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A Study of Some Enzymes of Glycerolipid Biosynthesis in Rat Liver after Subtotal Hepatectomy

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1. The accumulation of triglyceride in the liver remnant after subtotal hepatectomy (removal of 82% of the liver) exceeded that described for partial hepatectomy (removal of 70% of the liver). 2. Palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and diglyceride acyltransferase activities were measured in the microsomal fraction, and phosphatidate phosphohydrolase activity was measured in the particle-free supernatant fraction, prepared from the liver remnant at various times after subtotal hepatectomy. 3. The only enzyme showing a significant change in specific activity was phosphatidate phosphohydrolase. The specific activity was approximately fivefold that of the control value at 6h after operation and threefold that of the control at 10, 16 and 24h after operation. A smaller increase in the specific activity of the enzyme in sham-operated animals occurred only at 6h after operation. 4. However, at this time the total phosphohydrolase activity of the remaining liver in the sham-operated rats was approximately threefold that in hepatectomized rats. 5. Injection of actinomycin D prevented the increase in activity of phosphatidate phosphohydrolase but did not prevent the accumulation of triglyceride.

There is a dramatic accumulation of neutral lipid in parenchymal cells of the rat liver remnant after partial hepatectomy (Camargo *et al.*, 1966; Glende & Winfield, 1968; Delahunty & Rubinstein, 1970; Fex, 1970). The lipid that accumulates is predominantly triglyceride (Fex, 1970) and this may increase approximately fourfold 20h after partial hepatectomy (Glende & Winfield, 1968; Delahunty & Rubinstein, 1970). By approximately 48h after operation, however, the triglyceride concentration of the liver returns to normal (Glende & Winfield, 1968; Girard *et al.*, 1971). A detailed study of the composition of neutral lipids and phospholipids at various times after partial hepatectomy has been reported (Vaver *et al.*, 1969).

Lipid accumulation in the regenerating liver is apparently not due to a defect in lipoprotein transport (Fex & Olivecrona, 1968*b*; Infante *et al.*, 1969) or of synthesis of very low-density lipoprotein (Girard *et al.*, 1971). Infante *et al.* (1969) and Delahunty & Rubinstein (1970) suggested that the fatty liver observed shortly after partial hepatectomy was due to the rate of triglyceride biosynthesis temporarily exceeding the rate of its export from the liver as lipoprotein. There is evidence for an increased synthesis of new triglycerides in regenerating liver. Thus an increased incorporation of [^{14}C]glycerol into neutral lipids was observed after partial hepatectomy (Johnson & Alberts, 1960; Olivecrona & Fex, 1970).

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There is also an increased incorporation of plasma free fatty acids into liver triglycerides (Fex & Olivecrona, 1968*a*; Girard *et al.*, 1971).

In the present study the activities of palmitoyl-CoA synthetase (EC 6.2.1.3), glycerol phosphate acyltransferase (EC 2.3.1.15), phosphatidate phosphohydrolase (EC 3.1.3.4) and diglyceride acyltransferase (EC 2.3.1.20) were measured in the liver remnant at various times after subtotal hepatectomy (Weinbren & Woodward, 1964) to determine whether any of these activities changed during the increased synthesis of triglyceride in the regenerating liver.

Materials and Methods

Materials

Chemicals. CoA, grade I, *rac*-glycerol 3-phosphate (disodium salt), GSH, triolein (Sigma grade) and phospholipase C (type I from *Clostridium perfringens*) were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Dithiothreitol, (−)-carnitine and indoxyl acetate were purchased from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. and ATP (disodium salt) from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K. Bovine serum albumin (fraction V, fatty acid-poor) was obtained from Pentex Inc., Kankakee, Ill., U.S.A. Silicic acid (SilicAR CC-7; 100–200 mesh) was obtained from Camlab Ltd., Cambridge, U.K., alumina (aluminium oxide, M.C.F., Camag, 100–250 mesh, alkaline,

Brockmann activity 1) from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K., and actinomycin D (Lyovac Cosmegen) from Merck, Sharp and Dohme, West Point, Pa. 19486, U.S.A. (±)-[^{3}H]Carnitine, palmitoyl-(−)-carnitine and carnitine palmitoyltransferase (EC 2.3.1.23) were prepared by the method of Sánchez *et al.* (1973) and *sn*-[1,3-^{3}H]glycerol 3-phosphate was prepared as described by Smith & Hübscher (1966). [1-^{14}C]-Palmitate, [^{3}H]methyl iodide and [1,3-^{3}H]glycerol were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Animals. Male Wistar rats were supplied by the University of Nottingham Joint Animal Breeding Unit, Sutton Bonington, Leics., U.K. The animal weight (mean±1 S.D.) at the time of operation was 200±14g (96 animals).

Methods

Preparation of sn-1,2-diglyceride. The method was that of R. H. Michell (personal communication). Lipids were extracted from the yolks of 12 eggs with 300ml of chloroform and dried by rotary evaporation; the lipids were dissolved in 50ml of chloroform and applied to an alumina column (200g; 5cm×8cm). Neutral lipids were eluted with 700ml of chloroform and phosphatidylcholine with 1.2 litres of chloroform–methanol (5:1, v/v). Fractions containing phosphatidylcholine were combined, dried by rotary evaporation, the residues dissolved in 50ml of chloroform and applied to a silicic acid column (3.5cm×85cm) that had been previously washed with methanol and then chloroform. Any remaining neutral lipid was eluted with 1 litre of chloroform–methanol (11:2, v/v) and phosphatidylcholine was recovered with 2 litres of chloroform–methanol (3:2, v/v). The phosphatidylcholine was dried by rotary evaporation and stored in 10ml of benzene. No sphingomyelin could be detected in the preparation by t.l.c. on plates of silica gel G by using chloroform–methanol–water (95:35:5, by vol.) for development.

sn-1,2-Diglyceride was prepared from 300μmol of phosphatidylcholine after evaporation of the benzene and solution in 4ml of diethyl ether. To this solution was added 100ml of water and the mixture was shaken to emulsify. After this 10.4ml of 0.25M-Tris, adjusted to pH7.4 with HCl, and 12.7ml of 10mM-$CaCl_2$ were added. The mixture was shaken for 5h at 37°C with 25mg of phospholipase C. Diglyceride was extracted into 92ml of hexane and the solvent removed by rotary evaporation. The residue was dissolved in 15ml of chloroform and 8g of silicic acid was added to absorb phosphatidylcholine. After the mixture had been shaken and filtered, the diglyceride was recovered from the filtrate by rotary evaporation, dissolved in hexane and stored at −20°C. The diglyceride was shown to be mainly 1,2-diglyceride with a trace of 1,3-diglyceride after two-dimensional t.l.c. on silica gel H with isopropyl ether–acetic acid (96:4, v/v) and then light petroleum (b.p. 40–60°C)–diethyl ether–acetic acid (90:40:1, by vol.) used for development. Before chromatography the t.l.c. plates were washed with chloroform–methanol (4:1, v/v) and activated at 110°C for 1h.

The emulsion of diglyceride was prepared immediately before use in enzyme assays. A sample of diglyceride was taken and the solvent removed with a stream of N_2. Sufficient water–hexane (49:1, v/v) was added to give a final concentration of 21mM-diglyceride after emulsification. The mixture was sonicated four times for approx. 30s at 0°C and 21kHz until a uniform emulsion was obtained.

Preparation of membrane-bound phosphatidate. A microsomal fraction was prepared from several rat livers as described below. The membrane-bound phosphatidate labelled with [1-^{14}C]palmitate was prepared as described by Mitchell *et al.* (1971), except that approx. 4mg of microsomal protein/ml was used, incubation was for 60min and, after incubation, the mixture was layered over 0.3M-sucrose containing 20mM-EDTA (pH adjusted to 7.4 with $KHCO_3$) and was centrifuged for 80min at 4°C at 76000*g* ($r_{av.}$ 7.62cm). The pellets were combined, suspended by homogenization in 0.3M-sucrose containing 1mM-dithiothreitol and stored at −20°C. The membrane-bound phosphatidate preparation was thawed only once before use as a substrate for the supernatant phosphatidate phosphohydrolase, as loss of effectiveness as substrate was observed after successive freezing-and-thawing treatments.

Approx. 90% of the [1-^{14}C]palmitate incorporated into glycerolipids was recovered in the total phospholipid fraction of the membrane-bound phosphatidate preparation, and of this approx. 83% was in phosphatidate.

Hepatectomy. Rats were subjected to subtotal hepatectomy, which involved 81.5±1.5% (S.D.) of the liver, through removal of the two anterior lobes, two divisions of the caudate lobe and the inferior division of the right lobe (Weinbren & Woodward, 1964). Sham operations were carried out in a similar manner except that the liver lobes were not excised after exposure. Animals accustomed to normal light and dark periods were operated on between 9.00 a.m. and 1.00 p.m., whereas animals adapted to 'reverse daylight' were operated on between 9.00 p.m. and 1.00 a.m. Animals were fed *ad libitum* before operation. Thereafter they were kept singly in ordinary cages with free access to water but with no food supplied. They were kept in a warm room (approx. 28°C) until time of death. Animals which had sham operations and those on which no operation was performed were kept under the same conditions as hepatectomized animals.

In one series of experiments the activities of various

enzymes were measured at 6, 10, 16 and 24h after operations. In a second series of experiments the effect of actinomycin D on certain enzyme activities in the regenerating liver was studied. Actinomycin D (100μg) in 0.2ml of a mannitol solution (20mg of mannitol/ml) was injected intraperitoneally into control and hepatectomized animals. The antibiotic was given to the latter group of animals immediately after operation. In the same series of experiments some animals (both control and hepatectomized) were given injections of 0.2ml of mannitol (20mg/ml). All animals in this second series of experiments were killed 13h after injection.

Preparation of microsomal and particle-free supernatant fractions. Rats were killed by a blow on the head followed by decapitation. Livers were removed from sham-operated and control rats, washed with 0.9% NaCl soln., blotted and weighed. The right lobe, corresponding to that which was not removed from hepatectomized rats at the time of operation, was weighed and in addition a weighed portion of the median lobe was taken to give sufficient material for fractionation. The liver remnant of each hepatectomized animal was freed of atrophied remains of lobes that had been removed at the time of operation, washed with 0.9% NaCl soln., blotted and weighed. Each liver portion was cut into small fragments with scissors and homogenized by six up-and-down strokes of a Teflon pestle–glass homogenizer (radius clearance 0.0076cm; 590rev./min), in 7.5ml of 0.3M-sucrose (pH adjusted to 7.4 with $KHCO_3$) per g of liver. The crude homogenate was filtered through bolting cloth and the volume noted. A sample (5ml) of homogenate was centrifuged for 10min at 17850*g* ($r_{av.}$ 7.62cm). The supernatant (1) was decanted carefully. The pellet was washed by resuspension in 2.5ml of 0.3M-sucrose, pH7.4, with three up-and-down strokes of a pestle homogenizer (radius clearance 0.0117cm) and subsequent centrifugation for 10min at 17850*g*. The supernatant (2) was decanted gently. The combined supernatants (1 plus 2) were centrifuged for 60min at 104000*g* ($r_{av.}$ 6.25cm). The pellet was suspended in 5ml of 0.3M-sucrose, pH7.4, containing 1mM-dithiothreitol (microsomal fraction) and the supernatant (particle-free supernatant fraction) was retained.

For experiments in which the effect of actinomycin D on various enzyme activities was studied, the particle-free supernatant fraction was prepared as described above except that the last centrifugation step was for 35min at 180000*g* ($r_{av.}$ 6.25cm).

All stages of the fractionation were performed at 0–4°C.

Enzyme assays. Each enzyme was assayed at several protein concentrations to ensure that the reaction rate was proportional to enzyme concentration.

(a) Palmitoyl-CoA synthetase. The method was essentially that of Sánchez *et al.* (1973). Each assay mixture contained, in 0.25ml of 25mM-Tris buffer adjusted to pH7.4 with HCl: 5mM-dithiothreitol, 50μM-CoA, 2.5mM-ATP, 2.5mM-$MgCl_2$, 0.8mM-potassium palmitate, 1.5mg of fatty acid-poor bovine serum albumin, 2.4mM-(−)-[^{3}H]carnitine (0.4μCi/μmol) and excess (approx. 420μg of protein) of carnitine palmitoyltransferase. Between 25 and 50μg of microsomal protein was used per assay.

(b) Glycerol phosphate acyltransferase. The method of incubation and extraction was essentially that of Brindley (1973). Each assay contained, in 0.25ml of 25mM-Tris buffer adjusted to pH7.4 with HCl: 5mM-dithiothreitol, 70μM-CoA, 0.25mM-palmitoyl-(−)-carnitine, 1.5mg of fatty acid-poor bovine serum albumin, an excess (approx. 200μg of protein) of carnitine palmitoyltransferase and 15.6mM-*sn*-[1,3-^{3}H]glycerol 3-phosphate (1μCi/μmol). Between 50 and 100μg of microsomal protein was used per assay.

(c) Supernatant phosphatidate phosphohydrolase. This was assayed as described by Mitchell *et al.* (1971), except that a potassium phosphate buffer (pH7.4) was used and microsomal membrane-bound phosphatidate (approx. 2mg of protein per incubation) was used instead of mitochondrial membrane-bound phosphatidate. Incubation of membrane-bound phosphatidate in the absence of particle-free supernatant fraction was used as a control, as there was some phosphatidate phosphohydrolase activity in the membrane-bound phosphatidate preparation.

The reaction was terminated by addition of 3.75ml of chloroform–methanol (1:2, v/v). Chloroform (1.25ml), containing 0.24% (v/v) olive oil, and 1.25ml of 10mM-$CaCl_2$ were then added and, after gently mixing, the fractions were stored at −20°C. Neutral lipids were isolated from the reaction mixture as follows: after being warmed to room temperature each fraction was centrifuged for 10min (bench centrifuge), 2ml of bottom phase was applied to a column of 2.5g of alumina in chloroform and neutral lipids were eluted in 10ml of chloroform. After evaporation of chloroform the recovery of [1-^{14}C]palmitate in the neutral lipids was determined by liquid-scintillation counting with 5ml of xylene containing 4g of 2,5-diphenyloxazole/litre and a Tri-Carb Liquid Scintillation Counter, model 3375. Correction for counting efficiency was by the channels-ratio method.

(d) Diglyceride acyltransferase. This was determined by measuring the incorporation of [1-^{14}C]-palmitate into neutral lipid in the presence of optimum concentrations of cofactors and substrate. The enzyme reaction rate was found to be constant for 40min and constant with respect to protein at concentrations below 1.6mg of microsomal protein/ml.

Each incubation contained, in a final volume of 0.5ml of 25mM-Tris adjusted to pH7.4 with HCl: 5mM-GSH, 1mM-ATP, 0.16mM-CoA, 20mM-$MgCl_2$, 3mg of bovine serum albumin (fatty acid-poor),

0.1–0.6mg of microsomal protein and 4.2mM-*sn*-1,2-diglyceride. The reaction was started, after 2min preincubation at 37°C, with sufficient potassium [1-^{14}C]palmitate (0.2μCi/μmol) to give a final concentration of 2.0mM, and continued for 20min. The reaction was terminated by addition of 3.75ml of chloroform–methanol (1:2, v/v). Neutral lipids were isolated from the reaction mixture as described for phosphatidate phosphohydrolase.

(e) Arylesterase (EC 3.1.1.2). This enzyme was assayed as described by Shephard & Hübscher (1969).

Determination of DNA and protein. These were determined as described by Hübscher *et al.* (1965).

Determination of triglyceride. Duplicate samples (1ml) of each homogenate were thawed after storing at −20°C and the lipids extracted as follows. Chloroform (1ml) and methanol (1ml) were added. After mixing vigorously on a Vortex mixer, followed by centrifuging (bench centrifuge), the bottom phase was removed. The top phase was washed with 1ml of chloroform by mixing and centrifugation as before. The resultant bottom phase was combined with the first, the solvents were removed by evaporation under a stream of N_2, and the lipid extract was dissolved in 2ml of hexane. A triglyceride fraction was obtained free of phospholipid, cholesterol, cholesterol esters and mono- and di-glyceride by a modification of the method of Barron & Hanahan (1958). Neutral lipid in hexane (1ml) was applied to a column of 1g of silicic acid in hexane. Cholesterol esters were eluted with 5ml of hexane containing 15% (v/v) benzene. Triglycerides (and some free fatty acids) were eluted subsequently with 20ml of hexane containing 10% (v/v) diethyl ether. Mono- and di-glycerides remained on the column. The solvents were removed from the triglyceride fraction by evaporation under a stream of N_2, 2ml of ethanol–ether (3:1, v/v) was added and the triglyceride ester content determined by the method of Stein & Shapiro (1953).

Experimental and Results

In the present investigation subtotal hepatectomy (removal of 82% of the liver; Weinbren & Woodward, 1964) rather than partial hepatectomy (70% removal) was performed as the synthesis of DNA was found to be delayed by the former operation. Thus a maximum incorporation of ^{32}P into DNA by the liver remnant after subtotal hepatectomy was observed approx. 36h after operation compared with a maximum at about 20h for partial hepatectomy (Weinbren & Woodward, 1964). In the present investigation a significant difference of DNA concentration (μmol of DNA phosphorus/g wet wt.) was observed between control or sham-operated and hepatectomized animals at each time-interval (Table 1). The lower concentration in hepatectomized animals is probably due to the increase in water, protein and lipid content of the liver remnant. As there was a significant increase in weight of the liver remnant with time after subtotal hepatectomy (Table 1), the total DNA per liver remnant was calculated. With the small number of samples taken there were no significant differences in DNA content of the liver remnant from that at 6h and that at 10 or 16h, but a significant increase occurred by 24h compared with 6h.

The lipid accumulation in the liver remnant after subtotal hepatectomy, however, is not delayed compared with that after partial hepatectomy and may even begin earlier. The extent of lipid accumulation in the liver remnant after subtotal hepatectomy is two- to three-fold that in partially hepatectomized animals (Girard *et al.*, 1971). The period during which there is a high rate of lipid accumulation (less than 16h after operation) occurs before significant DNA synthesis begins.

The behaviour of various enzymes of lipid biosynthesis in the liver remnant after subtotal hepatectomy could thus be studied during the period of massive lipid accumulation and before significant cell division occurred. The specific activities of each enzyme studied were expressed in terms of DNA so that an estimate of the activity per cell could be obtained.

The activity of arylesterase, an enzyme found in the endoplasmic reticulum (Underhay *et al.*, 1956), was determined in each homogenate and in each corresponding microsomal fraction so that the recovery of endoplasmic reticulum membrane in the microsomal fraction could be estimated. Hence the total activity of the microsomal palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and diglyceride acyltransferase in each homogenate could be calculated from their activity in the microsomal fraction prepared from that homogenate.

The recovery of arylesterase in the microsomal fraction, expressed as a percentage of the corresponding homogenate activity, did not differ significantly between control or sham-operated and hepatectomized animals at each time-interval studied (Table 1). This suggests that the procedure was suitable for fractionating regenerating liver. The specific activity of arylesterase, expressed in terms of DNA (Table 2), did not differ between control or sham-operated and hepatectomized animals at each time-interval studied although there was an apparent decrease in activity, in each of the three groups of animals, between 6 and 10h after the operation.

There was no significant difference in the activity of palmitoyl-CoA synthetase between control or sham-operated and hepatectomized animals at each time-interval after operation, with the exception of a small difference at 24h (Table 2). Control values at 16 and 24h were significantly lower ($P<0.01$ and $P<0.02$ respectively) than at 10h. The activity of glycerol phosphate acyltransferase also did not differ signifi-

Table 1. *Effect of subtotal hepatectomy on liver wet weight, DNA, triglyceride, arylesterase activity and phosphatidate phosphohydrolase activity*

Values are given as mean ±1 S.D. The number of animals in each group is given in parentheses. Where results are significantly different from (*a*) control or (*b*) sham-operated animals the difference, calculated by Student's *t* test, is indicated as: $*P < 0.01$; $**P < 0.001$. Units of phosphatidate phosphohydrolase activity are μmol of phosphatidate hydrolysed/min.

Time after operation (h) ...	6			10			16			24		
Group ...	Control (4)	Sham-operated (7)	Subtotally hepatectomized (7)	Control (5)	Sham-operated (5)	Subtotally hepatectomized (8)	Control (4)	Sham-operated (6)	Subtotally hepatectomized (8)	Control (4)	Sham-operated (5)	Subtotally hepatectomized (9)
Wet wt. of liver at time of death	7.09 ± 0.39	7.79 ± 0.25	1.80 ± 0.22	6.29 ± 0.19	7.23 ± 0.80	2.05 ± 0.09	6.71 ± 0.39	7.53 ± 0.62	2.51 ± 0.17	6.53 ± 0.65	6.48 ± 0.37	2.76 ± 0.14
DNA phosphorus (μmol/g wet wt. of liver)	5.96 ± 0.34	5.57 ± 0.66	4.97 ± 0.48 *a**	6.30 ± 0.46	6.59 ± 0.60	4.74 ± 0.58 *a** *b***	6.19 ± 0.89	6.27 ± 0.92	4.55 ± 0.83 *a** *b**	7.11 ± 1.01	6.49 ± 1.14	4.75 ± 0.73 *a** *b**
DNA phosphorus (μmol/ liver remnant)	42.1 ± 1.4	43.3 ± 4.8	8.46 ± 1.32	39.7 ± 3.9	47.9 ± 8.8	9.72 ± 1.19	40.9 ± 4.3	47.0 ± 6.2	11.52 ± 2.7	46.1 ± 6.7	42.2 ± 8.8	13.18 ± 2.55
Recovery of arylesterase activity in microsomal fraction (%)	30.9 ± 4.5	40.7 ± 12.6	38.6 ± 10.5	50.3 ± 15.7	43.6 ± 18.5	46.8 ± 8.9	41.2 ± 4.9	35.1 ± 5.0	35.8 ± 4.6	52.3 ± 2.4	48.3 ± 9.5	44.7 ± 4.6
Triglyceride (μmol/μmol of DNA phosphorus)	0.713 ± 0.133	1.04 ± 0.317	4.80 ± 0.203 *a*** *b***	0.597 ± 0.140	0.450 ± 0.110	7.93 ± 1.73 *a*** *b***	0.717 ± 0.243	0.690 ± 0.157	17.9 ± 5.50 *a*** *b***	0.627 ± 0.073	0.727 ± 0.183	14.7 ± 3.60 *a*** *b***
Units of phosphatidate phosphohydrolase/total liver	0.78 ± 0.43	2.59 ± 0.52	0.85 ± 0.19 *b***	1.68 ± 0.26	2.86 ± 0.22	1.23 ± 0.35 *b***	1.75 ± 0.38	1.40 ± 0.46	1.32 ± 0.31	1.57 ± 0.11	1.32 ± 0.48	1.30 ± 0.37

Table 2. *Comparison of various enzyme activities in livers of rats after subtotal hepatectomy with those of control and sham-operated animals*

The activity of phosphatidate phosphohydrolase was measured in particle-free supernatant and the activities of the other enzymes were measured in the microsomal fraction. Activities are expressed as: acyl-CoA synthetase, nmol of palmitoyl-CoA formed/min per μmol of DNA phosphorus; glycerol phosphate acyltransferase, nmol of *sn*-glycerol 3-phosphate incorporated/min per μmol of DNA phosphorus; phosphatidate phosphohydrolase, nmol of phosphatidate hydrolysed/min per μmol of DNA phosphorus; arylesterase, μmol of indoxyl formed/min per μmol of DNA phosphorus. For diglyceride acyltransferase the specific activities of hepatectomized and sham-operated animals were expressed relative to the corresponding control values (see the text) and the mean control specific activity of each experiment was arbitrarily given the value of 1.0. The mean value ±1 S.D. of each enzyme activity is recorded. The number of animals in each group is given in parentheses. Where results are significantly different from (*a*) control or (*b*) sham-operated animals the difference, calculated by Student's *t* test, is indicated as: $*P < 0.01$; $**P < 0.001$.

	Specific activity											
Time after operation (h)	6			10			16			24		
Group	Control (4)	Sham-operated (7)	Subtotally hepatectomized (7)	Control (5)	Sham-operated (5)	Subtotally hepatectomized (8)	Control (4)	Sham-operated (6)	Subtotally hepatectomized (8)	Control (8)	Sham-operated (5)	Subtotally hepatectomized (9)
Palmitoyl-CoA synthetase	132 ± 45	91.9 ± 58.5	115 ± 46	114 ± 18	115 ± 21	120 ± 28	64.0 ± 13.1	82.3 ± 20.2	88.5 ± 22.2	72.7 ± 9.8	65.7 ± 8.4	84.8 ± 9.6 *b**
Glycerol phosphate acyltransferase	41.2 ± 7.3	26.0 ± 14.5	32.5 ± 13.3	17.7 ± 4.6	15.8 ± 3.0	22.0 ± 3.4	12.3 ± 4.3	16.0 ± 6.4	17.7 ± 7.4	23.1 ± 4.9	18.6 ± 3.8	35.1 ± 6.9 *b***
Phosphatidate phosphohydrolase	18.7 ± 10.5	56 ± 14 *a**	103 ± 30 *a*** *b**	42.3 ± 5.0	60.5 ± 7.5 *a***	125 ± 28 *a*** *b***	42.8 ± 9.5	30.6 ± 11.3	122 ± 45 *a** *b***	34.6 ± 5.9	31.3 ± 10.3	101 ± 30 *a** *b***
Diglyceride acyltransferase		1.01 ± 0.19	1.58 ± 0.67		0.94 ± 0.27	1.42 ± 0.31		1.24 ± 0.12	1.17 ± 0.27		0.98 ± 0.16	1.15 ± 0.22
Arylesterase	52.0 ± 11.9	41.1 ± 10.3	46.9 ± 15.0	24.5 ± 4.8	22.8 ± 4.1	27.3 ± 6.0	26.2 ± 4.4	31.1 ± 6.1	27.4 ± 5.3	25.2 ± 5.2	29.5 ± 4.4	27.8 ± 4.5

Table 3. *Effect of actinomycin D on DNA and triglyceride content and on the activities of arylesterase and phosphatidate phosphohydrolase in livers of control and hepatectomized rats*

The specific activities of arylesterase and phosphatidate phosphohydrolase are expressed as described in legend to Table 2. The number of animals in each group is indicated in parentheses. The significance of differences between results was calculated by the Student's t test and $P>0.05$ is shown as not significant (n.s.).

	Specific activity				
	1	2	3	4	
Group ...	Control	Control+ actinomycin D	Subtotally hepatectomized	Subtotally hepatectomized +actinomycin D	Significance of differences
DNA phosphorus (μmol/g wet wt.)	6.50 ±0.39 (6)	6.47 ± 0.29 (6)	4.98± 0.37 (6)	5.15± 0.16 (7)	1 v. 2 n.s. 1 v. 3 $P<0.001$ 1 v. 4 $P<0.001$ 3 v. 4 n.s.
Triglyceride (μmol/μmol of DNA phosphorus)	0.657±0.237 (6)	0.747± 0.257 (6)	6.80± 2.43 (6)	10.0 ± 3.07 (7)	1 v. 2 n.s. 1 v. 3 $P<0.001$ 1 v. 4 $P<0.001$ 3 v. 4 n.s.
Specific activity of arylesterase	27.2 ±7.9 (4)	30.1 ± 3.7 (6)	34.0 ±12.3 (6)	39.3 ± 9.4 (7)	1 v. 2 n.s. 1 v. 3 n.s. 1 v. 4 n.s. 3 v. 4 n.s.
Specific activity of phosphatidate phosphohydrolase	26.8 ±7.2 (6)	28.2 ±14.6 (6)	81.0 ±16.1 (6)	32.0 ±17.8 (7)	1 v. 2 n.s. 1 v. 3 $P<0.001$ 1 v. 4 n.s. 3 v. 4 $P<0.001$

cantly between control or sham-operated and hepatectomized animals at each time-interval studied, with the exception of a difference at 24h (Table 2). Control values at 10 and 16h were significantly lower ($P<0.01$ and $P<0.001$ respectively) than at 6h.

It was necessary to express the activity of diglyceride acyltransferase in sham-operated and hepatectomized animals relative to the mean control value of the corresponding experiment, because of the difficulty in preparing a standard diglyceride emulsion. As the latter was unstable it was freshly prepared for each experiment. In each particular experiment good agreement was obtained between the activity in each group of control animals, each group of sham-operated animals and each group of hepatectomized animals. For example, at 10h in the first experiment control values were 23.8 and 26.5nmol of palmitate incorporated into neutral lipid/min per μmol of DNA phosphorus and in the second experiment control values were 109, 143 and 90.7. In the first experiment values for hepatectomized animals were 36.7, 30.6, 46.4 and 48.7 and in the second experiment were 129, 134, 143 and 155nmol/min per μmol of DNA phosphorus. The enzyme reaction rate was found to be constant with protein concentration in each experiment. The difference between experiments was probably due to difference in the dispersion of diglyceride in the emulsions used as substrate for the enzyme. From the relative values of diglyceride acyltransferase shown in Table 2 it was calculated that there was no significant difference between the activity of the enzyme in control or sham-operated and hepatectomized animals at each time-interval studied.

Phosphatidate phosphohydrolase was the only enzyme studied whose activity altered dramatically after hepatectomy. The specific activity of this enzyme in the regenerating liver was significantly higher than in the liver of control and sham-operated animals at each time-interval studied (Table 2). At 6h the activity of phosphatidate phosphohydrolase was approximately fivefold the value for the control. An analogous rise in specific activity at 6h was observed in the sham-operated animal but this was significantly lower than in the hepatectomized animal. Subsequently the specific activity in the sham-operated animals returned to approximately control activity, but the enzyme activity in the hepatectomized animals remained approximately threefold that of the control at 10, 16 and 24h after operation.

Expressed in terms of the mean activity in the remaining liver at 6h after operation, the phosphohydrolase was approximately threefold as active in the sham-operated rats as in those subjected to subtotal hepatectomy (Table 1). This activity in the sham-operated animals was maintained at 10h but showed a significant ($P<0.01$) decrease at 16 and 24h after operation. After 24h the total activity in the livers of control, sham-operated and rats subjected to subtotal hepatectomy was not significantly different.

To investigate whether the increase in activity of phosphatidate phosphohydrolase was due to an activation of available enzyme or due to the synthesis of new enzyme protein the effect of actinomycin D on phosphatidate phosphohydrolase activity in regenerating liver was studied. Actinomycin D did not directly inhibit phosphatidate phosphohydrolase activity, as there was no significant difference between the activity in livers of controls and that of controls treated with actinomycin D (Table 3). The antibiotic did not alter the DNA concentration in the liver remnant after hepatectomy as there was no significant difference in DNA concentration between actinomycin-treated animals and those not given the antibiotic (Table 3).

Administration of actinomycin D at the time of operation abolished the increase in phosphatidate phosphohydrolase activity after 13h (Table 3). In contrast, arylesterase activity, which is not affected by subtotal hepatectomy (Table 2), was not decreased.

Discussion

In adult rats hepatocytes rarely divide. Removal of a large portion of the liver stimulates hepatocytes in the remaining portion to hypertrophy and hyperplasia. Many studies have been carried out on DNA and RNA metabolism in regenerating liver (Bucher & Malt, 1971). Various changes in enzyme activities may occur, with the activity of some enzymes decreasing and others increasing after partial hepatectomy (Lancker, 1969). In the first 12h after partial hepatectomy RNA synthesis increases; enzymes of DNA synthesis and DNA synthesis itself increase 12–24h postoperatively whereas mitoses are observed 24–30h after operation (Becker, 1970).

In the present investigation the only enzyme of triglyceride biosynthesis that showed a significant rise in specific activity after subtotal hepatectomy was phosphatidate phosphohydrolase (Table 2). An increase in phosphohydrolase activity was also observed in sham-operated rats 6h after operation. It is likely that both this increase and the one observed in liver remnants after subtotal hepatectomy were caused, at least in part, by stress and the increased requirement for hepatic glyceride synthesis caused by elevation of free fatty acids in blood (Girard *et al.*, 1971). The smaller increase in specific activity in the sham-operated rats compared with rats after subtotal hepatectomy is probably explained in terms of the smaller liver mass of the latter group and the greater requirement for triglyceride synthesis in each hepatocyte of this group. However, the increase in phosphatidate phosphohydrolase activity considered in the total liver of sham-operated rats was significantly greater between 6 and 10h after operation than in the rats subjected to subtotal hepatectomy (Table 1).

Injection of actinomycin D immediately after subtotal hepatectomy prevented the increase in phosphatidate phosphohydrolase activity (Table 3). These results suggest that the increased phosphohydrolase activity was due to an increased synthesis of enzyme protein. Although actinomycin D prevented the rise in phosphohydrolase activity it did not stop the accumulation of hepatic triglyceride. However, actinomycin D also inhibits the increased synthesis of low-density lipoprotein, which is produced in response to an elevation in serum free fatty acids (Alcindor *et al.*, 1970). This would impair the secretion of neutral lipids from the liver and tend to produce a fatty liver.

Changes in phosphatidate phosphohydrolase activity have been reported in other situations in which hepatic lipid metabolism is altered. Vavrečka *et al.* (1969) starved rats for 36–40h and reported an increased phosphohydrolase activity. In the present investigation there was a significant ($P<0.01$) increase in phosphohydrolase activity between 6 and 10h after feeding was stopped (Tables 1 and 2). Re-feeding with a high-carbohydrate diet after a period of starvation resulted in an approximately fivefold increase in liver glyceride concentration (Park *et al.*, 1972). The increase in glyceride synthesis caused by dietary carbohydrate was accompanied by a two- to three-fold elevation in phosphatidate phosphohydrolase activity (Lamb & Fallon, 1972).

The enzymes of triglyceride synthesis, other than phosphatidate phosphohydrolase, showed no significant changes in hepatectomized rats compared with controls. The activity of palmitoyl-CoA synthetase was decreased after starvation of rats for 16 and 24h. Aas & Daae (1971) and Lippel (1971) reported no change in synthetase activity during starvation, whereas Farstad (1968) reported an increase in synthetase activity in the homogenates of starved-rat liver. Recent studies have indicated that palmitoyl-CoA synthetase is probably not rate-limiting in the biosynthesis of glycerolipids in the microsomal (Lloyd-Davies & Brindley, 1973) and mitochondrial fractions (Sánchez *et al.*, 1973) of rat liver. The present results support this conclusion.

A decrease in glycerol phosphate acyltransferase activity was observed during starvation (Table 2), and this agrees with the work of Aas & Daae (1971). However, Fallon & Kemp (1968) reported no decrease in activity of the enzyme during starvation. If the activities of palmitoyl-CoA synthetase, glycerol phosphate acyltransferase and diglyceride acyltransferase measured *in vitro* in the present investigation reflect the activities *in vivo*, then these enzymes may have sufficient reserve activity to cope with the large increase in synthesis of triglyceride that occurs in the regenerating liver without an additional enzyme synthesis being required.

An increased mobilization of free fatty acids from the adipose tissue may be an important factor governing the production of fatty liver in carbon tetrachloride poisoning (Gravela *et al.*, 1971; Glaser & Mager, 1972), in ethanol intoxication (Nikkila & Ojala, 1963) and in the regenerating liver of rat (Girard *et al.*, 1971). The last-named authors showed that an increased mobilization of fatty acids from adipose tissue occurred in both sham-operated and hepatectomized animals but a fatty liver developed only in the regenerating liver. The same amount of fatty acid was taken up by the livers of sham-operated and hepatectomized animals, but because of the much smaller size of the regenerating liver the concentration of fatty acid available for esterification was higher in the latter (Girard *et al.*, 1971), and this was probably responsible for the greater increase in the specific activity of the phosphatidate phosphohydrolase (Table 2).

A greater proportion of the [^{14}C]palmitate mobilized from the fat-pad was incorporated into glycerides than into phospholipids in the liver remnant of rats 24h after partial hepatectomy, compared with sham-operated animals (Girard *et al.*, 1971). The increase in triglyceride content of regenerating liver may thus be due to a combination of increased fatty acid concentration in the liver and an altered distribution of fatty acid between triglyceride and phospholipid. Whether or not the phosphatidate phosphohydrolase has a function in regulating the latter is a subject for further research.

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