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Conversion of (U-^{14}C)-Glycerol, (2-^{3}H)-Glycerol and (1-^{14}C)-Palmitate into Circulating Lipoproteins in the Rat *

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The *in vivo* formation of labelled very low density lipoproteins (VLDL) from (U-^{14}C)-glycerol, (2-^{3}H)-glycerol and (1-^{14}C)-palmitate was studied in fed female rats. The rate of disappearance of radioactivity from plasma after the i.v. injection with these tracers was similar for (U-^{14}C)-glycerol and (1-^{14}C)-palmitate. With (2-^{3}H)-glycerol, plasma radioactivity at 10 min was lower than with the other substrates although it did not change thereafter. A certain proportion of radioactivity administered as glycerol appeared in plasma lipids, mainly in the VLDL glyceride glycerol fraction, although when (U-^{14}C)-glycerol was the substrate, a considerable portion also appeared in the esterified fatty acids of these lipoproteins. When using (1-^{14}C)-palmitate, practically all the circulating labelled esterified fatty acids appeared in the VLDL fraction, while the labelled free fatty acids appeared in lipoprotein of higher density, presumable free fatty acid-albumin complexes. This data is discussed in terms of the role of the liver in the rapid, continuous cycling of these substrates to yield VLDL-glycerides for their extrahepatic utilization.

The liver synthesizes and secretes to the blood triglycerides as a component of circulating very low density lipoproteins (VLDL) (17). These endogenous triglycerides are processed by extrahepatic tissues (11), mainly adipose tissue, for their deposition after being hydrolyzed by the action of lipoproteinlipase (23). Sources of the fatty acids of these triglycerides include dietary carbohydrates (via hepatic

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biosynthesis), dietary triglycerides, and free acids (FFA) derived from adipose tissue (21). For the synthesis of tryglycerides, the FFA are esterified with α-glycerophosphate generated either from the metabolism of glucose or from the direct phosphorylation of glycerol catalyzed by glycerokinase, the activity of which is greater in the liver than in any other organ (15).

In the present work, fed rats were injected i.v. with either ($U^{-14}C$)-glycerol, ($2^{-3}H$)-glycerol, or ($1^{-14}C$)-palmitate to study their comparative conversion into circulating VLDL-glycerides. The interest of this work has been potentiated by the frequent use of these tracers to determine lipoprotein kinetics in man (16, 19, 24, 27) which requires quantification of their precise transformation into different lipoprotein fractions.

Materials and Methods

Female Wistar rats weighing 170-190 g and fed standard rat chow were maintained in a temperature (22 ± 2° C) and light cycle (12 h on-off) controlled room. Each animal was injected i.v. in the tail at the beginning of the light cycle with either 15 μCi of ($U^{-14}C$)-glycerol (46 mCi/mmol) or 30 μCi of ($2^{-3}H$)-glycerol (200 mCi/mmol), dissolved in 0.5 ml saline. Other animals were injected with 15 μCi of ($1^{-14}C$)-palmitate (51.55 mCi/mmol) per rat, dissolved in 0.5 ml of 8 % bovine albumin.

The ^{14}C-palmitate solution was prepared as follows: the proper aliquot of ($1^{-14}C$) palmitic acid in hexane was dried under N_2, slowly mixed with 0.1N NaOH, and dissolved in 8 % bovine albumin purified by the method of Chen (5) by vigorous shaking for 60 min in a 37° C water bath. Blood was collected in heparinized receptors from the tip of the tail at 10 and 20 minutes and from the neck after decapitation at 30 min. Plasma was obtained from blood after centrifugation at 1.000 × g for 30 min at 4° C, and aliquots were used for the direct count of radioactivity and for lipid extraction (8) and fractionation following the method of KERPEL *et al.* (13) with minor modifications (3). Another aliquot of plasma (1 ml) was centrifuged under 0.15 M NaCl containing 0.001 M EDTA for 18 h at 143.000 × g in a 40.3 rotor of a Beckman preparative ultracentrifuge. The supernatant was recovered by tube slicing and designated VLDL, although it contained both chylomicrons and VLDL as shown by electrophoretic analysis in agarose gel and densitometry (7, 20) after being stained with black Sudan. The infranatant was designated «bottom» and was shown to contain high density lipoproteins (HDL), low density lipoproteins (LDL) and free fatty acids bound to albumin by the same criteria.

Lipids were purified and fractionated, as indicated above, in both the VLDL and bottom fractions, and the amount of radioactivity recovered in the summation of the lipids in these fractions was always above 90 % of that found in the lipids of whole plasma. Aliquots of plasma and all the lipid fractions were placed in a PPO/POPOP toluene/triton X-100 based scintillation cocktail (26) for counting their radioactivity in a scintillation counter. Radioactivity values were adjusted to 1×10^6 DPM for the injected tracer. Statistical analysis of the data was performed by the student «t» test.

Results

As shown in figure 1, the disappearance rate of radioactivity from plasma is very rapid and similar after i.v. injection of either ($U^{-14}C$)-glycerol or ($1^{-14}C$)-palmitate, while in rats receiving ($2^{-3}H$)-glycerol, the radioactivity in plasma is lower than with the other tracers at 10 min after its administration; this value remains almost constant until 30 min when it is higher than in the other groups. A certain proportion of the plasma radioactivity cor-

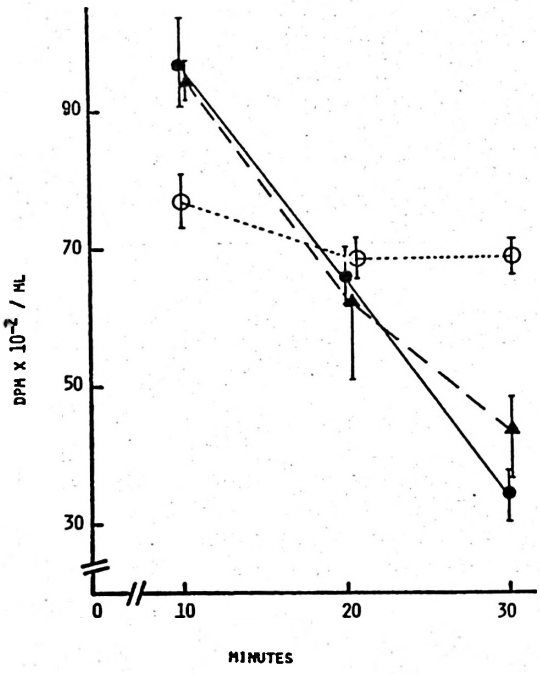

Fig. 1. *Total radioactivity in plasma after the i.v. administration of (U-^{14}C)-glycerol (●——●), (2-^{3}H)-glycerol (○·····○) or (1-^{14}C)-palmitate (▲— — —▲) in the fed rat.*
The radioactivity values were adjusted to 1×10^6 DPM for the injected tracer. The values correspond to mean ± S.E.M. of 5-7 animals/group.

responds to lipids (table I), the values obtained with (2-^{3}H)-glycerol being much lower than those with (U-^{14}C)-glycerol even at 30 min when total plasma radioactivity values were higher in the former. Using (1-^{14}C)-palmitate as a tracer, the change in radioactive circulating lipids (table I) corresponds very closely to that of total plasma radioactivity (fig. 1). The distribution of plasma lipidic fractions at 30 min varies among the groups (table I), a certain amount of radioactivity appearing as fatty acids (mainly in their esterified form) and a major proportion as glyceride glycerol when (U-^{14}C)-glycerol was the tracer, while almost all labelled lipids were in the glyceride glycerol form when (2-^{3}H)-glycerol was used, and most labelled lipids were in the form of esterified fatty acids when using (1-^{14}C)-palmitate.

With any of the labelled glycerols, most of the plasma lipidic radioactivity appeared in the VLDL fraction (table II), a minor portion being present in the lipoproteins of higher density («bottom»). Most of the radioactivity in the VLDL-lipids form from either (U-^{14}C)-glycerol or (2-^{3}H)-glycerol appeared in the form of glyceride glycerol (table II) from which over 93.8 % corresponds to triglycerides, as assessed by thin layer chromatography (data not shown). With the former tracer, a considerable amount of fatty acids (mostly esterified) is also formed (table II). With (1-^{14}C)-palmitate, almost all circulating, labelled esterified fatty acids appear in the VLDL fraction (over 95.4 % as triglycerides), while the free fatty acids are mainly found in the «bottom» (table II).

Discussion

Despite the parallel disappearance of

Table I. *Labelled plasma (DPM × 10^{-2}/ml) lipids after the i.v. injection of radioactive substrates in the rat.*
The radioactivity values were adjusted to 1×10^6 DPM for the injected tracer. The values correspond to mean ± S.E.M of 5-7 animals per group.

Minutes after the tracer:	10	20	30	30		
Tracer	Total lipids			FFA	Esterified fatty acid	Glyceride glycerol
(U-^{14}C) glycerol	0.32±0.10	4.46±0.72	7.71±0.80	0.58±0.25	1.40±0.57	4.34±0.91
(2-^{3}H) glycerol	0.009±0.001	—	3.57±0.59	0.06±0.005	0.20±0.036	3.48±0.35
(1-^{14}C) palmitate	80.75±23.14	55.96±9.33	38.65±8.76	13.99±2.76	24.39±7.64	—

Table II. *Distribution of labelled lipids in plasma lipoproteins at 30 min after the i.v. injection of different tracers in the rat.*
The radioactivity values were adjusted to 1×10^6 DPM for the injected tracer. Expressions of «VLDL» and «BOTTOM» are explained in the text. The values correspond to mean ± S.E.M. of 5-7 animals per group. % corresponds to the percentage fraction as related to the total lipids value.

	Total lipids (DPM $\times 10^{-2}$/ml)	FFA (%)	Esterified faccty acid (%)	Glyceride glycerol (%)
			«VLDL»	
(U-^{14}C) glycerol	5.12 ± 0.62	4.03 ± 0.54	28.14 ± 4.46	67.14 ± 4.47
(2-^{3}H) glycerol	2.64 ± 0.50	1.41 ± 0.54	7.83 ± 0.92	91.21 ± 2.06
(1-^{14}C) palmitate	22.10 ± 5.91	10.56 ± 2.5	85.03 ± 3.39	—
			«BOTTOM»	
(U-^{14}C) glycerol	1.80 ± 0.35	—	—	—
(2-^{3}H) glycerol	0.13 ± 0.12	—	—	—
(1-^{14}C) palmitate	14.52 ± 1.17	85.50 ± 3.27	10.53 ± 2.51	—

total radioactivity from the blood after i.v. administration of both (U-^{14}C) glycerol and (1-^{14}C) palmitate, their metabolic fate seems quite different as shown by their relative reappearance in the different circulating fractions. The amount of (U-^{14}C)-glycerol which appears in plasma as lipids is quite small, probably due to its rapid conversion into glucose by the liver, as has been amply demonstrated (4, 6, 10, 18) as well as to its utilization by extrahepatic tissue (3). The disappearance of labelled palmitate from the blood also corresponds to its rapid utilization by different tissues (2, 12). Thus with both substrates, the label found in the blood 30 min after their administration must correspond to their respective recycling after being taken up by the different tissues. From either substrate, most of the lipids returned to circulation appear in the form of VLDL-triglycerides which indicates that the main conversion may take place in the liver, the known site of synthesis of these lipoproteins from endogenous substrates (17).

Part of the (U-^{14}C)-glycerol is converted to fatty acid which appears mainly in the esterified form of VLDL triglycerides. This demonstrates that this metabolite, after being phosphorylated by glycerokinase, is partially transformed into acetyl-CoA and is used as a lipogenetic substrate in the liver. Most of the endogenous glycerol in plasma comes from adipose tissue as a result of the breakdown of stored lipids (15), and present results show evidence of a continuous cycle in which part of this glycerol is converted to triglycerides in the liver (both in the fatty acids and glyceride glycerol fraction) to return to the blood in the form of VLDL where it will again be taken up by extrahepatic tissues. A similar cycle is valid for fatty acids which are esterified in the liver with a α-glycerophosphate coming from either glucose or glycerol (3) and returning to the blood in the form of VLDL.

Because of the rapid and continuous recycling of fatty acids (14), the labelled free fatty acids remaining in plasma 30 min after injection of (1-^{14}C)-palmitate presumably correspond more to their release from preformed VLDL-triglycerides than from the substrate not yet utilized. Most of these labelled free fatty acids appear in the «bottom» fraction after ultracentrifugation; that is, the fraction of highest

density which corresponds to their complex with albumin (9, 25).

The different behavior of ($U^{-14}C$)- and ($2^{-3}H$)-glycerol with respect to *in vivo* utilization is in agreement with previous findings of tissue distribution of these substrates (3). The prolonged maintenance of radioactivity in plasma after administration of ($2^{-3}H$)-glycerol may correspond to the interchange of tritiated glycerol and water hydrogens (the detritiation effect) (1, 22) more than to a slower recycling of the molecule as compared with that of ($U^{-14}C$)-glycerol. In any event, compared with ^{14}C, much lower radioactivity from glycerol, as tritium, appears in total plasma lipids. This finding is related to the reduced utilization of glycerol for fatty acid synthesis, while the conversion of either substrate to glyceride glycerol is very similar. These results indicate the advisability of using ($2^{-3}H$)- glycerol for the *in vivo* preparation of VLDL-triglycerides specifically prelabelled in the glyceride glycerol moiety, although the actual amount of radioactive substance recovered is very low, probably due to the rapid utilization of glycerol throughout other pathways.

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Resumen

Se ha estudiado la formación *in vivo* de lipoproteínas radiactivas de muy baja densidad (VLDL) en ratas alimentadas, a partir de glicerol-($U^{-14}C$), glicerol-($2^{-3}H$) y palmitato-($1^{-14}C$). La velocidad de desaparición de radiactividad del plasma después de la administración intravenosa de los trazadores fue similar para glicerol-($U^{-14}C$) y palmitato-($1^{-14}C$), mientras que en las ratas que recibieron glicerol-($2^{-3}H$), la radiactividad en plasma era inferior que con los otros sustratos, a los 10 min, pero no cambiaba posteriormente. Una determinada cantidad de la radiactividad administrada como glicerol apareció en plasma en forma de lípidos, principalmente como glicerol de glicéridos de VLDL, aunque cuando el sustrato era el glicerol-($U^{-14}C$), una proporción considerable también apareció en los ácidos grasos esterificados de estas lipoproteínas. Cuando se utilizó palmitato-($1^{-14}C$), prácticamente todos los ácidos grasos esterificados circulantes aparecieron en las VLDL, mientras que los ácidos grasos libres radiactivos se encontraban en lipoproteínas de más alta densidad (probablemente en forma de complejos ácidos grasos-albúmina). Los resultados se discuten de acuerdo con la función del hígado en el continuo y rápido ciclaje de estos sustratos para la formación de glicéridos de VLDL y su posterior utilización por tejidos extrahepáticos.

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