

Endogenous triglyceride turnover in liver and plasma of the dog

RICHARD C. GROSS,* EDWARD H. EIGENBRODT,† and JOHN W. FARQUHAR

Departments of Medicine and Surgery, Stanford University School of Medicine, Palo Alto, California 94304

ABSTRACT Radioactive glycerol and $S_f > 20$ lipoproteins labeled with it were used to study turnover of plasma $S_f > 20$ and hepatic triglyceride in anesthetized dogs. From specific activity-time curves of these lipids after an injection of labeled material, a tentative and incomplete model for the kinetics of endogenous hepatic and plasma triglyceride was defined and partially validated. Pool sizes and turnover rates of triglyceride in liver and $S_f > 20$ lipoproteins of plasma were then calculated in seven dogs.

Hepatic triglyceride was composed of two compartments: 60% metabolically inert and 40% metabolically active. Although communication between these hepatic compartments surely occurred during the time course of these studies, it was not sufficient to be detected by our present methods. The metabolically active compartment turned over as a single pool but with two destinations: a quite variable proportion (an average of 61%) was secreted into plasma as $S_f > 20$ triglyceride, and an average of 39% was presumably hydrolyzed within the liver.

The fractional turnover rate of plasma $S_f > 20$ triglyceride was 2-3 times that of hepatic triglyceride. This finding, and the parallel decline of specific activities of plasma $S_f > 20$ and liver triglyceride after injection of labeled glycerol, confirm the rate-determining role of hepatic triglyceride. In this respect the dog differs importantly from man. Though turnover rates of plasma $S_f > 20$ triglyceride fell in the same range in men and dogs, the relationship of turnover rate to plasma concentration of this lipid differed greatly between them. The model for the dog does resemble that previously reported for man, however, in the lack of major recycling of intact plasma triglyceride between the liver and plasma. Lack of such recycling, however, does not exclude return of plasma triglyceride into a hepatic triglyceride sink. The amount of such unidirectional uptake, if any, could not be determined by these techniques.

KEY WORDS triglyceride · turnover · dog · radioactive glycerol · kinetic model · rate-determining compartment · liver · plasma · $S_f > 20$ lipoprotein · precursor-product relationship

THE ROLE OF endogenous hepatic and plasma triglyceride in the metabolism and transport of fat has been investigated in several species, including man, in many laboratories. Historically, the function of plasma triglyceride in the transport of esterified fatty acids was established early (1-4) and more recently the liver was found to be virtually the sole source of endogenous plasma triglyceride (2, 5-7). Moreover, Havel and his coworkers showed that hepatic triglyceride is delivered to plasma primarily in the very low density lipoprotein or $S_f > 20$ fraction of plasma (8-10).

Several authors have depicted the routes of fat transport between liver and adipose tissue and other tissues, and the subject has been extensively reviewed (11-13). Also, a few attempts have been made to quantify rates of triglyceride transfer between body compartments (9, 14-17), and recently Baker and coworkers devised a multicompartmental model to calculate rates of triglyceride turnover in liver and plasma of the rat under various conditions (18, 19).

In this laboratory a model for the kinetics of endogenous triglyceride metabolism in man was partially validated by use of intravenous infusion of prelabeled $S_f > 20$ triglyceride and this model was used to calculate turnover rates of this lipid in plasma and liver (20). These studies employed radioactively labeled glycerol as a precursor of endogenous triglyceride, in distinction from previous work which used labeled fatty acids. These methods were used to study hypertriglyceridemia in man, in particular the variety termed "carbohydrate-induced" hypertriglyceridemia by Ahrens and his associates (21) and it was shown that the elevated plasma triglyceride concentrations in this condition are the result of increased secretion of $S_f > 20$ triglyceride

* Present address: King Gustaf V Research Institute, Stockholm, Sweden.

† Present address: Department of Pathology, University of Texas, Southwestern Medical School, Dallas, Tex.

into plasma by the liver (22, 23). From calculations based on this model we also defined the relationship between input rates of newly synthesized triglyceride into plasma and the concentration of this triglyceride in plasma over a wide range of values in man, and made deductions therefrom regarding possible mechanisms of removal of plasma triglyceride in this species (24).

To study further the regulation of hepatic triglyceride synthesis and its subsequent secretion into plasma as $S_f > 20$ triglyceride, we turned to an experimental animal, the dog. This allowed serial sampling of both liver and plasma triglyceride compartments and permitted closer scrutiny of the dynamics of hepatic triglyceride. In these studies, as in those previously reported, radioactive glycerol was used to label newly synthesized triglyceride in liver and plasma.

In this report we propose a tentative model, consistent with our data, which includes two hepatic pools and a single plasma pool for the metabolism of newly synthesized triglyceride in liver and plasma of the dog. The model is similar to, but is also different in many respects from, that described in man (20).

MATERIALS AND METHODS

Experimental Animals and Procedures

Mongrel dogs of both sexes weighing 16–22 kg were kept in individual cages and fed once a day with Purina Dog Chow (Ralston Purina Co., St. Louis, Mo.) mixed with a small amount of bacon grease and cod liver oil. We reduced fat intake by one of two methods for 3 days before the studies, to decrease the contribution of dietary fat to plasma triglyceride. In experiments 1–3, dry chow alone was fed during this time, which provided approximately 29% of calories as protein, 45% as carbohydrate, and 26% as fat. In experiments 4–7, we reduced dietary fat still further by feeding a slurry of dried skim milk, matzo meal, and water in the amount of 75 cal/kg of body weight per day. This slurry contained 18% of calories as protein, 80% as carbohydrate, and 2% as fat. All animals maintained constant weight or gained weight slightly prior to each study. Food was removed from cages 12 hr before experiments.

Animals were anesthetized with intravenous sodium pentobarbital, 25 mg/kg of body weight, and 60–180 mg more was given as needed during the experiment. The trachea was intubated and good spontaneous respiration was maintained. A 10% glucose solution was infused in a hind leg vein at a rate of 7 mg/min per kg of body weight during each study and plasma glucose concentrations were maintained to within $\pm 10\%$ of the mean value. (We infused glucose to maintain positive glucose balance in the liver and to enable these studies to serve as controls for further work in which insulin

will be given in addition to glucose.) The abdomen was opened and a cannula was inserted into the porta hepatis via a mesenteric vein. This was kept open with a slow infusion of normal saline. Although cannulation was unnecessary for these studies, the procedure was a necessary control for planned future experiments. This initial preparation required about 1 hr; a 2nd hr followed without intraabdominal manipulation. Isotopically labeled materials (see below) were then injected into a peripheral vein; blood samples were taken and liver samples were resected from the liver edge at various intervals for the next 3–5 hr.

Blood samples, (8–14 in number, each of 15–20 ml) were drawn by venipuncture from the external jugular veins into syringes moistened with heparin (Liquamin Sodium, 1000 units/ml, The Upjohn Co., Kalamazoo, Mich.). From four to six biopsies, about 1 g each, were taken from the liver edge from widely spaced sites at intervals of 20–60 min. Blood for determination of glucose concentration was drawn hourly.

Seven experiments were done; in experiments 1, 3, 5, 6, and 7, glycerol-2- ^3H , 1 mc/ml, 3.05 mg/ml in sterile water (New England Nuclear Corp., Boston, Mass.) was used as the labeled substrate for incorporation into triglycerides of liver and plasma. Just before injection, labeled glycerol (100 μc /kg of body weight) was drawn into a syringe and diluted to 5 ml with sterile isotonic saline.

In experiment 1, 200 μc of palmitic acid-1- ^{14}C , specific activity 12.8 mc/mmmole (Volk Radiochemical Co., Skokie, Ill.) was injected simultaneously with tritiated glycerol to compare triglyceride turnover by measurement of each labeled substrate. The palmitic acid-1- ^{14}C was prepared as a complex with albumin before injection by neutralization with ethanolic 0.1 N NaOH, drying, and then mixing with 5 ml of human serum albumin (The Cutter Laboratories, Berkeley, Calif.).

In experiment 2, 50 μc of glycerol-1,3- ^{14}C in 10% isopropanol (New England Nuclear Corp.) was injected as a substrate for endogenous triglyceride synthesis. After 60 min, homologous $S_f > 20$ lipoproteins labeled with glycerol-2- ^3H and containing 9.2 μmoles of $S_f > 20$ triglyceride were injected intravenously into the same animal for measurement of the turnover of their triglycerides in plasma, and direct comparison of this with the turnover of the ^{14}C -labeled, endogenously synthesized, plasma $S_f > 20$ triglyceride.

The labeled lipoproteins were prepared as follows: a donor dog was placed on chow for 3 days and was without food for 14 hr just before the procedure. The dog was anesthetized with sodium pentobarbital and then given 4 mc of glycerol-2- ^3H intravenously. Five 25-ml samples of venous blood were drawn into heparinized syringes at intervals of 10 min beginning 30 min after

the injection. Plasma was rapidly separated from cells at 4°C and $S_f > 20$ lipoproteins were isolated under sterile conditions by methods previously described (20, 24). These labeled lipoproteins were aspirated from the top of the spun plasma, brought to a volume of 50 ml with sterile 0.9% saline, and stored at 4°C for 4 days before use. Before intravenous injection into the recipient, lipoproteins were warmed at 37°C for 30 min. Portions of the lipoprotein solution were retained for later measurement of radioactivity and triglyceride content.

In experiment 4, glycerol-1,3-¹⁴C (400 μ c) was injected intravenously as a label for endogenous liver and plasma triglycerides. At the same time, 200 ml of labeled whole blood from a donor dog was rapidly infused. The donor was prepared as follows: after being fed the low fat (2% of calories) diet for 3 days and after a 14 hr fast, the donor dog was anesthetized and 7 mc of glycerol-2-³H was injected intravenously. 70 min later 200 ml of blood was drawn rapidly into siliconized syringes and infused immediately into the recipient, from whom 100 ml of blood had just been removed. This maneuver took 5 min. In this experiment, ¹²⁵I-labeled, delipidized, homologous high density lipoprotein¹ was injected simultaneously as part of a separate study, but also to estimate the recipient's plasma volume.

Analytical Methods

Plasma was separated at 4°C and $S_f > 20$ lipoproteins were obtained by ultracentrifugation (20, 24). These lipoproteins were frozen at -20°C until extracted, or lyophilized at 60°C in a Virtis lyophilizer (Model 10-145MR-SA, Virtis Co. Inc., Gardiner, N.Y.) to reduce the volume of solvent required for extraction of lipids. These extracts were stored at -20°C.

Liver biopsies were immediately placed on ice and later blotted dry and weighed, and the total volume of sample was brought to 1.5 ml with 0.9% saline containing 5 mg of EDTA per 100 ml. The tissue was homogenized with 30 ml of chloroform-methanol 2:1 in glass homogenizers or in a Virtis "45" homogenizer (Virtis Co. Inc.).

Total lipids of plasma $S_f > 20$ lipoproteins and liver were then extracted by the method of Folch, Lees, and Sloane Stanley (25). Triglycerides were isolated by thin-layer chromatography (20), and triglyceride content was measured, in experiments 1-3, by the method of Stern and Shapiro (26). In experiments 4-7 the semi-automated procedure of Lofland (27) for the Autoanalyzer (Technicon Co., Chauncey, N.Y.) was used with the following modifications: since the triglyceride had already been isolated by thin-layer chromatography, initial removal of phospholipids by zeolite was not neces-

sary. The aliquot for colorimetric determination was dried under nitrogen, then saponified with 1 ml of 4% ethanolic KOH at 65°C for 20 min. Ethanol was evaporated and either 2.0 or 3.0 ml of 0.2 N sulfuric acid was added. The volume of acid was adjusted to the estimated amount of sample triglyceride, so that the absorption remained within the colorimeter's optimal range. The Autoanalyzer manifold (27) was also changed in that "DO" fittings were substituted for "DI" at points where pressure and subsequent "bumping" were noted. Also the line carrying 0.2 N sulfuric acid was removed from the steam and redirected into the rinse reservoir of Sampler II. Recovery of 0.8-2.0 μ eq of triolein in these experiments was 90-100%.

Content of ¹⁴C and ³H in triglycerides was determined as previously described (20). No quenching was noted. Activity of plasma ¹²⁵I in experiment 4 was measured in a gamma well counter (Packard Auto-Gamma Spectrometer, Series 410A).

Plasma glucose was determined by a specific enzymatic method (28) in earlier experiments and by Autoanalyzer (29) in later experiments.

RESULTS

Comparison of Glycerol and Fatty Acid in Hepatic and Plasma Triglyceride Metabolism

Fig. 1 shows a study in which glycerol-2-³H and palmitate-1-¹⁴C were used simultaneously as substrates for incorporation into liver and plasma triglyceride. Maximum specific activity of plasma $S_f > 20$ triglyceride occurred at the same time and decline of liver triglyceride and of plasma $S_f > 20$ triglyceride specific activities remained first order after injection of both labeled substrates. The slopes for decline of specific activity (calculated by the method of least squares by use of a digital computer program) were steeper after labeled glycerol. These values (hr^{-1}) were: liver -0.40 ± 0.036 (SE) versus -0.31 ± 0.008 ; plasma -0.43 ± 0.009 versus -0.34 ± 0.006 . The differences between these slopes are highly significant for plasma ($P < 0.01$) and somewhat less so for liver ($P < 0.05$). Similar differences in kinetics of glycerol- and fatty acid-labeled triglyceride were noted in human subjects in this laboratory (20). The implications of these data with respect to the use of labeled glycerol and fatty acids as precursors in studies of triglyceride kinetics have been discussed (20) and provided the initial basis for choice of glycerol as the preferred substrate in subsequent experiments.

Calculation of Pool Sizes of Total Liver Triglyceride and of Plasma $S_f > 20$ Triglyceride

Concentrations and estimated pool sizes of total liver triglyceride and of plasma $S_f > 20$ triglyceride ap-

¹ Prepared through the kindness of Dr. H. S. Sodhi.

TABLE 1 CONCENTRATION AND POOL SIZE OF LIVER TRIGLYCERIDE AND PLASMA $S_f > 20$ TRIGLYCERIDE

Expt. No.	Dog Wt.	Liver Total Triglyceride		Plasma $S_f > 20$ Triglyceride	
		Concentration*	Pool Size†	Concentration‡	Pool Size§
	kg	$\mu\text{moles/g liver}$	$\mu\text{moles/kg body wt}$	$\mu\text{moles/100 ml}$	$\mu\text{moles/kg body wt}$
1	22.5	1.87 ± 0.09	63.2 ± 3.04	8.4 ± 0.4	4.20 ± 0.2
2	20.2	2.88 ± 0.10	97.4 ± 3.39	12.3 ± 0.8	6.72 ± 0.4
3	15.9	1.80 ± 0.15	62.2 ± 5.05	11.6 ± 2.2	5.80 ± 1.1
4	20.0	3.90 ± 0.23	132.0 ± 7.80	14.0 ± 0.6	8.48 ± 0.38
5	20.0	2.76 ± 0.07	93.3 ± 2.38	14.1 ± 0.6	7.05 ± 0.30
6	27.3	3.58 ± 0.20	121.0 ± 6.80	30.9 ± 0.9	15.45 ± 0.95
7	19.1	3.69 ± 0.12	125.0 ± 4.06	14.9 ± 0.8	7.45 ± 0.4
Mean		2.93	99.2	15.2	7.80

* Mean triglyceride concentration of the 4 to 5 liver biopsies taken during study.

† Based on liver weight estimated as 3.38% of body weight (30).

‡ Mean $S_f > 20$ triglyceride concentration of the 5 to 8 plasma samples taken during first 2 hr of each study.

§ Based on plasma volume estimated as 5% of body weight (31), except experiments 2 and 5, in which volume of distribution of pre-labeled lipoproteins was used (see text).

|| Standard error of the mean. The number of values were as given above.

TABLE 2 SOME CHARACTERISTICS OF RADIOACTIVE GLYCEROL-LABELED TRIGLYCERIDE METABOLISM IN LIVER AND PLASMA OF DOGS

Expt. No.	Slope* of Liver Triglyceride Specific Activity-Time Curve	Delay in Appearance of ^3H in Plasma $S_f > 20$ Triglyceride after t_0	t_{max} of ^3H in Plasma $S_f > 20$ Triglyceride	Slope* of Declining Part of Plasma $S_f > 20$ Triglyceride Specific Activity-Time Curve	P Values for Difference of Slopes of Liver and Plasma Triglyceride
1	-0.40 ± 0.04 †	10	76	-0.43 ± 0.01	>0.60
2	-0.34 ± 0.09	7	67	-0.44 ± 0.07	>0.50
3	-0.81 ± 0.08	8	64	-0.69 ± 0.04	>0.20
4	-0.41 ± 0.05	10	102	-0.43 ± 0.002	>0.80
5	-0.68 ± 0.04	10	74	-0.42 ± 0.03	>0.05
6	-0.63 ± 0.07	10	68	-0.58 ± 0.03	>0.60
7	-0.72 ± 0.05	10	73	-0.69 ± 0.002	>0.80

* Fractional turnover rates were obtained as the slopes calculated from the plot of log specific activity vs. time.

† Standard deviation of the slope.

pear in Table 1. The mean concentration of the four or five biopsies taken during each study was multiplied by liver weight [estimated as 3.38% of body weight (30)] to give an estimate of total hepatic triglyceride. The pool size for plasma $S_f > 20$ triglycerides was calculated by multiplying mean plasma concentration by plasma volume. Plasma volume was estimated as 5.0% of body weight (31) in experiments 1, 3, 5, and 7, or calculated from the volumes of distribution either of pre-labeled $S_f > 20$ lipoprotein (experiment 2) or of pre-labeled delipidized high density lipoprotein (experiment 4).

The small standard errors (Table 1) of hepatic and plasma triglyceride concentrations (and pool sizes) determined at wide intervals during each experiment indicate that these triglyceride concentrations remained fairly constant during the experiments. Only in experiment 3 was the standard error appreciably greater than 5% of the mean values (Table 1). Therefore the requirement of a steady state of pool size appears to have been met reasonably well.

Incorporation of Labeled Glycerol into Liver Triglyceride
Specific activities of liver triglyceride in seven dogs after injection of labeled glycerol are plotted against time in Fig. 2. The decline of these specific activities appeared reasonably first order during the span of time liver biopsies were taken, from as early as 9 min to as long as 3 hr after administration of the isotope (Figs. 1 and 2). The slopes of these specific activity-time plots ranged from -0.342 to -0.811 hr^{-1} (Table 2).

Incorporation of Labeled Glycerol into Plasma $S_f > 20$ Triglyceride

Radioactivity began to appear in plasma $S_f > 20$ triglyceride about 7-10 min after injection of the isotope (Table 2). The amount of delay in appearance was estimated by plotting the curves of specific activity versus time on linear coordinates and extrapolating to zero specific activity. A delay of closely similar magnitude was also found in the rabbit (9) and the rat (18) in studies that used radioactive palmitate as a labeled

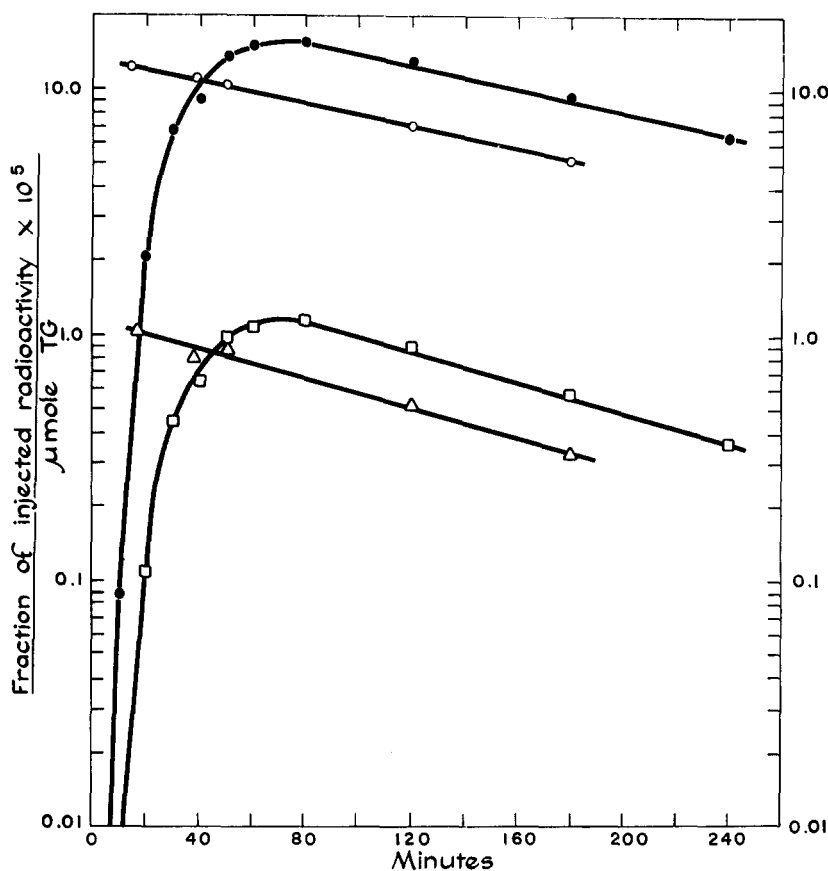


FIG. 1. Specific activity-time curves of plasma $S_f > 20$ triglyceride and hepatic triglyceride after simultaneous injection of glycerol-2- ^3H (lower pair) and palmitic acid-1- ^{14}C (upper pair) in a single dog (Expt. 1). Symbols: \square , plasma glycerol; Δ , liver glycerol; \bullet , plasma palmitic acid; \circ , liver palmitic acid. Specific activity here and in Figs. 2 and 3 is expressed as a fraction of injected radioactivity per μmole of triglyceride, i.e., $(\text{dpm in sample triglyceride} \times 10^5) \div (\mu\text{moles of triglyceride in sample} \times \text{total dpm injected at } t_0)$.

precursor. After the initial delay, specific activity rose rapidly, exceeding the corresponding liver triglyceride specific activity at 40 min and reaching a maximum 64–102 min (t_{max}) after injection of the isotope (Fig. 3). t_{max} occurred between 64 and 76 min in every experiment except number 4 (Table 2). Although t_{max} was not precisely defined by experimental points on the curve (which were 20 min apart during that portion of the experiments) it is clear from inspection of Fig. 3 that it can be estimated within a range of ± 5 min. After t_{max} , plasma specific activity declined in what appeared to be a first-order manner for the duration of the experiments, up to 4 hr after administration of the isotope (Fig. 3). Slopes of regression lines for decline of specific activity versus time ranged from -0.42 to -0.69 hr^{-1} (Table 2).

Interrelationships between Liver and Plasma Triglyceride Specific Activities

Since radioactivity appears in plasma only after a delay and then increases rapidly during a period of decline in specific activity of liver triglyceride (Figs. 1–3), a pre-

cursor-product relationship of hepatic and plasma triglyceride is possible (32–35). Also, since both liver triglyceride and plasma $S_f > 20$ triglyceride declined in a first-order manner (Figs. 1–3), a two-compartment model without recycling is suggested (34). However, if *all* hepatic triglyceride were a single precursor pool for the plasma $S_f > 20$ triglyceride compartment, the ratio of specific activities of the hepatic and plasma pools would be unity at the time of the maximum specific activity of the plasma $S_f > 20$ triglyceride pool (32–35). Since the system here includes a delay in secretion of precursor into the product pool, the t_{max} chosen for comparison of precursor and product specific activities must correct for these delays (listed in Table 2). However, these ratios, when calculated in this manner, are less than 1 (Table 3), which implies the presence of unlabeled triglyceride in the liver that is not an immediate precursor of the plasma triglyceride pool. This unlabeled lipid must then dilute the specific activity of the hepatic triglyceride compartment that is the precursor of plasma $S_f > 20$ triglyceride. Thus, the relative sizes of these two hepatic compartments can be derived from the ratio

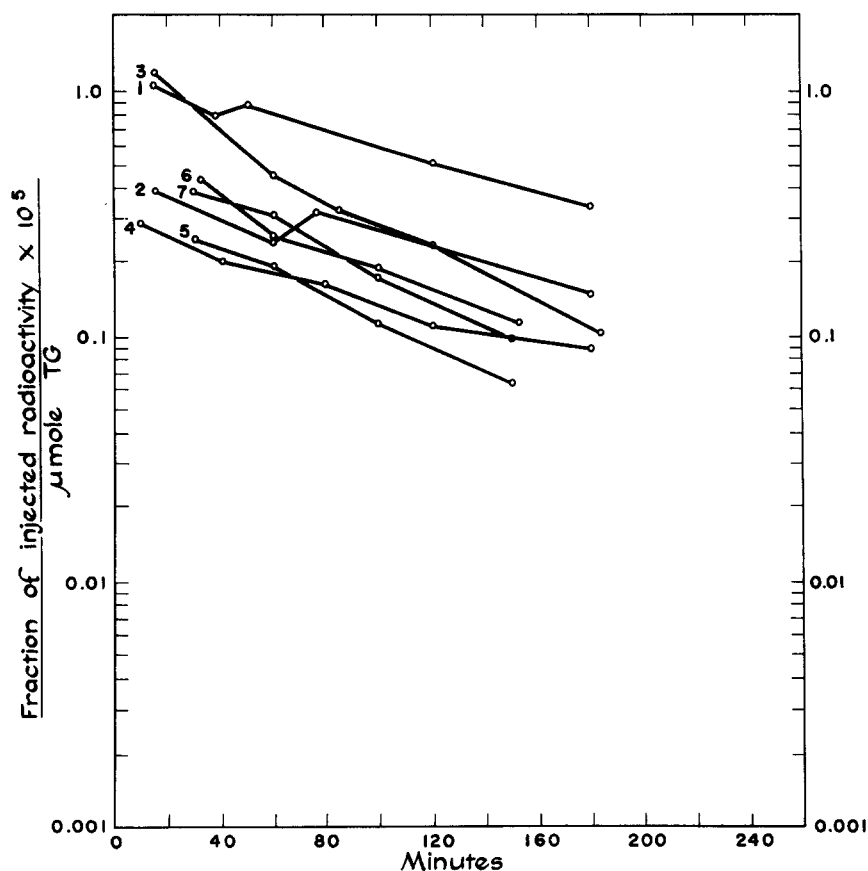


FIG. 2. Specific activity-time curves of liver triglyceride after injection of radioactive glycerol in seven experiments in seven dogs. Liver biopsies for determination of triglyceride specific activity were obtained concurrently with blood samples for determination of plasma $S_f > 20$ triglyceride specific activity (Fig. 3).

of the two specific activities at t_{max} . The size of the precursor pool of liver triglyceride was calculated by multiplying total hepatic triglyceride content by this ratio, i.e., [specific activity of liver triglyceride at (t_{max} minus plasma triglyceride delay time)] \div (specific activity of plasma $S_f > 20$ triglyceride at t_{max}). The remainder of total hepatic triglyceride was considered to constitute the inert pool. A similar calculation was made previously in man (20) and the derivation of this may be seen in Appendix E of that publication.

Pool sizes of the precursor pool of hepatic triglyceride varied somewhat less than did that of total liver triglyceride, ranging from 28.0 to 48.9 μ moles/kg of body weight compared to a range of 61.5 to 132 μ moles/kg for total triglyceride (Table 1). In four of seven experiments this precursor pool contained 28.0–36.9 μ moles/kg (Table 3). Thus, the size of this pool may remain relatively constant, and changes in the size of the inert pool would account for the wider variation of total liver triglyceride.

The declining portions of the plasma and liver specific activity curves are virtually parallel, as seen in Figs. 1–3 and from the similarity of their slopes in Table 2. This im-

plies that the fractional rate of turnover of hepatic triglyceride governs the rate of disappearance of radioactivity from the plasma $S_f > 20$ triglyceride compartment (i.e., hepatic triglyceride is the rate-determining compartment) or, less likely, that the fractional turnover rates of the two triglyceride compartments are equal (34).

Infusion of Labeled Lipoproteins

The specific activities of plasma $S_f > 20$ triglyceride following rapid injection of prelabeled homologous $S_f > 20$ lipoprotein (experiment 2) or of prelabeled whole blood (experiment 4) are shown in Fig. 4. We performed these studies to partially validate the proposed model for liver and plasma triglyceride kinetics by furnishing experimentally derived values for turnover of plasma $S_f > 20$ triglyceride for comparison with those calculated from the model. The volume of distribution of prelabeled $S_f > 20$ lipoprotein was 5.46% of body weight in experiment 2, a figure very close to the expected value for plasma volume of 5.0% of body weight (31). The volume of distribution of the labeled whole blood was not determined, but the volume of distribution of 125 I-labeled

TABLE 3 CALCULATIONS OF HEPATIC PRECURSOR TRIGLYCERIDE COMPARTMENT

Expt. No.	Ratio		Pool Size† of Liver Precursor Triglyceride
	SA liver TG*	SA S _f > 20 TG† at t _{max}	
1	0.60		μmoles/kg body wt
2	0.37		37.9 ± 1.8‡
3	0.45		36.0 ± 1.3
4	0.25		28.0 ± 2.3
5	0.36		33.0 ± 1.9
6	0.40		33.6 ± 0.9
7	0.36		48.4 ± 2.7
7	0.36		45.0 ± 1.5
Mean	0.40		37.4

* Specific activity of total liver triglyceride corrected for hepatic secretion delay time.

† Specific activity of plasma S_f > 20 triglyceride.

‡ Standard error of the mean. The data is derived from the 4 to 5 liver biopsies taken during each experiment.

delipidized homologous high density lipoprotein injected at the same time was 6.06% of body weight.

The decline of specific activity of plasma S_f > 2 triglyceride remained essentially first order during the time of both studies (Fig. 4) and slopes of disappearance were much steeper [-1.72 and -0.99 (Table 4)] than those of plasma S_f > 20 triglyceride specific activity following the simultaneous injection of labeled glycerol [-0.44 and -0.43 (Table 2)]. These data are needed for the calculation of fractional turnover rates and turnover rates of triglyceride of the various compartments (see below).

Calculation of Fractional Turnover Rates of Liver Triglyceride

If specific activity declines in a first-order fashion, the fractional turnover rates of metabolically active liver

TABLE 4 FRACTIONAL TURNOVER RATE OF PLASMA S_f > 20 TRIGLYCERIDE (k₂) CALCULATED FROM SPECIFIC ACTIVITY-TIME CURVES OF PLASMA S_f > 20 AND HEPATIC TRIGLYCERIDE AFTER LABELED GLYCEROL, AND FROM PLASMA S_f > 20 TRIGLYCERIDE CURVES AFTER PRELABELED S_f > 20 LIPOPROTEIN

Expt. No.	k ₂		
	(Calculation 1)*	(Calculation 2)†	(Calculation 3)‡
	hr ⁻¹	hr ⁻¹	hr ⁻¹
1	1.65 ± 0.13§	1.73 ± 0.21§	
2	1.90 ± 0.33	2.21 ± 0.52	1.72 ± 0.15
3	1.58 ± 0.20	1.39 ± 0.23	
4	0.94 ± 0.05	0.98 ± 0.13	0.99 ± 0.05
5	1.76 ± 0.20	1.25 ± 0.14	
6	1.67 ± 0.18	1.60 ± 0.26	
7	1.27 ± 0.10	1.24 ± 0.16	

* Calculation 1, based upon t_{max} of plasma S_f > 20 triglyceride and assumption that slope of plasma S_f > 20 triglyceride = slope of the liver triglyceride precursor compartment = k₁; relationship between k₂, k₁, and t_{max} is given in text (equation 1).

† Calculation 2, based upon t_{max} of plasma S_f > 20 triglyceride and assumption that observed slope of liver triglyceride equals that of the liver precursor compartment = k₁; k₂ was then calculated using equation 1 (see text).

‡ Calculation 3. k₂ measured directly from the initial slopes shown in Fig. 4 for pre-labeled plasma lipoprotein triglyceride.

§ Standard deviation of the slope.

|| "Standard error" estimated as 1/4 of range of k₂ as calculated in the text.

triglyceride (k₁) are given by the slopes of the semilogarithmic plots of specific activity of total liver triglyceride versus time after the injection of labeled glycerol (32). We want to make clear that k₁ obtained from the slopes of total liver triglyceride is in reality the k₁ of only the precursor portion of liver triglyceride, since this precursor pool is considered to be simply diluted by a variable amount of metabolically inert hepatic triglyceride. Since the slope of decline of specific activity of plasma S_f > 20 triglyceride is parallel to that of liver triglyceride

TABLE 5 TURNOVER RATE (t_r) OF ENDOGENOUS TRIGLYCERIDE IN LIVER AND PLASMA OF THE DOG*

Expt. No.	Plasma S _f > 20 Triglyceride				Ratio (t _r Plasma)/(t _r Liver)		
	Liver Precursor Triglyceride		Using k ₂ Calculated from Liver Curve k ₁	Using k ₂ Calculated from Plasma Curve k ₁	Using k ₂ Obtained from Labeled Lipoprotein Experiment	Using k ₁ and k ₂ from Liver Curve	Using k ₁ and k ₂ from Plasma Curve
	Calculated from Liver Curve k ₁	Calculated from Plasma Curve k ₁					
	μmoles/hr per kg body wt		μmoles/hr per kg body wt				
1	15.20 ± 1.55†	16.30 ± 0.85†	7.27 ± 0.95‡	6.93 ± 0.64‡	11.60 ± 1.25	0.48	
2	12.20 ± 3.39	15.80 ± 2.66	14.80 ± 3.62	12.80 ± 2.35		0.80	
3	22.70 ± 2.94	19.30 ± 1.95	8.06 ± 2.03	9.16 ± 2.09		0.47	
4	13.50 ± 1.67	14.20 ± 0.86	8.31 ± 1.17	7.97 ± 0.55	8.40 ± 0.58	0.62	
5	22.80 ± 1.33	14.10 ± 1.16	8.81 ± 1.05	12.40 ± 1.51		0.88	
6	30.50 ± 4.00	28.10 ± 2.09	24.70 ± 4.33	25.80 ± 3.21		0.92	
7	32.40 ± 2.35	31.00 ± 1.37	9.24 ± 1.29	9.46 ± 0.90		0.31	
Mean	21.30	19.80	11.60	12.10		0.59	

* See text for calculations.

† Standard deviation of the slope.

‡ "Standard error" of k₂ calculated as in text and Table 4.

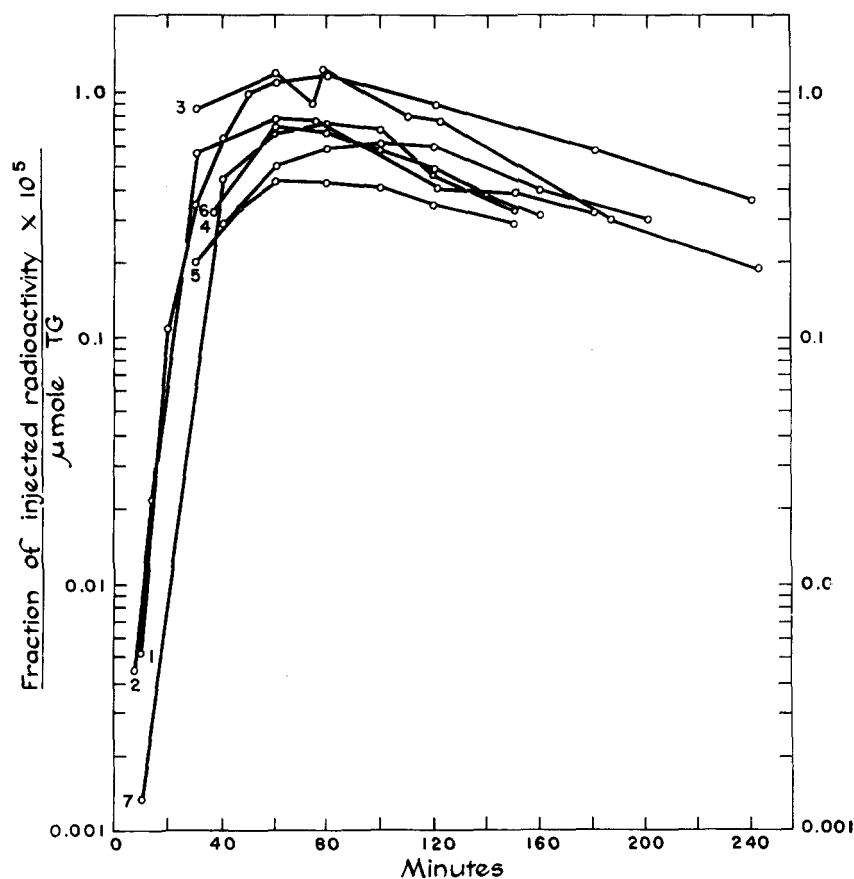


FIG. 3. Specific activity-time curves of plasma $S_f > 20$ triglyceride after injection of radioactive glycerol in the seven studies depicted in Fig. 2.

after administration of labeled glycerol (Table 2), k_1 could also be obtained from these measurements and the slopes from both liver and plasma triglycerides of Table 2 can therefore be considered synonymous with k_1 .

Calculation of Fractional Turnover Rates of Plasma Triglyceride

If liver and plasma triglyceride are related as a precursor and product in a nonrecycling, two-pool system, then fractional turnover rates of plasma $S_f > 20$ triglyceride (k_2) may be calculated from the relationship:

$$\ln(k_2/k_1) = t_{\max}(k_2 - k_1) \quad \text{equation 1 (20, 33, 44)}$$

An iterative procedure on the Stanford IBM 7090 Digital Computer was employed to solve equation 1 for k_2 . The fractional turnover rate (k_1) from both liver and plasma postglycerol specific activity-time curves were used. To estimate the variability of k_2 calculated in this manner, the equation was also solved for all combinations of $k_1 \pm 2$ SEM and $t_{\max} \pm 5$ min. The range of k_2 calculated from these combinations of extremes was divided by 4 to give a crude estimate of SEM for k_2 (Table 4).

In experiments 2 and 4, k_2 was also obtained experimentally from the slope of specific activity versus time

of $S_f > 20$ triglyceride after injection of prelabeled lipoproteins as described above. These experimental k_2 values agree well with the k_2 calculated (equation 1) from k_1 derived from plasma $S_f > 20$ triglyceride specific activity curves obtained from the simultaneous administration of labeled glycerol in the same animals (Table 4). Thus the calculation of k_2 from equation 1 is partly validated, although since only two experiments with prelabeled lipoproteins were done, further confirmation is required.

Calculation of Triglyceride Turnover Rates

Turnover rates of plasma $S_f > 20$ triglyceride and hepatic precursor triglyceride were calculated by multiplying pool size per kilogram of body weight by the corresponding fractional turnover rate.

Turnover rates of plasma $S_f > 20$ triglyceride derived from reinfusion of prelabeled $S_f > 20$ lipoproteins or from whole blood infusions (experiments 2 and 4) agree well with rates calculated from simultaneous labeled glycerol studies (Table 5). These results would be expected from the similarity of the experimental and calculated k_2 values (Table 4).

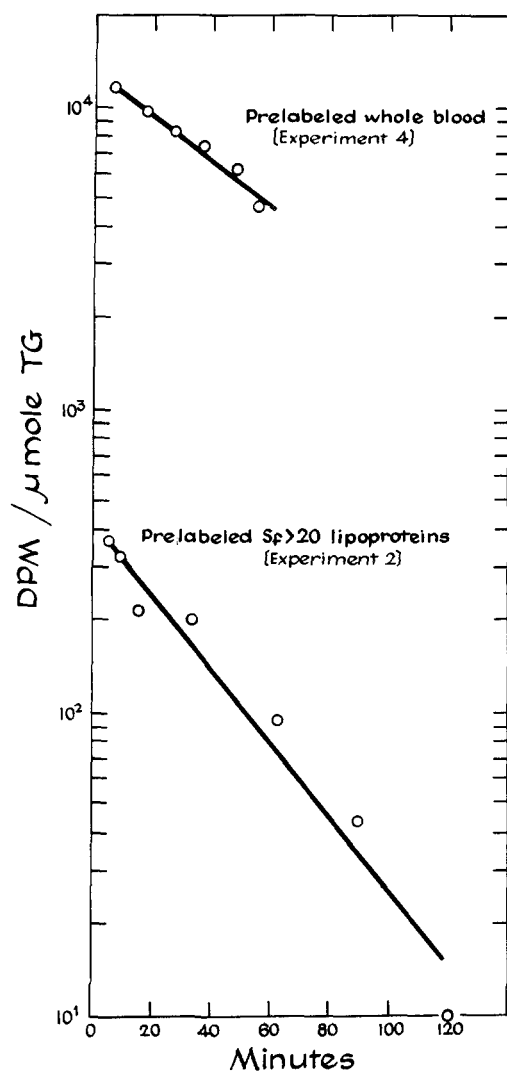


FIG. 4. Specific activity-time curves of plasma $S_f > 20$ triglyceride after injection of pre-labeled $S_f > 20$ lipoprotein (Expt. 2) or pre-labeled whole blood (Expt. 4). Specific activity in this figure is expressed as radioactivity per μ mole of triglyceride, i.e., (dpm in sample triglyceride) \div (μ moles triglyceride in sample). These labeled materials were injected concurrently with radioactive glycerol in Expts. 2 and 4.

From examination of the estimates of SEM (Table 4), it appears that turnover calculations based on the specific activity-time curves of plasma $S_f > 20$ triglyceride after glycerol administration are somewhat more precise than those based on the specific activity-time curve of liver triglyceride. This is due to the smaller variability of the slopes of the plasma specific activities (Table 2), and thus of the fractional turnover rates (k_1) derived from them.

Model for Endogenous Liver and Plasma Triglyceride Metabolism in the Dog

The model depicted in Fig. 5 has been formulated to account for the foregoing data. Additional pools and path-

ways are possible, and some for which the present studies provide no direct evidence are also shown. Pool sizes and rates of transfer between compartments are mean values from the seven glycerol experiments. Although there is no evidence for recycling of triglyceride from plasma to the liver precursor compartment under the present circumstances, this pathway is shown by a dashed line to indicate that some probably does return by this route (Fig. 5). Of course, return of a significant portion of plasma triglyceride to the liver to undergo hydrolysis is to be expected. This type of return is not considered to be recycling but is shown returning to a site termed "intrahepatic metabolism" in Fig. 5. Unfortunately the magnitude of this pathway could not be determined and is so labeled. We also show dashed lines between the two hepatic pools to indicate that interchange of triglyceride between them may well occur under these conditions. However, pathways shown by dashed lines, though likely on other grounds, are insufficient in magnitude to cause detectable deviations in the apparent first-order nature of the specific activity-time curves of precursor and product. More elaborate methods are required to set the maximal allowable rates for these "probable pathways."

An average of 61% of hepatic precursor pool triglyceride is secreted into plasma (Table 5 and Fig. 5). Therefore, the liver pool is the sole [or unique (36)] precursor of plasma $S_f > 20$ triglyceride, but this liver pool is not termed the absolute precursor (36) since not all of its substance enters the product pool (plasma) under study.

Relationship of Turnover Rate and Concentration of Plasma $S_f > 20$ Triglyceride

Fig. 6 shows turnover rates of plasma $S_f > 20$ triglyceride plotted against their corresponding concentrations in the dog. For comparison, the same relationship observed in studies in man (24) is also displayed. In the range of turnover rates found in the dog in the present studies, the function relating the two variables is linear, whereas it is not linear in man. Further, at similar turnover rates, the concentration of $S_f > 20$ triglyceride in plasma is consistently lower in the dog.

DISCUSSION

The tentative model described herein for the dog is similar to that proposed for man (20) on the basis of studies which also employed labeled glycerol as a tracer for hepatic and plasma triglyceride. In both species, only a part of hepatic triglyceride is metabolically active and in a precursor relationship to plasma $S_f > 20$ triglyceride. In addition, in both man and dog, only a portion of the hepatic precursor compartment is secreted

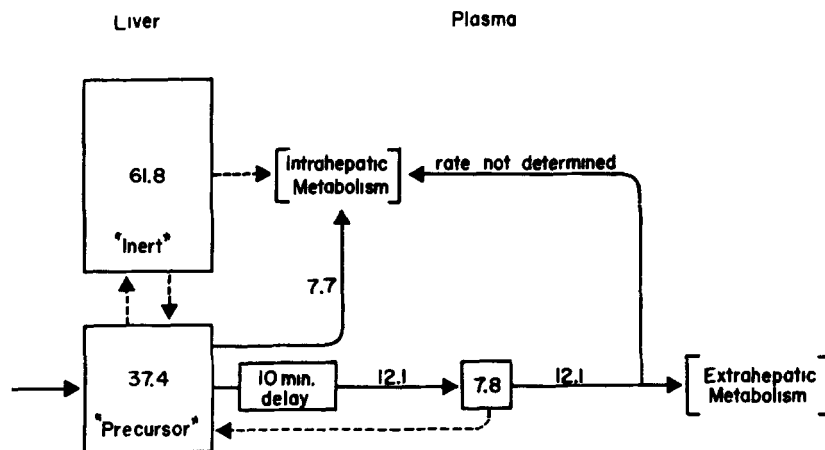


FIG. 5. A tentative model for turnover of endogenous triglyceride in liver and plasma of the dog. Numbers in boxes indicate compartment size in μ moles of triglyceride per kilogram of body weight. Dashed lines indicate pathways that are likely, yet not definable by techniques used. Transfer of triglyceride between compartments is indicated by arrows, and numbers with the arrows are transfer rates in μ moles of triglyceride per hour per kilogram of body weight. Values are means of data from seven experiments in seven dogs.

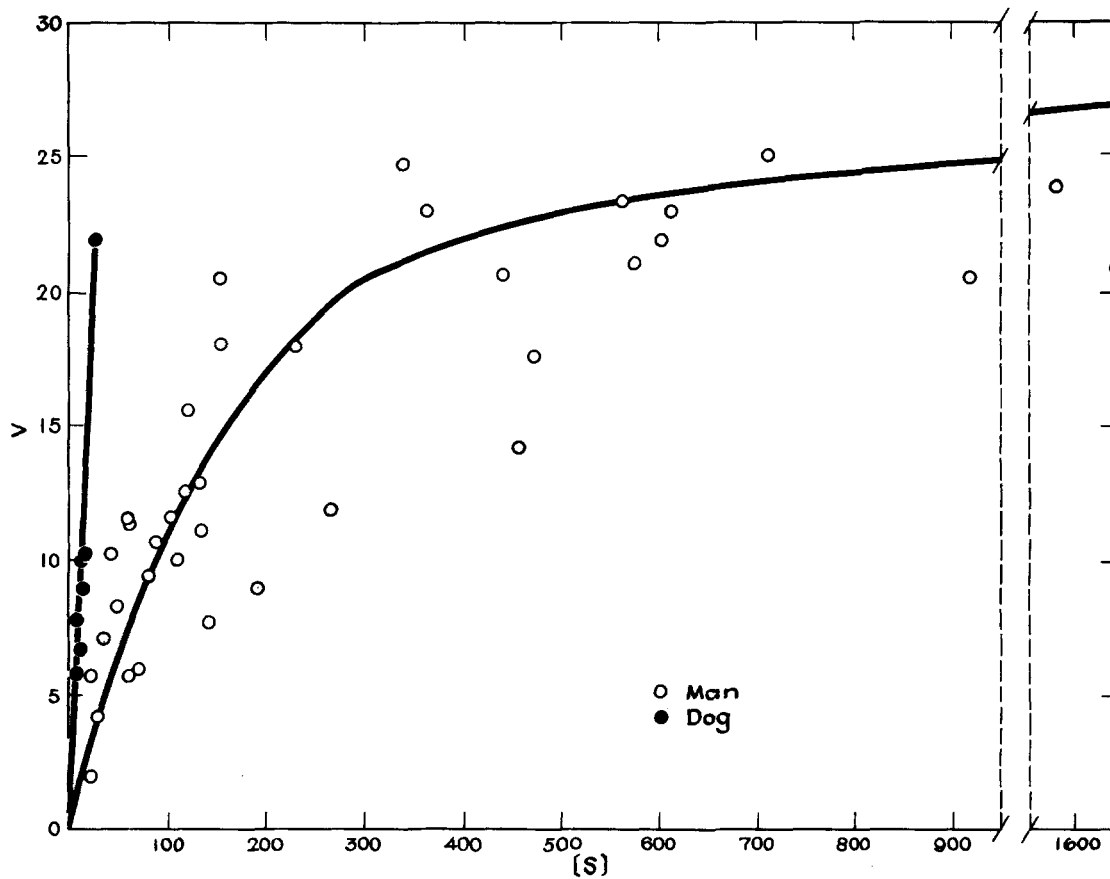


FIG. 6. Comparison of the functions relating turnover rate and concentration of plasma $S_f > 20$ triglyceride in men and dogs. V on the ordinate refers to velocity of reaction or turnover rate in milligrams per hour per kilogram of body weight. $[S]$ on the abscissa represents substrate concentration (concentration of $S_f > 20$ triglyceride) in mg per 100 ml plasma. The data for dogs are from the present studies; those for men are from reference 24 and from 11 additional studies.

into plasma, though this portion differs greatly in the two species. Further study of precursor and inert hepatic compartments in dogs by analysis of subcellular fractions would be of interest but have not been done. Such subcellular fractionation has identified liver triglyceride pools of widely different specific activity, first reported by Stein and Shapiro (37) in rats, and later by Havel, Felts, and Van Duyne (9) in rabbits.

A further similarity between the species is the absence of early recycling of labeled plasma $S_f > 20$ triglyceride back to the hepatic pool of precursor triglyceride. Recycling as defined here refers to triglyceride recycling into plasma from liver after its initial secretion into plasma. Unidirectional uptake of plasma triglyceride by the liver (which could not be measured by our methods) can well occur without recycling. Such uptake into a hepatic triglyceride "sink" would not be expected to result in apparent recycling of hydrolysis if triglyceride which has "returned" to the liver occurred prior to any preferential incorporation of this triglyceride (or its glycerol moiety) into the hepatic triglyceride synthetic system (20).

There are, however, several important differences between the two species studied in our laboratory. In the dog, hepatic triglyceride is the rate-determining compartment, since when only the plasma $S_f > 20$ triglyceride compartment is labeled (by injection of pre-labeled $S_f > 20$ lipoproteins) the rate of disappearance of radioactivity from it is much faster than when both liver and plasma triglyceride compartments are labeled by injection of radioactive glycerol. In man, plasma $S_f > 20$ triglyceride is apparently rate-determining since specific activity-time curves for $S_f > 20$ triglycerides after injection of pre-labeled $S_f > 20$ lipoproteins are parallel to those observed after labeled glycerol (20).

Another major difference is in the fraction of newly synthesized hepatic precursor triglyceride that is secreted into plasma. In the dog it averaged 60%. In man it is only 3% (20), though in man it has not yet been possible to be as certain of total hepatic triglyceride turnover. In both species the remaining turnover of hepatic precursor triglyceride is apparently metabolized within the liver. In the glucose-fed rat, in contrast, Baker and Schotz (18) have calculated that 97% of newly synthesized liver triglyceride is transferred to plasma. In a later study Schotz et al. (19) calculated that 46% of hepatic triglyceride synthesis was secreted into plasma of fasted rats.

These large differences in the fraction of hepatic triglyceride turnover that becomes plasma triglyceride could be the result of actual differences in species. They could also be due to differences in model formulations or experimental conditions (e.g., dogs were given glucose and anesthetized, men were neither fed nor anesthetized, and rats were anesthetized and were either

fed or fasted), or they may have arisen by chance, since this value is probably the most variable of all those calculated.

When turnover rates of plasma $S_f > 20$ triglyceride calculated from this model are plotted against concentration in the dog and compared with a similar relationship in man, another striking difference arises (Fig. 6). In man, as turnover rate increases beyond a certain level, concentration begins to rise with increasing rapidity as removal sites become "saturated" (24). In the dog this relationship remains linear throughout the range of turnover rates observed so far. Furthermore, at equivalent turnover rates, concentration of $S_f > 20$ triglyceride is much lower in the dog.

The reason for this difference is not yet known. When compared in this way, the dog appears to have a greater capacity for removal of $S_f > 20$ triglyceride than does man. Whether it is possible to increase influx of endogenous triglyceride in the dog sufficiently to saturate mechanisms for triglyceride removal and thereby to produce sharp increases in plasma triglyceride remains to be studied. The relationships of this difference in triglyceride metabolism to differences in the two kinetic models, such as the rate-determining compartment, and to the relative susceptibility of the two species to lipemia remain fascinating problems for further elucidation.

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REFERENCES

1. Pihl, A., and K. Bloch. 1950. *J. Biol. Chem.* **183**: 431.
2. Harper, P. V., Jr., W. B. Neal, Jr., and G. R. Hlavacek. 1953. *Metabolism*. **2**: 69.
3. Lipsky, S. R., A. Haaavik, C. L. Hopper, and R. W. McDivitt. 1957. *J. Clin. Invest.* **36**: 233.
4. Bates, M. E. 1958. *Am. J. Physiol.* **194**: 446.
5. Byers, S. O., and M. Friedman. 1960. *Am. J. Physiol.* **198**: 629.
6. Borgström, B., and T. Olivecrona. 1961. *J. Lipid Res.* **2**: 263.
7. Havel, R. J., and A. Goldfien. 1961. *J. Lipid Res.* **2**: 389.
8. Havel, R. J., 1961. *Metabolism*. **10**: 1031.
9. Havel, R. J., J. M. Felts, and C. M. Van Duyne. 1962. *J. Lipid Res.* **3**: 297.

10. Bezman-Tarcher, A., P. J. Nestel, J. M. Felts, and R. J. Havel. *J. Lipid Res.* **7**: 248.
11. Fredrickson, D. S., and R. S. Gordon, Jr. 1958. *Physiol. Rev.* **38**: 585.
12. Olson, R. E., and J. W. Vester. 1960. *Physiol. Rev.* **40**: 677.
13. Dole, V. P., and J. T. Hamlin III. 1962. *Physiol. Rev.* **42**: 674.
14. Laurell, S. 1959. *Acta Physiol. Scand.* **47**: 218.
15. Friedberg, S. J., R. F. Klein, D. L. Trout, M. D. Bogdonoff, and E. H. Estes, Jr. 1961. *J. Clin. Invest.* **40**: 1846.
16. Waterhouse, C., J. H. Kemperman, and J. M. Stormont. 1964. *J. Lab. Clin. Med.* **63**: 605.
17. Nestel, P. J., and E. Z. Hirsch. 1965. *Austral. Annals Med.* **14**: 265.
18. Baker, N., and M. C. Schotz. 1964. *J. Lipid Res.* **5**: 188.
19. Schotz, M. C., N. Baker, and M. N. Chavez. 1964. *J. Lipid Res.* **5**: 569.
20. Farquhar, J. W., R. C. Gross, R. M. Wagner, and G. M. Reaven. 1965. *J. Lipid Res.* **6**: 119.
21. Ahrens, E. H., Jr., J. Hirsch, K. Oette, J. W. Farquhar, and Y. Stein. 1961. *Trans. Assoc. Am. Physicians.* **74**: 134.
22. Farquhar, J. W., G. M. Reaven, R. C. Gross, and R. M. Wagner. 1963. *J. Clin. Invest.* **42**: 930. (Abstr.)
23. Farquhar, J. W., G. M. Reaven, R. M. Wagner, and R. C. Gross. 1964. *J. Clin. Invest.* **43**: 1299. (Abstr.)
24. Reaven, G. M., D. B. Hill, R. C. Gross, and J. W. Farquhar. 1965. *J. Clin. Invest.* **44**: 1826.
25. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **226**: 497.
26. Stern, J., and B. Shapiro. 1953. *J. Clin. Pathol.* **6**: 158.
27. Loffland, H. B., Jr. 1964. *Anal. Biochem.* **9**: 393.
28. *Glucose oxidase method "Glucostat."* Worthington Biochemical Corp., Freehold, N.J. 1965.
29. *Plasma glucose procedure N-9.* Autoanalyzer Manual. Technicon Instruments, Chauncey, N.Y. 1964.
30. Miller, M. E., G. C. Christensen, and H. E. Evans. 1964. *In Anatomy of the Dog.* W. B. Saunders, Philadelphia. 699.
31. Huggins, R. A., E. L. Smith, and S. Deavers. 1963. *Am. J. Physiol.* **205**: 351.
32. Zilversmit, D. B., C. Entenman, and M. C. Fishler. 1943. *J. Gen. Physiol.* **26**: 325.
33. Zilversmit, D. B. 1960. *Am. J. Med.* **29**: 832.
34. Strisower, E. H. 1951. Ph.D. Thesis. Univ. Calif. Berkeley, Calif. (Libr. Cong. Card No. 308-TS-9177).
35. Riggs, D. S. 1963. *In The Mathematical Approach to Physiological Problems.* Williams & Wilkins Co., Baltimore. 233-239.
36. Rescigno, A., and G. Segre. 1961. *J. Theoret. Biol.* **1**: 498.
37. Stein, Y., and B. Shapiro. 1959. *Am. J. Physiol.* **196**: 1238.