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The metabolism of palmitic acid-1-C^{14} in functionally hepatectomized rats*

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SUMMARY

Normal carbohydrate-fed rats and rats on which a functional hepatectomy had been performed by ligating the vessels of the porta hepatis were injected with C^{14}-labeled palmitic acid bound to albumin, and the distribution of activity studied. The concentration of free fatty acids (FFA) in plasma rapidly rises after the hepatectomy. The half life of the injected activity, however, does not seem to change significantly in these animals. In the normal rat a considerable fraction of the injected FFA is recirculated in the blood in glycerides and to a lesser extent in phospholipids. This recirculation is almost completely abolished after hepatectomy, indicating that the liver is the chief organ for plasma lipoprotein synthesis. The peripheral tissues are capable of esterifying the FFA but do not release any esterified fatty acids into plasma, with the possible exception of adipose tissue. There is less activity going into the adipose tissue after hepatectomy. The uptake of fatty acids into adipose tissue is discussed.

During recent years much interest has been focused on the metabolism of albumin-bound "free fatty acids" (FFA) in plasma (1, 2). They have a rapid turnover and seem to be the transport form of fatty acids mobilized from the fat depots (3). The flux of FFA from plasma, however, is considerably greater than the utilization of these acids for energy production (4). An equivalent flux of fatty acids back into adipose tissue is necessary if no net redistribution of fatty acids from adipose tissue to other tissues is to take place. Adipose tissue has been shown to take up only small amounts of the plasma FFA (5). A possible vehicle for transport of fatty acids back into adipose tissue might be lipoproteins synthesized by the liver. It is now well known that the liver takes up albumin-bound fatty acids (6) and gives them off as glycerides and phospholipids incorporated in plasma lipoprotein (7).

The purpose of the present study was to investigate the effect of functional hepatectomy on the recycling, as esterified fatty acid, of injected palmitic acid-1-C^{14} bound to albumin in plasma, and on the tissue distribution of the injected activity.

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EXPERIMENTAL

Fifty microcuries of palmitic acid-1-C^{14} (The Radiochemical Centre, Amersham, England; specific activity 28 mc/mmole) was dissolved in 1 ml ethanol, a moderate excess of NaOH was added, and the solution taken to dryness. Rat serum (5 ml) was added, the mixture stirred, and allowed to stand at room temperature for 1 hour before it was filtered. The filtrate was then stored deepfrozen until used. On analysis, more than 99% of the radioactivity was found in the form of FFA.

The experimental animals were male Sprague-Dawley rats, weighing 185 to 210 g at the time of injection. They were offered 10% glucose in 0.45% NaCl 12 to 20 hours before injection. No other food was given during this time. The functional hepatectomy was performed as follows: The rats were anesthetized with ether and the abdomen opened with a mid-line incision. The superior mesenteric artery was ligated to avoid engorgement of the splanchnic vessels with blood. Three minutes later the vessels entering and leaving the liver through the porta hepatis were ligated. Five minutes later 0.5 ml of palmitic acid-1-C^{14} serum mixture was injected into the left jugular vein and the rats were then kept anesthetized until sacrificed. Normal rats were injected in the same way but were not operated.

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At the time of sacrifice as much blood as possible was drawn from the abdominal aorta and immediately centrifuged. An aliquot of serum was measured and poured into 20 volumes of chloroform-methanol 2/1 (v/v). The liver, the heart, and a piece of the left epididymal adipose tissue were cut up in approximately 20 ml of chloroform-methanol 2/1 (v/v) per g of tissue. The tissues were then homogenized in a Waring Blendor and allowed to stand overnight. The chloroform-methanol extracts were filtered and washed once with 0.4 volume 2% KH_2PO_4 and taken to dryness.

The extracts were separated on silicic-acid columns using a slight modification of Borgström's method (8, 9). The serum lipids were fractionated into a cholesterol ester fraction, a fraction containing glycerides and FFA, and a phospholipid fraction. The lipids of the other organs were separated into one fraction containing cholesterol esters, glycerides, and FFA, and one containing the phospholipids. The FFA were extracted from the neutral fat by dissolving the lipids in petroleum ether and washing twice with equal volumes of 0.1 N KOH in 50% ethanol. The ethanol phases were then acidified and extracted twice with equal volumes of petroleum ether. The petroleum ether was then evaporated and FFA determined by dissolving the residue in absolute ethanol and titrating. The rest of the neutral fat fraction in the first petroleum ether was hydrolyzed by adding an equal volume of 4% KOH in absolute ethanol. After the acidification, the fatty acids were extracted by adding equal volumes of water and petroleum ether. The lower phase was extracted once more with petroleum ether, the petroleum ether phases were taken to dryness, the residue dissolved in absolute ethanol and titrated. This extract contains the nonphospholipid esterified fatty acids and is called "neutral fat" in this article.

To the methanol eluate from the silicic-acid column an equal volume of 4 N KOH in water was added and the mixture refluxed for 4 hours. After acidification, the phospholipid acids were then extracted twice with equal volumes of petroleum ether. The petroleum ether was evaporated, and the residue was weighed, or dissolved in absolute ethanol and titrated. Finally, all fractions were acidified again, extracted, taken to dryness, dissolved in toluol containing 3% 2,5-diphenyloxazole (DPO), and counted in a liquid scintillation counter. By the use of a standard and an internal standard, all activities were subsequently corrected to an injected dose of 75,000 cps and a body weight of 200 g. The specific activity of the FFA of plasma after complete mixing was approximately calculated to 10,000 cps/μeq in the normal rats, and 3,400 cps/μeq in the hepatectomized rats. Each point on the curves represents results from one animal, but similar figures have been obtained in other series of experiments.

An additional series of experiments was undertaken to study more accurately the half time of the injected isotope. In these experiments three normal, three sham-operated, and three hepatectomized rats were injected as above, and 0.5 ml of blood taken from the jugular vein 1, 2, 4, 6, 8, 12, and 16 minutes after the injection. Plasma (0.2 ml) was extracted and the total activity determined.

RESULTS

Disappearance of Injected Material. The concentration of FFA in plasma is 0.62 ± 0.12 μeq/ml in the normal rats and 2.76 ± 0.92 μeq/ml in the hepatectomized rats. The half life of the injected activity is 0.8 minute in the normal rats and 1.0 minute in the hepatectomized (Fig. 1). These half-life figures are approximate as they are calculated on the estimated zero-time dilution of injected label and the observed values after 5 minutes. In these calculations it has also been assumed that the blood volume is not changed after hepatectomy. In the experiments in which blood from the same animals was frequently sampled, half-life figures between 2 and 4 minutes were 1.6, 1.7, and 1.7 minutes and between 4 and 6 minutes 3.2, 3.4, and

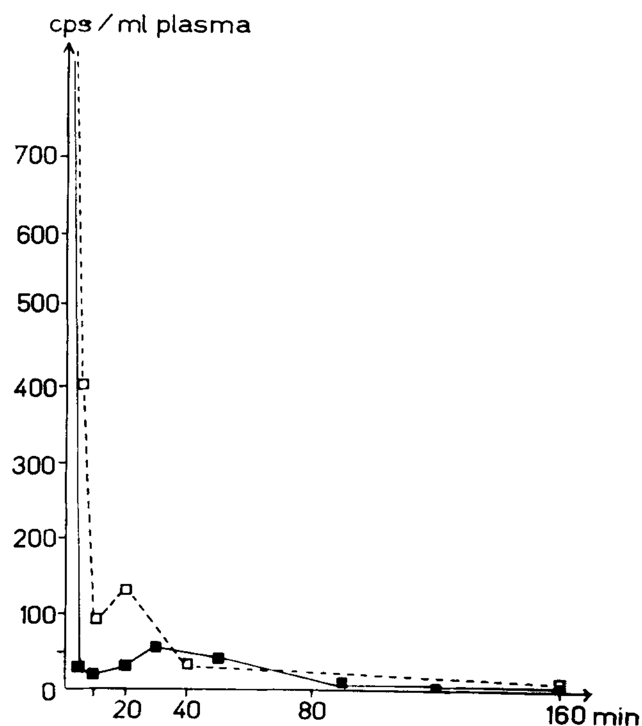

FIG. 1. Disappearance of injected albumin-bound palmitic acid-1-C^{14} from rat plasma. Solid lines: normal rats. Broken lines: hepatectomized rats.

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3.2 minutes for the normal, sham-operated, and hepatectomized rats, respectively. Calculating the zero-time dilution of injected label from a plasma volume of 12 ml in a 200-g rat gave half lives for the three types of rats during the first 2 minutes of 0.9, 0.8, and 1.4 minutes. If the half life during this time in the hepatectomized rats is assumed to be the same in the normal, their plasma volume can be calculated to about 8 ml.

Labeling of Other Plasma Lipids. In the normal rats the plasma neutral fat fraction soon becomes labeled (Fig. 2). After about 15 minutes its specific activity is higher than that of the FFA in the plasma, and it stays higher during the rest of the observed time. The phospholipids are labeled more slowly. Their maximum specific activity is 27 and is reached after 50 minutes; the maximum specific activity of the triglycerides is 300 and is reached 30 minutes after the injection, making up around 3.5% of the specific activity of the FFA fraction of plasma as calculated at zero time.

In the hepatectomized rats, on the other hand, no significant labeling of the plasma triglycerides or phospholipids can be shown. The low activities shown after 5 minutes can well be ascribed to contamination from the FFA fraction during the analysis.

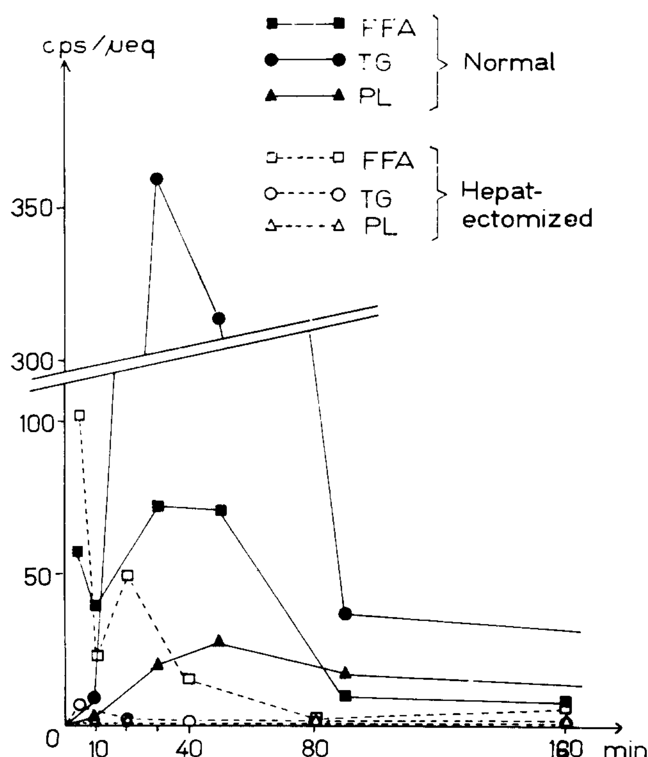

FIG. 2. Specific activity of the fatty acids in the plasma lipid fractions after the injection of albumin-bound palmitic acid-1-C^{14}.

Labeling of the Liver Lipids. The liver lipids are labeled soon after the injection of labeled FFA (Fig. 3). The triglycerides reach their maximum specific activity 10 minutes after the injection, and then fall off at a rather constant rate during the next 70 minutes. The phospholipids reach their maximum specific activity 90 minutes after the injection. At this time the lipid fractions seem to have equilibrated, and their specific activities then fall off at about the same rate. In the hepatectomized rats the livers were cut out after sacrifice, and were shown to contain less than 0.5% of the injected activity.

Labeling of the Heart Lipids. The heart was chosen as a typical peripheral tissue using FFA for energy production. It is obvious from Figure 4 that a large proportion of the label is contained in the neutral fat fraction, both in the normal and in the hepatectomized rats. This indicates that the fatty acids are esterified in the heart itself and not transported there from the liver, as the plasma in the hepatectomized rats was shown to contain almost no labeled esterified fatty acids.

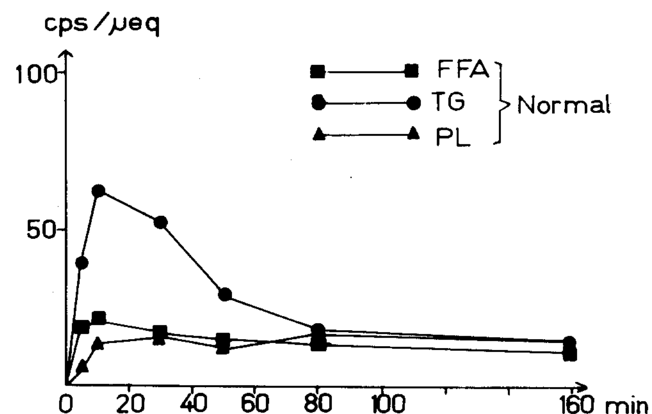

FIG. 3. Specific activity of the fatty acids in the liver lipid fractions after the injection of albumin-bound palmitic acid-1-C^{14}.

Labeling of the Adipose Tissue Lipids. The hepatectomized rats have more FFA in their depots than the normal ones: 0.5% to 2.0% of the total amount of fatty acids of the depots are free in the hepatectomized rats compared with 0.1% to 0.3% in the normal ones. Figure 5 shows the total amount of activity present in 1 g of epididymal adipose tissue in the different fat fractions rather than the specific activity. There is less activity going into the adipose tissue of the hepatectomized rats, which might well be due to the fact that the direction of fatty acid transport in these animals is largely out of the adipose tissue.

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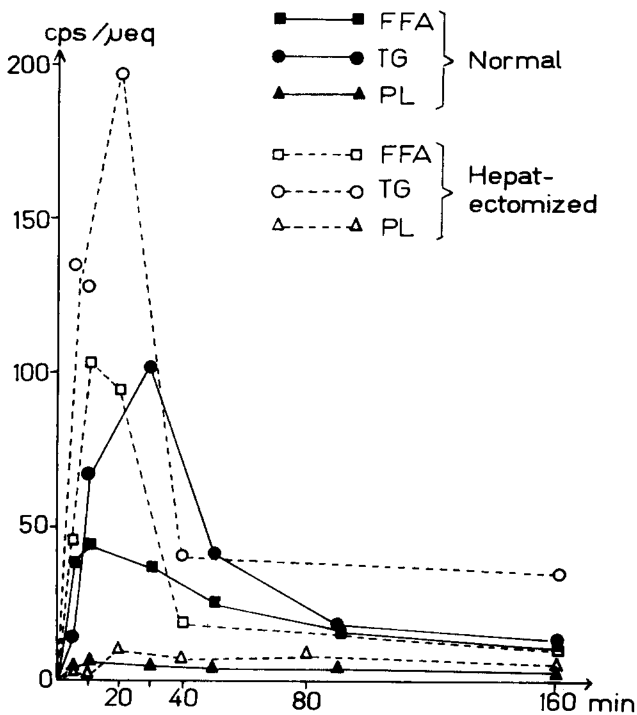

Fig. 4. Specific activity of the fatty acids in the heart lipid fractions after the injection of albumin-bound palmitic acid-1-C^{14}.

DISCUSSION

The increased concentration of plasma FFA in hepatectomized animals can be the result of an increased mobilization of fatty acids from the fat depots, or a decreased utilization of fatty acids, or a combination of both. The increase in the FFA pool of adipose tissue found in these animals might indicate an increased outflow of fatty acids from this tissue. A decrease in the plasma volume after hepatectomy also contributes to this increase of FFA concentration.

The most interesting conclusion to be drawn from this work is that the liver is necessary for the incorporation of FFA of plasma into plasma lipoprotein glycerides. Although other organs, such as the heart, synthesize triglycerides from the FFA fraction of plasma, they are not given off to the circulation. The possibility exists that the liver is the only organ, beside the small intestinal mucosa, which can synthesize lipoproteins of plasma type. That the same holds true for phospholipid has been shown by Fishler *et al.* (10). The possibility that the adipose tissue is liberating triglycerides into the plasma cannot, however, be ruled out, as the specific activity of the adipose tissue triglycerides remains very low during the observation time. The importance of the liver for the synthesis of plasma glycerides has also been stressed recently by Byers and Friedman (11), who found that hepatectomy abolished triglyceridemia after injection of Triton WR-1338 into rats.

A common feature for the liver and the heart is that the specific activity of the triglyceride fatty acids soon rises above that of the FFA from which they probably are derived. This fact may be explained in light of the findings of Stein and Shapiro (7), that within the liver cells there is a much higher specific activity in the particle-bound triglycerides than in those of the

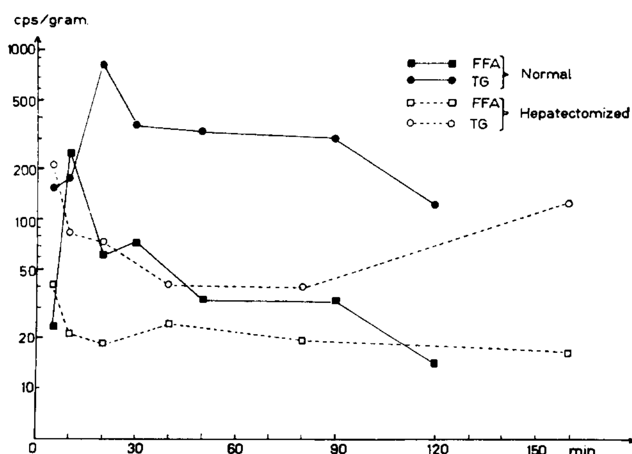

Fig. 5. Total activities of the fatty acids in the epididymal adipose tissue lipid fractions after the injection of albumin-bound palmitic acid-1-C^{14}. The figures are given per gram of wet tissue weight.

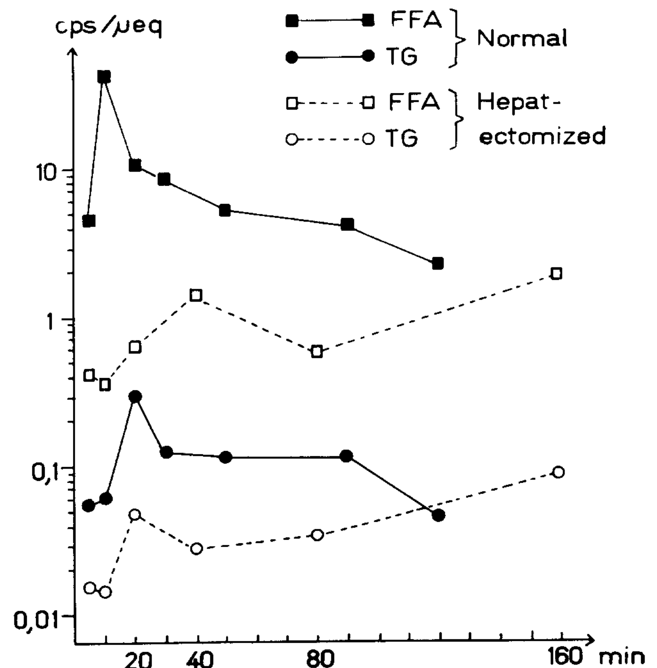

Fig. 6. Specific activities of the fatty acids in the epididymal adipose tissue lipid fractions after the injection of albumin-bound palmitic acid-1-C^{14}.

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floating fat. The same is in all probability true for lipid fractions of other organs. This means that the FFA coming into the cells from the plasma and having high specific activity is directed preferentially to the actively metabolizing sites in the cells, that is, the particles, where they are built into different forms of fatty acid esters. One must also assume that in the liver these newly synthesized triglycerides and phosphatides having higher specific activity than the bulk of the liver fat are preferentially built into lipoproteins. This may explain the fact that the plasma triglycerides have a higher specific activity than the total liver triglycerides.

In the adipose tissue of the normal rats the FFA fraction reaches its maximum activity 10 minutes after the injection of the labeled palmitic acid (Fig. 6). It then gradually falls off during the observation time. The triglycerides are rapidly labeled and reach their maximum specific activity 20 minutes after the injection. The activity then falls off during the first 2 hours. Other experiments carried out in this laboratory have shown that there is a secondary rise of activity in the adipose tissue glycerides in carbohydrate-fed rats. This starts after about 2 hours and continues for at least 24 hours.[1] This can be interpreted to mean that the FFA coming into the adipose tissue are directed to the "actively metabolizing sites," where they are rapidly built into esters. The main part of these esters is almost immediately broken down again, and the fatty acids mobilized from the adipose tissue. This means that a rather rapid exchange of fatty acids between plasma and adipose tissue takes place, but that only a small fraction of the adipose tissue fatty acids is readily exchangeable with those of plasma. However, a small fraction of the newly synthesized esters leaks out into the floating fat fraction and remains there, being turned over at a much lower rate than the fat on the "actively metabolizing centers." This fraction accounts for the secondary slow rise of activity.

In the hepatectomized rats the same trend can be seen. However, both the FFA and the triglycerides reach their maximum specific activity faster than in the normal rats, and the activity going into adipose tissue is lower. As was mentioned before, these rats seem to be mobilizing fatty acids from their depots at a rapid rate. This means that the rate of exchange between plasma and adipose tissue is higher than in the normal rats and that the direction of fatty acid transport is mainly out of adipose tissue. If this is so, it could well explain the fact that the activity rises and falls off faster and stays at a lower level in these rats than in the normal ones. Another explanation that cannot be ruled out is that the adipose tissue normally takes up preferentially plasma esterified fatty acids. Bragdon and Gordon (5) have shown that more activity goes into the adipose tissue after the injection of fatty acids incorporated into chylomicrons than after injection of FFA. In the normal rats in the present study a large fraction of the injected activity is recycled in the plasma as esterified fatty acids, while in the hepatectomized rats no such recycling takes place. If the adipose tissue is specialized on the uptake of esterified fatty acids, it is obvious that less activity should appear in the adipose tissue in the hepatectomized rats.

[1] Thomas Olivecrona, unpublished observation.

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