Lack of Uptake and Metabolism of the Triglyceride of Serum Lipoproteins of Density less than 1.006 by the Perfused Rat Liver

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Previous experiments on the utilization of chylomicron triglycerides by the perfused rat liver (Felts & Mayes, 1965) indicated that the liver did not assimilate triglycerides of dietary origin to an appreciable extent. Since the perfused liver metabolized FFA† extensively, it was suggested that, *in vivo*, chylomicron triglycerides were first hydrolysed to FFA before uptake into the liver cells. The results, which showed only minimal hydrolysis of the chylomicron triglyceride within the liver, indicated that most of the hydrolysis would have to take place in extrahepatic tissues to account for the rapid oxidation of chylomicron TGFA *in vivo*.

Triglycerides are present in plasma not only in chylomicrons, from the absorption of dietary fat, but also in lipoproteins secreted by the liver. Laurell (1959) noted that ^{14}C -labelled serum TGFA were taken up by the liver within 15 min. of their intravenous administration into rats. Stein & Shapiro (1960), using serum triglycerides labelled *in vitro* with [^{14}C]glycerol [^3H]trioleate, showed that 35% of the administered lipid was in the liver within 5 min. of injection. Little alteration of the $^{14}\text{C}/^3\text{H}$ ratio occurred, indicating little hydrolysis of the labelled molecules. Havel, Felts & Van Duyne (1962) found a large recovery of endogenously labelled serum TGFA ($d < 1.006$ lipoproteins) in the livers of rabbits 2 hr. after injection. These findings have led to the assumption that intact serum triglyceride molecules can enter freely into hepatic cells to form a common metabolic pool with the intracellular triglyceride already present in the organ (Stein & Shapiro, 1960; Havel *et al.* 1962).

The utilization of ^{14}C -labelled TGFA in serum lipoproteins of $d < 1.006$ by the isolated perfused liver of the rat is compared here with that of ^{14}C -labelled FFA. As in previous experiments with chylomicrons (Felts & Mayes, 1965) no heparin was used at any stage, to prevent the activation of lipoprotein lipase (Mayes & Felts, 1966a). The results of eight perfusion experiments are shown in Table 1. When ^{14}C -labelled FFA were infused, they were metabolized extensively, a large per-

centage of the ^{14}C being recovered in liver lipids. Oxidation to both labelled CO_2 and ketone bodies also occurred and the livers from fed rats esterified more and oxidized less of the infused FFA than the livers from starved rats. In contrast, labelled TGFA were not taken up or metabolized to any extent by livers from either fed or starved rats. No label was detected in ketone bodies and an average of only 0.4% of the infused ^{14}C was recovered in respiratory CO_2 . Very little labelling of liver lipids was evident, nor was there appreciable hydrolysis of the perfused ^{14}C -labelled TGFA to serum ^{14}C -labelled FFA. It is to be noted that some hydrolysis of ^{14}C -labelled TGFA is to be expected since, even in the absence of heparin, there is a small but significant amount of lipase activity in the perfusate (Felts & Mayes, 1967). In a subsequent experiment (P. A. Mayes & J. M. Felts, unpublished work), heparin was added to the perfusate and then appreciable ^{14}C was recovered in products resulting from the metabolism of the infused ^{14}C -labelled TGFA.

Analysis of the ^{14}C -labelled lipoproteins infused showed that 98% of the label was in TGFA, up to 1% in FFA and up to 2% in phospholipids. In experiments without heparin nearly all the ^{14}C -labelled TGFA was still present in lipoproteins of density < 1.006 after 90 min. of perfusion, no more than 2% being in lipoproteins of density > 1.006 . There was no increase in ^{14}C -labelled phospholipids during the perfusion and, after 90 min., these were distributed equally between the low-density and high-density lipoprotein fractions.

These results show clearly that triglycerides released from the liver *in vivo* do not re-enter active metabolic pools in the liver to a significant extent when infused into the isolated preparation. This conclusion is at variance with the implications drawn from earlier experiments *in vivo* in which a large fraction of serum triglycerides was found in the liver soon after injection (Laurell, 1959). Nevertheless it is possible that the radioactivity measured in the liver *in vivo* could have been present in extracellular spaces. Felts (1965) has shown that chylomicrons, which also accumulate in the liver *in vivo* immediately after injection, may be washed out readily as unhydrolysed particles. This entrapment in the liver of the intact animal

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† Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids.

Table 1. *Uptake and metabolism of ^{14}C -labelled FFA and TGFA of serum lipoproteins of $d < 1.006$ by perfused rat livers*

^{14}C -labelled FFA were prepared by combining the potassium salt of [^{14}C]oleic acid (32.5 mc/m-mole; The Radiochemical Centre, Amersham, Bucks.) and any carrier oleic acid (grade A, Calbiochem Ltd.; obtained from Kodak Ltd., Kirkby, Lancs.) with 1.0 g. of crystalline bovine serum albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) dissolved in 10.0 ml. of 0.9% NaCl solution at 37° and at pH 8.0. Serum lipoproteins ($d < 1.006$) containing ^{14}C -labelled TGFA were prepared by injecting albumin-bound trace quantities of [^{14}C]oleic acid (250 μC) intravenously into rats (250 g.) that had been maintained on a high-fructose diet for 1 week and that exhibited visible lipaemia. Then 40 min. after injection the rats were exsanguinated and, without delay, the serum was layered under 0.9% NaCl solution and centrifuged at 110000g for 90 min. at 13° (MSE Superspeed 50 centrifuge, rotor 2408). Serum lipoproteins ($d < 1.006$) were harvested from the top of the centrifuge tubes with the aid of a tube cutter, suspended in Krebs & Henseleit (1932) bicarbonate buffer and used in perfusion experiments immediately. Livers were perfused as described previously (Mayes & Felts, 1966b). Labelled FFA or TGFA were added to the perfusate as an infused priming dose, and then infused at a constant rate for a period of 90 min. Analyses of radioactivity in serum and liver lipids were carried out by the procedure of Havel *et al.* (1962). A correction for the contamination of the radioactivity of the liver lipids by ^{14}C -labelled lipids present in the perfusing blood was made by estimating the perfusate volume within the liver with ^{131}I -labelled serum albumin (The Radiochemical Centre) added to the perfusate. $^{14}\text{CO}_2$ was estimated as described previously (Mayes & Felts, 1966b), as was the radioactivity in blood ketone bodies (Mayes & Felts, 1967). Wistar-strain albino male rats were used throughout (Scientific Animal Service, Elstree, Herts.) and were maintained on a stock diet (M.R.C. 41B). Rats were starved for 24 hr. where indicated.

^{14}C -labelled substrate infused	Nutritional state of liver donor	Quantity of substrate infused (μmoles)	Radioactivity of substrate infused (μC)	Percentage of infused ^{14}C recovered in 90 min.					
				In total esterified lipids					Total
				In CO_2	In ketone bodies	In serum liver		In serum FFA	
						In serum	In liver		
FFA	Fed	0.8	25.0	2.9	1.6	26.2	45.6	14.5	90.8
FFA	Starved	0.8	25.0	24.4	6.7	2.2	10.0	22.0	65.3
FFA	Fed	177	25.0	10.4	4.8	22.0	25.2	20.5	82.9
FFA	Starved	177	25.0	7.9	32.7	3.7	16.0	20.0	80.3
TGFA	Fed	60	5.9	0.2	0.0	92.1	1.4	1.4	95.1
TGFA	Fed	32	2.2	0.1	0.0	94.1	1.5	0.8	96.5
TGFA	Starved	19	0.9	0.9	0.0	94.9	1.5	3.6	100.9
TGFA	Starved	16	1.4	0.2	0.0	91.6	1.1	2.2	95.1

might result from the lodging of triglycerides in the spaces of Disse or to intermittent blood flow through certain sinusoids, as proposed by Nakata, Leong & Brauer (1960).

The experiments reported demonstrate negligible uptake of ^{14}C -labelled TGFA of serum lipoproteins into the perfused liver compared with ^{14}C -labelled FFA. This confirms our previous proposition (Felts & Mayes, 1965) that FFA are the principal chemical form in which fatty acids are taken up by the tissues, not only in the periphery, but in the liver as well. Our experiments indicate that very little hydrolysis of ^{14}C -labelled TGFA to ^{14}C -labelled FFA occurs in the liver and suggest that much of the metabolism of serum lipoproteins *in vivo* must take place via hydrolysis of TGFA to FFA in peripheral capillary beds containing lipoprotein lipase (Robinson, 1964).

Belfrage (1967) and Schotz, Arnesjö & Olivecrona (1967), using intact rats, have attempted to account for the synthesis of ^3H -labelled fatty acid esters by the liver in terms of the calculated flux of ^3H -labelled

FFA that would result from the hydrolysis of ^3H -labelled lipids of chylomicrons or of ^3H -labelled serum lipoproteins at extrahepatic sites. Their results support our conclusions in starved rats injected with ^3H -labelled chylomicrons. However, they were unable to account completely for the synthesis of ^3H -labelled fatty acid esters in fed rats injected with ^3H -labelled chylomicrons and in either starved or fed rats injected with ^3H -labelled serum lipoproteins. It is not apparent why there was a difference between chylomicrons and serum lipoproteins. We find no differences between the metabolism of TGFA in chylomicrons and serum lipoproteins by the perfused liver from either the fed or starved animal. To account for their results, Belfrage (1967) and Schotz *et al.* (1967) suggest that the triglyceride, 'trapped' by the liver in the extra-cellular spaces, is hydrolysed *in situ*, liberating FFA. This conclusion does not conflict with our hypothesis that FFA are the principal chemical form in which fatty acids are taken up by the tissues. However,

neither chylomicrons nor serum lipoproteins are 'trapped', nor is there any appreciable hydrolysis of TGFA, in our perfused liver preparation. The reason for the 'trapping' observed in experiments *in vivo* remains to be determined.

Present results support the conclusion that the synthesis and secretion of endogenous triglyceride by the liver in the form of $d < 1.006$ lipoproteins is a unidirectional process. As the secretion of lipoproteins appears to be a unique property of the liver, if any significant hepatic uptake of $d < 1.006$ lipoproteins occurred, it would nullify this important function.

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