Accumulation and release of triglycerides by rat liver following partial hepatectomy

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ABSTRACT Regenerating liver accumulates lipid for about 20 hr following partial hepatectomy. During this time incorporation of intravenously administered palmitate-9,10-^{3}H into β-lipoprotein increased. 13 hr after partial hepatectomy, there was no change in the level of serum β-lipoproteins, but the specific activities of the triglycerides in the liver and β-lipoproteins were significantly diminished. Extension of these studies to the isolated perfused liver system demonstrated that 13 hr after partial hepatectomy the regenerating liver is capable of secreting greater quantities of the lipid, but not the protein, moiety of the β-lipoproteins in comparison with liver taken immediately from a partially hepatectomized animal, although there was no difference between the weights of the livers. However following addition of palmitate-^{3}H and ^{14}C-labeled amino acids to the perfusate, the specific activity of the hepatic and β-lipoprotein triglycerides of the liver excised 13 hr after partial hepatectomy was diminished, but that of the protein was not affected. Prelabeling of the accumulated triglyceride with palmitate-1-^{14}C in vivo revealed that the proportions of the accumulated triglyceride secreted as β-lipoproteins by perfused livers excised immediately and 13 hr after partial hepatectomy were identical. It is concluded that regenerating liver rapidly acquires the ability to mobilize triglycerides at a rate equal to that of the much larger normal liver, so that it can handle all free fatty acids presented to it.

SUPPLEMENTARY KEY WORDS regenerating liver · β-lipoprotein · free fatty acids · liver perfusion

One of the first changes seen after partial hepatectomy is the accumulation of lipid in the liver (1–4). This accumulation starts within 6 hr and reaches its peak 24 hr postoperatively, when the weight of liver has not as yet significantly increased, although mitosis is becoming most rapid (5). The lipid content of the whole liver then remains stable for 21 days (6) although its concentration decreases with the increase in liver weight (7). Bartsch and Gerber (8) have concluded that the accumulation of lipid, mainly as triglyceride, in the liver remaining after partial hepatectomy is due to a defect in transport rather than to an excess synthesis. However, this liver is unlike the fatty liver found in various toxic states, such as CCl_4 (9), orotic acid (10), or puromycin (11) intoxication, which can be attributed to a failure of secretion of the β-lipoproteins. By using Triton to prevent removal of triglycerides from the plasma pool, Fex and Olivecrona (12) have shown that there is no defect in secretion of lipoprotein by the partially hepatectomized rat liver. This conclusion is supported by data obtained recently by Infante et al. (13) with perfused livers. It has therefore been suggested that the accumulation of lipid by partially hepatectomized livers may be due to an increased uptake of free fatty acids per gram of liver, which is then stored as triglyceride (4, 12, 13).

Interpretation of data on lipid transport is complicated by the discrepancy in size between the livers from normal and partially hepatectomized animals, making it difficult to decide whether results should be compared on the basis of the whole liver or per gram of hepatic tissue. The problem is accentuated by the differences in specific activities between the lipids in livers taken from normal and partially hepatectomized animals when isotopic tracers are used to study the secretion of the triglyceride moiety of lipoproteins. In order to obviate some of these difficulties and to obtain a better indication of the ability of the liver from the partially hepatectomized animal to synthesize and release β-lipoproteins, we have studied in vivo and in the perfused liver the simultaneous synthesis of the lipid and protein moieties. In the perfusion experiments liver perfused immediately after surgery served as the control. With these maneuvers we have concluded that within the first 20 hr the regenerating liver develops the capacity to secrete lipid as β-lipo-

protein at three times the rate of liver excised immediately after partial hepatectomy, thus enabling it to cope with all of the free fatty acids presented to it by the circulation.

METHODS

Male hooded rats weighing 240–270 g, fasted overnight, were anesthetized by intraperitoneal injection of 5 mg of Nembutal per 100 g of body weight. For the in vivo experiments, 2 μCi of amino acid-U-^{14}C mixture and 0.5 μCi of albumin-bound palmitate-9,10-^{3}H, both in trace amounts, were injected via the jugular bulb $1^1/_2$ and $^1/_2$ hr, respectively, before the animals were killed. The secretion of labeled protein and lipid moieties of the β-lipoproteins are optimal after these intervals (14). Partial hepatectomy, resulting in the removal of 70% of the liver, was performed according to the procedure of Higgins and Anderson (15). Liver perfusions were carried out by the method of Miller, Bly, Watson, and Bale (16); the rate of flow of the perfusate was 210–250 drops/min with livers taken from both normal and partially hepatectomized animals. The perfusion medium consisted of 25 ml of packed, washed rat erythrocytes and 75 ml of serum or Krebs–Ringer bicarbonate solution, pH 7.2, containing 3% bovine serum albumin (Cohn fraction V), 500 mg of glucose, and 120 mg of an amino acid mixture (16) per 100 ml of perfusate. The first 25 ml of perfusate was collected and discarded; 6 μCi of albumin-bound palmitate-9,10-^{3}H and 10 μCi leucine-1-^{14}C were then added to the remaining medium, and the perfusion was carried on for 120 min. At the conclusion of the experiment all livers were washed by perfusion with 30 ml of saline, rinsed, blotted, weighed, and homogenized in 50 ml of 0.25 M sucrose.

β-Lipoproteins of the serum or cell-free perfusate were isolated by the heparin–calcium precipitation method of Jordan, Faulkner, and Knoblock (17), and the precipitate was washed twice with 0.4% calcium chloride. This procedure precipitates the β-lipoprotein (very low density and low density lipoproteins), which contains almost all of the serum triglycerides (18). Liver and lipoprotein lipids were extracted by the method of Folch, Lees, and Sloane Stanely (19). Neutral lipids and phospholipids were separated on silicic acid columns using chloroform and methanol, respectively, as the eluting solvents. Free fatty acids were separated from triglycerides by the extraction procedure of Borgström (20). Triglyceride levels were estimated by the procedure of Van Handel (21) while lipid phosphorus was determined according to Fiske and Subbarow (22). The lipid-extracted tissue homogenate was precipitated with 10% trichloroacetic acid, washed twice with 10% trichloroacetic acid containing an unlabeled amino acid mixture (1 mg/ml), and dissolved in 1 N NaOH for protein estimation according to Lowry, Rosebrough, Farr, and Randall (23). 0.2 ml of the protein solution was spotted on Whatman filter paper No. 2 and dried. The paper was then placed in 15 ml of toluene-based phosphor solution. Neutral lipid samples were dissolved directly in the phosphor solution while phospholipids were first solubilized in 1 ml of methanol. Radioactivity was measured in a Tri-Carb liquid scintillation counter (double-label settings), and corrections were made for self-absorption, overlapping of isotopes, and for quenching by using an external standard. Albumin was bound to palmitate by a modification of the procedure of Milstein and Driscoll (24). The radioactive isotopes were obtained from New England Nuclear Corp., (Boston, Mass.), the unlabeled amino acids from Nutritional Biochemicals Corporation (Cleveland, Ohio), and heparin from Fisher Scientific Company (Montreal, Canada).

RESULTS

In order to establish the optimum time for the projected studies on lipid accumulation and secretion by the livers from partially hepatectomized animals, hepatic triglyceride levels were measured during the first 20 hr postoperatively. These data are shown in Fig. 1. The level continued to rise for at least 20 hr and the increase resembled that reported earlier for total lipids by Camargo, Cornicelli, and Cardoso (4). The greater increment noted here is probably due to a lower initial level in the livers of this group of animals and the determination of triglycerides rather than total lipids, since the latter include phospholipids which do not increase to the same extent (25). The concentration of liver triglyc-

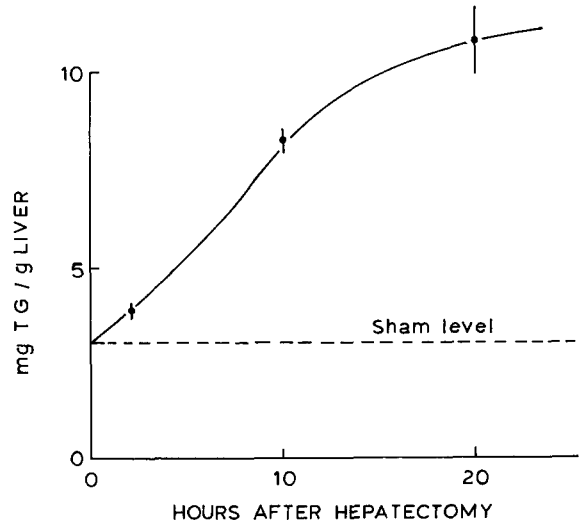

FIG. 1. Hepatic triglyceride levels following partial hepatectomy. Each point is the mean of seven animals ±SEM.

erides is increased fourfold 20 hr after partial hepatectomy, but the total amount of the triglyceride is approximately equal to that of the liver of an intact animal. As a result of this experiment a 13 hr postoperative period was selected as a suitable time for the study of lipid accumulation and secretion by the livers from partially hepatectomized rats. At this time most of the lipid has already accumulated. On the basis of hepatic RNA content, Simek, Rubin, and Lieberman (26) have found that the lipid accumulation is already leveling off. In addition, the first alteration in ribosomal configuration characteristic of regeneration is seen at this time (27), although no change in the weight of the liver has yet occurred.

The ability of the liver from the partially hepatectomized animal to secrete the lipid and protein moieties of β-lipoproteins in vivo was compared with that of sham-operated animals; intravenously injected amino acid-^{14}C and palmitate-9,10-^{3}H were used as tracers. The relevant hepatic triglyceride concentrations and palmitate-9,10-^{3}H incorporation data are shown in Table 1. There is an accumulation of triglyceride in the livers of the partially hepatectomized animals, which also incorporated approximately 33% more isotope into the triglyceride per gram of liver but contained less total radioactivity due to their smaller size. The accumulation of lipid following partial hepatectomy resulted in a lower triglyceride specific activity. In some animals the incorporation of the amino acid-^{14}C mixture in the liver was also examined. The data are shown in Table 2. The livers of the partially hepatectomized animals incorporated 45% more labeled amino acids into protein per gram of liver. This resulted in an elevated protein specific activity, although the total radioactivity incorporated by the smaller regenerating livers was less than that in sham-operated animals. The incorporation of the isotopes into serum β-lipoproteins under these conditions is shown in Tables 3 and 4. It will be noted that the levels of both the protein and lipid moieties of the β-lipoproteins are unaltered by the partial hepatectomy. However, incorporation of palmitate-^{3}H (Table 3) into the lipid moiety of the serum β-lipoproteins of partially hepatectomized rats is diminished by 50% with a concomitant decrease in specific activity. This may be a reflection of the lower specific activity of the liver triglycerides. The incorporation of the amino acids-^{14}C (Table 4) into the protein moiety of the lipoprotein was not significantly affected by the partial hepatectomy. The failure of the partial hepatectomy to decrease the serum β-lipoprotein concentration and the specific activity of the protein moiety suggests that the accumula-

TABLE 1 INCORPORATION OF PALMITATE-9,10-^{3}H INTO LIVER TRIGLYCERIDES IN VIVO

	Sham Operated	Partial Hepatectomy
No. of animals	12	9
Weights of livers, *g*	7.9 ± 0.2	2.2 ± 0.1*
Triglyceride		
Concentrations, *mg/g liver*	5.6 ± 0.5	10.7 ± 0.5*
Radioactivity,		
cpm × 10^{-4}/*g liver*	64.5 ± 4.5	86.6 ± 7.7†
Total radioactivity		
cpm × 10^{-4} *per liver*	529 ± 42	194 ± 165†
Specific activity		
cpm × 10^{-3}/*mg*	124 ± 14	81 ± 7†

Figure represent means ±SEM.
* Significantly different from control ($P < 0.01$).
† Significantly different from control ($P < 0.05$).

TABLE 2 INCORPORATION OF AN AMINO ACID-^{14}C MIXTURE INTO HEPATIC PROTEINS IN VIVO

	Sham Operated	Partial Hepatectomy
No. of animals	4	3
Weights of livers, *g*	7.4 ± 0.2	2.0 ± 0.1
Proteins		
Concentration, *mg/g liver*	150 ± 1.1	140 ± 4.8
Radioactivity,		
cpm × 10^{-4}/*g liver*	3.3 ± 0.4	4.8 ± 0.3*
Specific activity,		
cpm × 10^{-2}/*mg protein*	2.2 ± 0.2	3.5 ± 0.2*
Total radioactivity,		
cpm × 10^{-4} *per liver*	24.5 ± 2.7	9.7 ± 0.5*

Figures represent means ± SEM.
* Significantly different from control, $P < 0.05$.

TABLE 3 INCORPORATION OF PALMITATE-9,10-^{3}H INTO TRIGLYCERIDES OF β-LIPOPROTEINS IN VIVO

	Sham Operated	Partial Hepatectomy
Triglycerides		
Concentration, *mg/100 ml serum*	60.0 ± 5.0	62.1 ± 5.5
Radioactivity,		
cpm × 10^{-2}/*ml serum*	52.0 ± 5.4	26.8 ± 2.3*
Specific activity,		
cpm/μ*g triglyceride*	8.3 ± 0.7	4.1 ± 0.3*

There were 11 animals in each group. Values of means ± SEM.
* Significantly different from control ($P < 0.01$).

TABLE 4 INCORPORATION OF AMINO ACID-^{14}C INTO THE PROTEIN OF THE β-LIPOPROTEINS IN VIVO

	Sham Operated	Partial Hepatectomy
No. of animals	7	8
Proteins		
Concentration, *mg/100 ml serum*	22 ± 2.7	24 ± 1.7
Radioactivity,		
cpm × 10^{-2}/*ml serum*	12.7 ± 1.2	15.5 ± 0.9
Specific activity,		
cpm/μ*g protein*	6.2 ± 0.8	6.5 ± 0.7

Figures represent the means ±SEM.

tion of hepatic triglycerides is not due to an inability of the regenerating liver to secrete β-lipoproteins.

This was further studied by following the ability of livers from partially hepatectomized animals to convert pulses of palmitate-9,10-^{3}H into triglycerides of β-lipoproteins over a 20 hr period. The results are shown in Fig. 2. It can be seen that 2 hr after partial hepatectomy the livers incorporated fatty acids into lipoprotein triglycerides at about one-third the rate of the sham-operated animals, although on the basis of liver weight the incorporation corresponded with that of the control animals. As the postoperative period advanced, the ability of the livers from the partially hepatectomized animals to incorporate palmitate into the triglycerides of the serum β-lipoprotein increased, so that after 20 hr it was similar to that of the control animals, although the weight of the liver in the partially hepatectomized animals remained unchanged.

To avoid the complication of removal of the triglycerides by other tissues, the perfused liver was used to study the mechanism of lipid accumulation and secretion after partial hepatectomy. Fig. 3 shows typical patterns of incorporation of palmitate-9,10-^{3}H into the triglycerides of serum used as the perfusate. It will be noted that the most rapid incorporation occurred with normal liver. This was probably due to the more rapid uptake of the palmitate and the larger size of the liver. The release of labeled neutral lipids by livers from animals which had been partially hepatectomized either immediately before perfusion or 13 hr earlier was considerably lower, although the radioactivity released by the two types of

Mean Liver Weights

hr	2	10	20	sham
g	2·76	2·94	2·96	9·5

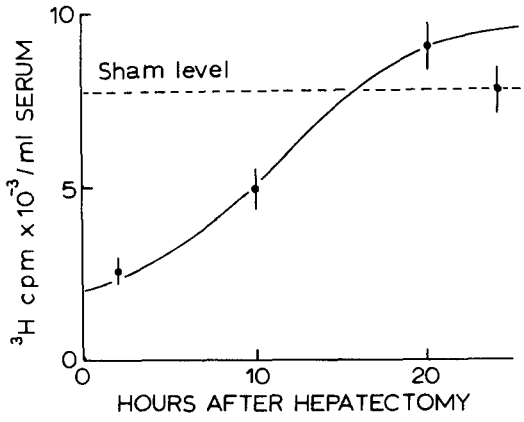

FIG. 2. Incorporation of palmitate-^{3}H into the triglycerides of the serum β-lipoproteins at various intervals after partial hepatectomy. Each point is the average of seven animals ± SEM.

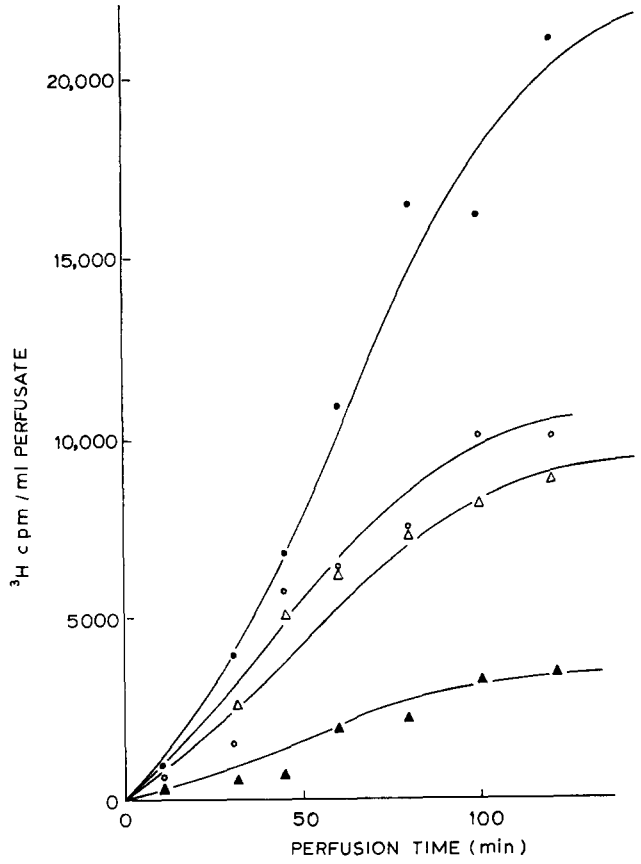

FIG. 3. Incorporation of palmitate-^{3}H into the triglyceride of the liver perfusate β-lipoproteins. ●, normal liver; ○, liver removed from a partially hepatectomized rat immediately before perfusion; △, liver removed 13 hr after partial hepatectomy; ▲, liver from animal given 0.1 ml of CCl_4 per 100 g body weight 12 hr earlier. The data cited in the figure are typical of three such experiments. The weights of the livers and the $t_{\frac{1}{2}}$ for the uptake of the palmitate-^{3}H respectively during the perfusions were as follows: normal, 11.1 g and 20 min; removed immediately after partial hepatectomy, 3.5 g and 40 min; removed 13 hr after partial hepatectomy, 2.8 g and 40 min; CCl_4-treated, 12.0 g and 30 min.

hepatectomized preparations apparently did not differ from each other. The secretion of labeled triglyceride (per gram of liver) was not decreased by partial hepatectomy. A liver from a CCl_4-treated animal (0.1 ml/100 g of body weight administered intraduodenally 12 hr earlier) was also examined and showed a greater diminution in secretion of labeled triglyceride than the smaller livers from the partially hepatectomized animals.

While the perfused liver experiment cited in Fig. 3 confirms that, on the basis of liver weight, there is no decrease in the ability of livers from partially hepatectomized animals to synthesize and secrete labeled triglycerides, it does not demonstrate the increased ability of regenerating liver to secrete lipoproteins suggested by the in vivo experiments cited in Fig. 2. In order to measure both the total quantity and the incorporation of fatty acids and amino acids into the lipid and protein

moieties of the β-lipoproteins, livers were excised immediately or 13 hr after partial hepatectomy and perfused for 120 min with a medium consisting of 4% albumin in Krebs–Ringer bicarbonate buffer which replaced the serum of the perfusate. Tables 5 and 6 show the chemical and isotopic analyses of the livers following the perfusion, and the data resemble those seen for the experiments in which the isotopic precursors were administered in vivo (see Tables 1 and 2). There was no difference in weight between the livers excised immediately and 13 hr after partial hepatectomy, although the latter contained a great deal more lipid (Table 5). The incorporation of palmitate into the liver triglycerides did not differ significantly between the two types of liver preparations but due to the large amount of lipid present in the 13 hr regenerating liver its specific activity was considerably lower than that in the liver from the newly partially hepatectomized animal. The data in Table 6 indicate that the 13 hr regenerating liver incorporated amino acids more rapidly into hepatic protein than one excised immediately after partial hepatectomy. This resulted in an increase in specific activity, since the protein content did not differ. Analyses of the corresponding β-lipoprotein secreted into the perfusate after 120 min are shown in Tables 7 and 8. Table 7 indicates that the release of triglycerides as β-lipoproteins per gram of tissue by the liver removed 13 hr after partial hepatectomy was twice as great, although the specific activity was decreased. There was no significant difference between the two liver preparations in the amount of the protein moiety of the β-lipoprotein (Table 8). The data suggest an increased incorporation of amino acid and specific activity of the protein in the regenerating liver (see Table 6). The lipoproteins secreted by livers excised 13 hr after partial hepatectomy have a higher ratio of triglyceride to protein than the lipoprotein secreted by livers removed immediately after partial hepatectomy. In a number of experiments the total amount of and the incorporation of palmitate-^{3}H into phospholipids of the β-lipoproteins were also followed (not shown here). A pattern similar to that of the triglycerides, namely, increased secretion but decreased specific activity of phospholipids, was found in the β-lipoproteins secreted by the livers removed 13 hr after partial hepatectomy.

The specific activity of the triglycerides in the β-lipoproteins secreted into the perfusate by the livers removed

TABLE 5 INCORPORATION OF PALMITATE-9,10-^{3}H INTO HEPATIC TRIGLYCERIDES DURING PERFUSION OF LIVERS FROM PARTIALLY HEPATECTOMIZED RATS

	Time of Excision of Liver	
	Immediately after Partial Hepatectomy	13 hr after Partial Hepatectomy
No. of animals	7	8
Weights of livers, *g*	2.7 ± 0.5	2.9 ± 0.2
Triglyceride		
Concentration, *mg/g liver*	6.0 ± 0.5	13.4 ± 1.8*
Radioactivity, *cpm* × 10^{-3}/*g liver*	604 ± 160	667 ± 233
Specific activity, *cpm/μg triglyceride*	117 ± 23	43 ± 7.6†

Figures represent means ± SEM. See text for details.
* Significantly different from control ($P < 0.01$).
† Significantly different from control ($P < 0.05$).

TABLE 6 INCORPORATION OF AMINO ACID-^{14}C INTO HEPATIC PROTEINS DURING PERFUSION OF LIVERS FROM PARTIALLY HEPATECTOMIZED RATS

	Time of Excision of Livers	
	Immediately after Partial Hepatectomy	13 hr after Partial Hepatectomy
Weight of liver, *g*	2.4 ± 0.2	2.8 ± 0.2
Proteins		
Concentration, *mg/g liver*	134 ± 10	130 ± 12
Radioactivity, *cpm* × 10^{-3}/*g liver*	54.1 ± 7.0	93.2 ± 11.7*
Specific activity, *cpm/μg protein*	0.39 ± 0.02	0.74 ± 0.08*

Figures represent the mean of four animals ± SEM. See text for details.
* Significantly different from control ($P < 0.05$).

TABLE 7 INCORPORATION OF PALMITATE-9,10-^{3}H INTO TRIGLYCERIDES OF PERFUSATE β-LIPOPROTEINS

	Time of Excision of Livers	
	Immediately after Partial Hepatectomy	13 hr after Partial Hepatectomy
No. of animals	6	8
Triglycerides		
Concentration, *mg/g liver*	113 ± 26	217 ± 17*
Radioactivity, *cpm* × 10^{-3}/*g liver*	41 ± 16	34 ± 12
Specific activity, *cpm/μg triglycerides*	349 ± 68	143 ± 28†

Figures represent the mean ± SEM.
* Significantly different from control ($P < 0.01$).
† Significantly different from control ($P < 0.05$).

TABLE 8 INCORPORATION OF AMINO ACID-U-^{14}C INTO PROTEINS OF PERFUSATE β-LIPOPROTEINS

	Time of Excision of Livers	
	Immediately after Partial Hepatectomy	13 hr after Partial Hepatectomy
Protein		
Concentration, *mg/g liver*	568 ± 200	633 ± 260
Radioactivity, *cpm* × 10^{-3}/*g liver*	220 ± 12	465 ± 176
Specific activity, *cpm/μg protein*	0.39 ± 0.05	0.71 ± 0.16

Figures represent the means of four experiments in each group ± SEM.

13 hr after partial hepatectomy is lower than that of the β-lipoproteins secreted by livers taken immediately after partial hepatectomy. While this observation may be a reflection of the lower specific activity of the triglycerides in the former liver, it suggests that the accumulated triglycerides are not sequestered as a relatively inert cytoplasmic pool but may be directly secreted. To check this point, the triglyceride pool of the liver was labeled by the intravenous administration of palmitate-1-^{14}C 12 hr before the livers were excised for perfusion. The secretion of this accumulated labeled triglyceride pool by perfused livers removed immediately and 13 hr after partial hepatectomy, was studied. Since lipids accumulate in relatively inert cytoplasmic droplets following CCl_4 intoxication, it was interesting to compare the secretion of triglycerides by livers from these animals with those released by liver from partially hepatectomized animals. As was the case with the control animals, the CCl_4-treated rats were partially hepatectomized immediately before removal of the liver for perfusion. The results are shown in Table 9. The specific activities of the hepatic triglycerides from the various groups of animals cannot be compared directly since the palmitate-1-^{14}C was injected when the livers of the control and CCl_4-treated animals (groups 1 and 3) were intact, but the animals undergoing partial hepatectomy 13 hr before the liver perfusion (group 2), were given the palmitate-1-^{14}C 1 hr after 70% of the liver had been removed. Thus the hepatic specific activities correspond more closely to those seen after the in vivo experiments (see Table 1) than those observed with perfused livers. It is of interest however to compare the specific activities of the hepatic and perfusate triglycerides from the same group of animals. In the untreated livers removed either immediately or 13 hr after partial hepatectomy, the specific activities of the hepatic and perfusate triglycerides were similar. On the other hand, the specific activity of the perfusate triglyceride secreted by the CCl_4-treated liver was considerably lower than that of the liver, suggesting that little of the accumulated triglyceride contributed to the secretory pool. To permit comparison between various groups of animals, the percentage of the accumulated labeled triglycerides secreted is also shown. There was no significant difference in the percentage release between the livers removed immediately and 13 hr after partial hepatectomy. There was, however, a 30-fold decrease in the release of accumulated triglyceride radioactivity from the CCl_4-intoxicated liver, although the actual amount of triglyceride secreted (not shown here) was from one-half to one-fourth that of the control. These data are consistent with the concept that the accumulated triglycerides in the regenerating liver are secreted as readily as those of normal livers. The experiments cited in Table 9 differed from those reported in Tables 1–8 since it was necessary to obtain the animals from a different supplier, and perfusate triglycerides rather then β-lipoproteins were determined. Therefore, during these perfusions, palmitate-9,10-^{3}H was added to the Ringer-based perfusate. The results (not shown here) indicated that the pattern reported in Tables 3 and 7 was nevertheless reproduced.

DISCUSSION

The present data and the earlier studies of Fex and Olivecrona (28) indicate that livers from partially hepatectomized animals are capable of taking up more free fatty acids per gram of tissue than are normal livers. Although there is some difference in opinion as to whether or not serum free fatty levels are elevated after partial hepatectomy (4, 28), the greater hepatic blood flow following surgery (29, 30) probably accounts for the increased uptake of the labeled free fatty acids (28) and the accumulation of lipid during the first 15–20 postoperative hr. The accumulated lipid per se has no apparent affect on the uptake of fatty acids.

TABLE 9 RELEASE INTO PERFUSATES OF LABELED TRIGLYCERIDE ACCUMULATED IN VITRO LIVERS FROM NORMAL OR CCl_4-TREATED PARTIALLY HEPATECTOMIZED RATS

	Treatment of Animals		
	Group 1: Control, Liver Excised Immediately after Partial Hepatectomy	Group 2: Liver Excised 13 hr after Partial Hepatectomy	Group 3: CCl_4-Treated 13 hr before Partial Hepatectomy; Liver Removed Immediately after Surgery
No. of animals	4	5	3
Liver triglycerides			
Concentration, *mg/g liver*	4.4 ± 0.6	9.9 ± 0.8	27.1 ± 3.0
Specific activity, *μg triglyceride*	1.5 ± 0.5	2.3 ± 0.2	3.0 ± 0.8
Perfusate triglycerides			
Specific activity, *μg triglyceride*	1.6 ± 0.6	1.8 ± 0.5	0.6 ± 0.2
Hepatic triglycerides-^{14}C secreted, %	5.6 ± 0.4	6.3 ± 0.3	0.3 ± 0.05

Values represent means ± SEM. Palmitate-1-^{14}C injected intravenously 12 hr before removal of the livers in each case. Perfusion time: 120 min.

While the liver of a partially hepatectomized animal takes up free fatty acids in vivo in increased amounts relative to its size (28), it does not appear to be able to secrete the resulting triglycerides at a correspondingly greater rate. Thus the rate of incorporation of palmitate-^{3}H into the triglycerides of the β-lipoproteins within 2 hr of partial hepatectomy is only one-third of that seen with a normal liver. A similar phenomenon is seen with perfused livers which have been excised immediately after partial hepatectomy. Since the decrease in secretion is proportional to the decrease in size of the liver, there is no failure in secretion of lipoprotein by the remaining hepatocytes. With increasing time intervals following partial hepatectomy, the ability of the liver to resecrete the free fatty acids obtained from the circulation as triglycerides of β-lipoproteins increases. After 20 hr the hepatic remnant, which has still not increased in weight, can cope with the relatively elevated flux of free fatty acids. A morphological basis for the increased lipoprotein secretion is provided by the observations of Trotter (31) who showed the presence of osmiophilic bodies in the cisternae of the endoplasmic reticulum, which may represent an increased rate of export of very low density lipoprotein. An increase in secretory function during the premitotic period has also been reported for albumin and fibrinogen (32). However, the partially hepatectomized liver appears to secrete the greater quantities of lipid primarily by increasing the amount of triglyceride added to the protein moiety of the β-lipoprotein, the rate of secretion of the latter being slightly elevated during the 20 hr post-partial hepatectomy period. Likewise Infante et al. (13) could find no difference between the perfused livers of normal and partially hepatectomized rats with regard to the incorporation of labeled amino acids into the protein moiety of the $d < 1.063$ lipoproteins. Since the β-lipoprotein included the very low density and low density components, it is possible that an increase in protein content of the former, which carries most of the triglycerides, has been overlooked. However, in the rat the VLDL protein makes up about 40% of the protein of the $d < 1.063$ lipoproteins (33, 34) and is 90% precipitable by heparin (33). It is likely, therefore, that a significant change in the amount of VLDL protein would be detected by our procedure. Nevertheless, the possibility of a change in amounts of a specific peptide such as the C apoprotein, said to be characteristic of human VLDL (35) especially under conditions involving transport of large amounts of lipid (36), cannot be ruled out. The ability of liver to increase the amount of lipid bound to the protein moiety of the β-lipoprotein when presented with a large free fatty acid load has also been demonstrated in perfused normal liver by Ruderman, Richards, Valles de Bourges, and Jones (37), and in diabetic livers by Wilcox, Dishmon, and Heimberg (38). The increase in phospholipid secretion which accompanies the increased release of triglycerides by the regenerating liver is consistent with the concept that various species of lipid in lipoproteins are secreted in discrete molar ratios (39). The factor which initiates the increased association of lipid with the protein moiety of the lipoproteins has not yet been determined.

The decrease in the specific activity of the triglycerides released into the perfusate by the 24 hr regenerating liver when labeled acetate is used as a precursor, noted by Bartsch and Gerber (8), is consistent with a decrease in lipoprotein secretion. However, since the actual amount of triglyceride secreted is not altered in spite of the smaller liver, it is apparent that the decreased specific activity of the lipid moiety of the perfusate β-lipoprotein is related to the lower specific activity of the triglyceride in the liver excised 13 hr after partial hepatectomy. Thus a large portion of the accumulated triglyceride serves as a pool which can provide lipoprotein-lipid even though it has accumulated for a period of at least 13 hr. Trotter (31) noted that 7 hr after partial hepatectomy the fat droplets in the liver (usually associated with stored triglycerides) are only slightly larger than normal. Most of the accumulated lipid is present as osmiophilic bodies in the endoplasmic reticulum, which ultimately appear in the spaces of Disse. It is tempting to speculate that most of the triglyceride is temporarily stored in these bodies and is removed from the regenerating liver as its capacity to secrete lipoprotein increases during the first 20 hr after partial hepatectomy. These osmiophilic bodies disappear after 24 hr (31). This is in contrast with the situation following CCl_4 intoxication where lipid accumulates as fat droplets in the cytoplasm of the hepatocyte, and is not released to any significant degree during perfusion (see Table 9).

The present data suggest the following sequence of events in hepatic lipid metabolism after partial hepatectomy. In the earliest stages after surgery the serum free fatty acids are esterified to form triglycerides which accumulate in excess of the liver's ability to secrete them as β-lipoproteins. As time progresses, the secretion of triglyceride is increased, primarily by coupling more lipid to the protein of the β-lipoproteins. After 20 hr the small partially hepatectomized liver can secrete lipid at a rate equal to the normal liver, thereby coping with the free fatty acid flux and preventing further accumulation.

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