Biosynthesis and secretion of triacylglycerol in rat liver after partial hepatectomy

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Partially hepatectomized rats were used to investigate the mechanism of fatty-liver development in the regenerating rat liver. After partial hepatectomy the amount of hepatic triacylglycerol increased by almost 4-fold compared with sham-operated rats. The activities of both cytosolic and microsomal phosphatidate phosphohydrolase were enhanced at 12 h after surgery. The activity of diacylglycerol acyltransferase was increased at a later stage of regeneration. Analysis of plasma lipoproteins showed a significant decrease of lipids associated with very-low-density lipoproteins (VLDL). Relative to control, the rate of hepatic triacylglycerol synthesis from [³H]glycerol *in vivo* was stimulated at 22 h after partial liver resection. However, secretion of glycerol-labelled triacylglycerol in VLDL was the same in control and hepatectomized rats. In cultures of hepatocytes from hepatectomized donor rats, the concentration of triacylglycerol and the biosynthesis of this lipid from [³H]glycerol or from [³H]oleate were enhanced. The secretion of total triacylglycerol into the medium was not affected, resulting in a net accumulation of intracellular triacylglycerol. The rate of secretion of leucine-labelled apolipoproteins B and E associated with VLDL was similar in cell cultures from hepatectomized and sham-operated rats. The results of this study show that the enhancement of the biosynthesis of triacylglycerol in hepatectomized livers is not accompanied by an increase of the secretion of VLDL.

INTRODUCTION

Rats subjected to partial hepatectomy (PH) rapidly develop a fatty liver [1,2]. Several factors have been proposed to contribute to this phenomenon. These include: excessive increase of the rate of fatty acid synthesis [3], highly elevated mobilization of fatty acids from the adipose tissue [4], impaired secretion of lipoproteins by the liver, and increased channelling of fatty acids into liver triacylglycerol [4,5].

Potentially important regulatory sites in the intracellular synthesis of triacylglycerol are (1) glycerolphosphate acyltransferase (EC 2.3.1.15), which catalyses the first committed step in glycerolipid synthesis [6], (2) phosphatidate phosphohydrolase (EC 3.1.3.4), which controls the rate of diacylglycerol production [7], and (3) diacylglycerol acyltransferase (EC 2.3.1.20), which may regulate the channelling of diacylglycerol into triacylglycerol [8]. There have been very few studies on the effects of PH on these key enzymes, except for an early report by Mangiapanè *et al.* [9], who showed that the activity of phosphatidate phosphohydrolase was increased in liver homogenates from sub-totally hepatectomized rats.

Discrepant results have been published with respect to the effects of liver resection on lipoprotein secretion. Although several investigators showed that the secretion of lipoproteins was not inhibited after PH [4,5], Trentalance *et al.* [10] recently reported that the plasma concentration of very-low-density lipoprotein (VLDL) was decreased at 16 h after surgery. Hitherto the effects of PH on the synthesis and secretion of triacylglycerol have been investigated *in vivo* by determining the incorporation of radioactive precursors into hepatic lipids and into components of VLDL secreted by the liver [4,5]. Interpretation of such studies is hampered by further catabolism of VLDL and by differences in liver mass and blood flow through the liver of PH and control

rats. By using isolated liver cells, such problems can be circumvented. In primary cultures of isolated hepatocytes it is possible to examine many metabolic parameters more easily and efficiently. In addition, cultured hepatocytes have been shown to be a useful system to study lipoprotein secretion [11,12].

It was the purpose of the present study to obtain more insight into the mechanisms of triacylglycerol accumulation in regenerating liver. We measured the rates of triacylglycerol synthesis after PH or sham operation (SO) both *in vivo* and in primary cultures of hepatocytes from partially hepatectomized or sham-operated rats. In view of the discrepancies mentioned above (ref. [10] versus refs. [4,5]), we also measured lipid content and composition of the major lipoproteins (VLDL, LDL and HDL) as a function of post-surgery time. In addition, we examined the time course of alterations in the activities of important regulatory enzymes of triacylglycerol synthesis in subcellular fractions isolated from regenerating liver. Taken collectively, our observations demonstrate that in hepatocytes from regenerating liver the synthesis of triacylglycerol is increased without a concomitant enhancement of VLDL secretion.

EXPERIMENTAL

Materials

[methyl-³H]Choline, [13-³H]glycerol, [methyl-¹⁴C]carnitine and [1-¹⁴C]palmitoyl-CoA were obtained from Amersham International, Amersham, Bucks., U.K. [9,10-³H]Oleic acid, [U-¹⁴C]glycerol 3-phosphate and L-[4,5-³H]leucine were purchased from New England Nuclear, Dreieichenhain, Germany. [³H]-Phosphatidic acid was prepared from [³H]glycerol as described by Martin *et al.* [13]. Collagen (type III, from calf skin), collagenase (type I), palmitoyl-CoA and fumed silica (Cab-O-Sil) were from

Abbreviations used: apo, apolipoprotein; DMEM, Dulbecco's modified Eagle's medium; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PC, phosphatidylcholine; PH, partial hepatectomy; SO, sham operation; VLDL, very-low-density lipoprotein.

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Sigma, St. Louis, MO, U.S.A. Dulbecco's modified Eagle's medium (DMEM) and foetal-calf serum were obtained from Gibco, Paisley, Scotland, U.K. The origin of other chemicals has been described previously [14].

Preparation of biological samples and enzyme assays

Male Wistar rats (180–200 g) were kept under an inverted light–dark cycle (darkness from 06:00 to 18:00 h) for at least 2 weeks before surgery. Two-thirds PH or SO was routinely performed between 09:00 and 10:00 h under light diethyl ether anaesthesia [15]. Before and after surgical procedures the rats had free access to water and food.

At the appropriate times the animals were anaesthetized with diethyl ether, blood was collected from the aorta after opening the abdominal cavity, and the livers were excised. Several pieces of each liver were homogenized [25 % (w/v) homogenate] for subsequent subcellular fractionation or for extraction of liver lipids [16]. Buffers and procedures for homogenizing the liver tissue and subsequent isolation of microsomes and cytosol have been reported previously [14]. Activities of diacylglycerol acyltransferase in microsomes and phosphatidate phosphohydrolase in cytosol and microsomes were measured as described previously [14]. Glycerolphosphate acyltransferase in microsomes was assayed as described by Batenburg *et al.* [17], and the activity of carnitine palmitoyltransferase I (EC 2.3.1.21) was determined in mitochondria as reported previously [18].

Lipoprotein isolation and secretion *in vivo*

To blood samples collected from the aorta, EDTA was added such that the final concentration was 1 mg/ml. Chylomicrons were removed from the plasma by centrifugation at 20 000 *g* for 15 min at 20 °C. The remaining plasma lipoproteins were fractionated by density-gradient ultracentrifugation [19]. Cholesterol, triacylglycerol and phospholipids in VLDL ($d < 1.006$), in LDL ($1.019 < d < 1.063$) and in HDL ($1.063 < d < 1.21$) were determined by using test combinations supplied by Boehringer, Mannheim, Germany.

To determine the rate of lipoprotein secretion *in vivo*, rats were anaesthetized with pentobarbital at 22 h after surgery. The abdominal cavity was opened, and rats were injected intraportally with 0.25 ml of 0.9 % NaCl containing 75 μ Ci of [1,3-³H]glycerol or 75 μ Ci of [³H]choline. At the time points indicated in the legends, blood samples were collected by cardiac puncture and the experiment was terminated by excising the liver. Plasma lipoproteins were fractionated by ultracentrifugation as described above. The liver was homogenized in 4 vol. of ice-cold 0.9 % NaCl and lipids were extracted [16].

Preparation and incubation of hepatocytes

Rat hepatocytes were prepared by a collagenase perfusion technique similar to that described previously at 22 h after surgery [20,21]. Freshly isolated hepatocytes were suspended in DMEM supplemented with 10 % (v/v) foetal-calf serum. The cells were plated in 3 ml of medium on collagen-coated dishes at a density of 2.5×10^6 cells per dish (60 mm diam.). Cells to be used for measurement of the secretion of apoproteins were plated in leucine-free medium. After the cells had been allowed to adhere for 2 h, the medium was replaced with 2 ml of serum-free DMEM supplemented with 0.5 % fatty-acid-free BSA [22] and 1 mM-glycerol. In most experiments the medium also contained 0.5 mM-oleate. Hepatocytes were further incubated for 6 h.

Quantitative analysis of triacylglycerol isolated from cells and medium

At the end of the incubation period, the medium was collected.

Triacylglycerol was extracted from the medium [16] after centrifugation at 10 000 *g* for 15 min to remove any dead cells and cell debris. The cells were taken up in 2.5 ml of methanol/water (1:1, v/v). Lipids were extracted from the cells as described in [23].

In experiments with labelled precursors, triacylglycerol from cells or medium was isolated by t.l.c. [21] and assayed for radioactivity.

For determination of the triacylglycerol content of cells and medium, this lipid fraction was eluted from the silica with 3×2 ml of freshly distilled ether. After evaporation of the solvent, triacylglycerol was quantified by measuring the fatty acyl content, with triolein as a standard [24].

Isolation and characterization of VLDL secreted in the culture medium

Hepatocytes were incubated in the presence of 0.5 mM-oleate, 1 mM-glycerol and 25 μ Ci of 0.05 mM-[4,5-³H]leucine for 6 h. At the end of the incubation period, medium from three dishes was combined and the density adjusted to 1.006 g/ml with NaCl. The samples were centrifuged at 40 000 rev./min in a Beckman SW 40 rotor for 38 h. The floating VLDL fraction was collected from the top of the tube. VLDL were concentrated by adsorption on to Cab-O-Sil [25,26]. The apolipoproteins were solubilized from the silica, and samples were separated on a gradient polyacrylamide gel (3–15 %) containing SDS. The positions of apolipoproteins on the gel were confirmed by using standards of VLDL prepared from rat serum [27]. Bands containing apolipoproteins (apo) B and E were cut from the gels and incubated overnight with 0.5 ml of 30 % (v/v) H₂O₂ at 50 °C. The radioactivity incorporated was determined by scintillation counting.

To determine the incorporation of labelled amino acids into cellular protein, the latter was precipitated with 10 % trichloroacetic acid, followed by washing of the precipitate with 10 % trichloroacetic acid. The proteins were solubilized in 0.5 M-NaOH and assayed for radioactivity.

Further analytical procedures

Separation and quantification of triacylglycerol and phosphatidylcholine (PC) isolated from liver homogenates were carried out as described in [21]. Free glycerol levels were analysed in deproteinized serum samples [14]. Protein was determined with BSA as a standard [28]. Results are presented as means $\pm$ S.D. or S.E.M. for the numbers of experiments indicated in the legends. Student's two-tailed *t* test was used for statistical analysis.

RESULTS

Accumulation of hepatic triacylglycerol and plasma glycerol after PH

The increase in liver weight in the PH group compared with SO animals indicated rapid regeneration of the liver during the experimental period (Table 1). In accordance with other studies [1,2], the regenerating livers of our animals accumulated triacylglycerol (Table 1).

Stress associated with surgery or with the complex hormonal changes during the initial 48 h after PH [29,30] may cause lipolysis in adipose tissue, which may contribute to the hepatic fat deposition following resection. Therefore the levels of serum free glycerol (indicator of lipolysis in adipose tissue) were determined at intervals throughout the experimental period. In both the sham-operated and the PH group, the level of serum free glycerol was increased at 4 h after surgery as compared with control (*t* = 0 h) rats. However, the response in the hepatectomized animals was greater and was sustained much longer.

Table 1. Effect of PH on relative liver weight, hepatic triacylglycerol and plasma glycerol levels

Rats were subjected to PH or SO. At the time points indicated blood was collected, the liver was removed and liver weight, hepatic triacylglycerol and serum glycerol levels were determined. Data represent means $\pm$ S.E.M. of six animals: * significantly different from sham-operated rats ($P < 0.001$); n.d., not determined.

Time after surgery (h)	Type of surgery	Liver wt./100 g body wt. (g)	Triacylglycerol (nmol/mg of protein)	Plasma glycerol (μ M)
0	None	4.5 $\pm$ 0.1	15.7 $\pm$ 1.1	67.4 $\pm$ 2.2
4	SO	4.7 $\pm$ 0.1	16.2 $\pm$ 1.2	135.6 $\pm$ 2.7
	PH	1.5 $\pm$ 0.1	28.6 $\pm$ 2.6*	196.1 $\pm$ 6.0*
22	SO	4.1 $\pm$ 0.1	9.5 $\pm$ 0.7	n.d.
	PH	2.0 $\pm$ 0.1	35.1 $\pm$ 1.7*	n.d.
30	SO	5.0 $\pm$ 0.2	24.0 $\pm$ 0.9	96.9 $\pm$ 3.1
	PH	2.4 $\pm$ 0.1	41.4 $\pm$ 2.0*	177.7 $\pm$ 5.3*
36	SO	4.9 $\pm$ 0.1	24.1 $\pm$ 1.2	n.d.
	PH	2.7 $\pm$ 0.1	48.2 $\pm$ 2.3*	n.d.
48	SO	4.8 $\pm$ 0.1	28.7 $\pm$ 2.8	90.5 $\pm$ 2.6
	PH	3.1 $\pm$ 0.1	37.2 $\pm$ 2.9*	129.0 $\pm$ 2.5*

Effect of partial hepatectomy on the enzymes involved in triacylglycerol synthesis

We investigated the activities of the enzymes involved in triacylglycerol synthesis as a function of post-surgery time.

The activity of microsomal glycerolphosphate acyltransferase was not affected by partial liver resection (results not shown). The activities of cytosolic and microsomal phosphatidate phosphohydrolase in livers from SO and hepatectomized rats showed a marked increase at 4 h after surgery, probably owing to stress associated with the treatment (Figs. 1a and 1b). At 12 h after surgery the activities of both cytosolic (Fig. 1a) and microsomal (Fig. 1b) phosphatidate phosphohydrolase were significantly increased in the hepatectomized rats as compared with SO animals.

Fig. 1(c) illustrates that the activity of diacylglycerol acyltransferase increased in regenerating liver. The stimulation was maximal between 22 and 36 h after surgery and coincided with changes in hepatic triacylglycerol levels observed after liver resection (cf. Table 1). Since the enzyme activity presented in Fig. 1(c) was assayed with endogenous microsomal diacylglycerol, the changes observed in diacylglycerol acyltransferase activity may reflect changes in microsomal levels of diacylglycerol. However, at one time after surgery (30 h) we also measured diacylglycerol acyltransferase activity using dispersions of exogenous diacylglycerol. Although the specific activities of the enzyme are obviously much higher when measured with exogenous diacylglycerol, the activity of the enzyme was again significantly higher in PH rats than in SO animals (1.36 ± 0.07 versus 0.91 ± 0.02 nmol/min per mg of protein; $P < 0.001$).

Synthesis and secretion of glycerolipids *in vivo*

Partial resection of the liver resulted in a marked decrease in the lipid components of VLDL in plasma as early as 4 h after surgery (Table 2). The observed decrease was more pronounced in VLDL triacylglycerol than in cholesterol or phospholipids associated with VLDL. The level of plasma triacylglycerol was minimal at 22 h after surgery. The data in Table 2 also show that, at 22 and 48 h after surgery, the concentration of LDL cholesterol was significantly higher in plasma from hepatectomized than

Fig. 1. Profile of the activities of phosphatidate phosphohydrolase and diacylglycerol acyltransferase after PH

The activities of cytosolic (a) and microsomal (b) phosphatidate phosphohydrolase and the activity of diacylglycerol acyltransferase (c) were determined in livers of SO (●) and PH animals (○). Data represent means $\pm$ S.E.M. for five animals. Significantly different from corresponding SO group: * $P < 0.001$; ** $P < 0.05$.

from SO rats. The amounts of cholesterol and phospholipids associated with HDL were significantly lower during the first 48 h after surgery in rats subjected to PH.

In order to study the secretion of triacylglycerol in VLDL after partial resection of the liver, rats were injected intraportally with [3 H]glycerol. Table 3 demonstrates that the incorporation of [3 H]glycerol into liver triacylglycerol was increased by almost 4-fold at 22 h after surgery. However, radioactivity associated with triacylglycerol of plasma VLDL from PH rats was not significantly different from that of SO rats. It should be noted that labelled glycerol is diluted by plasma glycerol released from adipose tissue (cf. Table 1). Thus the specific radioactivity of glycerol injected into PH rats is lower than in controls. Using oleate as precursor of triacylglycerol [4,5], one encounters the same problem. Therefore, we searched for another radioactive precursor to quantify VLDL secretion *in vivo*.

[3 H]Choline is incorporated into phosphatidylcholine (PC) via the CDP-choline pathway, and subsequently secreted in lipoproteins [31]. Rats subjected to PH or SO were intraportally injected with [3 H]choline. Resection of the liver resulted in a stimulation of the synthesis of hepatic PC by 4.4-fold (Table 3). The incorporation of labelled choline into VLDL was not appreciably affected by PH. However, per g of tissue, the hepatectomized livers, being much smaller, produced more plasma PC than did those of SO rats (Table 3).

Secretion of VLDL by cultured hepatocytes after partial hepatectomy

The interpretation of studies of the biosynthesis and secretion

Table 2. Effects of PH on the composition of plasma lipoproteins

At the time points indicated blood samples were collected from PH or SO rats, plasma lipoproteins fractionated and triacylglycerol, cholesterol and phospholipids were determined. Data represent means $\pm$ S.E.M. of six animals. Significantly different from SO: ^a $P < 0.001$, ^b $P < 0.01$; ^c $P < 0.05$; n.d., not detectable.

		Composition (mm)								
		Time after surgery ...	0 h		4 h		22 h		48 h	
			SO	PH	SO	PH	SO	PH		
VLDL	Triacylglycerol	0.36 ± 0.02	0.42 ± 0.02	0.12 ± 0.01 ^a	0.46 ± 0.04	0.10 ± 0.02 ^a	0.47 ± 0.07	0.23 ± 0.03 ^b		
	Cholesterol	0.37 ± 0.04	0.22 ± 0.02	0.12 ± 0.01 ^b	0.07 ± 0.02	0.05 ± 0.02	0.22 ± 0.03	0.21 ± 0.03		
	Phospholipids	0.33 ± 0.05	0.14 ± 0.01	0.05 ± 0.01 ^a	0.08 ± 0.01	0.03 ± 0.01	0.18 ± 0.03	0.15 ± 0.02		
LDL	Triacylglycerol	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01 ^c	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.14 ± 0.01 ^a		
	Cholesterol	1.05 ± 0.04	0.97 ± 0.04	1.00 ± 0.04	1.04 ± 0.06	1.30 ± 0.06 ^c	0.94 ± 0.05	1.36 ± 0.10 ^b		
	Phospholipids	0.54 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	0.43 ± 0.03	0.56 ± 0.03 ^c	0.44 ± 0.03	0.66 ± 0.06 ^b		
HDL	Triacylglycerol	0.03 ± 0.01	n.d.	n.d.	n.d.	n.d.	0.02 ± 0.01	n.d.		
	Cholesterol	1.52 ± 0.01	1.29 ± 0.04	1.17 ± 0.03 ^c	1.36 ± 0.06	0.83 ± 0.02 ^a	1.30 ± 0.04	0.97 ± 0.04 ^a		
	Phospholipids	1.06 ± 0.03	0.92 ± 0.03	0.77 ± 0.03 ^b	0.85 ± 0.03	0.66 ± 0.01 ^a	0.86 ± 0.02	0.76 ± 0.01 ^b		

Table 3. Incorporation of [³H]glycerol or [³H]choline into glycerolipids in liver and VLDL *in vivo*

Rats were subjected to PH or SO. At 22 h after surgery the animals were intraportally injected with 75 μ Ci of either [³H]glycerol or [³H]choline. After 45 min and 60 min, blood was collected and the liver was excised in glycerol- and choline-injected rats respectively. Data represent means $\pm$ S.E.M. of three to five animals. The weights of the livers were 7.2 $\pm$ 0.6 g (SO) and 3.6 $\pm$ 0.3 g (PH). Significantly different from SO: ^a $P < 0.01$, ^b $P < 0.02$.

	10 ⁻⁵ $\times$ Incorporation into liver (d.p.m./g of liver)	Incorporation into VLDL	
		(d.p.m./ml of plasma)	(d.p.m./ml of plasma per g of liver)
[³ H]Glycerol			
SO	1.34 $\pm$ 0.19	15800 $\pm$ 4900	2192 $\pm$ 595
PH	5.20 $\pm$ 1.23 ^b	9580 $\pm$ 2950	2662 $\pm$ 355
[³ H]Choline			
SO	11.9 $\pm$ 4.2	1458 $\pm$ 303	203 $\pm$ 42
PH	52.5 $\pm$ 5.9 ^a	1981 $\pm$ 372	550 $\pm$ 103 ^b

of triacylglycerol and phospholipids *in vivo* is hampered by distinction in liver mass and blood flow in the livers of hepatectomized and SO rats. The use of cultured hepatocytes elegantly circumvents these problems. Thus, we isolated hepatocytes from rats subjected to PH or SO and compared the biosynthesis of triacylglycerol and the secretion of VLDL.

Consistent with previous work *in vivo* [1,2] and the data in Table 1, the concentration of triacylglycerol 22 h after surgery was significantly higher in hepatocytes from PH rats than in cells from SO donors (147.8 $\pm$ 16.5 versus 67.5 $\pm$ 14.7 nmol/mg of cell protein; $P < 0.02$). However, the secretion of triacylglycerol into the medium during an incubation period of 6 h was of the same magnitude in both cell types (45.4 $\pm$ 11.3 and 39.8 $\pm$ 7.6 nmol/mg of cell protein for SO and PH respectively; means $\pm$ S.E.M. for four independent cell preparations). Thus the secretion of triacylglycerol is not impaired after partial resection of the liver.

Incubation of cultured hepatocytes with [³H]glycerol showed that the synthesis of triacylglycerol was 1.6-fold higher in cells from PH rats than in cells from SO animals (Table 4). The secretion of newly synthesized triacylglycerol into the medium was similar in both cell types. This implies that in cells from PH

Table 4. Biosynthesis and secretion of triacylglycerol in cultured hepatocytes after PH

Hepatocytes isolated from PH or SO rats 22 h after surgery were incubated in 2 ml of DMEM containing 0.5% BSA, 1 mM-glycerol and either 15 μ Ci of [1,3-³H]glycerol or 3 μ Ci of [³H]oleate. The final concentration of [³H]oleate was 0.5 mM. After 6 h the medium was collected and the cells were harvested. Data represent means $\pm$ S.E.M. of four cell preparations. Significantly different from control: ^a $P < 0.05$; ^b $P < 0.02$; ^c $P < 0.001$.

	10 ⁻⁴ $\times$ [³ H]Glycerol incorporation (d.p.m./mg of protein)		10 ⁻⁵ $\times$ [³ H]Oleate incorporation (d.p.m./mg of protein)	
	Cells	Medium	Cells	Medium
SO	4.04 $\pm$ 0.58	2.59 $\pm$ 0.64	6.25 $\pm$ 0.12	3.44 $\pm$ 0.03
PH	6.70 $\pm$ 0.76 ^a	2.99 $\pm$ 0.70	10.39 $\pm$ 0.04 ^b	0.96 $\pm$ 0.11 ^c

rats a relatively smaller part of newly synthesized triacylglycerol was secreted than in cells from SO animals.

When hepatocytes were treated with [³H]oleate, the amount of radioactive triacylglycerol recovered in cells from PH rats was enhanced as compared with control cells (Table 4). Concomitantly, the secretion of newly synthesized triacylglycerol was significantly lower in PH cells than in hepatocytes isolated from SO animals. Although control cells secreted 35.8 $\pm$ 4.8% of labelled triacylglycerol, cells isolated from PH rats only secreted 8.45 $\pm$ 1.81% of the total amount of newly synthesized triacylglycerol (Table 4). Thus the experiments with [³H]glycerol and [³H]oleate in cultured hepatocytes clearly show that after partial hepatectomy the synthesis of triacylglycerol is increased and that a relatively smaller part of newly synthesized triacylglycerol is secreted into the medium. The data in Table 4 reveal that PH leads to a decrease in the secretion of triacylglycerol labelled with [³H]oleate, but not with [³H]glycerol. The fact that oleate incorporation into triacylglycerol does not exclusively reflect synthesis *de novo*, but also deacylation/reacylation reactions, might be the reason for this difference.

The effect of partial hepatectomy on the secretion of apolipoproteins was studied in cultured hepatocytes by measuring the incorporation of labelled leucine into apolipoproteins at 22 h after surgery. Hepatocytes isolated from SO rats incorporated [³H]leucine into total cellular protein at a rate of 6.51 $\pm$

Table 5. Secretion of [³H]leucine-labelled apoproteins in VLDL from cultured hepatocytes after PH

Hepatocytes isolated 22 h after surgery were incubated for 6 h in 2 ml of DMEM containing 0.5% BSA, 1 mM-glycerol, 0.5 mM-oleate and 0.05 mM-[³H]leucine (25 μ Ci). At the end of the incubation period, the medium of three dishes was combined and VLDL were isolated. VLDL were concentrated, and the apoproteins were separated by SDS/PAGE. Results are presented as means $\pm$ S.D. of three different cell preparations.

	Secretion (d.p.m./mg of protein)		
	Apo B _h	Apo B _l	Apo E
SO	1146 $\pm$ 212	1128 $\pm$ 138	2408 $\pm$ 708
PH	1007 $\pm$ 115	1454 $\pm$ 133	2934 $\pm$ 388

1.10 nmol/6 h per mg of protein. Partial liver resection increased the incorporation of labelled leucine by almost 1.7-fold, to 10.9 ± 0.8 nmol/6 h per mg ($P < 0.01$). Medium of leucine-treated cells was collected and VLDL were isolated. It is generally assumed that apo B is essential for the assembly of triacylglycerol-rich lipoproteins [12]. VLDL from rat liver contain two forms of apo B of different molecular masses, a larger form (apo B_h) and a smaller form (apo B_l). Analysis of apolipoproteins associated with VLDL showed that the synthesis and secretion of leucine-labelled apo B_h, apo B_l and apo E were similar in SO cells and in cells from PH rats (Table 5).

DISCUSSION

In this study we examined the mechanism of fat accumulation in rat liver after partial hepatectomy. We determined the activities of enzymes involved in triacylglycerol synthesis as a function of time after surgery and measured the rate of synthesis and secretion of triacylglycerol *in vivo* as well as in primary cultures of hepatocytes isolated from SO or PH donor rats. These different experimental approaches offer the opportunity to distinguish between alterations in lipid synthesis and secretion at the level of hepatocytes and alterations in hepatic lipid metabolism due to changes in other organs.

We examined the plasma lipoprotein pattern at different time points after surgery (Table 2). Partial liver resection resulted in a marked decrease in the concentration of VLDL lipid components, particularly triacylglycerol. These data are in agreement with those of Trentalance *et al.* [10], who reported a decrease of plasma VLDL by approx. 50% at 16 h after surgery. However, there are several studies demonstrating a moderate hyperlipaemia following partial hepatectomy [4,5]. The discrepancy between the latter observations and our data (Table 2) may be explained by differences in the experimental outline. In one of the above-mentioned studies [4], rats subjected to surgery had free access to water containing 5% glucose, whereas in our experiments the drinking water had no glucose added. It is well established that the intake of glucose results in a marked induction of hepatic lipogenic enzymes [32], and consequently an increase in VLDL secretion.

In regenerating liver there is a tremendous synthesis of cellular phospholipids. It can be hypothesized that the requirement of phospholipids for the assembly of new membranes may result in a decreased availability of, particularly, PC for VLDL assembly. In this respect it is noteworthy that choline deficiency leads to a decline in VLDL secretion and the development of fatty liver [26]. We investigated the secretion of PC associated with VLDL. Our observations demonstrate that the biosynthesis of hepatic PC from [³H]choline was increased by more than 4-fold (Table

3). This is probably due to an increase of the activity of CTP:choline-phosphate cytidyltransferase, the key enzyme of PC synthesis *de novo* (M. Houweling, unpublished work). The secretion of [³H]choline-labelled PC in VLDL, expressed per g of liver, was significantly higher in serum from PH rats than in controls (Table 3). Thus, we conclude that the secretion of PC in VLDL is not impaired in PH rats.

Comparison of the data in Fig. 1 with those in Table 1 shows that there was about a 2-fold increase in hepatic triacylglycerol after 4 h in the PH animals as compared with the SO ones, yet there were no significant changes in any of the regulatory enzymes at this time. This rapid increase in hepatic triacylglycerol probably results from an enhanced influx of non-esterified fatty acids into the liver. The increase in plasma glycerol in PH animals (Table 1) indicates that these fatty acids are generated during lipolysis in adipose tissue. However, at later time points enhanced activities of key enzymes in triacylglycerol formation, such as phosphatidate phosphohydrolase and diacylglycerol acyltransferase (Fig. 1), contributed to the elevated synthesis of hepatic triacylglycerols in resected animals. This notion is supported by the observation in the isolated hepatocyte system, in which the synthesis of triacylglycerol is increased in the absence of added fatty acids (Table 4).

In line with the observation that the activity of diacylglycerol acyltransferase was enhanced in regenerating liver at 22 h after surgery, the biosynthesis of triacylglycerol from [³H]glycerol *in vivo* at that time was significantly elevated in livers from PH rats as compared with SO animals (Table 3). However, the secretion of labelled triacylglycerol associated with VLDL was very similar in hepatectomized rats and in controls (Table 3). The reason for this might be either a decrease in the total capacity of the liver remnant to secrete VLDL or an intracellular alteration of triacylglycerol metabolism in hepatocytes isolated from PH rats. The values for VLDL synthesis *in vivo* reported in Table 3 may not be true reflections of VLDL synthesis, since lipoprotein lipase activity was not inhibited in these experiments. However, the data on glycerol-labelled triacylglycerol in VLDL *in vivo* are supported by those *in vitro* (Table 4). In the latter system, secretion of glycerol-labelled triacylglycerol was unaffected by PH.

To distinguish between decrease in capacity for VLDL secretion or alteration in triacylglycerol metabolism, we examined the biosynthesis and secretion of triacylglycerol in hepatocytes isolated from PH or SO rats at 22 h after surgery. The cellular content of triacylglycerol in hepatocytes from PH rats was more than twice that of cells isolated from SO animals. The secretion of this lipid into the medium was similar in both cell types. It is assumed that the total secretion of triacylglycerol into the medium is a good estimate of VLDL secretion of this lipid [11]. Thus we conclude that the observed decline in the amount of VLDL triacylglycerol after liver resection (Table 2) is the result of a decrease of liver mass.

Schofield *et al.* [33] suggested that the development of fatty liver in regenerating liver is due to an increase in redistribution of fatty acids to triacylglycerol and away from fatty acid oxidation. Consistent with their suggestion, our data show that the biosynthesis of triacylglycerol, assayed by the incorporation of labelled glycerol and oleate, was significantly enhanced in hepatocytes from PH rats as compared with SO rats (Table 4). However, the activity of carnitine palmitoyltransferase I, the rate-controlling step in fatty acid oxidation, was not affected in livers from hepatectomized rats throughout 48 h following surgery (results not shown).

There have been several studies demonstrating a relation between the rate of triacylglycerol synthesis and subsequent secretion of this lipid. Exogenous fatty acids stimulated triacyl-

glycerol synthesis in perfused rat liver [34] and cultured hepatocytes [35] with a concomitant increase of VLDL secretion. When the supply of endogenous fatty acids was enhanced in isolated liver by inhibition of the oxidation of long-chain fatty acids, the secretion of triacylglycerol increased by approx. 5-fold [36]. Our data, however, demonstrate that in hepatocytes from hepatectomized rats the observed increase in triacylglycerol synthesis was not accompanied by an elevation of VLDL secretion. This may indeed lead to an accumulation of fat in the cells. Comparable observations have been made in livers of diabetic animals and in suckling-rat liver. In both conditions the supply of serum non-esterified fatty acids, the major precursor of triacylglycerol, is high and the capacity for VLDL secretion is low, resulting in cytosolic storage of triacylglycerol in hepatocytes [12].

We examined whether this uncoupling of synthesis and secretion was due to a decreased availability of apoproteins. Our hypothesis was that the requirement of newly synthesized proteins in regenerating liver might result in a temporary shortage of apolipoproteins for VLDL secretion. It is obvious from the data presented in Table 5 that the synthesis and secretion of the major apoproteins of VLDL were similar in hepatocytes from hepatectomized donors and SO rats.

Davis & Boogaerts [35] demonstrated that stimulation of the synthesis and secretion of triacylglycerol by fatty acids was not accompanied by a simultaneous induction of the formation of apoproteins in short-term cultures of hepatocytes. Rather, the size of the lipoprotein particles might increase in fatty acid-treated cells. In hepatocytes from PH rats, however, the observed stimulation of triacylglycerol formation does not induce an elevation in the secretion of this lipid. Apparently, the synthesis of triacylglycerol in hepatocytes isolated from resected livers temporarily exceeds the secretion, which may result in the development of fatty liver. Further studies are needed to investigate the influence of partial hepatectomy on the assembly and transport of nascent VLDL in hepatocytes.

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