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Metabolism of Palmitate in Perfused Rat Liver

ISOLATION OF SUBCELLULAR FRACTIONS CONTAINING TRIACYLGLYCEROL

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1. The metabolism of [1-^{14}C]palmitate in rat liver was studied in a single-pass perfusion system at concentrations of 0.2 or 1 mM. 2. After the perfusion the liver was homogenized and the floating fat was isolated. The incorporation of [1-^{14}C]palmitate into triacylglycerol in this pool increased 9-fold when the palmitate concentration in the medium was increased from 0.2 to 1 mM. In time studies with 1 mM-[1-^{14}C]palmitate 75% of the total accumulation of triacylglycerol occurred in this pool. Our results support the concept that the floating-fat fraction contains the storage pool of triacylglycerol, i.e. the cytoplasmic lipid droplets. 3. In a particulate preparation consisting mainly of mitochondria and microsomal fraction the incorporation of [1-^{14}C]palmitate into triacylglycerol was proportional to the fatty acid concentration. Triacylglycerol in the perfusate medium and in the particulate fraction was in isotopic equilibrium, which indicates that the particulate fraction contained the precursor pool for secreted triacylglycerol, i.e. the pool in endoplasmic reticulum and Golgi apparatus. 4. The oxidation to labelled water-soluble products and to CO_2 was increased 14-fold by the 5-fold increase in palmitate concentration.

Several investigators have described the existence of different hepatic pools of triacylglycerol. Some workers have calculated the flux of fatty acids between these pools (Nikkilä *et al.*, 1966; Haude *et al.*, 1972). The existence of the pools, however, was suggested solely on the basis of computer calculations on isotope studies with intact rats. Other workers have separated triacylglycerol in floating fat from microsomal triacylglycerol and have described the time-dependence of the specific radioactivity of these pools after pulse labelling (Stein & Shapiro, 1959; Glaumann *et al.*, 1975). These studies, however, were not planned to calculate the flux of fatty acids between the pools.

The present study reports an approach to the quantitative isolation of two major triacylglycerol pools from rat liver; the study deals with the separation, purity and function of the two pools, investigated by 20min perfusion with 0.2mM- or 1mM-[1-^{14}C]palmitate, and by perfusion with 1mM-[1-^{14}C]palmitate for 90min. In a subsequent paper the data are used for evaluating possible precursor–product relationships between these pools by means of computer models.

The single-pass perfusion system is advantageous compared with the recirculating system, because the composition of the medium entering the liver is well-defined and constant with time, which facilitates the interpretation of the results.

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Experimental

Animals

Female Wistar rats of our own breed weighing 165–175g were used throughout. The animals had free access to water and chow, containing (in terms of energy) 11% fat, 16% protein and 73% carbohydrate until 07:00h on the day of experiment and thereafter to water only. The start of the experiment was at 13:00h. The animals used for perfusion were anaesthetized by intraperitoneal injection of 20mg of sodium pentothal. The animals from which the non-perfused livers were taken were killed by a blow on the neck, the livers were quickly excised and otherwise treated like the perfused livers.

Materials

[1-^{14}C]Palmitate was from The Radiochemical Centre, Amersham, Bucks., U.K. T.l.c. showed that 98–99% of the radioactivity was fatty acids and 0.2–0.4% was triacylglycerol and phospholipid; the [1-^{14}C]palmitate was used without further purification. Albumin (fraction V from bovine plasma) was purchased from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. Activated untreated charcoal was Norit A from Sigma Chemical Co., St. Louis, MO, U.S.A. Silica gel G was from Merck, Darmstadt, Germany. Glycerol 3-phosphate dehydrogenase and glycerol kinase used for determination of glycerol in acylglycerols were from C. F. Boehringer and Soehne G.m.b.h., Mannheim, Germany.

Perfusion technique

Albumin was purified to remove fatty acids by the method of Chen (1967). The perfusion medium consisted of Krebs–Ringer bicarbonate buffer that contained washed bovine erythrocytes (haematocrit 0.30), heparin (20000 units/litre) and albumin (0.35mM) as described by Sestoft (1974). The palmitate–albumin complex was prepared as follows. On the day before use the albumin was dissolved in one-third of the buffer to be used, at a concentration of 1.05mM; the solution was kept for 2h at 37°C and left overnight at 5°C. On the day of the experiment the solution was centrifuged twice at 1.6×10^6*g*-min at $r_{av.}$ 7cm and 25°C to precipitate undissolved material and then heated to 37°C. Under magnetic stirring a heated (60°C) 200mM solution of palmitate in 0.4M-KOH was added in small volume and the final clear solution was added to the other part of the perfusate. The perfusion medium was aerated with O_2/CO_2 (19:1, v/v). The saturation of haemoglobin with O_2 in the perfusion medium was about 98–100%. The perfusions were carried out at 37°C. The operation technique and perfusion chamber was the same as used by Sestoft (1974). A double cannula was used for cannulating the portal vein, whereby the portal flow was stopped for a few seconds only. The duration of the operation ranged from 11 to 14min. The flow was kept constant at 5ml/min per liver and the perfusion pressure was about 7cm of water. The preparation was discarded if the pressure rose above 12cm of water.

Experiments were started with a 26min equilibration period when the livers were perfused with the desired palmitate concentration, whereupon the perfusion was continued with another identical medium, but containing [1-^{14}C]palmitate. [1-^{14}C]Palmitate was mixed with unlabelled palmitate before complexing to albumin.

The perfusate was precipitated in a glass bottle that contained 0.2M-$HClO_4$ and 0.1M-citrate buffer, pH3, in a volume equal to the amount of perfusate medium. CO_2, made to escape by magnetic stirring and suction, was trapped in three consecutive bubbling flasks containing 120mM-ethanolamine, total volume 400ml. The CO_2 collection was continued for 2h after the end of the perfusion. As a test of recovery, $^{14}CO_2$ as $NaH^{14}CO_3$ was added to a glass bottle containing the precipitated perfusate from the equilibration period only. The added radioactivity was recovered in the bubbling flasks at a yield of 90–95%.

Isolation of subcellular fractions

At the end of the experiment a lobe of the liver was removed without stopping the perfusion. The subcellular fractions of hepatic lipids were separated by the following procedure (at 0–5°C). The lobe was rinsed three times with the homogenization medium (0.15M-KCl in 10mM-potassium phosphate buffer, pH7.4). The lobe was weighed and approx. 2g was transferred to a beaker containing 5ml of homogenization medium and cut with scissors. The preparation was homogenized in a Potter–Elvehjem homogenizer by five strokes of a loosely fitting pestle (difference in diameter approx. 0.3mm; diameter of pestle 18mm); then 15ml of medium was added and the liver was further homogenized by ten strokes at 600rev./min.

This homogenate was centrifuged in a Sorvall RC 2-B centrifuge [200*g* for 5min at 1000*g*-min ($r_{av.}$ = 7cm)] at 4°C, and the supernatant was decanted into another centrifuge tube. The floating fat appearing during the centrifugation was immersed in the supernatant by a glass spatula before decanting. The pellet was rehomogenized and recentrifuged three times, and each time 6ml of medium was added. After the first rehomogenization the pestle was changed to a more tightly fitting one, the difference in diameter being about 0.08mm and the diameter of the pestle was 15mm. Each time 15 strokes were applied at a speed of 600rev./min.

The supernatants were combined, overlayered with 6ml of diethyl ether and centrifuged in the same centrifuge to separate floating fat from fat in the particulate fraction, known to consist mainly of mitochondria and microsomal fraction. The rotor was operated for 100min at 4°C and 40000*g* (4×10^6*g*-min).

After the centrifugation, the diethyl ether fraction with the dissolved floating fat was removed by a pipette and the supernatant was decanted. The 1000*g*-min and the 4×10^6*g*-min pellets were precipitated with 200μl of 0.7M-$HClO_4$ and 100μl of 1M-citrate buffer, pH3. A pH value of approx. 3 was thus obtained in the acidified pellets. These pellets will be referred to below as the low-speed and high-speed pellets respectively. Samples (2ml) of the high-speed supernatant were precipitated with 100μl each of 0.7M-$HClO_4$ and 1M-citrate buffer, pH3. Samples (2ml) of the perfusion medium were precipitated in 500μl of 0.7M-$HClO_4$ and 100μl of 1M-citrate buffer, pH3. The experimental basis for the procedure is described below.

Extraction of lipid

Lipid was extracted at 5°C from samples of the precipitated perfusion and perfusate media, from the low- and high-speed pellets and from precipitated samples of the supernatant by the procedure described by Hajra *et al.* (1968), except that a higher pH was employed (see below). The extraction of lipid from the high-speed pellet yielded 98–99% of the total lipid present in the pellet. The lipid extracts were evaporated in a stream of N_2 and washed by the

method of Folch *et al.* (1957). The lipid dissolved in diethyl ether was dried in a stream of N_2 and submitted to the washing procedure only.

Separate samples were used for determination of radioactivity in water-soluble products of palmitate metabolism. Acidified samples (2ml) of supernatant and perfusate were washed twice with 10ml of water-saturated chloroform and 500μl were taken for determination of radioactivity.

Analytical techniques

For separation of lipid classes the t.l.c. system described by Breckenridge & Kuksis (1968) was used. The bands, made visible by I_2 vapour, were scraped off into small beakers, the I_2 was evaporated at 110°C and the silica gel was transferred to counting vials. The band containing phospholipid was bleached overnight in the vial with 25μl of H_2O_2 (40%) in 1ml of 96% ethanol. H_2O_2 and ethanol were evaporated off before counting for radioactivity. The radioactivity applied was recovered in phospholipid, diacylglycerol, non-esterified fatty acids and triacylglycerol with a recovery of 96±6% (mean±S.D., $n = 15$).

When individual neutral acylglycerols were determined chemically, the silica gel containing mono-, di- or tri-acylglycerol respectively was extracted three times with 4ml of Folch's lower phase for 1h at 70°C. By this procedure 87–102% of the radioactive isotope was recovered in solution.

Acylglycerol was hydrolysed by the method of Eggstein & Kuhlmann (1970) in 0.5M-KOH in 85% (v/v) ethanol. After addition of 2vol. of 0.15M-$MgSO_4$, glycerol was determined by the method of Wieland (1962) in the clear supernatant after centrifugation. Phospholipid was determined as P_i by the method of Bartlett (1959) as modified by Kates (1972).

O_2 uptake was calculated from the difference in saturation of haemoglobin in the perfusion and perfusate media as described by Sestoft (1974).

Radioactivity was counted for 10min in a model 2002 Packard Tri-Carb scintillation spectrometer in the scintillation liquids devised by Anderson & McClure (1973). The counting efficiency was 60%, as determined with an external standard. Water-soluble products and ethanolamine containing $^{14}CO_2$ was counted for radioactivity in the same liquid that contained in addition 25% Triton X-114.

Calculations

The specific radioactivity of palmitate in the perfusion medium was calculated from the known addition of unlabelled palmitate and from the amount of radioactivity present in non-esterified fatty acids after t.l.c. of the lipid extract from the perfusion medium. This calculation was permitted by the fact that purified albumin contains negligible amounts of fatty acids (Chen, 1967). The specific radioactivity was about 150000d.p.m./μmol of palmitate in experiments with 1mM-palmitate and about 750000d.p.m./μmol in experiments with 0.2mM-palmitate. The uptake of palmitate was calculated from the difference in radioactivity present in non-esterified fatty acids in the perfusion and perfusate media. The radioactivity recovered in the various fractions is expressed as μmol or nmol of palmitate incorporated. No quench corrections were necessary.

To allow for possible changes in water content of the single liver during perfusion the radioactive isotope results are expressed per liver. The post-perfusion weight of the liver perfused with 0.2mM-palmitate was 6.45±0.59g (mean±S.D., $n = 3$), and with 1mM-palmitate was 6.75±0.64g (mean±S.D., $n = 6$). Non-perfused liver from animals of the same weight weighed 7.00±0.78g (mean±S.D., $n = 6$).

For evaluation of statistical significance Student's t test was used.

Reliability of the methods

The high-speed pellet was isolated at 4×10^6 or $6\times10^6 g$-min without difference in recovery of radioactive isotope in the fraction. Liberation of more lipid from the pellet was attempted by various means. The pellet was rehomogenized with loosely or tightly fitting pestles or subjected to osmotic shock (Schlunk & Lombardi, 1967) or washed with 0.15M-Tris buffer, pH8 (Glaumann *et al.*, 1975), and recentrifuged. These procedures caused only about 1–2% additional radioactivity to be liberated from the pellet and did not cause significantly more radioactivity to appear in the diethyl ether. Furthermore homogenizing the liver with ten or 20 additional strokes did not affect the recovery in diethyl ether, neither did the use of an even more loosely fitting pestle.

When sucrose was used instead of KCl less radioactivity appeared in the diethyl ether, whereas more was found in the high-speed pellet. However, rehomogenization and recentrifugation of the pellet isolated in sucrose caused the remaining radioactivity to appear in the diethyl ether.

The recovery of the radioactive isotope, expressed as a percentage of the perfusion medium−perfusate medium difference, was 104±12% (mean±S.D., $n = 18$), the range being 87–117%. In six experiments the fractions obtained from one half of the homogenate were kept at −20°C for 6–8 weeks before extraction, whereas the fractions from the other half were extracted and chromatographed as soon as possible. The reproducibility for extraction and t.l.c. of the major lipid fractions, i.e. triacylglycerol in the diethyl ether, triacylglycerol, diacylglycerol and phospholipid in the high-speed pellet was 94±1% (mean±S.D.; $n=24$).

The radioactivity present in lipid in the combined supernatants, after removal of the low-speed pellet, was recovered in lipid in diethyl ether, high-speed pellet and supernatant at a yield of 98–100%. When compared with radioactivity in lipids extracted from a frozen biopsy sample the described procedure gave a recovery of 102%.

When the extraction of palmitate from the perfusion medium was performed at pH< 1, as devised by Hajra *et al.* (1968), 50–80% of the radioactive label was found to migrate with triacylglycerol in t.l.c. This was not found when the precipitated perfusion medium was extracted with diethyl ether or with chloroform/methanol at pH 3. It is likely that at the low pH palmitate would form methyl esters with the methanol present and it was confirmed that methyl esters of fatty acids migrated as triacylglycerol in this system. When a pH value of 3 was used, 99% of the extracted radioactivity migrated as non-esterified fatty acids, 0.2–0.3% migrated with phospholipids and 0.6–0.8% migrated with triacylglycerol. These values were identical after 40 and 80 min incubation of palmitate in the perfusion medium, indicating that no metabolism of palmitate took place in the medium during the perfusion period.

Results

Variation of the concentration of palmitate

The metabolism of [1-^{14}C]palmitate was investigated at concentrations of 0.2 and 1 mM, which cover the physiological range in rat serum (Johnson, 1974; Fex & Olivecrona, 1969).

Table 1 shows the content of glycerolipids in the subcellular fractions from non-perfused liver and from liver perfused with 0.2 or 1 mM-palmitate for 47 min, including the 26 min period before the [1-^{14}C]palmitate was introduced.

About 80% of hepatic phospholipid was present in the high-speed pellet. The phospholipid content of the low-speed pellet was higher in the non-perfused liver, probably because of the higher blood content.

The low-speed pellet contained about 10% and the high-speed pellet about 40% of total acylglycerol. In perfused liver di- and tri-acylglycerol accounted for 80–95% of total acylglycerol in the high-speed pellet as opposed to 50% in non-perfused liver. The content of monoacylglycerol in the high-speed pellet from non-perfused liver was 0.22 ± 0.10 (mean ± S.D., $n = 6$) μmol/g wet wt. compared with 0.14 ± 0.05 (mean ± S.D.; $n = 3$) μmol/g wet wt. in perfused liver. The alkali-labile acylglycerol not accounted for as mono-, di- or tri-acylglycerol in non-perfused liver was found in the phospholipid band after t.l.c. No further characterization of this compound was attempted.

The amount of acylglycerol recovered in diethyl ether accounted for about 50% of total acylglycerol. Diacylglycerol accounted for 0.3–4% of total acylglycerol in this pool and the remainder was triacylglycerol.

Table 1. *Contents of phospholipid and acylglycerol in subcellular fractions isolated from perfused or non-perfused liver of fed female rats*

The subcellular fractions were isolated as described in the text. The high-speed pellet consisted mainly of mitochondria and microsomal fraction. The diethyl ether fraction is suggested to contain lipid from cytoplasmic lipid droplets. The livers were perfused for 47 min; experimental details are given in the text. The results are expressed as means ± S.D. The values in parentheses refer to numbers of observations.

	Contents (μmol/g wet wt.)					
	Non-perfused (6)		Perfused with 0.2 mM-palmitate (3)		Perfused with 1 mM-palmitate (6)	
	Phospholipid	Acylglycerol	Phospholipid	Acylglycerol	Phospholipid	Acylglycerol
Low-speed pellet	10.79 ± 0.80	0.83 ± 0.32	7.70 ± 0.90*	0.68 ± 0.28	5.69 ± 0.93*‖	0.67 ± 0.11
High-speed pellet	32.60 ± 1.78		29.61 ± 4.84		31.70 ± 3.50	
Total acylglycerol		3.04 ± 0.25		2.75 ± 0.35		4.52 ± 0.70§¶
Diacylglycerol		0.45 ± 0.14		0.92 ± 0.13†		0.75 ± 0.16†
Triacylglycerol		1.10 ± 0.36		1.73 ± 0.28‡		2.84 ± 0.38*¶
Diethyl ether fraction	0.18 ± 0.05	4.51 ± 1.18	0.22 ± 0.04	4.24 ± 1.21	0.22 ± 0.08	5.50 ± 1.36

* P<0.001 compared with value for non-perfused.
† P<0.005 compared with value for non-perfused.
‡ P<0.05 compared with value for non-perfused.
§ P<0.02 compared with value for non-perfused.
‖ P<0.05 compared with value for 0.2 mM-palmitate perfusion.
¶ P<0.005 compared with value for 0.2 mM-palmitate perfusion.

Table 2. *Metabolism of exogenous [1-^{14}C]palmitate in perfused liver from female rats*

The experiments are the same as those presented in Table 1. The radioactive label was introduced after an equilibration period of 26min and its metabolism was followed for 21 min. See the text for experimental details. The results are expressed as μmol/min per liver and they are given as means ± S.D. for three experiments with 0.2mM-palmitate and for six experiments with 1mM-palmitate.

	Rate (μmol/min per liver)	
	Perfusion with 0.2mM-palmitate	Perfusion with 1mM-palmitate
Uptake of [1-^{14}C]palmitate	0.74 ± 0.11	3.42 ± 0.29
Total esterification	0.68 ± 0.12	2.71 ± 0.26
Total oxidation	0.05 ± 0.02	0.69 ± 0.12
Water-soluble products in the liver	0.015 ± 0.004	0.163 ± 0.045
Water-soluble products in the perfusate	0.013 ± 0.007	0.335 ± 0.091
CO_2 in the perfusate	0.021 ± 0.007	0.142 ± 0.042

	Perfusion with 0.2mM-palmitate			Perfusion with 1mM-palmitate		
	Phospholipid	Diacylglycerol	Triacylglycerol	Phospholipid	Diacylglycerol	Triacylglycerol
Low-speed pellet	0.033 ± 0.007	0.008 ± 0.003	0.013 ± 0.004	0.055 ± 0.015	0.018 ± 0.005	0.046 ± 0.005
High-speed pellet	0.157 ± 0.045	0.081 ± 0.028	0.209 ± 0.031	0.352 ± 0.082	0.197 ± 0.050	0.849 ± 0.133
Diethyl ether fraction	0.005 ± 0.005	0.010 ± 0.004	0.099 ± 0.043	0.007 ± 0.002	0.034 ± 0.004	0.870 ± 0.182

Perfusion with 1mM-palmitate caused acylglycerol to accumulate in the high-speed pellet, which was reflected by an increase in di- and triacylglycerol. The increase in the triacylglycerol content of the diethyl ether fraction was not statistically significant. Table 2 shows that the uptake of [1-^{14}C]-palmitate increased almost in parallel with the fatty acid concentration, the fractional uptake being about 70%.

Total esterification (Table 2) is a summation of the incorporation into lipids in all fractions (after t.l.c. less than 4% of total lipid radioactivity was found in non-glycerolipid fractions). Total oxidation is the sum of metabolism to labelled water-soluble products in the liver and in the perfusate medium and to $^{14}CO_2$ in the perfusate medium. The $^{14}CO_2$ remaining in the liver was considered negligible. Total oxidation increased 14 times by the 5-fold increase in fatty acid concentration and accounted for 7% of the palmitate uptake at the low concentration compared with 19% at the high concentration. The increase was most pronounced in water-soluble products in the perfusate medium. The proportion of palmitate recovered as $^{14}CO_2$ was 3% at the low and 4% at the high concentration.

In Table 2 is also presented the rate of incorporation of palmitate into various lipid classes. The values shown comprise 90% of the 'total esterification', the remaining radioactivity being present in the supernatant (1–2%), in non-glycerolipids (<4%) and in loss during chromatography (about 4%).

Incorporation of palmitate into triacylglycerol in the high-speed pellet increased almost in parallel with the increased uptake, whereas incorporation into triacylglycerol recovered in the diethyl ether fraction was increased nearly 9-fold. The esterification to triacylglycerol in all fractions was 321 nmol/min per liver at 0.2mM-palmitate and 1765nmol/min per liver at 1mM. The chemically estimated difference in triacylglycerol content between the two groups (Table 1) corresponds to an increased triacylglycerol formation of 1128nmol of fatty acids/min per liver in the 1mM group. This is 80% of the observed increase in incorporation of radioactivity.

The O_2 uptake at 0.2mM-palmitate was 2.02 ± 0.61 μmol/min per g wet wt. ($n = 4$) compared with 2.83 ± 0.20 μmol/min per g wet wt. at 1mM ($n = 6$, $P < 0.02$). On the basis of the assumption that each mol of palmitate oxidized to CO_2 requires 23mol of O_2 and that each mol of palmitate oxidized to acetoacetate requires 7mol of O_2 it was calculated that the observed increase in oxidation of [1-^{14}C]palmitate to CO_2 and water-soluble products would require additionally 0.89 μmol of O_2/min per g wet wt., which is very close to the observed increase in O_2 uptake of 0.81 μmol/min per g wet wt.

Studies of time-dependence

Experiments were performed to study the time-dependence of palmitate metabolism in the two major fractions and to allow calculations of the fatty acid flow in liver accumulating triacylglycerol (Figs. 1–5). These experiments were all done with 1mM-palmitate. Three livers were perfused for 10min with [1-^{14}C]palmitate after the equilibration period, three livers were perfused for 60min and three livers were perfused for 90min. The six 21min perfusions

with 1mM-palmitate shown in Table 1 were also included in the time study. The rates presented below are the slopes from analysis of linear regression performed with the experimental data without time-point zero. The rates were calculated from 15 individual experiments.

The mean rate of uptake of [1-^{14}C]palmitate was 3.21 μmol/min per liver, the rate of total esterification was 2.83 μmol/min per liver and the rate of total oxidation was 0.53 μmol/min per liver. These rates are similar to those given in Table 1 and appeared constant with time.

The rates of incorporation of [1-^{14}C]palmitate into phospholipid, diacylglycerol and triacylglycerol in the high-speed pellet were 290, 66 and 850nmol/min per liver respectively. The rates of incorporation into phospholipid and triacylglycerol were similar to those given in Table 1 and appeared constant with time from time-point zero. The rate of incorporation into diacylglycerol was about one-third of the rate given in Table 1 and it appeared that the rate within the first 10min was considerably higher than that of the rest of the perfusion period. As will be discussed in the following paper (Kondrup *et al.*, 1979*a*) this suggests a fast turnover of diacylglycerol and its precursors.

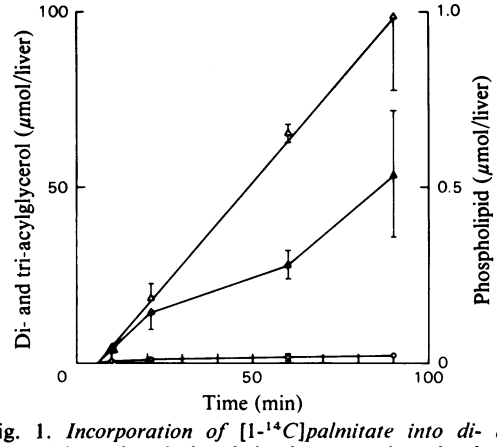

Fig. 1. *Incorporation of* [1-^{14}C]*palmitate into di- and tri-acylglycerol and phospholipid recovered in the diethyl ether fraction*

The livers from fed female rats were perfused with 1mM-palmitate. After a 26min equilibration period [1-^{14}C]palmitate was introduced and the perfusion was continued for the times indicated whereupon hepatic lipid was isolated in subcellular fractions as described in the text. In this and the following Figures the symbols represent the mean for three experiments, except those at 21min that represent the mean for six experiments. The bars indicate ±S.D. Lines not connecting the measured mean values were drawn from a linear regression analysis. Symbols: ○, diacylglycerol; △, triacylglycerol; ▲, phospholipid.

On some occasions the silica gel containing the diacylglycerol was subjected to alkaline hydrolysis and a lipid extract from the acidified hydrolysate was rechromatographed. Of the original activity 90–92% now migrated as non-esterified fatty acids.

Fig. 1 shows the incorporation of [1-^{14}C]palmitate into lipid classes in the diethyl ether fraction. The incorporation into triacylglycerol, diacylglycerol and phospholipid proceeded at rates of 1180, 22 and 6nmol/min per liver respectively. It appears that about 5min elapsed before the radioactive isotope was stored in acylglycerol and phospholipid in this fraction. [1-^{14}C]Palmitate was incorporated into phospholipid, diacylglycerol and triacylglycerol in the low-speed pellet at rates of 68, 13 and 97nmol/min per liver respectively (results not shown).

The oxidation of [1-^{14}C]palmitate is shown in Fig. 2. Water-soluble products accumulated in the liver during the first 20min. [1-^{14}C]Palmitate was converted into labelled water-soluble compounds and into $^{14}CO_2$ in the perfusate medium at rates of 318 and 184nmol/min per liver respectively.

Fig. 3 shows the chemical estimations of acylglycerol in the high-speed pellet. The contents of di- and tri-acylglycerol increased at rates of 30 and 160nmol of glycerol in acylglycerols/min per liver respectively. Compared with the values for [1-^{14}C]palmitate incorporation, 2.2 and 5.3nmol palmitate were incorporated/nmol of glycerol in di- and tri-acylglycerol respectively. The plots were extended (broken lines) to the values measured in non-perfused liver and it appears that neutral acylglycerols accumulated from the beginning of the experiment.

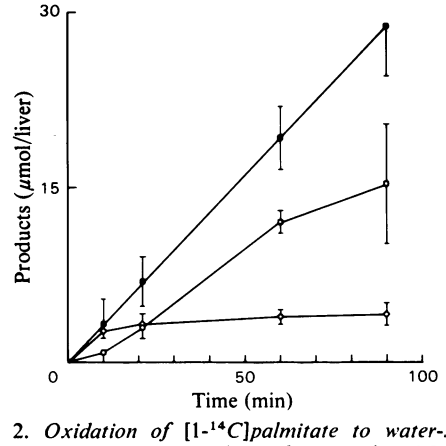

Fig. 2. *Oxidation of* [1-^{14}C]*palmitate to water-soluble products in the liver and in the perfusate and to* CO_2 *in the perfusate*

The experiments are the same as those described in the legend to Fig. 1. Symbols: ◇, water-soluble products in the liver; ■, water-soluble products in the perfusate; □, CO_2 in the perfusate.

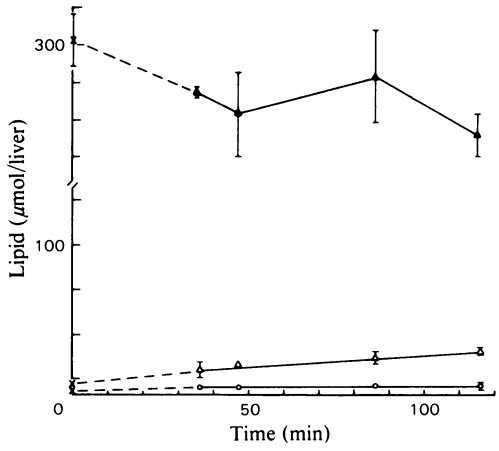

Fig. 3. *Chemical estimations of di- and tri-acylglycerol in the high-speed pellet and of phospholipid in the low- and high-speed pellets*

The experiments are the same as those described in the legend to Fig. 1. The plots were extrapolated (broken lines) to values for non-perfused liver. The time-point zero in the preceding Figures corresponds to 26min in this Figure. Symbols: ○, diacylglycerol; △, triacylglycerol; ▲, phospholipid.

The total content of phospholipid in the high-speed and in the low-speed pellets is shown in Fig. 3. It appears that there was a slight decrease in the total content of phospholipid during the perfusion (180nmol/min per liver). However, the slope was not significantly different from zero. The relative specific radioactivity of phospholipid in the two pellets was similar during the experimental period (results not shown).

Secretion of triacylglycerol into the perfusate medium was estimated chemically in some experiments. The secretion rate was 43 ± 17 (mean $\pm$ S.D., $n = 6$) nmol/min per liver. At 10min after introduction of the radioactive label radioactivity appeared in triacylglycerol in the perfusate medium at a rate of 7 ± 3 (mean $\pm$ S.D.; $n = 6$) nmol of palmitate/min per liver and, 85min after, the mean secretion rate was 208nmol/min per liver ($n = 3$, range 85–410). On the basis of the appearance of radioactivity at 10 and 20min in six experiments the radioactive label seemed delayed 5min in the liver.

Fig. 4 shows the accumulation of triacylglycerol and phospholipid in the fraction recovered in the diethyl ether fraction. Triacylglycerol accumulated at a rate of 530nmol/min per liver. When compared with the accumulation of [1-^{14}C]palmitate it can be calculated that 2.2nmol of [1-^{14}C]palmitate accumulated/nmol of glycerol. Phospholipid accumulated at a rate of 20nmol/min per liver. The plots were extrapolated (broken lines) to the values found in non-perfused liver.

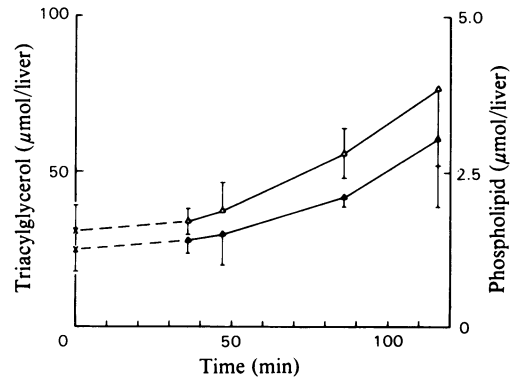

Fig. 4. *Chemical estimations of triacylglycerol and phospholipid recovered in the diethyl ether fraction*

The experiments are the same as those described in the legend to Fig. 1. The plots were extrapolated (broken lines) to values for non-perfused liver. The time-point zero in Fig. 1 corresponds to 26min in this Figure. Symbols: △, triacylglycerol; ▲, phospholipid.

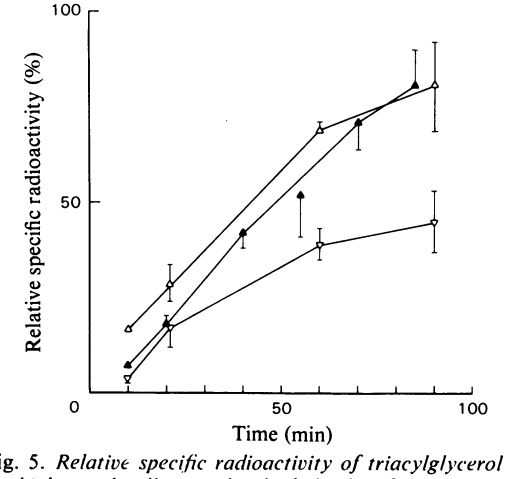

Fig. 5. *Relative specific radioactivity of triacylglycerol in the high-speed pellet, in the diethyl ether fraction and in the perfusate*

The relative specific radioactivities (μmol of [^{14}C]palmitate × 100/μmol of fatty acid) were calculated from the same experiments as presented in Figs. 1–4 (specific radioactivity of exogenous palmitate = 100). Symbols: △, triacylglycerol in the pellet; ▽, triacylglycerol in the diethyl ether fraction; ▲, triacylglycerol in the perfusate.

Fig. 5 shows the relative specific radioactivity of various acylglycerol fractions. The relative specific radioactivity of triacylglycerol in the perfusate medium was estimated in the three 90min perfusions. It followed closely the relative specific radioactivity

of triacylglycerol in the high-speed pellet. The relative specific radioactivity of triacylglycerol in the high-speed pellet increased faster than that of triacylglycerol recovered in the diethyl ether fraction indicating a faster turnover of the former pool.

Discussion

The uptake and esterification of [1-^{14}C]palmitate was largely proportional to the palmitate concentration and to the perfusion time. This is similar to results obtained in a recirculating perfusion system with unlabelled oleate (Heimberg *et al.*, 1969). Therefore the single-pass perfusion system appears suitable for studying hepatic fatty acid metabolism, especially when a well-defined constant composition of the perfusion medium is essential.

The oxidation of exogenous palmitate increased much more than proportionally to the palmitate uptake and the increased oxidation was associated with an increased O_2 consumption. The latter is an unexpected finding since it is generally assumed that O_2 consumption is not regulated by the substrate availability. However, since the contents of adenylates and P_i were not determined a discussion of this finding is omitted.

Triacylglycerol in the endoplasmic reticulum and Golgi apparatus is believed to be the precursor pool for secreted triacylglycerol (Glaumann *et al.*, 1975). Therefore the similarity in specific radioactivity of triacylglycerol in the high-speed pellet and triacylglycerol in the perfusate medium (Fig. 5) suggests that the high-speed pellet mainly contained triacylglycerol from the endoplasmic reticulum and Golgi apparatus. A possible mitochondrial pool of triacylglycerol was either small or followed the same kinetics as did triacylglycerol in endoplasmic reticulum and Golgi apparatus. The similarity also suggests that the high-speed pellet triacylglycerol was not to any large extent contaminated by triacylglycerol from cytoplasmic lipid droplets, since this pool exhibited a much lower specific radioactivity.

The lipid recovered in the diethyl ether fraction is suggested to be dissolved floating fat derived from cytoplasmic lipid droplets. The ratio between the contents of triacylglycerol and phospholipid was similar to the ratio found in floating fat by DiAugustine *et al.* (1973) and likewise we found the fraction to contain little diacylglycerol compared with triacylglycerol (0.2–4%). DiAugustine and his coworkers suggested that the floating-fat layer represented cytoplasmic lipid droplets, mainly because of morphological similarity to the droplets seen in intact liver.

Stein & Shapiro (1959) suggested that the floating-fat layer was a storage pool of triacylglycerol. In accord with this suggestion we found that 4 weeks of ethanol feeding increased the triacylglycerol content of the diethyl ether fraction about 4-fold, whereas the triacylglycerol content of the high-speed pellet was unchanged (Kondrup *et al.*, 1979*b*). The results presented in Figs. 3 and 4 showed that the rate of triacylglycerol accumulation in the diethyl ether fraction was three times higher than that in the high-speed pellet, indicating that the newly formed triacylglycerol was mainly deposited in the pool trapped in diethyl ether. The fractional deposition of [1-^{14}C]palmitate in triacylglycerol in the diethyl ether fractions increased with an increased palmitate concentration: 13% of the palmitate taken up was incorporated into triacylglycerol in the diethyl ether fraction at 0.2mM-palmitate compared with 25% at 1mM-palmitate (Table 2). All these observations agree with the concept of this pool as a storage pool.

In the diethyl ether fraction the chemically determined rate of phospholipid formation relative to triacylglycerol formation was 0.038 (μmol/nmol; Fig. 4), which is similar to the ratio between phospholipid and triacylglycerol in the similar fraction isolated from non-perfused liver (0.040; Table 1). This suggests that the lipid trapped in diethyl ether was not contaminated by membranous lipid. The ratio between phospholipid and triacylglycerol formation would be lower than the ratio found in non-perfused liver if the fraction from the beginning contained membranous phospholipid.

In the high-speed pellet [1-^{14}C]palmitate was incorporated into triacylglycerol in excess of the chemically determined accumulation of triacylglycerol, indicating replacement of pre-existing triacylglycerol by newly formed molecules. The opposite was found in triacylglycerol in the diethyl ether fraction, indicating that pre-existing fatty acids contributed to triacylglycerol formation in this pool. The metabolic interrelationship between these triacylglycerol pools is investigated in the following paper (Kondrup *et al.*, 1979*a*).

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