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THE EFFECT OF HEPARIN ON THE METABOLISM OF TRIGLYCERIDES BY THE LIVER*

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Abstract—The output of triglyceride by the perfused rat liver was depressed by heparin (Upjohn) added to the medium *in vitro*.

The impairment in output of triglyceride by the liver which resulted from fasting, alloxan diabetes, and from poisoning of the animal with carbon tetrachloride was observed whether or not heparin was used as an anticoagulant.

The output of triglyceride by the liver was inhibited significantly by crystalline heparin (Sigma) only at the smallest concentration (0·42 USP units/ml perfusate) of heparin added to the medium. The release of lipolytic activity by the liver was stimulated as the concentration of heparin in the medium was increased; the maximal effect was obtained at a concentration of 42 units heparin/ml perfusate. Paradoxically, as the lipolytic activity in the medium increased, the inhibition of output of triglyceride by heparin diminished.

The uptake and utilization by the liver of chylomicra labeled with palmitic acid-1-^{14}C was accelerated by crystalline heparin, presumably as a result of the increased lipolytic activity. There was, however, a real, albeit smaller, utilization of chylomicron triglyceride when heparin was omitted from the medium. One can surmise from these observations that the output of triglyceride into the very low density lipoprotein (VLDL) by the liver and the uptake of either chylomicron triglyceride or the VLDL-triglyceride occur by different cellular mechanisms, since heparin appears to affect each of these processes in diverse manners.

HEPARIN, when administered to man and animals, accelerates the clearing of alimentary lipemia and induces the appearance in the serum of a lipolytic enzyme, lipoprotein lipase.[1, 2] Postheparin lipoprotein lipase activity has been detected also in tissues such as heart and adipose tissue.[2–4] It has been postulated that postheparin lipoprotein lipase is required by different tissues for the uptake of chylomicra from the blood.[2] Lipoprotein lipase had been thought previously not to be present in livers from rats and several other animal species.[2, 5] More recently, however, it was observed that lipoprotein lipase was present in rat liver in an inactive form which became activated and released by heparin.[6, 7] The enzyme was also reported to be present in the hepatic

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circulation of dogs and rabbits after treatment of the animals with heparin.[8] The total activity of lipoprotein lipase in liver appears, however, to be less than in other tissues.[7] Although there is considerable uncertainty that hydrolysis of chylomicron triglycerides[3] or administration of exogenous heparin is necessary for the uptake of chylomicra by liver,[9, 10] the uptake of triglycerides from chylomicra or from the very low density lipoprotein (VLDL) by the isolated perfused rat liver *in vitro* was reported by Felts and Mayes[11, 12] not to occur in the absence of heparin in the medium.

In view of these conflicting reports about the presence of lipoprotein lipase in the liver and the controversy concerning the role of heparin in the hepatic metabolism of lipids, and in view of many studies from this and other laboratories with the perfused liver in which heparin was utilized as an anticoagulant, it became a necessity to investigate the effects of heparin on the output of triglycerides, the uptake of chylomicra, the release of lipolytic activity into the perfusate, and the production of glucose, urea and ketone bodies by the isolated perfused rat liver. A preliminary report of this work has appeared.[13]

METHODS

Normal male fed rats weighing 250–475 g (obtained from the Holtzman Company, Madison, Wis.) were used as the source of the livers, blood and serum in most of these experiments. In certain experiments, livers for perfusion were obtained from alloxan-diabetic rats,[14] rats fasted for 48 hr, and from rats treated with CCl_4 by mouth.[15] The livers were perfused *in vitro* using the apparatus[16] and procedures[17] which have been described previously. The livers were removed surgically from the animals and were perfused for 20 min with a basal medium consisting of 47% (v/v) defibrinated rat blood and 53% Krebs–Henseleit bicarbonate buffer, pH 7·4.[18] The volume of the basal medium was 70–100 ml, depending on the experimental group. Heparin was used during surgical removal of the liver[17] or was added to the perfusate only in those experiments in which it was so indicated. After a 20-min period of equilibration, a complex consisting of 30 mg palmitic acid, 15 ml rat serum, and sufficient 0·9% NaCl to make 30 ml was added to the perfusate.[15] Appropriate aliquots of the perfusate were removed for chemical analyses 3 min after addition of the fatty acid complex to the medium and at intervals thereafter; glucose was also measured in the medium before the liver was placed into the perfusion system.

The lipids of the erythrocyte-free perfusate were extracted as described previously[14] and suitable aliquots were taken for analysis of triglycerides[19] after separation from phospholipids on 3·0-g silicic acid columns. Free fatty acids (FFA) in the chloroform eluates of the silicic acid columns were estimated colorimetrically.[20] Additional aliquots of perfusate were precipitated with 0·15 N $Ba(OH)_2$ and 2·5% $ZnSO_4.7\ H_2O$.[14] The protein-free filtrate was analyzed for glucose,[21] urea,[22] and ketones; ketone bodies were estimated colorimetrically[23] after oxidation in sealed glass tubes at 150° for 75 min.[24] At the termination of the perfusions, the livers were washed with ice-cold 0·9% NaCl, trimmed of nonhepatic tissue, blotted, and weighed.

Measurement of lipolysis. In certain experiments, perfusate was removed at intervals, centrifuged to sediment the erythrocytes, and the lipolytic activity of the cell-free perfusate was estimated as follows. Cell-free perfusate (3·0 ml) was incubated with 0·4 ml of 2% bovine serum albumin, 0·7 ml of 1% Intralipid (v/v) in 0·2 M potassium phosphate buffer (pH 7·4), 0·2 ml rat serum, and 0·7 ml buffer (total volume, 5·0 ml)

for 1 hr in air at 37° in a Dubnoff metabolic incubator.[25] Samples (1·0 ml) of the incubation medium were analyzed for FFA by the Trout[26] modification of the Dole[27] titrimetric procedure, using Nile Blue as an acid–base indicator. Lipolytic activity in the cell-free perfusate was expressed as microequivalents of FFA per milliliter per 10 g of liver per hour.

Utilization of chylomicron triglyceride. Chylomicra were collected from the cysterna chyli of rats[28] after administration of 50–100 μc palmitic acid-1-^{14}C in 1–2 ml of corn oil. The chyle was collected at room temperature in flasks containing 20 mg streptomycin sulfate and 15,000 units of penicillin G potassium salt. The chyle was isolated and washed by ultracentrifugation.[14] The washed, concentrated chylomicra were stored in the refrigerator and were used within 8 days of collection. When required, chylomicra (total radioactivity, $4{\cdot}0 \times 10^6$ dpm; content of triglyceride, 110 μmoles) were added to the perfusate with the palmitate–rat serum complex. The perfusions were terminated after 2 hr, after which the livers were washed, weighed and the lipids extracted as described earlier.[29] Radioactivity of the lipid fractions was measured in toluene containing 5·0 g of 2,5-diphenyloxazole per liter in a Beckman CPM 100 liquid scintillation counter at ambient temperature.

The heparin used in these experiments was either a solution of heparin sodium (1000 USP units/ml, obtained from the Upjohn Company, Kalamazoo, Mich.) or crystalline heparin (155 USP units/mg, obtained from the Sigma Chemical Company, St. Louis, Mo.). Intralipid, an emulsion containing 20% soybean oil, 2·5% glycerol, 1% phosphatides, and water, was obtained from Riker Laboratories, Northridge, Calif. Palmitic acid-1-^{14}C was obtained from the New England Nuclear Corp., Boston, Mass.

The statistical significance of the differences between experimental groups was evaluated by means of a two-tailed table of Student's distribution for t.[30]

RESULTS

Effect of heparin on hepatic output of triglyceride and on lipolytic activity. The heparin used in this group of studies was the commercial preparation obtained from the Upjohn Company. The output of triglyceride by the liver was depressed by heparin given to the animal from which the liver was obtained, added to the medium *in vitro*, or both (Fig. 1). It is doubtful that this effect could have resulted from any action of heparin on the uptake of FFA by the livers, since the small amount of palmitate was removed completely from the medium by livers in all groups within the first 30 min of perfusion. The output of glucose and urea by liver was unchanged by heparin. The rate of ketogenesis was quite small in the control group ($0{\cdot}65 \pm 0{\cdot}08$ mg acetone/g liver/3 hr), since very little FFA was supplied;[24] the output of ketone bodies was actually somewhat less ($0{\cdot}42 \pm 0{\cdot}08$) when heparin (group B) was added. The presence or absence of heparin had no effect on the rate of perfusate flow through the liver or output of bile by the liver (Table 1).

It was reported in publications from this laboratory that the output of triglyceride (TG) by perfused livers from fasted rats,[17] from rats made diabetic with alloxan,[14] and from rats treated with CCl_4[15] was reduced considerably in comparison to the output by livers from normal, fed animals. In these earlier experiments, heparin (Upjohn) had been administered to the animals from which the livers were removed and was added also to the medium *in vitro*. It was desirable, therefore, to repeat these

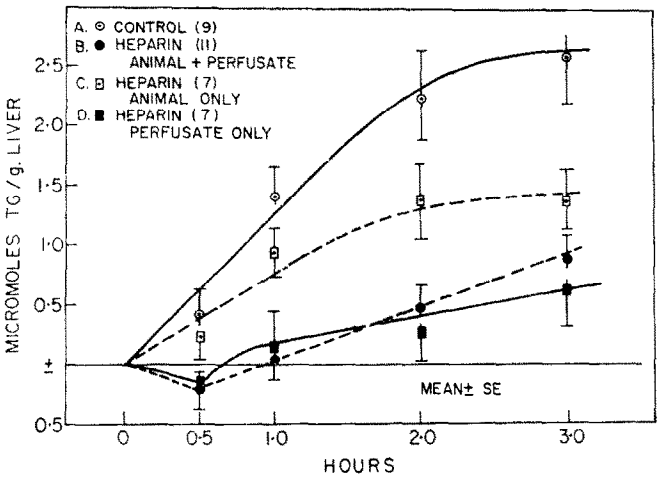

FIG. 1. Effect of heparin on the hepatic output of triglyceride. Heparin (Upjohn, 100 USP units) was either administered intravenously 3 min before the liver was removed from the animal for perfusion or was added to the basal medium *in vitro* (500 USP units), or both. (Please refer to the text for details.) The figures in parentheses indicate the number of experiments and all values are means ± standard error.

Statistical analysis:

	P value	
Comparison	1 hr	3 hr
A vs. B	<0.001	<0.001
A vs. C	<0.200	<0.05
A vs. D	<0.005	<0.005

TABLE 1. PERFUSATE FLOW RATE AND PRODUCTION OF BILE BY PERFUSED LIVERS

	Treatment	
Measurement	None (10)	Heparin* (12)
Production of bile (ml/10 g liver/hr)	0·41 ± 0·01	0·43 ± 0·01
Perfusate flow rate (ml/g liver/min)	1·91 ± 0·28	2·03 ± 0·21

* Heparin (Upjohn) was given to the animal from which the liver was obtained and was also added to the medium *in vitro*. Refer to text for details. Figures in parentheses indicate number of experiments. All values are means ± standard error. Production of bile was calculated from the output during the entire experimental period. The perfusate flow rate is that rate observed at the third hour of the experiment. There is no significant difference between treatments.

experiments under conditions in which heparin was excluded completely. The results obtained when heparin was omitted were identical qualitatively with those when heparin had been administered (Fig. 2). Clearly, the decrease in hepatic output of TG seen in fasting, alloxan diabetes, and poisoning with CCl_4 is a function of these specific conditions and is not related to any action of heparin.

So that we might ascertain whether the depression of output of TG resulting from the use of heparin (Upjohn) in the preceding experiments was related causally to a

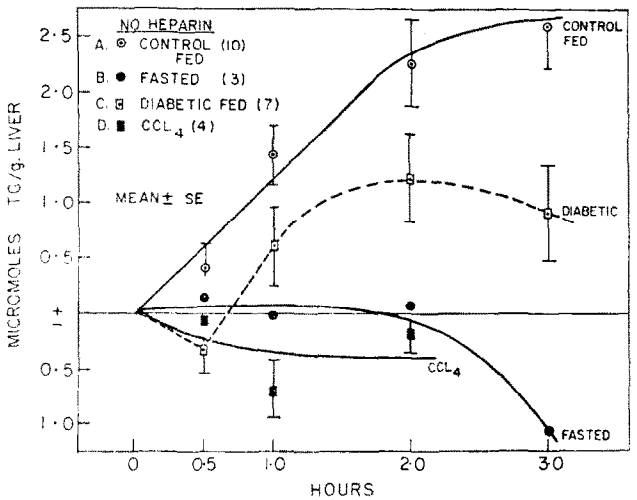

FIG. 2. Output of triglyceride by liver. Livers from alloxan-diabetic rats, fasted rats and rats treated with CCl_4 were perfused in the absence of heparin. The figures in parentheses indicate the number of experiments and all values are means ± standard error.

Statistical analysis:

	P value	
Comparison	2 hr	3 hr
A vs. B	$<0{\cdot}02$	$<0{\cdot}01$
A vs. C	$<0{\cdot}10$	$<0{\cdot}01$
A vs. D	$<0{\cdot}005$	

heparin-induced increase in hepatic lipolytic activity, livers were perfused with a medium containing crystalline heparin (Sigma); the lipolytic activity in the perfusate and the output of TG were measured simultaneously as a function of the concentration of heparin (Fig. 3). The output of TG was inhibited significantly only with 50 units of heparin; although output of TG appeared to be less than the control when 500 units was used, this difference was not statistically significant ($P < 0{\cdot}2$). It is indeed most curious that the output of TG by the liver was less than the control when 50 units of heparin was added to the medium, and that this inhibitory effect appeared to be lost as the concentration of heparin was increased but reappeared again as an exceedingly large quantity of heparin (50,000 units) was added. It was feared that the effect of heparin on the output of TG was not real, but may have resulted from reaction of heparin in the method employed for measurement of triglyceride. When, however, an

amount of heparin equivalent to that which was present theoretically in the samples of perfusate was added to a standard solution of triglycerides, extracted and analyzed by the usual procedures, no interference with the colorimetric procedure for triglyceride was detected at any concentration of heparin. The effect of heparin on lipolytic activity in the perfusate can be seen in Fig. 3B. It is curious that 5000 units of heparin induced maximal lipolytic activity and maximal output of TG, whereas 50 units of heparin resulted in minimal lipolytic activity and minimal output of TG.

Effect of heparin on uptake and utilization of chylomicron triglyceride by liver. The

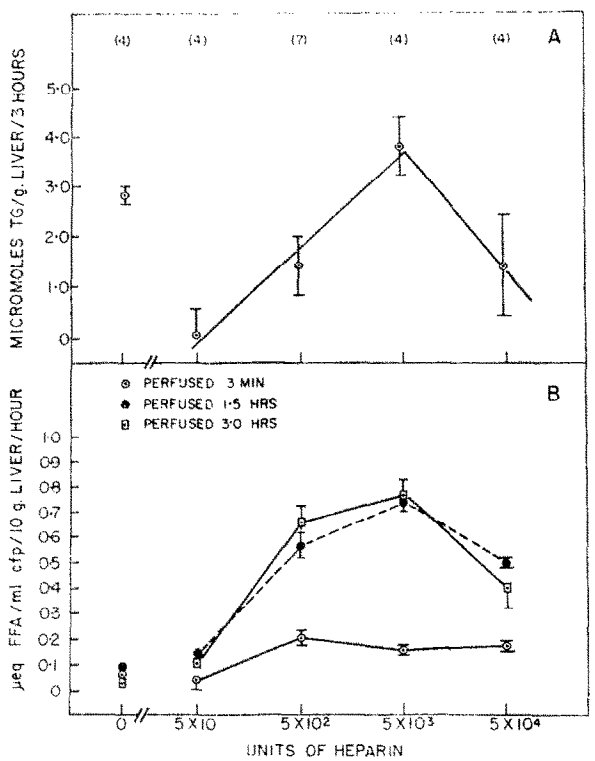

FIG. 3. Effect of heparin on hepatic triglyceride output and perfusate lipolytic activity. Crystalline heparin (Sigma) was used in these experiments. Heparin was added to the perfusate with the fatty acid–rat serum complex at the beginning of the experiment. (Please refer to the text for details.) Under the conditions of these experiments, the initial concentration of heparin in the perfusate was 0, 0·42, 4·17, 41·67 or 416·67 USP units/ml (1·0 USP unit = 0·82 i.u. = 6·3 μg). All values are means ± standard error. The number of experiments performed at each concentration of heparin is shown in parentheses. The output of triglyceride (A) is that amount secreted during the 3-hr experimental period. Lipolytic activity (B) was measured in the cell-free perfusate (cfp) at intervals throughout the experiment. The lipolytic activity in the medium before introduction of the liver into the system was negligible. Lipolytic activity within the liver was not measured. The output of triglyceride when 50 units of heparin was added differed significantly from the control ($P < 0{\cdot}005$); the output at other concentrations of heparin was not different from the control ($P \geqslant 0{\cdot}2$). Lipolytic activity after 1·5 hr of perfusion differed significantly from the control at all concentrations of heparin ($P < 0{\cdot}001$) except 50 units ($P < 0{\cdot}30$). Lipolytic activity after 3 hr of perfusion was significantly different from control at all concentrations of heparin.

uptake of chylomicra by liver was estimated by measurement of appearance of radioactivity in the liver (Table 2). Heparin (5000 USP units, Sigma) increased the quantity of radioactivity appearing in the liver after perfusion for 2 hr with chylomicra labeled with palmitate-1-^{14}C. Although only 2·1 per cent of the radioactivity in the administered chylomicra was in the phospholipid-containing fraction (F_2), about one-third of the counts in the liver was present as phospholipid whether or not heparin was added to the medium. Similarly, although 97·9 per cent of the radioactivity in the administered chylomicra was in the F_1 fraction (of which 68·6 per cent was triglyceride), only two-thirds of the counts in the liver were present in the F_1 fraction (of

TABLE 2. UPTAKE OF CHYLOMICRA BY LIVER*

Fraction	Treatment		P values†
	None	Heparin	
A. % of counts administered incorporated into total liver lipids	7·98 ± 0·41	11·22 ± 0·54	< 0·001
B. % of counts administered incorporated into F_1 fraction of liver lipids‡	5·52 ± 0·24	7·35 ± 0·49	< 0·01
C. % of total counts incorporated into liver appearing in the F_1 fraction	69·51 ± 0·99	65·52 ± 1·21	< 0·05
D. % of counts administered incorporated into F_2 fraction of liver lipids§	2·42 ± 0·18	3·78 ± 0·07	< 0·001
E. % of total counts incorporated into liver appearing in the F_2 fraction	30·48 ± 0·99	34·40 ± 1·21	< 0·05
F. % of counts administered incorporated into Triglyceride of livers‖	4·05 ± 0·26	5·85 ± 0·41	< 0·005
G. % of total counts incorporated into liver appearing in triglyceride‖	51·27 ± 3·50	52·27 ± 2·32	< 0·90

* Uptake of chylomicra per liver was estimated by measurement of incorporation of radioactivity into hepatic lipid fractions. All values are means ± standard error. Uptake was measured in the absence and presence of heparin during a 2-hr experimental period; 5000 USP units of crystalline heparin (Sigma) was added to the medium with the fatty acid–rat serum complex, giving an initial concentration of 37·88 USP units/ml of perfusate. Twelve experiments were performed, with six in each group. The liver weights (wet) were 11·01 ± 0·30 and 11·65 ± 0·31 g for the control and heparin-treated groups respectively. Of the radioactivity added to the perfusate ($4·0 \times 10^6$ dpm), 97·9% (of which 68·6% was triglyceride) was present in the F_1 fraction and 2·1% was present in the F_2 fraction.

† P value indicates significance of difference between treatments.

‡ The F_1 fraction, which is the $CHCl_3$-soluble fraction eluted from silicic acid columns, contains all hepatic lipids other than phospholipids.

§ The F_2 fraction, which is the CH_3OH-soluble fraction eluted from silicic acid columns, contains phospholipids.

‖ Triglycerides were isolated from the F_1 fraction thin-layer silicic acid chromatography.

which 76·4 per cent was triglyceride) whether or not heparin was added to the perfusate. The small percentage increase of incorporation into the F_2 fraction and decrease into the F_1 fraction in the presence of heparin may reflect contamination of the F_2 with FFA. This point was, unfortunately, not verified. The incorporation of ^{14}C into hepatic TG purified by thin-layer chromatography was, however, the same whether or not heparin was used. One may surmise from these data that, although heparin accelerates the rate of uptake of chylomicra by the liver, heparin does not alter their metabolic fate in that organ. It is also obvious that chylomicra were taken up and metabolized by the liver, albeit slowly, when heparin was excluded from the medium.

DISCUSSION

It is probable that the hepatic release of triglyceride into the very low density lipoprotein of serum is suppressed and that the uptake of chylomicron triglyceride from the blood is accelerated by a direct action of heparin on the liver. In the first series of experiments (Figs. 1 and 2) and in earlier work, 500 USP units (0·5 ml, Upjohn) of heparin was added to the perfusate as an anticoagulant and 100 units was administered intravenously to the animals from which the livers were removed; the output of triglyceride by the liver was reduced by this amount of heparin. These experiments were repeated to reconfirm earlier data reported from our laboratory and to assure ourselves that the interpretation of the data was not altered when heparin was not utilized. Since the Upjohn preparation of heparin contained benzyl alcohol (9 mg/ml), the possibility must be considered that the inhibition of release of triglyceride was caused, in part, by the alcohol. This is, however, irrelevant to the conclusion that the differences in the metabolism of triglyceride by livers from normal fed and fasted rats, alloxan-diabetic rats, and rats poisoned with CCl_4 remained whether heparin (Upjohn) was added[14, 15, 17] or was omitted from the experiments.

The experiments carried out with the Upjohn heparin cannot be compared directly with the second series of experiments in which crystalline heparin (Sigma) was used. In the latter experiments, statistically significant impairment of hepatic release of triglyceride was observed only at the lowest concentration of heparin (0·42 USP units/ml of perfusate). As the concentration of heparin in the medium was increased, so was the lipolytic activity of the perfusate, in confirmation of the observations of Felts and Mayes.[6] The lipolytic activity measured reflects most probably the activity of a triglyceride lipase and not a phospholipase;[31] the latter is unlikely because the pH (7·4) at which lipolytic activity was estimated differed from the optimal pH (8·8–9·1) for the phospholipase, and because the substrate (Intralipid) in the incubation mixture contained approximately twenty times more triglyceride than phospholipid on a weight basis (7·0 vs. 0·35 mg). The quantity of triglyceride and phospholipid present in the complete incubation mixture with addition of Intralipid, serum, and cell-free perfusate was 10·2 and 2·6 μmoles, respectively, giving a molar ratio of triglyceride:phospholipid of 4:1. It is doubtful whether the increased lipolytic activity in the perfusate as a result of crystalline heparin can in any way be responsible for an impairment of release of triglyceride. There was an apparent dissociation between lipolytic activity and output of triglyceride. As the concentration of heparin and the lipolytic activity in the medium increased simultaneously, the output of triglyceride increased as well, being impaired only at the lowest concentration of heparin. It is quite evident, however, that the uptake of triglycerides from chylomicra or from very low density lipoproteins[11, 12] is accelerated by heparin. It may be inferred from these observations that the metabolic pathways for release or uptake of triglyceride by liver differ, and that the effects of heparin on these divergent pathways must operate through distinct mechanisms.

It was stated by Felts and Mayes[11] that the isolated, perfused rat liver did not hydrolyze or utilize chylomicron triglyceride to any significant extent in the absence of heparin. It is clear that the rate of uptake and utilization of chylomicron triglyceride by liver is less in the absence than in the presence of heparin. Although the preferred lipid substrate for the liver is probably long-chain free fatty acids,[32] exogenous triglyceride can nevertheless be utilized. Felts and Mayes reported that about 2 per cent of ^{14}C-labeled chylomicron triglyceride[11] and about 1·5 per cent in the VLDL

triglyceride[12] were recovered from perfused livers in the absence of heparin, and about 12 per cent was recovered when lipoprotein lipase was added.[11] In this laboratory, 7·98 and 11·22 per cent of ^{14}C-labeled chylomicron triglyceride was recovered from lipids of perfused livers in the absence or presence of heparin respectively (Table 2). It has been proposed that chylomicron triglyceride enters the hepatic cell intact and undergoes hydrolysis within the cell.[33–36] Whether the site of this hydrolysis is within the cell or at the cell membrane, or occurs intravascularly *in vivo*, and whether a heparin-activated hepatic lipoprotein lipase is physiologically important in the utilization of triglyceride by liver cannot be answered with certainty at this time. The major metabolic function of the liver under normal conditions may be to convert FFA to hepatic and VLDL triglyceride of the serum; this sequence of reactions appears to be analogous to the hepatic synthesis of glycogen and blood glucose from lactate and other gluconeogenic precursors. Whether or not the utilization of chylomicron triglyceride by liver can be regulated by hormonal mechanisms, as can the output of VLDL triglyceride,[37] remains to be established. Suggestions for such control may be derived from the observation that in intact fasted animals a larger fraction of injected chylomicra labeled with ^{14}C appears in the liver than does under fed conditions,[38] and that the uptake of chylomicron triglyceride by perfused livers from alloxan-diabetic rats may exceed that by livers from normal fed animals.[14]

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