterol ester was delayed with respect to phospholipids (Fig. 2). These results are in keeping with those obtained in rats and rabbits injected with labeled palmitate (19, 20) or in man following intravenous injection of labeled acetate (21).

For both glycerol-2-H^3 and palmitate-1-C^{14} most of the label incorporated into triglyceride was in the $S_f >$ 20 fraction. Almost all the plasma triglyceride turnover was in this very low density lipoprotein fraction (Table 2). (Calculations of turnover are possible only after validation of the proposed models. These validation steps and calculations are explained in later sections.) Table 2 also includes similar data from subject R. N. given glycerol only. These results are consistent with previous observations using palmitate alone (19, 20, 22, 23).

Analysis of the hydrolysate of $S_f > 20$ triglycerides from one subject E. C. demonstrated that essentially all the H^3 was in the glycerol moiety, and all the C^{14} in the fatty acid portion at 1, 6, and 12 hr after the infusion

TABLE 1 CALCULATED INCORPORATION OF LABELED COMPOUNDS AND MOLAR RATIOS* OF H^3/C^{14} IN VARIOUS $S_f > 20$ LIPIDS OF ONE SUBJECT† FOLLOWING SIMULTANEOUS INTRAVENOUS INFUSION OF PALMITATE-1-C^{14} AND GLYCEROL-2-H^3

Lipid Fraction of $S_f > 20$ Lipoproteins	Molar Ratios H^3/C^{14} at Times (Hr) after t_0					% Dose Incorporated at t_0‡		Percentage of Total Lipid of $S_f > 20$ Lipoproteins
	1	3.5	12	24	48	H^3	C^{14}	
Triglyceride	100	93.8	78.1	62.5	40.6	90.0	80.5	53
Phospholipid	100	86.7	76.7	66.7	63.3	10.0	18.6	20
Cholesterol ester	—§	—	—	—	—	0.1	0.8	13

* Measured ratio corrected to 100 at the 1 hr sample.
† Subject E. C., a 41 yr old male.
‡ Calculation of percentage dose incorporated requires assumptions that each lipid turns over independently and that no recycling of label occurs within that lipid.
§ H^3 specific activities were too low to allow accurate calculation of H^3/C^{14} ratios.

TABLE 2 DISTRIBUTION OF RADIOACTIVITY IN TRIGLYCERIDE (TG) OF PLASMA LIPOPROTEINS AFTER INTRAVENOUS INJECTION OF PALMITATE-1-C^{14} OR GLYCEROL-2-H^3 IN TWO SUBJECTS*

	Amount Incorporated into TG			Half-Time ($t_{1/2}$) TG			Total TG in Plasma‡			Amount of Total TG in Each Lipoprotein			Turnover Rate (t_r§)			Amount of TG Turnover for Each Lipoprotein		
Subject	VLD	LD	HD	VLD	LD	HD	VLD	LD	HD	VLD	LD	HD	VLD	LD	HD	VLD	LD	HD
	%†			*hr*			*mg/100 ml*			%			*mg/hr/kg*			%		
E. C. H^3	1.7	0.03	0.02	5.0	11.3	7.6	309	13.0	6.1	94.1	4.0	1.9	19.3	0.36	0.25	97.0	1.75	1.20
E. C. C^{14}	13.5	0.31	0.16	7.5	13.0	11.3							12.9	0.31	0.17	96.5	2.70	0.80
R. N. H^3	1.0	0.36	0.01	8.6	13.6	26.4	506	80.2	14.8	91.8	5.5	2.7	18.4	0.69	0.18	99.6	0.38	0.09

* Both subjects were males, 41 and 42 yr old, respectively.
† Calculated as percentage of administered dose present at t_0 in total plasma volume.
‡ Mean of all values of the study.
§ Turnover rate is expressed here in different units from those used in explanation of the validation of the proposed model.
Nomenclature of lipoprotein classes: VLD = very low density = $S_f > 20$ = d < 1.006; LD = low density = S_f 0–20 = d 1.006–1.063; HD = high density = d 1.063–1.21.

(Table 3). These data indicated that significant re-entry of either label from either acetate or hydrogen exchange does not occur up to at least 12 hr.[5]

Although both labeled compounds served as adequate precursors for $S_f > 20$ triglycerides, the specific activity–time curves were not identical (Figs. 3 and 4). Major differences were noted in the $S_f > 20$ triglyceride specific activity ($SA_{(y)}$)–time curves that led to the general conclusion that recycling of labeled palmitate in plasma triglyceride was much greater than that of labeled glycerol. For example, the $SA_{(y)}$–time curve of palmitate-labeled $S_f > 20$ triglyceride (of subject E. C.) was clearly no longer linear by 24 hr, whereas the disappearance of glycerol-labeled molecules remained first-order up to 48 hr (Fig. 3). Calculations based upon the observed H^3/C^{14} molar ratios (Table 1) demonstrate that there is considerably greater retention in $S_f > 20$ triglyceride of palmitate-1-C^{14} as compared to glycerol-2-H^3 48 hr following the infusion of both labeled compounds. From these data one gains a qualitative measure of the degree of triglyceride fatty acid recycling that results from triglyceride hydrolysis and reesterification with unlabeled glycerophosphate molecules.

Interpretations

In this section we describe steps for validation of simple multicompartmental models and we follow conventions outlined by Zilversmit, Entenman, and Fishler (24) Strisower (25), and Rescigno and Segre (26).

From various lines of evidence it seems likely that plasma lipoprotein triglyceride is a secretory product of a precursor triglyceride pool in the liver (19, 20). Thus, as a simple yet most useful first step in model validation, one may infuse prelabeled lipoprotein triglyceride into the product pool (plasma). If this triglyceride distributes only into the plasma volume and if it disappears from plasma in a first-order manner, then one particular simple model of the product pool has been defined; i.e., a single pool with a single entrance and a single exit and without recycling from other pools. Our results (see below) define the product ($S_f > 20$ triglycerides) as following this simple model (Model 1, Fig. 5) with plasma as the single product pool *B*, containing *b* grams of $S_f > 20$ triglyceride. In such a simple model, if the disappearance of prelabeled triglyceride from plasma is more rapid than

TABLE 3 DISTRIBUTION OF H^3 AND C^{14} IN $S_f > 20$ TRIGLYCERIDE OF ONE SUBJECT* FOLLOWING SIMULTANEOUS INFUSION OF PALMITATE-1-C^{14} AND GLYCEROL-2-H^3

		Hr after Infusion		
	Label	1	6	12
TG-glycerol	H^3	79.1	83.1	74.8
	C^{14}	0	0	0
TG-fatty acid	H^3	0	0	4.1
	C^{14}	84.3	76.2	81.0

Values are expressed as percentage of counts in original triglyceride recovered as H^3 in aqueous hydrolysate (glycerol) or as C^{14} fatty esters after transmethylation. Recovery of label in model compounds was approximately 85%. Amounts of ~3% would have been detected, but values <10% are approximate because of the low absolute radioactivity in such samples.
* Subject E. C., a 41 yr old male.

[5] Such redistribution has been noted at later hours, for example about 5% of $S_f > 20$ triglyceride-H^3 was in the fatty acid moiety in subject J. P. (Fig. 1) and 6% in subject E. C. (Fig. 3) 24 hr after infusion. It has also been noted that this redistribution is present to a greater extent in samples analyzed from the distal and relatively flat portions of the $S_f > 20$ triglyceride specific activity–time curves. Although relocation of C^{14} or H^3 in triglyceride most probably follows their entry into the acetate pool, other routes are possible.

that of endogenously labeled triglyceride, then the precursor pool turns over more slowly than does the product pool; if the disappearance is at the same rate, the product pool turns over more slowly (25). Our results define a product pool which turns over more slowly than its precursor pool (and is therefore the rate-determining pool with a rate constant k_2 less than the precursor rate constant k_1).

In Model 1, an *assumed* single hepatic precursor pool *A* containing *a* grams of triglyceride is also shown, and is drawn as completely coupled to the product pool. This precursor is therefore known as an absolute precursor (26). However, the dimensions and other features of this precursor pool remain mysterious unless directly measured. Nevertheless, the first model remains correct for the product pool and allows one to calculate turnover rates (p_2 in g/hr) of the product from "endogenous labeling" studies such as those described below after glycerol-2-H^3 injection (see Figs. 1, 3, 6, and 7). Formulae for such product pool calculations appear in Appendices *A*, *B*, and *C*. With use of product pool calculations the size *a*, rate constant k_1 and turnover rate p_1 of the assumed hepatic precursor pool may also be calculated from the "endogenous labeling" studies. Formulae for these precursor pool calculations appear in Appendix *D*.

Any further information on the precursor pool(s) requires direct sampling of their contents. One must determine *if* hepatic triglyceride could be a precursor of plasma lipoprotein triglyceride and to determine which class of precursor (such as absolute or unique) it may be. The simplest model would place *all* of the hepatic triglyceride in a single precursor pool turning over with a single rate constant. Evidence for this would come from an "endogenous labeling" experiment if the precursor specific activity $SA_{(x)}$ closely approximates (or coincides) with the descending slope of $SA_{(y)}$ at any point—but particularly at its crossover with the product $SA_{(y)}$ at $_{max}$ of the product (24). One would do this as an "endogenous labeling" study following an intravenous injection of a labeled triglyceride precursor, such as glycerol, and subsequent calculation of product specific activity–time curves $SA_{(y)}$. During such a study one must guess the probable t_{max} of the product specific activity and sample the liver at or near this time. Since product t_{max} varies (Figs. 1, 3, 6, 7, and 8), only once in 4 studies was our biopsy taken at exactly this time (see below). In the other three studies the biopsy was taken slightly after the

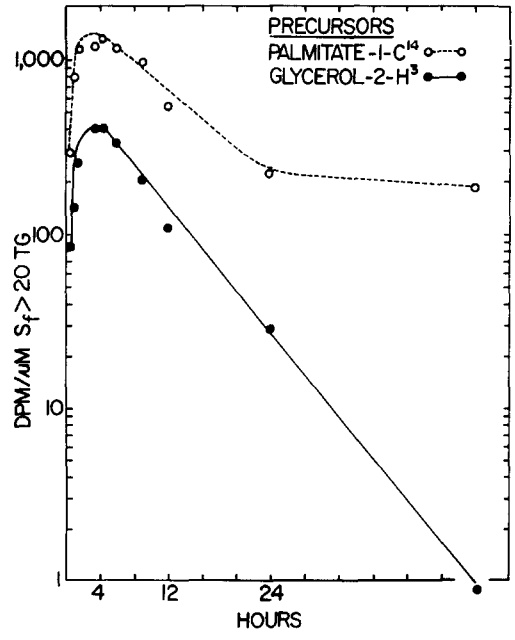

FIG. 3. Specific activity–time curves of $S_f > 20$ triglycerides ($SA_{(y)}$) following simultaneous infusion of glycerol-2-H^3 and palmitate-1-C^{14} (subject E. C.). SA–time curves of H^3 and C^{14} adjusted on basis of an assumed administered dose of 3.34 μc and 1.1 μc/kg body weight.

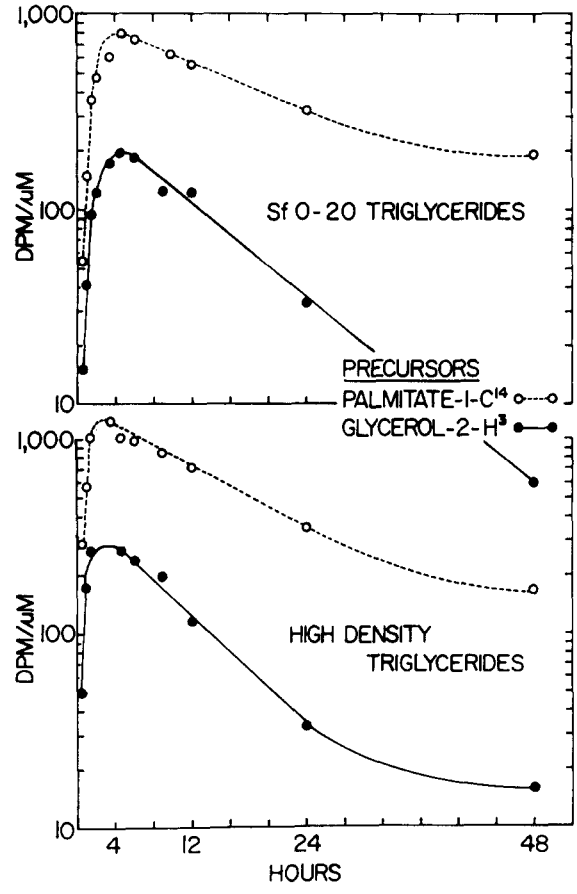

FIG. 4 Specific activity–time curves of triglycerides of S_f 0–20 lipoproteins (d 1.006–1.063), upper figure, and of high density lipoproteins (d 1.063–1.21), lower figure, after simultaneous intravenous infusion of glycerol-2-H^3 and palmitate-1-C^{14} (subject E. C.). SA–time curves of H^3 and C^{14} adjusted to values based on an assumed administered dose of 3.34 μc and 1.1 μc/kg body weight, respectively.

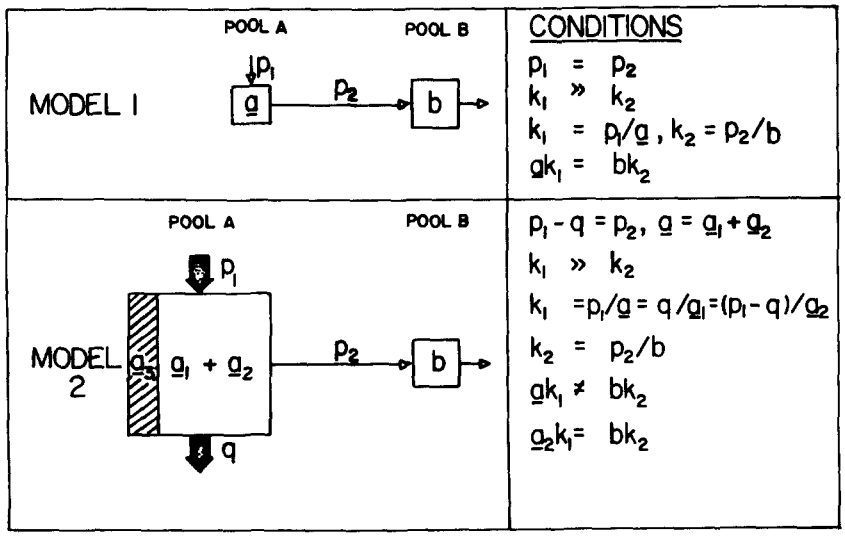

FIG. 5. Two proposed models for liver and plasma triglyceride turnover. Model 1. completely coupled, catenary, nonrecycling model. Product pool *B* is larger and rate-determining. Because the pools are completely coupled, the first pool *A* is an *absolute* precursor of second pool *B*. *a* and *b* = contents (grams of TG) of pools *A* and *B*, respectively. Model 2. Incompletely coupled, catenary, nonrecycling model. Product pool *B* is smaller and rate-determining, and, because the pools are incompletely coupled, the first pool *A* is classed as a unique precursor of the second pool. $a_1 + a_2$ and *b* are contents (grams of TG) of pools *A* and *B*, respectively. a_3 is not a part of pool *A*. p_1, p_2, and q = turnover rate of TG in g/hr. k_1 and k_2 = rate constants or fractional turnover rates of pools *A* and *B*, respectively; units = fraction of pool turning over/hr = 1/hr.

t_{max} of product activity. Our calculations from liver biopsy data allowed the more complex Model 2 of Fig. 5 to be proposed. In Model 2 almost all the hepatic triglyceride (listed in Fig. 5 as $a_1 + a_2$) was a precursor of the $S_f > 20$ triglycerides in plasma, but the data were also consistent with the presence of a small, relatively inert hepatic triglyceride compartment (a_3 of Fig. 5).

Two exits from this more complex precursor pool are required to account for the calculated hepatic triglyceride turnover. (Methods for this calculation are outlined in Appendix *E*.) These two exits (in g/hr) are termed p_1 and q and represent entry into plasma and into an intrahepatic "sink," respectively (Fig. 5).

Product Pool Rate Constants (k_2) from $S_f > 20$ Triglycerides of "Endogenous Labeling" and "Reinfusion" Studies

Two experiments were done (subjects J. P. and R. L.) as follows:

Study 3-A. "*Endogenous labeling.*" During the 3 hr after intravenous injection of 1000 μc of glycerol-2-H^3, in addition to blood drawn to obtain $S_f > 20$ triglyceride specific activity–time curves, an extra 140 ml of blood was removed, and an aliquot of $S_f > 20$ lipoprotein was used to determine the percentage of label incorporated into various lipid classes. Triglycerides contained more than 90% of the total H^3 of this lipoprotein. The remainder of the intact $S_f > 20$ lipoproteins was stored at 4° for use in reinfusion studies.

Study 3-B. "*Reinfusion.*" The $S_f > 20$ lipoproteins that had been previously isolated and stored were warmed to 37° for 30 min and then given by rapid intravenous infusions into the original donors. Specific activity–time curves of reinfused $S_f > 20$ triglycerides were then determined. These studies were performed 3 days (subject J. P.) and 7 days (subject R. L.) after zero time of study 3-A. The amounts of triglyceride infused were less than 15 mg and therefore were a negligible increment to the total plasma lipids.

The volumes of distribution (Table 4) of labeled $S_f > 20$ lipoprotein were calculated as outlined in Appendix *A* and were very close to 4.5% of body weight reported by Gregersen as the average plasma volume of normal adult males (27). $S_f > 20$ triglyceride concentrations were 72 and 575 mg/100 ml of plasma in subjects J. P. and R. L. respectively. This 8-fold variation was purposely sought in order to test the postulated model (Fig. 5) within wide ranges of plasma triglyceride concentration. From these data the product pool sizes *b* of plasma $S_f > 20$ triglyceride were calculated by the methods outlined in Appendix *A* (Table 4). One can also calculate *b* from $SA_{(y)}$–time curves of "endogenous labeling" studies (see Appendix *A*). Table 4 shows that values of *b* were remarkably similar, whether calculated from the "reinfusion" or from the "endogenous labeling" studies.

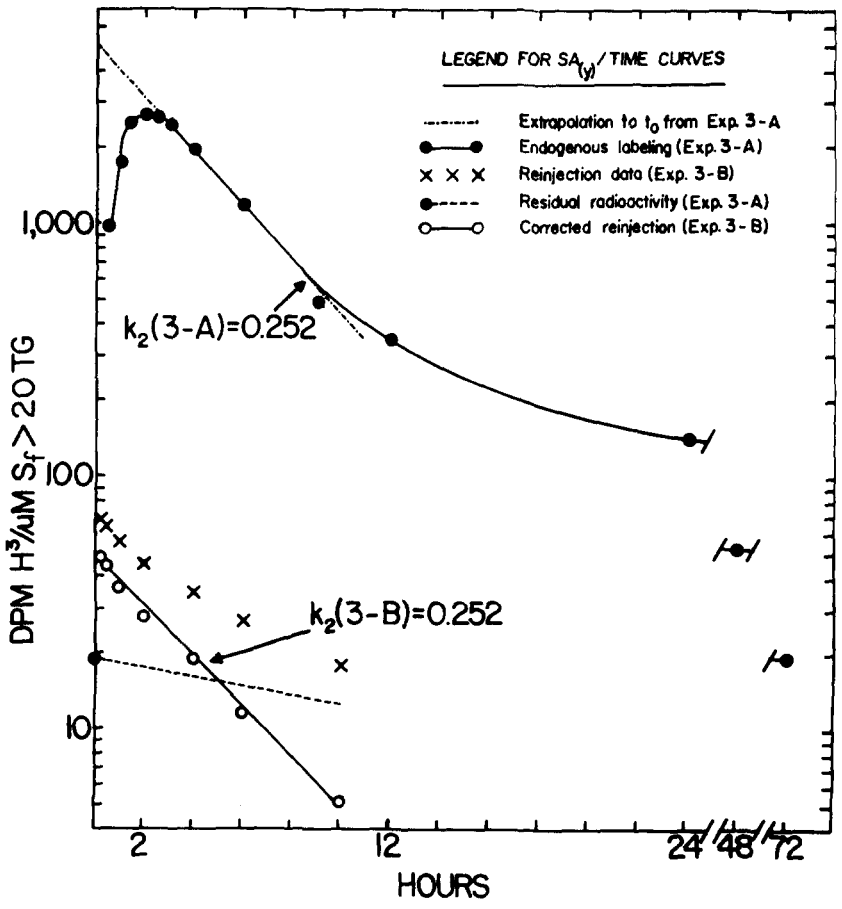

FIG. 6. Data demonstrating rate constants of the product (k_2) derived from $SA_{(y)}$ of Experiment 3-A on subject J. P. ($S_f > 20$ TG labeled "endogenously" after infusion of glycerol-2-H^3) and from corrected $SA_{(y)}$ of Experiment 3-B (labeled $S_f > 20$ TG of Experiment 3-A "reinfused" into subject J. P.—the donor). SA–time curves adjusted on of an assumed administered dose of 1.94 μc/kg body weight.

After rapid intravenous infusion, prelabeled $S_f > 20$ triglycerides disappeared in an apparent first-order manner to less than 5% of peak activity (Figs. 6 and 7). Therefore virtual absence of recycling from product pool to precursor pool is established (25), the volume of distribution will include only the plasma volume, and k_2 (the rate constant of *b*) is obtained from the slope from plots on semilogarithmic graph paper of $SA_{(y)}$ with time of reinfusion of $S_f > 20$ triglycerides. (This calculation of k_2 is outlined in Appendix *B*).

Both the observed $SA_{(y)}$ data and the corrected $SA_{(y)}$ curves of the two "reinfusion" studies are shown in Figs. 6 and 7. As stated, the initial specific activity of reinfused lipoproteins of both experiments declined in a first-order manner in both experiments. Radioactivity at later times was too low to detect curvilinearity similar to that noted after 24 hr in the "endogenous labeling" studies. In calculating corrected $SA_{(y)}$–time curves of reinfused triglyceride, corrections were made for residual H^3 activity remaining from the preceding experiments. Despite an interval of as long as 7 days (subject R. L., Fig. 7), the amount of residual radioactivity of plasma was large in comparison to that present after reinfusion of the endogenously labeled materials. Since the increment due to the latter was small, counting errors may influence the results (obtained by subtraction of the activity of the extrapolated curves of residual radioactivity from individual points of observed activity) and account for the scatter of points in the corrected $SA_{(y)}$–time curve in Fig. 7. However, despite scatter, all points were within one (log) standard deviation of the calculated line of best fit determined by least squares analysis (28).

The reinfusion study is essential in validation of the models described in Interpretations and in Fig. 5. If the rate constant derived from the $SA_{(y)}$ of "reinfused" $S_f > 20$ triglyceride had been greater in magnitude than that derived from the "endogenous" $S_f > 20$ triglyceride, the *precursor* would be rate-determining in a two-pool system (25). However the data in Table 4 clearly demonstrate that the rate constants k_2 from "endoge-

nous" and "reinfusion" studies are very similar in magnitude. This similarity indicates that the *product* compartment is rate-determining and that the similarity occurs despite wide variations in product (plasma $S_f > 20$ triglyceride) pool size. Therefore $SA_{(y)}$ of other "endogenous labeling" experiments can be used to determine k_2 directly by extrapolation of $SA_{(y)}$ to time of injection t_0 and subsequent graphic calculation as described in Appendix *B*. Values for the product turnover rates can then be obtained by methods outlined in Appendix *C*.

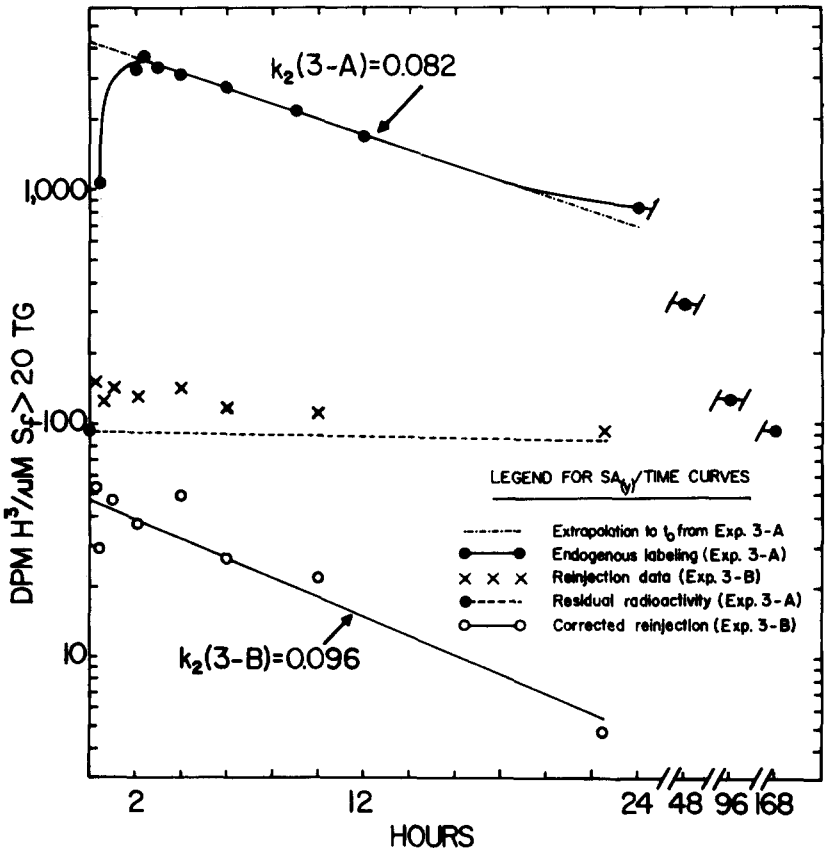

FIG. 7. Data demonstrating rate constants of the product (k_2) derived from $SA_{(y)}$ of Experiment 3-A on subject R. L. ($S_f > 20$ TG labeled "endogenously" after infusion of glycerol-2-H^3) and from corrected $SA_{(y)}$ of Experiment 3-B (labeled $S_f > 20$ TG of Experiment 3-A "reinfused" into donor subject). SA–time curves adjusted on basis of an assumed administered dose of 1.94 μc/kg body weight.

TABLE 4 RATE CONSTANTS, SIZES OF PLASMA AND HEPATIC TG POOLS, VOLUMES OF DISTRIBUTION AND TURNOVER RATES OF $S_f > 20$ TG, FROM "ENDOGENOUS LABELING" (PROCEDURE 3-A) AND "REINFUSION" (PROCEDURE 3-B) EXPERIMENTS*

Subject	Procedure	Rate Constant of $S_f > 20$ TG (k_2)	Pool Size of $S_f > 20$ TG (b)	Rate Constant of Hepatic Precursor (k_1) Equation (5)	(k_1) Graph	Hepatic TG Pool† (a_2)	Turnover Rate of $S_f > 20$ TG (p_2)	Hepatic TG Pool (a_1)	Hepatic TG Pool (a)	Vol Dist of b, Equation (1)‡	Estimated Plasma Volume§
		hr^{-1}	g	hr^{-1}		g	g/hr	g	g	ml	ml
R. L.	3-A	0.082	15.9	1.18	1.74	1.10	1.30	13.85	14.95	—	3470
	3-B	0.096	19.5	—	—	—	1.87	—	—	3390	
J. P.	3-A	0.252	2.34	0.75	1.34	0.80	0.60	13.50	14.30	—	3350
	3-B	0.252	3.42	—	—	—	0.72	—	—	3800	

* See text for full description of terms.
† a_2 is calculated from k_1 derived from *equation 6*—see Interpretations and Appendix *D*.
‡ See Appendix *A*.
§ Plasma volume (in ml) estimated as 4.5% of body weight (27).

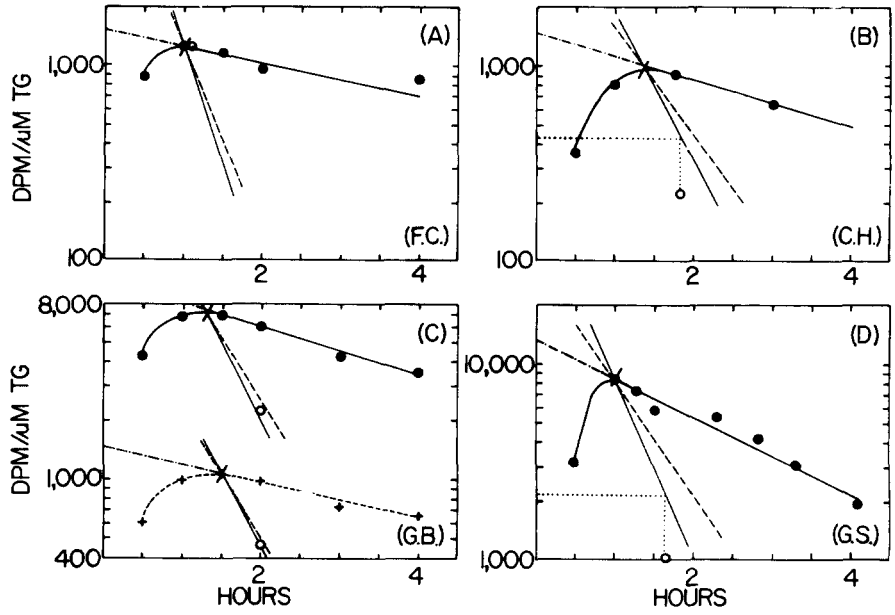

FIG. 8. Liver and plasma triglyceride labeling studies after infusion of glycerol-2-H^3 or palmitate-1-C^{14}. Data from five studies on four subjects with liver biopsy done near the time of maximum SA of plasma $S_f > 20$ TG to compare SA of the presumed precursor (liver TG) with that of the product at the peak activity of the latter ($SA_{(y)max}$) or with the calculated SA–time curves of an assumed precursor ($SA_{(x)}$) derived from rate constants (k_1) calculated by *equation 6* (first k_1) or by graphic method (second k_1). SA liver TG = O, $SA_{(y)}$ after glycerol-2-H^3 = ●—●—●, $SA_{(y)}$ after palmitate-1-C^{14} = +- -+- -+, $SA_{(x)}$ from first k_1 = ——, $SA_{(x)}$ from second k_1 = - - -, t_{max} = /. Extrapolation of $SA_{(y)}$ toward t_0 = ·····. Intersects of SA liver TG and of expected $SA_{(x)}$ with ordinate = ...

Such data from the two paired "reinfusion" and "endogenous labeling" studies are listed in Table 4, and similar data from many other experiments in patients with arteriosclerotic heart disease have been compiled and will be reported.[6]

Validation of Precursor Nature of Hepatic Triglyceride

The results are shown in Fig. 8. In one subject (F. C.) the measured specific activity of total liver triglyceride nearly coincided with $SA_{(y)}$ at the time of maximum specific activity of plasma $S_f > 20$ TG at t_{max}. In the four experiments on the remaining three subjects this experimentally determined hepatic triglyceride specific activity nearly coincided with or was slightly less than one or both *calculated* slopes of hepatic triglyceride specific activity. These two slopes were derived from the rate constants k_1 calculated by two methods (see Appendices *D* and *F*). Subject G. B. was given palmitate-C^{14} in addition to glycerol-H^3; in both studies on this subject, specific activity of liver triglyceride was bracketed by these slopes (Fig. 8). Interpretation of these data, although only in five experiments, requires one to assume most of the hepatic triglyceride to be a single pool in a precursor relationship to plasma $S_f > 20$ triglyceride (see Appendices *E* and *F* and Discussion).

Calculation of Pool Sizes and Turnover Rates of Hepatic Triglyceride Compartments

The size of the total hepatic triglyceride pool, calculated from measurement of content of total triglyceride per wet weight of the biopsy, and assuming liver to be 2.43% of body weight (29), is 11.33, 9.52, 10.55, and 15.10 g in subjects F. C., C. H., G. B., and G. S., respectively. Calculation of the proportion of this total hepatic triglyceride which is turning over as a single unique precursor of plasma $S_f > 20$ triglyceride is obtained from *equations 10* and *11* of Appendix *E* and corrects these figures to 11.33, 4.04, 10.55, and 8.23 g. These values equal $a_1 + a_2$ of Table 5. It is evident that a_2 represents a quite low (though variable) fraction of the total of $a_1 + a_2$. These calculations include estimates of the potential daily caloric equivalent of the triglyceride turning over as p_2 and q (Table 5).

Calculations of Rate Constants (k_1) and Sizes of Precursor Pool Subcompartments (a_1 and a_2) from Studies without Liver Biopsy

After validation of Model 2 (Fig. 5), data obtained by "endogenous labeling" of the product pool ($S_f > 20$ TG

[6] G. M. Reaven, R. C. Gross, R. M. Wagner, and J. W. Farquhar. Data in preparation.

TABLE 5 RATE CONSTANTS, POOL SIZES, TURNOVER DATA AND CALORIC EQUIVALENTS OF TURNOVER IN FIVE LIVER BIOPSY EXPERIMENTS

Subject*		Rate Constant of $S_f > 20$ TG (k_2)	Pool Size of $S_f > 20$ TG (b)	Rate Constant of Hepatic Precursor TG		Hepatic TG Subcompartments		Turnover Rate of $S_f > 20$ TG (p_2)	Turnover Rate Hepatic TG (q)	Caloric Turnover from Triglyceride		Liver TG as % of Wet Weight‖
				(k_1)†	(k_1)‡	(a_2)§	(a_1)			In $S_f > 20$	In Liver	
		hr^{-1}	g	hr^{-1}		g		g/hr	g/hr	cal/day		
F. C.		0.198	0.45	2.85	2.32	0.03	11.30	0.089	32.10	20	6,950	0.95
C. H.		0.233	3.83	1.71	1.23	0.52	3.52	0.820	6.02	177	1,478	0.46
G. B.	H^3	0.248	2.42	1.75	1.54	0.342	10.21	0.600	16.75	129	3,749	0.65
G. B.	C^{14}	0.172		1.71	2.17	0.244	10.31	0.416	17.65	90	3,900	
G. S.		0.465	0.53	2.10	1.26	0.272	7.96	0.246	16.70	54	3,664	1.03

* All subjects received glycerol-2-H^3 and subject G. B. also received palmitate-1-C^{14}.

Initials	Age, yr	Condition
F. C.	71	Terminal carcinoma of lung.
C. H.	33	Subsiding jaundice secondary to chlorpromazine.
G. B.	52	Arteriosclerotic heart disease.
G. S.	21	Bronchiectasis and membranous glomerulonephritis.

† Derived from *equation 5* (see Interpretations and Appendix *D*).
‡ Derived from graphic subtraction (see Interpretations and Appendix *F*).
§ a_2, a_1, q, and calculations of potential caloric equivalent of turnovers are all derived from k_1 obtained from *equation 6*.
‖ Liver wet weight assumed to be 2.43% of body weight (29).

of plasma) allow one to calculate rate constants k_1 and sizes of the *assumed* hepatic precursor pool. This may then be done without direct sampling of liver tissue in each study if one assumes an average hepatic triglyceride content of 1.02% of hepatic weight (30) and a hepatic weight of 2.43% of body weight (29). Rate constants may be calculated by two techniques (*Equation 6*, see Appendix *D*, or by the graphic procedure, Appendix *F*). Precursor pool sizes (a, a_1, or a_2) may then be calculated (Appendix *E*). These data, from two "endogenous labeling" studies, are listed in Table 4 and reveal a, a_1, and a_2 to be similar in both studies. In one case the total hepatic precursor triglyceride a was 7% smaller than the plasma triglyceride pool b, and in the other, it exceeded b 6-fold. In both experiments, $k_1 \gg k_2$.

DISCUSSION

These results indicate that glycerol-2-H^3 and glycerol-1,3-C^{14} are equivalent as precursors for newly synthesized plasma triglyceride in man. Palmitate-1-C^{14} can also be used for this purpose. Other compounds, including acetate, could be used, but all would probably result in more complex specific activity–time curves. Comparison of specific activity–time curves of triglyceride following administration of labeled free glycerol and of albumin-bound palmitate-1-C^{14} demonstrate that the use of fatty acid results in much earlier curvilinearity of plasma triglyceride specific activity–time curves (Figs. 3 and 8). It is also evident that, despite the "apparently" straight line that may be drawn through the early portions of the specific activity–time curves of palmitate-labeled triglycerides (Figs. 3, 4, and 8), the half-time, and turnover rate data derived from extrapolation of this line to t_0 are influenced by recycling.

These results are not surprising in view of other data indicative of considerable plasma free fatty acid recycling (31–34). There are several possible explanations for the greater recycling apparent in plasma triglyceride labeled with fatty acid. For example, the fate of most of the plasma glycerol is hepatic removal (35), probably by the action of glycerokinase in the liver (36). Plasma free fatty acids, however, are removed not only by the liver, but also by muscle, adipose tissue, and other organs (11, 32, 34, 37, 38). Much of this may be recycled as free fatty acid from these sites, either directly or following temporary storage as esterified lipid (32, 33), and could be one factor responsible for the greater recycling of triglyceride fatty acid. The extent of plasma triglyceride fatty acid recycling will also depend upon the proportion of total labeled fatty acid which is reesterified following intrahepatic hydrolysis of previously labeled triglyceride. Indeed, our data suggest that the major cause of plasma triglyceride fatty acid recycling results from its behavior

within a large hepatic triglyceride pool. Regardless of causes, it is clear that use of glycerol provides a more valid estimate of plasma triglyceride turnover than does free fatty acid. Labeled glycerol was accordingly used in the majority of our studies, but the observed differences do furnish a basis for contrast to the published animal and human studies where fatty acids have been used as precursors.

Direct evidence for lack of significant triglyceride recycling when glycerol is used as a precursor is derived from the studies after the infusion of endogenously labeled $S_f > 20$ lipoproteins into the donor subject (Figs. 6 and 7). If significant triglyceride recycling had occurred, disappearance from plasma of these endogenously labeled "reinfused" triglycerides would be more rapid than that observed in the initial experiment after glycerol infusion, and would not be first-order. Also, the graphic plot of this decline in activity observed in the reinfusion studies documents the virtual identity of the rate constants of "reinfused" and endogenously labeled $S_f > 20$ triglyceride. Consequently, the turnover of the plasma compartment is established as being slower than its absolute precursor pool and therefore rate-determining in a liver–plasma two-pool system. This was observed in both "reinfusion" studies despite an 8-fold variation in plasma triglyceride concentration. These concentrations were chosen to test the validity of this model over a wide range (from "normal" to "lipemic") of product pool size. Accordingly, calculation of plasma triglyceride turnover rate can be made with some confidence from endogenously labeled triglyceride without recourse to the more difficult reinfusion procedures.

Further experiments are in progress to define causes for the small degree of recycling of triglyceride glycerol apparent from the curvilinearity (in some studies) of specific activity–time curves by 24 hr (Fig. 1). This curvilinearity was most marked in plasmas containing low concentrations of $S_f > 20$ triglyceride, and accordingly containing a large proportion of phospholipid. It is possible, from our data which show greater incorporation of labeled glycerol into phospholipids at later hours (Fig. 2), to consider re-entry of glycerol released upon intra- or extrahepatic hydrolysis of phospholipids as a more important source of precursor for newly synthesized hepatic triglyceride at these later hours. The observation that H^3 appeared in plasma sterols and in fatty acids, although not in appreciable amounts until 24 hr after infusion of glycerol-2-H^3 (Table 3, Fig. 2), indicates that other sources of label for delayed recycling exist. One may compensate for recycling by resolving a data curve into its major exponents by successive graphic subtraction from a straight line drawn through the tail of the curve. Although this will certainly allow calculation of more accurate rate constants in simple systems (39), alteration of our data does not seem warranted in view of the sources of *delayed* recycling that have been discussed, and in view of the confidence in the data derived from the close similarity of rate constants of "endogenous" and "reinfused" lipid.

Although glycerol has been previously used in the rat as a precursor for plasma and liver esterified lipid (40, 41), the kinetics of lipid turnover were not studied. In most attempts to estimate liver and/or plasma triglyceride turnover rates in man and other animals, labeled fatty acids complexed to albumin were used as the precursors of these esterified lipids (20, 22, 33, 42–44). A general conclusion of these investigators was that the vast majority of newly synthesized plasma triglyceride originated in the liver.

However, an explicit model for turnover of liver and plasma triglyceride was proposed in only one study—that of Havel and co-workers in the rabbit (20). They demonstrated a precursor–product relationship between the triglyceride of all the hepatic subcellular elements and plasma $S_f > 20$ triglyceride. Despite these observations and evidence of a very rapid removal rate of triglycerides after intravenous injection of C^{14} palmitate labeled $S_f > 20$ lipoproteins, they suggested a single-pool system as best fitting their data. Our calculations from their data and from data of Laurell (33) suggest the existence in the rabbit (20) and in the rat (33) of a *two*-pool system with the precursor rate constants less than the product rate constants obtained from intravenous infusion of labeled $S_f > 20$ lipoproteins. We have recently found a similar relationship in the dog.[7] This is consistent with the liver rather than the plasma being the rate-determining compartment in a model otherwise similar to the one that we propose for man. The reason for this difference between man and the rat, rabbit, and dog is unknown. However, it may be noted that the $t_{1/2}$ of reinfused $S_f > 20$ triglycerides in man, though dependent on pool size (Table 4), is measured in hours—that of rabbit (20), rat (33), or dog[7] in minutes.

Our contention that the plasma compartment is rate-determining in man receives some support from the studies of Friedberg and co-workers (43) in which they reported incorporation of labeled palmitate into total plasma triglycerides. Since their plasma specific activity–time curves are a composite of all lipoprotein classes, their results cannot be compared directly with ours. However, in two experiments, they compared the rate constants of triglyceride specific activity–time curves obtained from an initial palmitate infusion with those derived from infusion of whole plasma containing palmitate-labeled lipoproteins. The rate constants were similar in both situations and despite the differences in

[7] R. C. Gross, and J. W. Farquhar. Unpublished data.

conditions, these results are analogous to those we obtained in our "reinfusion" studies.

Although the turnover of the plasma triglyceride pool is rate-determining in the model we have proposed for liver and plasma triglyceride turnover, it is evident from the liver biopsy experiments that the hepatic triglyceride pool is considerably larger than the plasma pool. Although further studies are required to confirm our tentative conclusions on this point, the liver biopsy studies suggest that only a very small proportion of newly synthesized hepatic triglyceride is delivered to the plasma and that most of it is delivered to an intrahepatic "sink" (see Interpretations and Table 5).

If this model is correct (Model 2, Fig. 5), the fate of this large intrahepatic triglyceride pool becomes important for several reasons. In the first place, one might expect the major determinant of glycerol or fatty acid recycling in plasma triglycerides to be the extent of their reutilization in intrahepatic resynthesis of triglyceride following turnover of this pool. The experimental observation that there is less recycling when glycerol is used as a precursor is compatible with the assumption that there is less reutilization of glycerol than of fatty acid in the reesterification of these potential precursors in the liver. Besides being consistent with our observed data, the assumption that there is less recycling of glycerol is supported by the work of several investigators who found much greater rates of reutilization of fatty acid than of glycerol. Their studies traced the incorporation of label in hepatic triglyceride following intravenous administration (23, 45) or perfusion in vitro (46) of doubly labeled triglyceride supplied as either chylomicrons or low density lipoproteins.

Secondly, and of prime physiological importance, the implication from our limited liver biopsy data is that an average of 97% of newly synthesized hepatic triglyceride turns over only in the liver, perhaps to furnish fatty acid for reesterification reactions after hydrolysis, or to serve as a source of energy, or to be returned to plasma. From our data (the five glycerol experiments of Table 5) one can calculate that the potential caloric equivalent from intrahepatic triglyceride turnover averages 3815 cal/day, with an average of only 95 potential calories (as triglyceride) secreted into plasma daily. These 3815 potential calories are a 6- to 7-fold excess of estimated hepatic caloric needs (48). Therefore one can predict that recycling as plasma free fatty acid is the fate of approximately 75% of the triglyceride fatty acid turning over within the liver. There is evidence in the rat that oxidation of fatty acids furnishes the principal fuel of the liver in the post-absorptive state (47). Much of this could come from the hepatic triglyceride fatty acid pool.

Although there are no reports of the magnitude or origin (i.e., from synthesis in situ or from plasma) of the free fatty acid efflux from the liver in man, Fredrickson and Gordon have estimated that as much as 5450 (average of 2400) potential calories, as free fatty acid, pass through the plasma daily (32). Although considerable variation is expected, dependent on nutritional state and degree of physical activity, one can estimate that about 35% of man's total energy requirements are derived from fatty acids when he is at rest and in the post-absorptive state (20, 32). Therefore, there is sufficient "excess" of plasma free fatty acid flux to explain the large magnitude of hepatic triglyceride turnover if there is continued recycling from liver triglyceride to plasma free fatty acid.

In one hyperlipemic subject (E. C.) 14% of administered free fatty acid was incorporated into plasma triglycerides (Table 2), and in this instance the estimated plasma triglyceride pool size was approximately equal to that in the liver. Since the hepatic pool turned over with a rate constant 3.3 times that of plasma, approximately 50% of the administered label was incorporated into hepatic triglyceride. This is not unexpected, since in studies in rats (19) from 30 to 60% and in rabbits (20) approximately 30% of administered palmitate-C^{14} was recovered in liver triglyceride 15 min (rats) or 2 min (rabbits) after injection. Indeed, it was apparent in these same studies that the liver was the major site of incorporation of labeled palmitate into lipid, and that, soon after injection, most of this was in triglycerides. This evidence of temporary storage of palmitate as hepatic triglyceride supports our proposal that a large hepatic triglyceride pool exists in man, and suggests that a large proportion of plasma free fatty acids enters and leaves this pool. Finally, at least as much of an administered amount of glycerol as of fatty acid is removed by the liver (35). However, from our data (Table 2) only one-eighth the amount of glycerol compared to fatty acid is incorporated into plasma triglyceride, and presumably a similar ratio applies to hepatic triglyceride. Consequently, the explanation for lesser glycerol recycling from the hepatic pool into plasma $S_f > 20$ triglyceride is most probably related to the fact that only one-eighth as much glycerol as fatty acid is reincorporated into newly synthesized triglycerides after intrahepatic hydrolysis. A paradox exists, therefore, in which one finds the "best" choice of labeled compound for entry into a unique precursor pool (the large hepatic triglyceride pool, Model 2, Fig. 5) is one which has the lowest ratio of total uptake to amount entering the pool.

Although the mechanisms controlling size and rate of turnover of this hepatic triglyceride pool remain to be determined, one can speculate that it is part of a system uniquely adapted to the liver's central role in maintenance of glucose homeostasis. Since oxidation of glucose

has been shown to be particularly low in liver (49), triglyceride fatty acid may provide an abundant substrate for hepatic oxidative metabolism and consequently spare carbohydrate for export. Further investigations will be needed to study this and other issues; nevertheless the size and turnover rate of this large hepatic pool and particularly the proportion of hepatic triglyceride selected for transfer into plasma all become interesting questions for study.

Our current studies (4, 5) have defined dietary-induced variations in hepatic triglyceride secretion rates in man and we are pursuing the causes of these effects. It will be of particular interest in these studies to determine the interdependence of rates of hepatic triglyceride synthesis and secretion.

APPENDICES

Appendix A

Method of calculation from data of "reinfusion" studies of volume of distribution of plasma $S_f > 20$ TG (volume of pool *B*) and of amount of TG (*b*) in pool *B*.
Data of "reinfusion" studies appear in Figs. 6 and 7.

Equation 1: Volume of distribution (ml) of $S_f > 20$ TG at $t_0 = \frac{\text{SA of infused } S_f > 20 \text{ TG}}{SA_{(y)}/\text{ml plasma at } t_0}$, where $SA_{(y)}$ is the specific activity of the $S_f > 20$ TG and its value at t_0 is obtained by extrapolation from semilogarithmic plots of the major linear portion of the $SA_{(y)}$–time curve to t_0.

Equation 2: $b = \left(\frac{\text{grams } S_f > 20 \text{ TG}}{\text{unit volume plasma}}\right)\left(\text{vol. dist. (ml) infused } S_f > 20 \text{ TG at } t_0\right)$.

Appendix B

Graphic method of calculation of product rate constants (k_2) of the $S_f > 20$ TG in the product pool (Pool *B*). Extrapolate the linear portion of $SA_{(y)}$ to t_0 on semilogarithmic paper. Half-time ($t_{1/2}$) is then obtained directly and k_2 is calculated from *equation 3:* $k_2 = 0.693/t_{1/2}$. (Derivation, p. 1298 of ref. 50.)

Appendix C

Method of calculation of turnover rates of product (plasma $S_f > 20$ TG) from the product pool size and rate constant.

Since product pool size *b* is in grams, and the product rate constant k_2 has units of 1/hr, a turnover rate t_r of g TG per hr is obtained simply from *equation 4:* $t_r = bk_2$.

In *equation 4* one may use a k_2 derived either from the $SA_{(y)}$ of prelabeled lipoprotein triglyceride (a "reinfusion" study) or from the $SA_{(y)}$ derived from a glycerol injection (an "endogenous labeling" study). In either case the method of calculation of Appendix *B* is used.

Appendix D

Calculations from $SA_{(y)}$–time curves of "endogenous labeling" studies of the rate constant (k_1) and pool size in grams, *a*, of the assumed precursor (pool *A*) of Model 1 (Fig. 5).

Equation 5: $ak_1 = bk_2$.

Equation 5 requires these assumptions: (a) *b* is known [from *equation 2* substituting an *assumed* plasma volume of 4.5% of body weight (27)], (b) pool *A* contains only *a* and therefore, p_1 (turnover rate of pool *A*) = p_2 (turnover rate of pool *B*), (c) there is no recycling (none of *B* returns to *A*), and (d) *all* of *A* goes to *B* and *all* of *B* is derived from *A* [*A* and *B* are completely coupled, i.e., *A* is an *absolute* precursor of *B* (26)].

The calculations of *a* and k_1 are obtained as follows: given the assumptions of $ak_1 = bk_2$, and of either $k_1 > k_2$ or $k_2 < k_1$ but not with $k_1 = k_2$, then:

Equation 6: $\ln \frac{k_1}{k_2} = (k_1 - k_2)\, t_{max}$ [from Zilversmit, equation 16 (51)].

Since t_{max} is known from the peak of $SA_{(y)}$ obtained from "endogenous labeling" studies, and k_2 is known from *equation 3*, then k_1 is calculated by trial and error substitution. Since *b* is known, then *a* (for model 1) is obtained directly from *equation 5*.

Appendix E

Derivation of terms and methods of calculation of components of Model 2 of Fig. 5.

This model assumes $p_1 = q + p_2$ = total flow (g/hr) of newly synthesized hepatic triglyceride into the hepatic pool *A*, with q = intrahepatic removal and p_2 = flow into plasma (pool *B*). Now, although *A* is no longer completely coupled to *B*, it is yet the unique precursor. In this model, the classical criteria of Zilversmit and co-workers of "crossover" of precursor–product specific activities at the t_{max} of the product (24) for a precursor–product relationship remain valid (26). Accordingly, k_1 is still calculated directly from *equation 6*. In this model (Fig. 5) since $q + p_2$ = total flow (g/hr) out of *A*, then $k_1 = (q + p_2)/a$.

But one must yet calculate a_1, a_2, and a_3. It was defined (Model 2, Fig. 5) that:

Equation 7: $a = a_1 + a_2$, where a_1 = the proportion of *a* diverting its product into the intrahepatic "sink" and a_2 = the proportion of *a* entering plasma. From liver biopsy data one can calculate a_1 and a_2 as follows: in Model 2 (Fig. 5) $ak_1 \neq bk_2$, and since a_2 is the absolute precursor of *b*,

Equation 8: $a_2k_1 = bk_2$.

Equation 8 can be solved for a_2 using the known *b*, k_1, and k_2. One can estimate total hepatic TG as follows: if the average liver weight is assumed to be 2.43% of body weight (29) the total hepatic triglyceride is calculated from the measured triglyceride concentration per wet weight of the liver biopsy specimen. If all of the liver triglyceride were in compartment *a* (i.e., if a_3 were negligible, see Model 2, Fig. 5) the total hepatic triglyceride specific activity should theoretically equal $SA_{(y)}$ max at t_{max}. If time of biopsy (t_{biopsy}) is slightly before or slightly after t_{max} of $SA_{(y)}$, the calculated total liver TG may still equal *a* if the SA of liver TG coincides with a point on the

calculated slope from k_1 (*equation 6*) at t_{biopsy}, since this slope represents the precursor specific activity $SA_{(y)}$. Therefore, since a_2 was calculated from *equation 8* and a is known, then a_1 can be calculated from *equation 7*.

Solution of a, a_1, and a_2 for any liver biopsy TG specific activity less than observed $SA_{(y)max}$ or less than $SA_{(x)}$ of the slope k_1 at t_{biopsy} (if t_{biopsy} is after t_{max}) is also possible as follows: assuming A is the unique precursor of B as stated, from the criteria for definition of an immediate precursor of Zilversmit and co-workers (24):

Equation 9: $SA_{(x)} > SA_{(y)}$ before t_{max} but $SA_{(x)} = SA_{(y)max}$ and $SA_{(x)} < SA_{(y)}$ after t_{max}.

Of course, if $SA_{(x)}$ intersects $SA_{(y)}$ prior to t_{max}, and is therefore $< SA_{(y)max}$, one has not identified an absolute or unique precursor of B unless one assumes dilution of a (total grams of TG in A) by another pool of relatively inert triglyceride in the liver (a_3). Calculation of the total grams of liver triglyceride in a is then:

$$\textit{Equation 10: } a = \frac{\text{SA of total liver TG at } t_{max}}{SA_{(y)max}} \times \text{total liver TG}$$

or, if t_{biopsy} is later than t_{max}, then:

$$\textit{Equation 11: } a = \left(\frac{\text{SA of total liver TG at } t_{biopsy}}{SA_{(x)} \text{ at } t_{biopsy}}\right) \times \text{total liver TG.}$$

Finally, to calculate a_3:

Equation 12: $a_3 = \text{total liver TG} - a$.

Appendix F

Method of calculation of precursor rate constant (k_1) by graphic method.

A second method (in addition to that presented in *equation 6*) of calculation of k_1 is as follows: "reinfusion" experiments (Procedure 3-B) give k_2 directly since the labeled $S_f > 20$ triglycerides disappear exponentially (in a space approximating the plasma volume) with an observed $SA_{(y)}$ virtually identical to the slope obtained from extrapolation to t_0 of $SA_{(y)}$ obtained in the preceding free labeled glycerol study in the same subject (Procedure 3-A)—(see Results). The identical or closely similar rate constants establish $k_2 < k_1$ and establish B as the rate-determining compartment (25) and allow use of labeled glycerol infusion studies (Procedure 3-A) to determine k_2 from the slope of the $SA_{(y)}$–time curve (see Discussion). In these glycerol infusion studies, a complete curve, including an ascending limb is obtained (Figs. 1, 3, 6, 7, and 8). Subtraction of the early points of $SA_{(y)}$ from the slope resulting from extrapolation of $SA_{(y)}$ to t_0 will result in a slope representing k_1 (51). The two methods of obtaining k_1 therefore allow independent calculations of this precursor rate constant and can be used in establishing the validity of the two models presented in Fig. 5. This is of value in analysis of liver biopsy studies (Fig. 8) since the biopsies were done at times either at t_{max} or after t_{max}, necessitating plotting of hepatic TG SA in reference to a calculated k_1 in four of the five studies.

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Note Added in Proof:

Baker and Schotz (*J. Lipid Res.* **5**: 188, 1964) have recently suggested a very different and more complex model for liver and plasma triglyceride turnover in the rat than the model we herein propose for man. Major differences include the following. In man 5%, in the rat 96% of newly synthesized hepatic triglyceride is secreted into plasma; in man none, in the rat 33%, of plasma triglyceride recycles through the liver to plasma without intervening hydrolysis. It is not yet clear if differences are due to assumptions in model building, methods, or species. Our preliminary findings[7] suggest that the dog more closely resembles man in the two parameters mentioned above. In any event, wide variations in triglyceride metabolism between species may be expected.

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