Biochem. J. (1978) **172**, 219–226
Printed in Great Britain

Rate of Release of Hepatic Triacylglycerol into Serum in the Starved Rat

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(*Received* 11 *July* 1977)

After an intravenous injection of a pulse of [U-^{14}C]palmitate to starved rats, the time-dependent radioactivity profiles were determined in the triacylglycerol (triglyceride) of hepatic microsomal fractions, floating fat, mitochondria and nuclei. The profile of activity in serum gave a value of 0.08 mg/min per 100 g body wt. for the irreversible disposal rate of triacylglycerol from serum. This value, combined with the previously estimated rate of movement of triacylglycerol from serum to liver, and the reported rate from intestine to serum, gave a calculated value of 0.35 mg/min per 100 g body wt. for release rate of triacylglycerol from liver to serum. The rate of release of hepatic triacylglycerol into serum was also measured by the widely used Triton WR-1339 method. The rate obtained with this technique (0.15 mg of triacylglycerol/min per 100 g body wt.) was identical with that reported previously. During the interval from 45 min to 3 h after ethanol administration this rate increased to 0.18 mg/min per 100 g body wt. It was concluded that the use of Triton underestimates the true rate of movement of triacylglycerol from liver to serum.

Abrams & Cooper (1976) found that serum fatty acid was converted into liver triacylglycerol (triglyceride) at a rate of 0.06 mg/min per 100 g body wt. in 48 h-starved rats. Lipkin *et al.* (1978) subsequently showed, by injection of serum labelled *in vitro* with radioactive trioleoylglycerol, that serum triacylglycerol recycles to liver at a rate of 0.29 mg/min per 100 g body wt. and thus contributes a significant fraction to the total hepatic triacylglycerol turnover rate. These two input rates totalling 0.35 mg/min should be matched by the output rate of hepatic triacylglycerol, yet estimates of the latter by the Triton method have given values of only about 0.15 mg/min. Triton inhibits lipoprotein lipase (Recknagel, 1967) and thereby causes triacylglycerol derived from liver to accumulate in serum. The rate of accumulation is interpreted as measuring input rate from liver. However, if triacylglycerol is recycling back to liver at the same time, the Triton method would be expected to underestimate input rate because only the net accumulation is being observed.

Hepatic triacylglycerol turnover rates have been estimated previously by use of a pulse injection of [U-^{14}C]palmitate and observation of the movement of label into hepatic and serum triacylglycerol (Havel *et al.*, 1962; Baker & Schotz, 1964; Farquhar *et al.*, 1965; Baker, 1969; Shames *et al.*, 1970; Quarfordt *et al.*, 1970). The mathematical analysis of such data has been complicated by the apparent existence of at least two hepatic triacylglycerol pools (Baker & Schotz, 1964; Farquhar *et al.*, 1965; Jones *et al.*, 1967; Stein & Stein, 1967). These two pools of triacylglycerol may be separated by subcellular fractionation. To identify the hepatic precursor pool for serum triacylglycerol by this means the following criteria should be met. (1) All radioactivity that enters the total liver triacylglycerol pool should be accounted for in the subcellular fractions to avoid selective loss of a relevant triacylglycerol pool, and (2) data must be obtained at enough time points to describe accurately the radioactivity profiles. Previous investigations have not met all of these criteria, but we have attempted to do so in the study described below.

The following study was designed to estimate the release rate of hepatic triacylglycerol to serum by a method that used precursor fatty acid as tracer material so that hepatic triacylglycerol would be labelled *in vivo* rather than *in vitro* as was done by Lipkin *et al.* (1978).

Experimental

The animal procedures, lipid analyses and measurement of the rate of release of hepatic triacylglycerols with Triton WR-1339 were as described by Abrams & Cooper (1976).

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Subcellular fractionation and triacylglycerol extraction

Livers were removed and rinsed twice in cold 0.15M-NaCl, blotted dry, and the small lobe, located on the right side dorsal to the large bifurcated lobe, was removed. This lobe, weighing approximately 1g, was immediately homogenized at 0°C in 8ml of freshly prepared 0.35M-sucrose/2mM-EDTA with seven strokes of a 10ml Potter–Elvehjem homogenizer [clearance 0.203mm (0.008in)]. The homogenates from all rats of the same experimental group were combined and rehomogenized with three strokes of the same homogenizer and the total volume was measured.

A 1ml portion of pooled homogenate was added immediately to each of three 12ml glass-stoppered conical test tubes containing 3.75ml of methanol/chloroform (2:1, v/v) at 4°C. The tubes were vortex-mixed for 1min. Redistilled chloroform (1.25ml) and triple-distilled water (1.25ml) were added and the tubes remixed; the phases were allowed to separate overnight at room temperature (20°C).

An 8ml portion of pooled homogenate was added to each of three 12ml glass Sorvall centrifuge tubes and fractionated. The tubes were centrifuged for 5min at 2°C and 500*g* in a Sorvall RC2B centrifuge to sediment the nuclei. The floating fat was displaced from the sides of the tubes with a glass rod and each supernatant solution added to another 12ml glass Sorvall tube. The second set of tubes was centrifuged at 600*g* for 10min at 2°C to sediment the mitochondria. Each of the latter supernatants were put into a 12ml Spinco centrifuge tube and centrifuged in a type-40 rotor in a Spinco model L ultracentrifuge at 4°C for 2h at 105000*g*.

Cold 0.35M-sucrose/2mM-EDTA (1ml) was added to each of the nuclear, mitochondrial and microsomal pellets and they were suspended uniformally with a Potter–Elvehjem homogenizer. To each suspended pellet was added 3.75ml of methanol/chloroform (2:1, v/v) and the tube contents were vortex-mixed for 1min. Then 1.25ml of triple-distilled water and 1.2ml of redistilled chloroform were added and the tube contents again mixed. The methanol-plus-water and the chloroform phases were separated by centrifugation at 500*g* for 15min at room temperature. The chloroform phase was removed entirely with a 5ml glass syringe, added to 15ml glass acid-washed tubes, the extracts were evaporated to dryness under N_2 at 50°C, and 500μl of redistilled chloroform was added to each tube. A 100μl portion was applied to a thin-layer plate and the remaining 400μl assayed for triacylglycerol (Soloni, 1971).

Immediately after ultracentrifugation, the floating fat was displaced gently from the sides of the tube and removed with a Pasteur pipette. The total volume of each supernatant fraction was measured, and 4ml portions transferred to 40ml glass-stoppered extraction tubes containing 15ml of methanol/chloroform (2:1, v/v). The tubes were shaken vigorously for 1ml and 5ml each of triple-distilled water and chloroform added, the tubes inverted three times, and the phases allowed to separate overnight at room temperature. A 5ml portion of the chloroform phase was evaporated to dryness, and the residue then dissolved in 500μl of redistilled chloroform. A 100μl portion was applied as a spot in a 2cm lane to a thin-layer plate, as described previously, and the remaining 400μl assayed for triacylglycerol.

Analysis of fatty acid composition of triacylglycerol from floating-fat and microsomal fractions

A pooled liver homogenate was made as described above from groups of six experimental animals by using the large bifurcated lobe from each. This gave about 12g wet wt. of liver per group. Lipids were extracted and purified by t.l.c. as described above, except that the entire 500μl chloroform solution was streaked across the thin-layer plate.

To locate the triacylglycerol spots, a 1cm vertical lane from each side of the chromatographic plate was scored, broken off, sprayed with 10% (w/v) sulphuric acid, and charred. A horizontal lane corresponding to triacylglycerol was scored on the uncharred portion of the plate and the silicic acid from each plate scraped into a 40ml glass-stoppered centrifuge tube. The scrapings from each plate were suspended in 20ml of methanol/chloroform (2:1, v/v) and the tube shaken manually for 1min. This was followed by the addition of 10ml of chloroform and 10ml of water, the tubes were inverted two to three times and the chloroform phases separated by centrifugation at 500*g* for 15min at room temperature.

The total chloroform extracts from the microsomal triacylglycerol pools were removed with a glass syringe, pooled and then divided into three equal portions, representing 4g of liver and thus 3mg of triacylglycerol each. From the total chloroform extracts of the floating-fat triacylglycerol, three pooled samples containing approx. 5mg of triacylglycerol each were obtained. Since the molecular weight of glycerol in triacylglycerol is negligible, this also represents 3 and 5mg of fatty acid per sample respectively. This allowed us to examine each sample in triplicate.

Fatty acid methyl esters were formed from triacylglycerols as described by Morgan *et al.* (1963). A 0.5μl portion of the fatty acid methyl ester solutions was analysed by gas-chromatographic separation on a 1.83m (6ft) stainless-steel column packed with 10% SP-2330 on 100/120 Chromosorb W AW in a Hewlett–Packard (5711A) gas chromatograph. The detector temperature was set at 250°C and the injector temperature at 200°C. Carrier gases were a mixture of N_2 (flow rate 12.5ml/min), H_2 (10ml/min) and air (100ml/min). After 5min of analysis, the

column temperature was programmed to rise (at 4°C/min) from 170 to 200°C.

Methyl esters were identified by comparison with 0.5 μl of a 5mg/ml solution of fatty acid methyl esters, standard RM-3, supplied by Supelco, Bellefonte, PA, U.S.A.

Areas under the appropriate peaks were calculated by an automatic integrator.

Glucose 6-phosphatase was assayed as described by Swanson (1955).

Results

Movement of radioactive label through serum and liver

Livers were fractionated as described above and the distribution of triacylglycerol in the various subcellular fractions determined (Table 1). In all cases, approx. 100% of the chemically determined triacylglycerol in the total homogenate was accounted for in the isolated fractions. The radioactivity in triacylglycerol in the homogenate was also completely accounted for in the triacylglycerol of the subcellular fractions (Table 1). These data indicate that no sizable triacylglycerol pool was lost during the subcellular fractionation. The results in Table 1 were obtained with livers removed 15min after injection of labelled palmitate. Similar results with regard to recovery of triacylglycerol and radioactivity were obtained at all times after injection of [U-^{14}C]palmitate shown in Figs. 1 and 2.

The specific radioactivity of triacylglycerol in each of the subfractions is also shown in Table 1. The specific radioactivities of triacylglycerols in the nuclear, mitochondrial and floating-fat fractions are identical and are considerably lower than those in the microsomal triacylglycerol pool. The triacylglycerol appearing in the nuclear and mitochondrial pellets probably results from contamination by lipid droplets of the cytosol and microsomal fraction rather than the presence of discrete pools of triacylglycerol. This is supported by the finding that the triacylglycerol and radioactivity in triacylglycerol could be largely removed by washing the nuclear and mitochondrial pellets twice, whereas washing the microsomal pellet produced no significant change (Table 2). Only about 8% of the triacylglycerol and about 5% of the radioactivity remained in the nuclear and mitochondrial fractions after washing. Since these probably do not represent distinct physiological pools of triacylglycerol, and, since their specific radioactivity is the same as that of the floating fat, the radioactivity and triacylglycerol in these fractions were included with that of the floating-fat pool for the results presented below.

Fig. 1 shows the movement of radioactive triacylglycerol through the relevant pools in liver and serum. Radioactivity in the microsomal triacylglycerol

Table 1. *Distribution of triacylglycerol and ^{14}C-labelled triacylglycerol among subcellular fractions of liver 15min after intravenous injection of [U-^{14}C]palmitate*

[U-^{14}C]Palmitate was injected into a tail vein, the livers were removed 15min later, homogenized in sucrose/EDTA, and the subcellular fractions were isolated and triacylglycerol was extracted as described in the text. Specific radioactivity is expressed as the percentage of injected dose/mg of triacylglycerol. The triacylglycerol concentration in the total homogenate before subfractionation was 4.34±0.11mg/g of liver. The percentage of injected dose in the total homogenate triacylglycerol was 3.82±0.09. The number of experimental animals was 51.

	Total triacylglycerol (%)	Total radioactivity (d.p.m.) in triacylglycerol (%)	Specific radioactivity
Nuclei	12.0±1.4	10.2±0.2	0.13±0.01
Mitochondria	9.7±0.7	7.8±0.2	0.13±0.01
Microsomal fraction	17.0±0.6	33.5±1.5	0.31±0.04
Cytosolic droplets	61.0±2.5	51.8±1.2	0.13±0.01
Recovery	99.7±2.9	103.3±1.9	

Table 2. *Effect of washing on triacylglycerol content of subcellular fractions*

Nuclear, mitochondrial and microsomal fractions obtained from six livers 5min after [U-^{14}C]palmitate injection were resuspended in 6ml of sucrose/EDTA buffer, followed by recentrifugation at the appropriate speed, and analysed for triacylglycerol and radioactivity in triacylglycerol or, alternatively, resuspended in 6ml of buffer, recentrifuged, resuspended in 4ml of buffer, recentrifuged, and then analysed for triacylglycerol and radioactivity in triacylglycerol. The percentages given below were obtained by dividing the recovered values by the total amount (4.2mg of triacylglycerol/g of liver) or dose of radioactivity (4.3% of injected dose). These percentages do not add up to 100 because the major triacylglycerol pool in the cytosolic droplets is not included.

Subfraction	Percentage of total triacylglycerol	Percentage of total hepatic radioactivity
Nuclei		
Original pellet	19.0	12.1
First wash	7.2	6.3
Second wash	3.9	4.2
Mitochondria		
Original pellet	12.3	6.7
First wash	6.4	2.3
Second wash	4.4	1.2
Microsomal fraction		
Original pellet	18.0	54.0
First wash	16.0	52.0
Second wash	17.0	52.0

pool reached a maximum in about 3min, and label began to appear in serum triacylglycerol about 10min after intravenous injection of [U-^{14}C]palmitate. Radioactivity did not reach a peak in the floating-fat pool until later and then declined.

Fig. 2 shows how the specific radioactivities of liver and serum triacylglycerol pools change with time. The maximum specific radioactivity reached in liver microsomal triacylglycerol was slightly higher than that in serum triacylglycerol, whereas the floating-fat fraction had a considerably lower specific radioactivity. It is possible that a higher-specific-radioactivity pool exists within the microsomal pellet. Triacylglycerol formed 2min after the palmitate pulse may have a higher specific radioactivity than that formed between 2 and 10min. It is not known whether the very-low-density lipoprotein complexes are released from liver randomly or in the order in which they are synthesized. It is likely that these complexes vary widely in specific radioactivity, depending on when their constituent triacylglycerol molecules were formed during the 10min interval. Since the microsomal pellet includes rough and smooth endoplasmic reticulum and Golgi, all of which are involved in lipoprotein production, the specific radioactivity in each of these fractions is probably not uniform. We assume that the average specific radioactivity of the total microsomal pool provides a reasonable approximation of the specific radioactivity of the serum triacylglycerol-precursor pool.

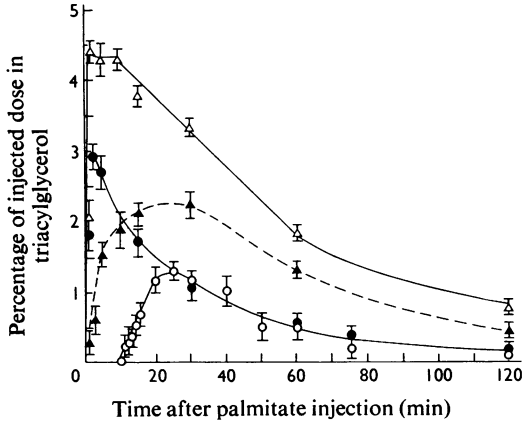

Fig. 1. *Distribution of triacylglycerol at different times after intravenous injection of [U-^{14}C]palmitate in 48h-starved rats*

The number of experimental animals per time point was ten, except for the 2, 10 and 15min points, where 50 animals per time point were used. Results are expressed as percentage of injected dose in triacylglycerol/rat in serum (○), cytosolic lipid (▲), microsomal fraction (●) and total homogenate (△). Rats weighed 200±10g (mean±S.E.M.).

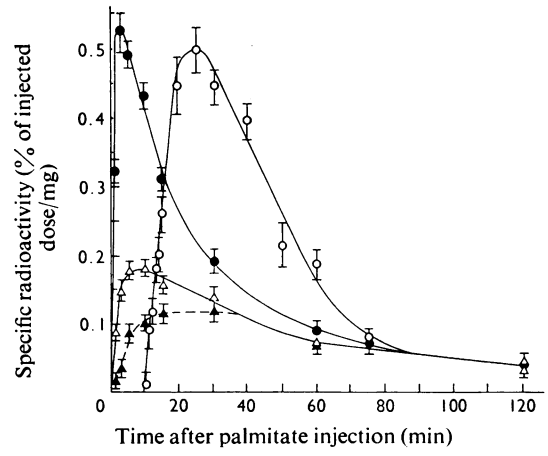

Fig. 2. *Specific radioactivity of triacylglycerol in liver and serum after [U-^{14}C]palmitate injection*

Results are expressed as specific radioactivity (mean±S.E.M.) (as percentage of injected dose/mg of triacylglycerol) in serum (○), cytosolic lipid (▲) microsomal fraction (●) and total homogenate (△).

Gas-chromatographic analysis of triacylglycerol fatty acid methyl esters from microsomal and floating fat in fed and starved rats

The specific radioactivity of triacylglycerol in the microsomal fraction was higher than that in floating fat during the first 25min (Fig. 2). This difference is to be expected when a small precursor pool donates tracer to a large recipient pool. It is also possible that microsomal triacylglycerol may contain a greater proportion of palmitate relative to the other fatty acids than does the triacylglycerol in the floating-fat pool. We were unable to find data in the literature regarding the fatty acid composition of triacylglycerol in these two fractions. Therefore the fatty acid composition of triacylglycerol was determined in each.

Livers from six fed and six 48h-starved rats were removed, and microsomal and floating-fat triacylglycerol fatty acid methyl esters were obtained as described in the Experimental section. When the samples were analysed by gas chromatography, there were no significant differences between microsomal and floating-fat triacylglycerol fatty acids. There was, however, a significant difference between fed and 48h-starved animals, as Table 3 shows. Although the percentage of palmitic acid in each was nearly the same, the fed animals had more palmitoleic ($C_{16:1}$). The starved animals had relatively less oleic ($C_{18:1}$), more linoleic ($C_{18:2}$) and also more arachidonic ($C_{20:4}$) than did the fed animals. Such differences probably reflect differences in dietary and adipose-tissue fatty acid composition. These differences might also have been reflected in floating-fat and

Table 3. *Fatty acid composition of floating fat and microsomal triacylglycerol*

Fatty acids were isolated and analysed as described in the Experimental section. An unidentified peak, designated '?' appeared in all experimental samples, but did not correspond to any of our standard peaks. The fed animals each weighed approx. 230g on the day of the experiment and the starved animals each weighed 200g. Values given below are means ± S.E.M. and the numbers of experimental animals used are given in parentheses. *P (fed versus starved) < 0.001.

Fatty acid	Composition (% of total) Fed animals	Starved animals
$C_{14:0}$	0.63 ± 0.08 (6)	0.37 ± 0.03 (6)
?	0.32 ± 0.07 (6)	0.12 ± 0.05 (6)
$C_{16:0}$	30.76 ± 0.33 (5)	30.00 ± 0.91 (5)
$C_{16:1}$	5.29 ± 0.20 (5)	0.55 ± 0.55* (6)
$C_{16:2}$	0.46 ± 0.31 (6)	0.21 ± 0.16 (6)
$C_{16:3}$	0.28 ± 0.06 (6)	0.39 ± 0.15 (6)
$C_{18:0}$	1.98 ± 0.18 (6)	3.55 ± 0.19* (6)
$C_{18:1}$	34.62 ± 0.35 (5)	27.62 ± 0.61* (6)
$C_{18:2}$	24.27 ± 0.26 (5)	33.35 ± 0.74* (6)
$C_{18:3}$	0.17 ± 0.11 (6)	0.39 ± 0.10 (6)
$C_{20:4}$	0.10 ± 0.06 (6)	3.75 ± 0.37* (6)

microsomal triacylglycerol if a shorter period of food deprivation had been examined, since these two pools appear to turn over at different rates.

Calculation of irreversible disposal rate of triacylglycerol from serum

In our working model (Fig. 3) radioactively labelled triacylglycerol molecules that have entered the serum compartment have two possible fates. (1) They may return to liver, or (2) they may be taken up by extrahepatic tissues and not returned to serum over the course of the experiment. The second movement is termed the irreversible disposal rate of serum triacylglycerol, or F_{os}. The rate constant k_{os} may be calculated from the percentage of injected dose of palmitate that reaches serum triacylglycerol divided by the integral of the serum triacylglycerol equation for percentage of dose versus time (Shipley & Clark, 1972). The latter equation is given in Fig. 4. A maximum estimate of the percentage injected dose that will reach serum triacylglycerol is the percentage rapidly converted into liver triacylglycerol from serum palmitate and held there for 10min before loss occurs. This is 4.3% of dose (Fig. 1). Since this

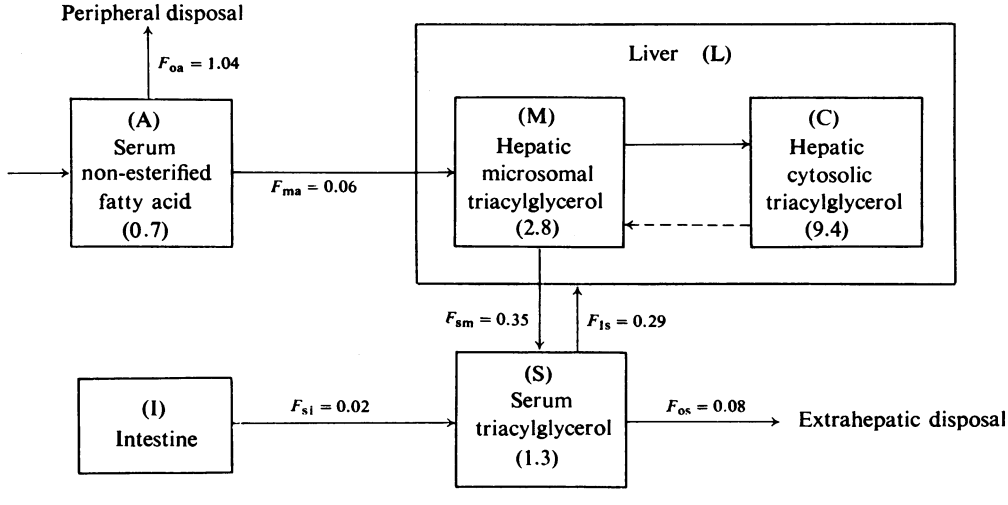

Fig. 3. *Rates for triacylglycerol turnover*

The numbers within the compartments are pool sizes expressed as mg/100g body wt., and F values with attending subscripts are rates expressed as mg/min per 100g body wt. The serum pool was determined by direct measurement. The sizes of the liver pools were estimated as follows. Total liver triacylglycerol = 4.34m/g of liver (Table 1) × 2.8g of liver/100g body wt. (Abrams & Cooper, 1976) = 12.2g/100g body wt. Microsomal triacylglycerol is 17% of that (Table 1), but this must be corrected for incomplete recovery when the nuclear and mitochondrial pellets are not washed. Microsomal recovery was estimated by measuring glucose 6-phosphatase activity (Hers *et al.*, 1951) and the value was 74 ± 9% (n = 6). Segal & Washko (1959) reported 75% with a similar fractionation procedure. The corrected microsomal value is 22.7% or 2.8mg. The remainder, 9.4mg, is attributed to the cytosolic pool.

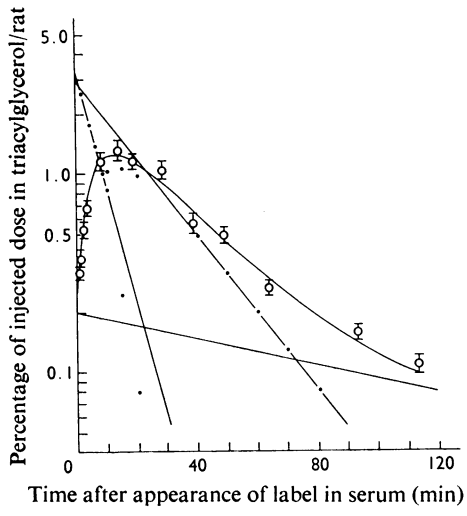

Fig. 4. *Appearance in serum of [U-^{14}C]palmitate in triacylglycerol*

Data are plotted semilogarithmically as percentage of injected dose in triacylglycerol/rat (mean±S.E.M.) at different times after palmitate injection. The solid curve was obtained by graphical analysis of the data and represents the closest fit to a triple exponential function. The 'dots' are the values obtained by subtraction from the curve. The curve drawn is represented by the equation:

$$q_s = -3.2e^{-0.13t} + 3.0e^{-0.045t} + 0.2e^{-0.008t}$$

The time of onset as estimated by the point at which coefficients added to zero was approx. 11.2min.

value does not change significantly during the interval of 2–10min after injection (Fig. 1), it may be assumed that overestimation caused by diversion to other pathways, such as oxidation, is probably small.

$$k_{os} = 4.3 \Big/ \int_0^\infty q_s dt$$

$$= 4.3 \Big/ \left(\frac{-3.2}{0.13} + \frac{3.0}{0.045} + \frac{0.2}{0.008}\right) = 0.064$$

The integral in the denominator also was determined by planimetric measurement of the subtended area under the smoothed curve from onset to 120min. To this was added a residual area under the tail from 120min to infinity as calculated by the equation in Fig. 4. These two values were 56.4 and 10.8 respectively (total 67.2). This is the same total area as calculated by the foregoing integral. Since the size of the pool of serum triacylglycerol is 1.3mg/100g body wt., the rate of irreversible disposal will be:

$F_{os} = (1.3)(0.064) = 0.08$ mg/min per 100g body wt.

Intestinal contribution to serum triacylglycerol

This rate was not measured directly in these experiments. It may, however, be estimated from literature values that indicate that intestine contributes about 0.02mg/min per 100g body wt. to serum triacylglycerol turnover in the starved rat (Baxter, 1966; Windmueller & Levy, 1968; Ockner *et al.*, 1969; Mistlis & Ockner, 1972).

Estimation of rate of movement of triacylglycerol from liver to serum

Examination of the model in Fig. 3 shows that serum triacylglycerol has the two channels of input, F_{sm} and F_{si}, and two routes of exit, F_{os} and F_{ls}. Three of these are known. A value of 0.08 for F_{os} is provided by the present study, and F_{si} is known from other published work to be 0.02. A rate of 0.29 for F_{ls}, the movement to liver from serum, has been determined by Lipkin *et al.* (1978). Thus in steady state, F_{sm}, the unknown rate from hepatic microsomal fractions to serum is calculable.

$$F_{sm} = F_{os} + F_{ls} - F_{si}, \text{ or } 0.08 + 0.29 - 0.02 = 0.35$$

All rates are mg of triacylglycerol/min per 100g body wt.

The zero-time derivative of the equation for activity in serum would give k_{sm} directly if certain conditions were met. The first of these is that a pulse dose of a known quantity of tracer be delivered to microsomal fractions to serve jointly thereafter for input to both serum and cytosolic droplets. Although a peak quantity of 2.9% of dose appears in microsomal fractions within 3min (Fig. 1), another 0.6% is found also in cytosol by this time. Thus there is not a known 'instantaneous' quantity in the labelled precursor pool before loss begins. Another assumption required by theory is that tracer reaches serum, not through a mixed intermediary compartment, but by movement of a series of sequentially labelled 'packets' carrying triacylglycerol to sites of release on the cell membrane, and, after about 10min in transit, being released in a temporal pattern that mimics undelayed discharge from a mixed microsomal pool. Whether this assumption is tenable is uncertain. A final problem is related to the early upslope of the serum curve. It is ambiguous to the extent that no firm position can be established for an adjusted zero time for onset of rise. This can be estimated indirectly by mathematical extrapolation of a curve fitted to observed points, but such an approach gives mathematical solutions that differ considerably and is much less satisfactory than having an independently fixed zero time to anchor the starting point of the curve.

Hepatic triacylglycerol release measured by use of Triton

The rate of release of hepatic triacylglycerol was also estimated by use of Triton WR-1339. The rate was measured in control animals and also in animals whose hepatic triacylglycerol metabolism was altered

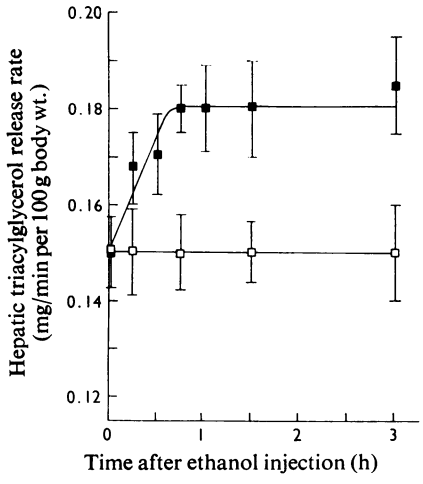

Fig. 5. *Hepatic triacylglycerol release rate at different times after ethanol injection in control and ethanol-treated rats*
Triton was administered at the indicated times after ethanol injection (2.1g/kg). Triacylglycerol release rates were determined as described in the text. Each data point represents a mean±S.E.M. for at least 20 experimental animals. □, Control; ■, ethanol-treated.

by the acute administration of ethanol (2.1g/kg). The reason for studying both types of animals was that Abrams & Cooper (1976) have shown that 45min after administration of this dose of ethanol the hepatic release rate, as measured with Triton, was increased slightly from 0.15 to 0.18mg of triacylglycerol/min per 100g body wt. We decided to examine the time course of this effect to learn whether the rate ever increased to the value of 0.35 described above. The results, shown in Fig. 5, demonstrate that the small effect of ethanol is developed over a 45min period and remains constant between 45min and 3h and at no time does it reach significantly higher values. Since Triton may be interfering with the release of triacylglycerol, the significance of the small increase observed after ethanol administration is uncertain.

Discussion

By consideration of only the hepatic triacylglycerol precursor pool and serum triacylglycerol product pool, a relatively simple two-pool model was used in the present study to calculate the hepatic triacylglycerol release rate by use of a pulse injection of [U-^{14}C]palmitate.

Fig. 3 summarizes the rates calculated in the present study together with some obtained from the literature. The value of 0.06mg of triacylglycerol/min per 100g body wt. for F_{ma} obtained by Abrams & Cooper (1976) is rather closely confirmed by combining their rate constant of total fatty acid disposal from serum (1.59min^{-1}) with the fraction of dose disposed to liver in the present study (0.043). Thus (1.59)(0.043) = 0.068 for k_{ma} and (0.067)·(0.7) = 0.05 for F_{ma}. Another reassuring finding is that the rate of irreversible disposal of serum triacylglycerol is 0.08mg/min, as it was when triacylglycerol was labelled by a procedure *in vitro*. This strongly suggests that the overall kinetic behaviour of triacylglycerol is the same with the two types of labelling.

The rate of release of hepatic triacylglycerol in 48h-starved rats was 0.15mg of triacylglycerol/min per 100g body wt. when measured by the use of Triton and 0.35 when measured by the tracer procedure. There are several possible explanations for the discrepancies between these results. The use of Triton requires the assumption that this substance has no effect on the release rate of hepatic triacylglycerol. Kissebah *et al.* (1974) found, by use of commercial preparations of lipids injected intravenously, that an increase in serum triacylglycerol concentration retards the release of hepatic triacylglycerol. It is therefore possible that the Triton-induced increase in serum triacylglycerol concentration may affect the release rate in a similar manner. Further, Triton has been shown to have effects on certain enzymes involved in lipid metabolism. It inhibits the esterification of cholesterol in plasma *in vitro* (Klauda & Zilversmit, 1974) and enhances the activity of hydroxymethylglutaryl-CoA reductase (Goldfarb, 1975). It is thus possible that it may affect enzymes important in either hepatic triacylglycerol synthesis or release. Finally, at best, Triton could only provide an estimate of net movement of triacylglycerol into serum. In the preceding paper Lipkin *et al.* (1978) showed that about 75% of the total turnover of serum triacylglycerol involves triacylglycerol returning to liver. The recycled component of hepatic release would not be reflected in the rate estimated with Triton because the calculation depends on net build-up in serum.

We greatly appreciate the technical assistance of Ksenija Dimitrov and Rebecca Coapman. This work was supported by grants from the Northeast Ohio Chapter American Heart Association, the Department of Health of Ohio, and a training grant (GM-00035) from the National Institutes of Health. Some of this work forms part of a thesis presented by J. F. P. in partial fulfillment of the requirements for the Ph.D. degree at Case Western Reserve University.

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