Selective channelling of arachidonic and linoleic acids into glycerolipids of rat hepatocytes in primary culture

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Rat hepatocytes in primary culture were incubated with a mixture of linoleic and arachidonic acid at various total fatty acid/serum albumin molar ratios. Mixed fatty acids were taken up at the same rate and distributed with the same pattern as fatty acids added separately. The rates of total uptake, incorporation into hepatocyte and secreted triacylglycerols and β-oxidation were linearly related to the fatty acid/albumin ratios, whereas the rate of incorporation into phospholipids was saturable. Neither the uptake rate nor the distribution of both fatty acids considered together varied with the arachidonic acid/linoleic acid molar ratio. Changes in this ratio and in the uptake rate led to significant variations in the respective fate of the fatty acids. The preferential channelling of arachidonic acid versus linoleic acid into β-oxidation and phosphatidylinositol was greatest at a low uptake rate and then decreased as the uptake rose. Conversely, the preferential channelling of arachidonic acid versus linoleic acid into phosphatidylcholine, but not phosphatidylethanolamine, increased with the uptake rate. Moreover, both arachidonic acid and linoleic acid were preferentially incorporated into the 1-palmitoyl molecular species of phosphatidylcholine and phosphatidylethanolamine at a low uptake rate, and of phosphatidylcholine at a high uptake rate. This could be related to the synthesis of biliary phosphatidylcholine, of which 1-palmitoyl-2-linoleoyl and 1-palmitoyl-2-arachidonoyl are the main molecular species. Linoleic and arachidonic acid were selectively distributed into distinct metabolic pools of triacylglycerol, the intrahepatocyte pool which preferentially incorporated linoleic acid at a low uptake rate and the secreted pool in which the relative enrichment of arachidonic acid increased with the uptake rate. This strengthens the central role of hepatic secretion in the supply of arachidonic acid to peripheral tissues.

INTRODUCTION

Essential polyunsaturated fatty acids (PUFA) are used as precursors of lipidic mediators in most mammalian cells (Hammarström, 1983). Studies performed during hepatic diseases (Owen *et al*, 1982; Johnson *et al.*, 1985) and cholestatic diseases (Picard *et al.*, 1972; Dupont *et al.*, 1986) established that the liver plays a central role in providing the organism with PUFA. But little is still known about the mechanisms involved in the hepatic storage and secretion of specific pools of PUFA-containing lipids.

It has been previously shown that PUFA of the $n-6$ series behave like other fatty acids in terms of uptake and triacylglycerol synthesis by rat hepatocytes in primary culture (Lamb *et al.*, 1977; Chambaz *et al.*, 1986). As reported in experiments using perfused liver (Kohout *et al.*, 1971; Weisiger *et al.*, 1981), fatty acid uptake and triacylglycerol synthesis were linearly correlated to the free fatty acid/serum albumin molar ratio, at least through the steady state (i.e. after 14 h of incubation) and whatever the fatty acid tested (Chambaz *et al.*, 1986). Fatty acid incorporation into phospholipids was not related to an increase in the phospholipid content of hepatocytes and therefore must be due to a deacylation–reacylation cycle.

The dependence of this cycle on the type of fatty acid has been questioned by various authors (Holub, 1976; Miki *et al.*, 1977; Hasegawa-Sasaki & Ohno, 1980; Holub, 1980; Chambaz *et al.*, 1986). Though most of this work concerned the specificity of glycerolipid metabolism for PUFA, they could not be predictive of cell behaviour *in vivo*, since Lands *et al.* (1982) have shown that it requires competition studies with a mixture of fatty acids. Whereas we did not evidence any selectivity in triacylglycerol synthesis, we have observed a preferential channelling of arachidonic acid into phospholipids in cultured hepatocytes in the simultaneous presence of arachidonic and linoleic acid, which could not be deduced from experiments carried out with fatty acids added separately (Chambaz *et al.*, 1986).

In the present work, we focused on the selective channelling of arachidonic acid versus linoleic acid into the major pathways of fatty acid metabolism: β-oxidation, phospholipid and triacylglycerol synthesis and secretion. For this purpose, rat hepatocytes in primary culture were incubated for 3 h with arachidonic and linoleic acid at various fatty acid/albumin and arachidonic/linoleic acid molar ratios. We found a preferential channelling of arachidonic acid into phospholipids, especially phosphatidylinositol (PI) and the 1-palmitoyl molecular species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and into the secreted triacylglycerol in contrast with intrahepatocyte tri-

Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PUFA, polyunsaturated fatty acids; MEM, minimal essential medium; FCS, fetal calf serum.

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acylglycerol enriched in linoleic acid. Such a selective channelling was related to the fatty acid uptake which varied with fatty acid/albumin ratio.

MATERIALS AND METHODS

Animals and chemicals

Male adult Wistar rats weighing 180–200 g, normally exposed to daylight, were fed on a standard pelleted diet (U.A.R., Villemoisson, France) containing 51% carbohydrate, 22% protein, 5% fat, 4% crude fibre, vitamins and salt mixture according to the recommendations of the *ad hoc* Committee on Standards for Nutrition of the American Institute of Nutrition. Food and water were available *ad libitum*. The animals were used for hepatocyte preparation between 9:30 and 10:00 h

For culture, MEM, antibiotics and FCS were obtained from Gibco. Unlabelled fatty acids, insulin and hydrocortisone hemisuccinate were from Sigma. Bovine serum albumin, fatty-acid-free serum albumin and collagenase were from Boehringer. [5,6,8,9,11,12,14,15-^{3}H] arachidonic acid and [1-^{14}C]linoleic acid were purchased from Amersham International.

Hepatocyte culture preparation

Hepatocytes were isolated by collagenase perfusion of the liver (Guguen *et al.*, 1975). Cells were plated (10^6 cells/ml) in medium no. 1 [MEM containing kanamycin (50 mg/l), penicillin (8000 i.u./l) and streptomycin (60 mg/l)] supplemented with bovine serum albumin (1g/l), FCS (100 ml/l) and insulin (5 mg/l). The plated cells were incubated at 37 °C in air/CO_2 (19:1) as gas phase; 18 h after plating the medium was removed and the hepatocytes were incubated for 4 h in medium no. 2. This medium, composed of MEM and the proportions of antibiotics given above, was supplemented with hydrocortisone hemisuccinate (32 mg/l) but did not include FCS or albumin.

Incubation procedure

Incubations were carried out for 3 h in the medium no. 3. This medium, composed of MEM, antibiotics and hydrocortisone as indicated above, was supplemented with insulin (80 m-i.u./l) and fatty-acid-free albumin (10 g/l). Experiments were started by adding a mixture of arachidonic and linoleic acid, in a molar ratio of 0.3–2.0. [1-^{14}C]Linoleic acid (1–2 μCi/dish) and [^{3}H] arachidonic acid (3–6 μCi/dish) were diluted with the corresponding unlabelled fatty acids in ethanol (< 1% v/v) to obtain a final concentration of 37.5–300 μM, i.e. a total fatty acid/albumin of 0.3–2.0.

After a 3 h incubation period, the medium was collected. Plated cells were washed twice with phosphate-buffered saline, pH 7.4. Medium and washing were collected and centrifuged for 5 min at 1000 ***g*** to discard detached cells. Plated cells were scraped with a rubber policeman into 1.5 ml of phosphate-buffered saline and sonicated for 10 s at 4° C.

The integrity of hepatocytes (more than 85%) was routinely assessed by measuring release of lactate dehydrogenase into the medium.

Analysis

The free fatty acid concentration was determined by enzymic assay (Nefac test, Wako, Japan) and the proportion of arachidonic and linoleic acid was analysed by means of g.l.c. (Van Wijngaarden, 1967) using a 25 m capillary column filled with Carbowax 20 M operating between 200 °C and 220 °C at 1 °C/min. Detection was by flame ionization. Media and disrupted cells were extracted by the Bligh & Dyer (1959) procedure. Lipids were separated by double monodimensional t.l.c. on silica gel G (Schleicher and Schuell, Germany). Plates were run with chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) to separate phospholipid classes (PC, PE and PI) then, after drying. with light petroleum/ diethyl ether/acetic acid (90:30:1, by vol.) to separate neutral lipids from the others (Skipski, 1965). Spots were revealed by autoradiography and scraped off to measure radioactivity by means of liquid-scintillation counting. Lipid phosphorus was directly assayed on silica gel after t.l.c. (Chen, 1956).

In order to determine the respective incorporation of [^{3}H]arachidonic acid and [1-^{14}C]linoleic acid in various molecular species, PE and PC were treated with phospholipase C and their diacylglycerol derivatives were benzoylated according to Blank *et al.* (1984). The benzoyl derivatives were separated by h.l.p.c as described by Colard *et al.* (1987).

In order to determine the rate of β-oxidation, we have measured the formation of radioactive acid-soluble products. Aliquots of medium and disrupted cells were treated with 1 M-$HClO_4$, centrifuged for 10 min at 2500 ***g*** and supernatants were collected for radioactivity measurement (Beynen *et al.*, 1980).

Proteins were determined according to Lowry *et al.* (1951).

For a range of fatty acid/albumin ratios or of arachidonic acid/linoleic acid molar ratios, the different points were in triplicate from the same rat to avoid discrepancies

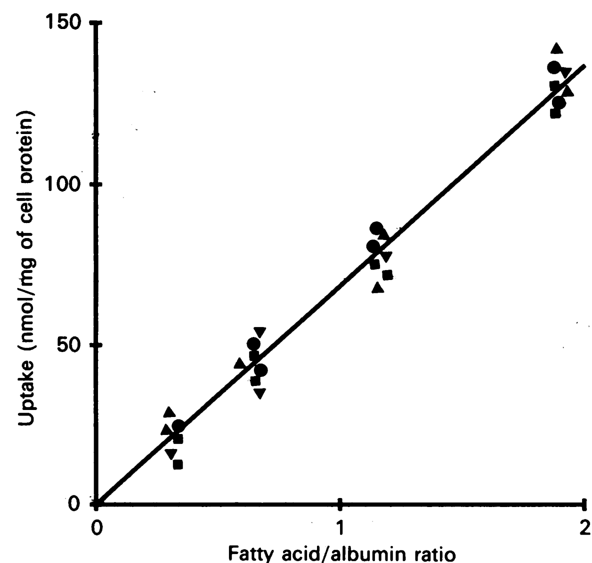

Fig. 1. Total fatty acid uptake

The total fatty acid uptake was calculated by adding the amounts of each fatty acid esterified in hepatocyte and secreted lipids or oxidized. These amounts were estimated from the specific radioactivity of each fatty acid. Arachidonic acid and linoleic acid were added in different molar ratios: 0.3 (■), 0.6 (●), 1.0 (▲) and 2.0 (▼). The regression curve was determined by means of least-square fitting.

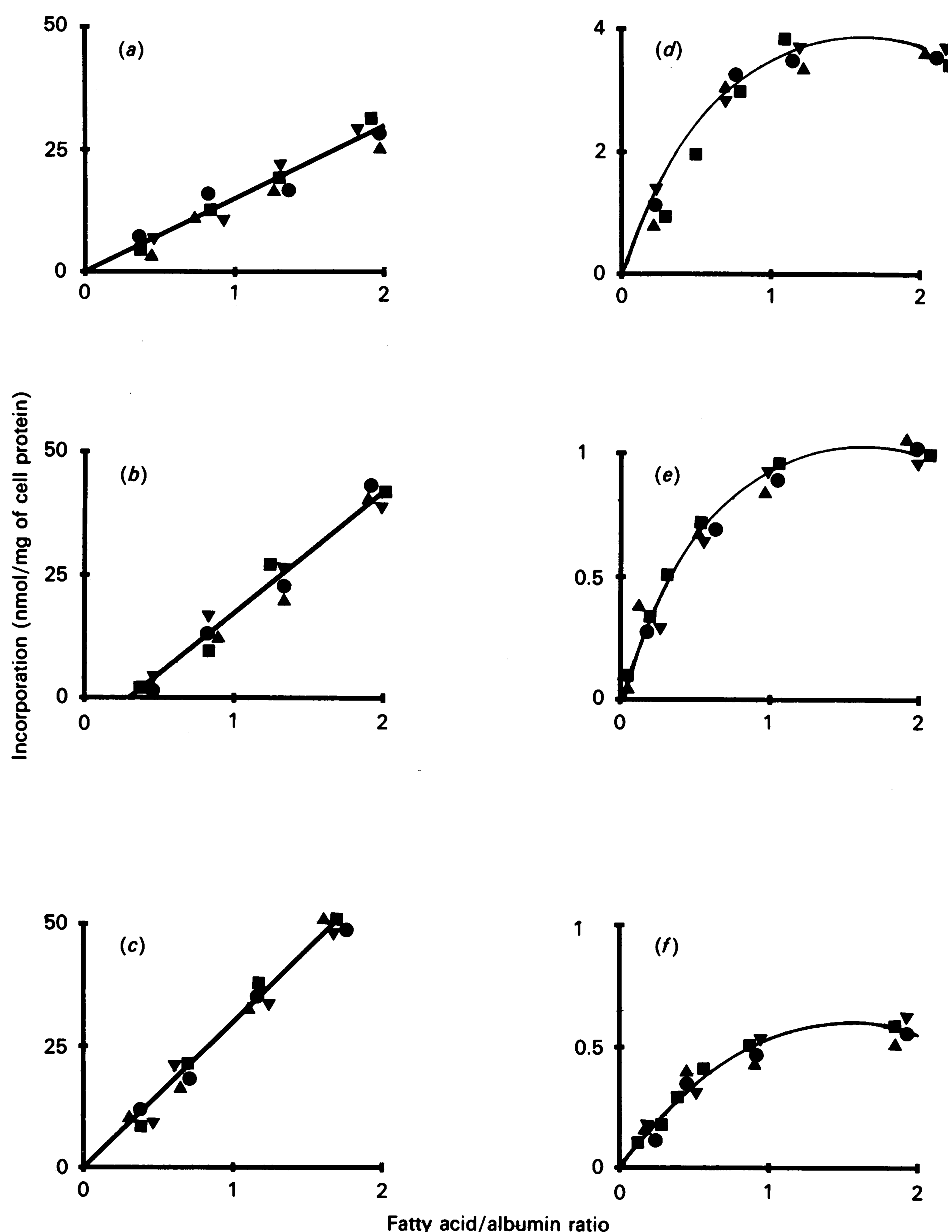

Fig. 2. Fate of total fatty acids taken up by hepatocytes

The amounts of total fatty acids incorporated into hepatocyte triacylglycerol (*a*), secreted triacylglycerol (*b*), oxidation products (*c*), hepatocyte phosphatidylcholine (*d*), phosphatidylethanolamine (*e*) and phosphatidylinositol (*f*) were calculated at the following arachidonic/linoleic acid molar ratios: 0.3 (■), 0.6 (●), 1.0 (▲) and 2.0 (▼). The regression curve was determined by means of least-square fitting.

related to the metabolic status of the rat. In addition, experiments were performed twice and three identical points were systematically performed from each rat liver preparation ($n = 10$) to allow us to compare the results obtained from the different rat liver preparations.

RESULTS

Rat hepatocytes took up total fatty acids linearly with time during the 3 h incubation (results not shown). The increase in total fatty acid uptake as a function of fatty acid/albumin ratio is displayed in Fig. 1. It might have been slightly underestimated because we could not discriminate between the fatty acids added and those released by the hepatic triacylglycerol lipase from secreted glycerolipids. The synthesis and secretion of active hepatic triacylglycerol lipase becomes significant after 2 h of primary culture (Parkes *et al.*, 1986). However, the enzyme action must have been low, since little radioactivity was associated with medium mono- or diacylglycerols (results not shown).

The total fatty acid uptake was linearly related to the fatty acid/albumin ratio added to the medium at the beginning of the incubation ($r^2 = 0.92$; Fig. 1). The uptake rate was similar when linoleic acid and arachidonic acid were added alone or when they were incubated together, and whatever the arachidonic acid/linoleic acid molar ratio in the range 0.3–2 (Fig. 1). This

Table 1. Effect of the initial arachidonic/linoleic molar ratio on the relative enrichment in arachidonic acid of lipid classes at an uptake rate of 44 nmol/3 h per mg of protein (initial fatty acid/albumin ratio = 0.6)

The amounts of arachidonic acid and linoleic acid incorporated in each class were measured and their ratio was divided by the initial arachidonic acid/linoleic molar ratio to obtain the arachidonic relative enrichment (E), which expressed the selective orientation of these fatty acids in the different pathways (For details, see the Results section). E values were means ± S.D. of triplicate experiments. * $P < 0.05$ and ** $P < 0.01$, as compared with the value for an initial arachidonate/linoleate ratio of 0.33; † $P < 0.05$ and †† $P < 0.01$, as compared with the value for an initial arachidonate/linoleate ratio of 0.62. Other statistical comparisons are not significant.

Initial arachidonate/ linoleate ratio ...	E 0.33±0.05	0.62±0.04	2.00±0.65
Acid-soluble products	2.4±0.1	2.3±0.2	2.9±0.2*,**
Secreted acylglycerols	1.6±0.3	2.5±0.6**	1.7±0.1††
Hepatocytes			
Triacylglycerol	0.5±0.1	1.0±0.1	1.0±0.1
PC	1.5±0.1	1.7±0.1	1.5±0.1
PE	2.9±0.2	2.6±0.3	2.2±0.2*
PI	4.7±0.7	3.8±0.4	3.9±0.5

linear regression curve reflected the strictly linear relationship between the flux of total fatty acids driven through the main metabolic pathways and the fatty acid/albumin ratio, i.e. synthesis of triacylglycerol stored in hepatocytes ($r^2 = 0.89$; Fig. 2*a*) or secreted in the medium ($r^2 = 0.87$; Fig. 2*b*) and acid-soluble product formation ($r^2 = 0.96$; Fig. 2*c*).

Total fatty acid incorporation into phospholipids was lower than into triacylglycerol and acid-soluble products. Furthermore, the incorporation into phospholipids was not linearly related to the fatty acid/albumin molar ratio but reached a plateau as soon as fatty acid/albumin reached 1.5–2.0. The maximal incorporations into PC, PE and PI were respectively 4.0, 1.0 and 0.6 nmol/3 h per mg of hepatocyte protein (Figs. 2*d*, 2*e* and 2*f*). We did not evidence any modification of total fatty acid incorporation into hepatocyte and secreted triacylglycerol, acid-soluble products and phospholipids when the arachidonic acid/linoleic acid molar ratio varied from 0.3 to 2.0 (Fig. 2).

In order to display the selective channelling of arachidonic acid or linoleic acid when they were added together to cultured hepatocytes, results were expressed in terms of relative enrichment in arachidonic acid (E), evaluated as follows: first, we calculated the molar ratio of arachidonic acid and linoleic acid incorporated into each lipid class or transformed into acid-soluble products after 3 h of incubation. Then E was obtained by dividing

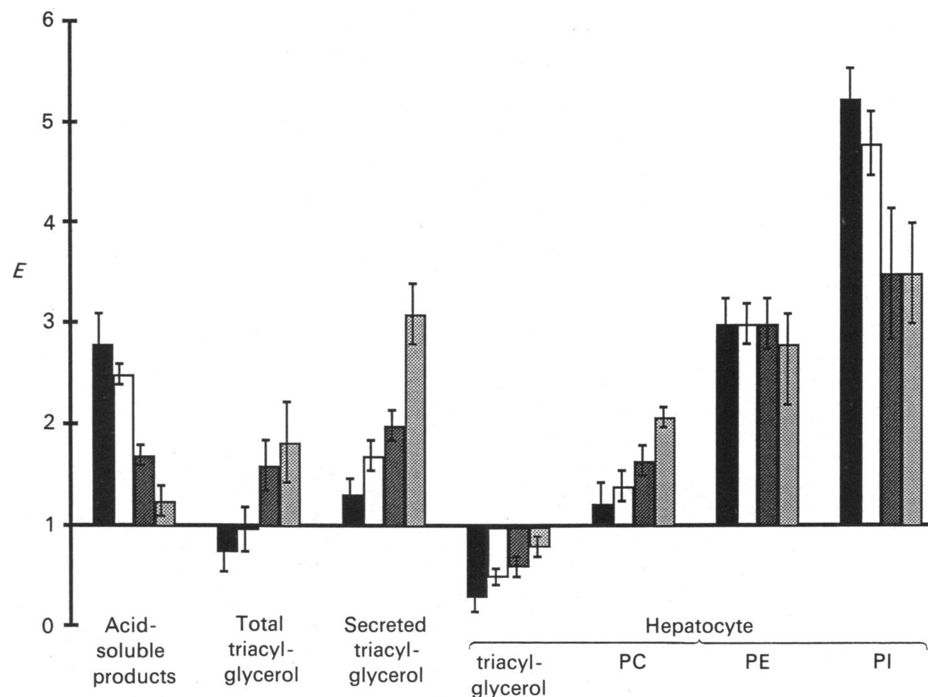

Fig. 3. Effect of the uptake rate on the relative enrichment in arachidonic acid of the different lipid classes (at an initial arachidonic acid/linoleic acid molar ratio of 0.3)

The amounts of arachidonic acid and linoleic acid incorporated into each lipid class were measured, and their molar ratio was divided by the initial arachidonic acid/linoleic acid molar ratio (0.3) to obtain the relative enrichment in arachidonic acid (E) values expressing the selective orientation of these fatty acids into the different metabolic pathways. E values were means ± S.D. of triplicate determinations for different uptake rates: 22 (■), 44 (□), 85 (■) and 130 (▩) nmol/3 h per mg of protein, corresponding respectively to initial fatty acid/albumin ratios of 0.3, 0.6, 1.1 and 2.0.

Table 2. Renewal of arachidonic acid and linoleic acid in the main molecular species of PC and PE at an initial arachidonic acid/linoleic molar ratio of 0.3

The concentrations of the different molecular species were calculated from the total level of hepatocyte phospholipids (110±5 nmol/mg of protein), the percentages of PC and PE measured by phosphorus determination after a t.l.c. separation (52.0±3.0 and 25.7±1.1% respectively), and the h.p.l.c. profile of the molecular species of each phospholipid. The specific turnover of arachidonic acid and linoleic acid in each molecular species was evaluated from the h.p.l.c.-coupled radioactivity profiles. Results are means±S.D. of triplicate experiments. Abbreviations used: 16:0, palmitoyl; 18:0, stearoyl; 18:1, oleoyl; 18:2, linoleoyl; 20:4, arachidonoyl, n.d., not determined.

Phospholipid	Molecular species	Amount (nmol of phospholipid/mg of protein)	Initial fatty acid/albumin ratio... Uptake rate (nmol of fatty acid/3 h per mg of protein)...	Turnover (pmol of fatty acid/nmol of molecular species of phospholipid) 0.3 / 22	2.0 / 130	Increase in turnover (-fold)
Phosphatidyl-choline						
	2-Linoleoyl					
	16:0–18:2	10.6±0.4		7.3±0.3	16.9±0.4	2.3
	18:0–18:2	11.2±0.5		2.0±0.1	5.6±0.2	2.8
	18:1–18:2	2.1±0.7		n.d.	n.d.	
	18:2–18:2	5.8±0.4		1.9±0.2	10.8±0.7	5.7
	2-Arachidonoyl					
	16:0–20:4	8.8±0.2		2.6±0.1	9.4±0.2	3.6
	18:0–20:4	12.3±1.0		1.4±0.1	5.5±0.4	3.9
	18:2–20:4	1.9±0.9		n.d.	n.d.	
	20:4 species/18:2 species ratio:					
	1-Palmitoyl			0.36	0.56	
	1-Stearoyl			0.70	0.98	
Phosphatidyl-ethanolamine						
	2-Linoleoyl					
	16:0–18:2	3.4±0.3		5.6±0.5	7.1±0.6	1.3
	18:0–18:2	3.4±0.5		2.4±0.2	9.4±0.6	3.9
	18:2–18:2	3.6±0.7		n.d.	n.d.	
	18:2–18:2	3.6±0.5		n.d.	n.d.	
	2-Arachidonoyl					
	16:0–20:4	5.2±0.1		2.0±0.1	2.5±0.1	1.2
	18:0–20:4	9.3±1.8		1.4±0.3	4.3±0.8	3.1
	18:2–20:4	n.d.		n.d.	n.d.	
	20:4 species/18:2 species ratio:					
	1-Palmitoyl			0.36	0.35	
	1-Stearoyl			0.58	0.46	

this ratio by the arachidonic acid/linoleic acid molar ratio added to the medium at the beginning of the incubation. Values of $E > 1$ reflected a preferential channelling of arachidonic acid through a given metabolic pathway whereas $E < 1$ reflected a preferential channelling of linoleic acid; $E = 1$ meant that no selectivity occurred.

Table 1 shows the selective orientation of arachidonic acid and linoleic acid for fatty acid/albumin of 0.6 corresponding to a total uptake of 44 nmol/3 h per mg of protein. At the lowest arachidonic acid/linoleic acid molar ratio (0.33±0.05), arachidonic acid was preferentially oriented into PI ($E = 4.7 \pm 0.7$), PE (2.9±0.2) and acid-soluble product formation (2.4±0.1). Intrahepatocyte PC and secreted triacylglycerol were only slightly enriched in arachidonic acid relative to linoleic acid ($E = 1.5 \pm 0.1$ and 1.6±0.3 respectively). Conversely, we observed a preferential channelling of linoleic acid into intracellular triacylglycerol at the end of the incubation ($E = 0.5 \pm 0.1$, i.e. a 2-fold linoleic acid enrichment).

The preferential channelling of arachidonic acid into hepatocyte PI and PC, secreted triacylglycerol and acid-soluble products was not modified when the arachidonic acid/linoleic acid molar ratio was increased (Table 1). Conversely, we observed a slight decrease in PE arachidonic acid for an initial molar ratio of 2.0 ($E = 2.2 \pm 0.2$). At the same time, the preferential channelling of linoleic acid into hepatocyte triacylglycerol was abolished, and then the pattern of fatty acid incorporation reflected exactly the initial arachidonic acid/linoleic acid ratio (Table 1). Similar results were obtained with an initial fatty acid/albumin ratio of 2.0, corresponding to a total fatty acid uptake of 135 nmol/3 h per mg of protein (results not shown).

Fig. 3 displays the influence of the fatty acid uptake

rate on the selective channelling of arachidonic and linoleic acids. Hepatocytes were incubated at a low initial arachidonic acid/linoleic acid molar ratio (0.3) and at total fatty acid/albumin ratios from 0.3 to 2, overlapping the physiological fatty acid/albumin ratio (0.4–0.9) (Abumrad *et al.*, 1978).

The preferential channelling of arachidonic acid into the β-oxidation pathway was the highest for an uptake rate of 22 nmol/3 h per mg of protein, then varied inversely to the uptake rate to be abolished at an uptake rate of 130 nmol/3 h per mg of protein. The selective channelling into total triacylglycerol synthesis (intracellular + secreted) favoured linoleic acid at a low uptake rate but arachidonic acid at a high rate. This inversion resulted from the increase of the relative arachidonic enrichment in both secreted (from $E = 1.3 \pm 0.1$ to 3.1 ± 0.4) and hepatocyte (from $E = 0.3 \pm 0.1$ to 0.8 ± 0.1) triacylglycerol as the total uptake rose from 22 to 130 nmol/3 h per mg of protein (Fig. 3).

As expected from the results shown in Table 1, the preferential channelling of arachidonic acid was higher in PI and PE than in PC. But the increase in the total uptake (from 22 to 130 nmol/3 h per mg of protein) was accompanied by an increase in the arachidonic acid enrichment in PC (from 1.2 ± 0.1 to 2.1 ± 0.1) and a simultaneous decrease in PI (from 5.2 ± 0.2 to 3.5 ± 0.5) whereas no change was observed in PE (Fig. 3).

To investigate further the fate of the arachidonic acid and linoleic acid incorporated into phospholipids, we determined the specific turnover of PC and PE molecular species by means of h.p.l.c. The amounts of extracted PI were too small to perform such an analysis. Table 2 displays the concentrations of the different molecular species of PC and PE in hepatocytes in primary culture and the specific turnover of arachidonic and linoleic acid from exogenous labelled fatty acids. Linoleic and arachidonic acid are more associated with the 1-saturated fatty acyl molecular species of PC than with the 1-unsaturated fatty acid species. This assymetrical distribution of both fatty acids between the molecular species of PE is less pronounced than in PC.

One can see from Table 2 that [1-^{14}C]linoleic acid and [^{3}H]arachidonic acid were only incorporated into 1-saturated molecular species of PC and PE and into dilinoleoyl-PC. At a low fatty acid uptake (fatty acid/albumin = 0.3), the respective turnover rate of linoleic and arachidonic acid in the 1-palmitoyl species of PC and PE (1-palmitoyl-2-arachidonoyl/1-palmitoyl-2-linoleoyl = 0.36) reflected the initial molar ratio of the fatty acids in the medium (arachidonic acid/linoleic acid = 0.3). Conversely, the stearoyl species of PC and PE were enriched in arachidonic acid (1-stearoyl-2-arachidonoyl/1-stearoyl-2-linoleoyl = 0.70 and 0.58 respectively). When the fatty acid uptake rose (fatty acid/albumin = 2.0), we observed the same preferential channelling of arachidonic towards the 1-palmitoyl PC, whereas the preferential channelling was inverted in favour of 1-stearoyl species of PE. At the same time, the labelling of dilinoleoyl PC was 5.7-fold increased.

DISCUSSION

Mixed fatty acids were taken up at the same rate and distributed with the same pattern as fatty acids added separately (Figs. 1 and 2). The total fatty acid uptake by hepatocytes in primary culture was linearly related to the initial total fatty acid/albumin ratio during a 3 h incubation, as already established for a 14 h incubation under our experimental conditions (Chambaz *et al.*, 1986) or with isolated hepatocytes incubated for 1 h (Groener & Van Golde, 1978) or with perfused liver (Kohout *et al.*, 1971; Weisiger *et al.*, 1981). Triacylglycerol synthesis (Figs. 2*a* and 2*b*) was also linearly related to the fatty acid/albumin ratio, as already shown with cultured (Lamb *et al.*, 1977; Chambaz *et al.*, 1986) or isolated (Sundler *et al.*, 1974; Groener & Van Golde, 1978) hepatocytes.

Whatever the fatty acid/albumin ratio, 42–44% of fatty acids taken up were recovered as acid-soluble products, in agreement with Ontko (1972) who found 28% with isolated hepatocytes obtained from fed rats. The constancy between acid-soluble product formation and fatty acid uptake might be due to a progressive disinhibition of the acyl-CoA:carnitine acyltransferase by malonyl-CoA (Saggerson & Carpenter, 1984), since malonyl-CoA production by acetyl-CoA carboxylase is inhibited as the concentration of fatty acyl-CoA increases (Lane & Moss, 1971).

The variation of the arachidonic acid/linoleic acid molar ratio from 0.3 to 2.0 did not modify the incorporation of total fatty acids into triacylglycerol phospholipids and acid-soluble products, and only slightly modified the selective channelling of arachidonic and linoleic acid through these different metabolic pathways (Table 1). But changes in the uptake rate by means of the fatty acid/albumin ratio led to significant variations of the respective channelling of both fatty acids (Fig. 3).

The saturable incorporation into phospholipids has already been shown by Lamb *et al.* (1977), Arachidonic acid was preferentially channelled into phospholipids, as already observed in steady-state conditions (Chambaz *et al.*, 1986), but also into β-oxidation and secreted triacylglycerol. A first explanation may be that the acyl-CoA synthetase specific for very polyunsaturated fatty acids which activates arachidonic acid (Laposata *et al.*, 1985) exhibits a greater affinity than the non-specific one which activates linoleic acid. In addition, arachidonic acid enrichment of glycerolipids may be due to the behaviour of the enzymes involved in their synthesis towards arachidonoyl- and linoleoyl-CoA.

PI enrichment in arachidonic acid could be explained by two mechanisms. First, the well-known preference of PI for arachidonoyl-CoA in hepatocyte microsomes (Holub, 1976; Neumüller *et al.*, 1987) was clearly involved at a high uptake rate. In addition, the higher PI enrichment observed at a low uptake rate might be due to the different affinity of the two acyl-CoA synthetases mentioned above, since PI reacylation would then be dependent on acyl-CoA availability (Fig. 2*f*). The preferential orientation of arachidonic acid into PE was unrelated to the uptake rate (Fig. 3 and Table 2). This was consistent with the sparing of arachidonoyl-PE observed in the liver during essential fatty acid deficiency (Lefkowith *et al.*, 1985) or in hepatocytes during long term culture deprived of essential fatty acids (Strum-Odin *et al.*, 1987; Pépin *et al.*, 1988). When the uptake rose, the preferential orientation of arachidonic acid into PC was enhanced (Fig. 3 and Table 2) whereas variations of the arachidonic acid/linoleic acid molar ratio had no effect. These results could not be explained by the kinetic data reported by Miki *et al.* (1977). But Okuyama *et al.* (1975) have shown, by incubating liver microsomes with

both fatty acids, that arachidonic acid is incorporated into PC more than is oleic acid when lyso-PC availability decreases, which might occur in our experiments when the uptake rose.

Another important point concerns the linoleic acid and arachidonic acid incorporation in the respective molecular species of PC and PE (Table 2). At a low fatty acid uptake, we observed a preferential incorporation of the two fatty acid in the 1-palmitoyl species of PC and PE as well. Though Miki *et al.* (1977) reported no difference in the reacylation pattern of either 1-linoleoyl, 1-palmitoyl or 1-stearoyl lysoPC by linoleoyl-CoA or arachidonoyl-CoA, Holub *et al.* (1979) have shown that the incubation of microsomes with a mixture of 1-palmitoyl or 1-stearoyl lysoPC greatly favours the reacylation of 1-palmitoyl lysoPC. The preferential channelling of both fatty acids into 1-palmitoyl-PC, whatever the fatty acid uptake rate, could be related to the synthesis of biliary lecithins, in which 1-palmitoyl-2-linoleoyl and 1-palmitoyl-2-arachidonoyl PC are the main molecular species (Balint *et al.*, 1967).

The incorporation pattern of arachidonic acid and linoleic acid into total triacylglycerol exhibited a progressive preferential channelling of arachidonic acid as a function of the uptake rate (Fig. 3). This masked strong differences between two apparently distinct pools of triacylglycerol: (1), the intracellular pool, which preferentially incorporated linoleic acid at a low uptake rate, but reflected the proportions of added fatty acids at a high uptake rate, and (2), the secreted pool, in which the preferential channelling of arachidonic acid rose with the uptake rate.

By incubating hepatocytes with a mixture of arachidonic acid and linoleic acid, we were able to show a preferential orientation of arachidonic acid into β-oxidation, phospholipids and secreted triacylglycerol. Such a specific orientation supports the sparing mechanism of hepatic arachidonoyl PE, as well as the preferential synthesis of both 1-palmitoyl-2-linoleoyl or 1-palmitoyl-2-arachidonoyl PC involved in bile secretion. It also strengthens the central role of hepatic very low density lipoprotein secretion in the arachidonic acid supply to peripheral tissues. This could partly explain why hepatocyte triacylglycerol became very quickly devoid of arachidonic acid under essential deprivation *in vivo* (Lefkowith *et al.*, 1985; Prasad *et al.*, 1987) or in long term culture (Pépin *et al.*, 1988).

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