THE ROLE OF LIVER AND SPLEEN IN THE METABOLISM OF INTRAVENOUSLY INJECTED FAT IN RABBITS*

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Intravenously injected artificial fat emulsions rapidly disappear from the blood stream. A controversy as to the mechanism of this phenomenon is going on in the literature.

Murray and Freeman reported that considerable quantities of injected fat emulsion were rapidly taken up by the reticuloendothelial cells of the liver and spleen, whereas the hepatic parenchymal cells failed to show a distinct accumulation of stainable lipids (1). They concluded that artificial fat particles are treated by the organism as foreign bodies, and that utilization of this fat by the liver chiefly depends on preceding uptake by the Kupffer cells.

Waddell et al. studied the influence of extirpation of several organs on the disappearance of artificial fat emulsions from the blood (2). They confirmed that the liver and also the spleen take up most of the injected triglycerides, other organs playing a subordinate role in this respect. In a further investigation the same authors demonstrated that after injection of stained emulsions all parenchymal cells of the liver were stained within 5 minutes—a fact which suggests direct uptake of the fat without passage through the Kupffer cells (3). This interpretation was supported by the observation that blocking of the reticuloendothelial cells by means of carbon, trypan blue, or lithium carmine excluded fat particles from the cells of this system, but did not affect the disappearance of injected fat from the blood or its uptake by the parenchymal cells of the liver.

Studies made by Felton et al. (4) with radioactively labeled triolein also demonstrated that the liver and spleen take up the highest concentrations of the injected fat, and showed that atherosclerotic animals were normal in this respect.

None of these experiments measured the quantity of triglyceride taken up by the various organs, making it impossible to compare the quantity of triglyceride disappearing from the blood and the quantities found in the tissues.

The purpose of our first series of experiments was to study the quantitative distribution of artificial fat emulsions among plasma, liver, and spleen at different times after intravenous administration to rabbits. It was found

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possible to follow the injected fat by gas-liquid chromatography of the fatty acids of plasma and tissues, since the coconut oil emulsion used in these experiments had a high content of lauric and myristic acids, of which only traces are found in the tissues of normal animals. The influence of a previous injection of the detergent Triton or administration of a high cholesterol diet on the distribution of this emulsion was also studied.

The second series of experiments concerned the remobilization of triglyceride from the liver into the circulation, by means of an injection of Triton 6 hours after the administration of the emulsion.

**Materials and Methods**

Rabbits were injected intravenously with an artificial coconut oil emulsion (Ediol®, Schenley, New York), an emulsion manufactured for oral use, but well tolerated by rabbits when given intravenously in a dose of 4 ml/kg body weight (2 gm oil/kg). The injections were given undiluted at a rate of about 10 ml in 2 minutes. Blood samples from the ear vein were collected in heparinized tubes.

The rabbits were sacrificed by a blow on the neck and immediately dissected. The inferior vena cava was ligated above and below the hepatic vein and cut between the ligatures; the liver was then perfused *in situ* with 500 ml chilled dextran plasma substitute (Macrodex®, Poviet, Amsterdam) via the portal vein. This perfusion removed most of the blood from the liver. The spleen was not perfused. The liver and spleen were then removed, weighed, and ground up. Aliquots were further fragmented in Potter tubes.

The lipids of plasma, liver, and spleen were extracted with cold methanol:methanol 4:1 v/v. This method proved to give the same yield of lipids as extraction in hot chloroform:methanol 2:1 v/v during 5 hours.

The fat emulsion used contained 71.5 gm of lauric and myristic acids per 100 gm of triglyceride. Since these fatty acids were injected in large amounts and only traces of these fatty acids are normally found in the tissues, the quantities recovered could be assumed to have come from the injection. To measure the absolute amounts of fatty acids a known quantity of heptadecanoic acid was added to an aliquot of the total lipid extract; this was then evaporated and the fatty acids converted into their methyl esters according to Stoffel and Ahrens (5). After extraction with petroleum ether the esters were chromatographed in a beta ray argon gas chromatograph (W. G. Pye Ltd., Cambridge, England) on polyethylene as stationary phase, at 160°C. The concentration of each fatty acid in the sample was calculated from the ratio of the area of its peak to that of the heptadecanoic standard.

Similar gas-liquid chromatographies were initially performed on the three lipid fractions obtained by separation of the total lipid extract on silicic acid columns following the simplified elution scheme of Hirsch and Ahrens (6). Nearly all lauric and myristic acid present in the total extract could be recovered from the fraction eluted by diethyl ether, which contains triglycerides, partial glycerides, and non-esterified fatty acids. Further separation of this fraction was not attempted. Since neither the cholesteryl esters nor the phospholipids contained appreciable amounts of lauric and myristic acids, the separation of the total lipid extract was omitted for the majority of our analyses, and all lauric and myristic acid was regarded as part of the glycerides (although a fraction may have been present as nonesterified fatty acid).

The absolute quantity of lauric and myristic acids in the plasma of each rabbit at the end of the experiments was calculated on the basis of the equation:

$$x = \frac{C_0 \cdot F}{C_t}$$
in which $F =$ the quantity of lauric and myristic acids injected. $C_0 =$ the concentration of these fatty acids at zero time. This value was calculated by extrapolation from the concentrations in samples taken 3 to 5 minutes after the start of the injection, and after 1 hour. $C_x =$ the concentration at the end of the experiment.

The absolute quantities in the liver and spleen were calculated from the fatty acid concentration and the weight of the organs.

Plasma total esterified fatty acids were determined by the method of Morgan and Kingsbury (7).

For the determination of plasma glycerides aliquots of total lipid extract were evaporated, taken up in light petroleum ether, and applied to silicic acid columns, which were eluted with 1 per cent anhydrous ether in petroleum ether to remove the cholesteryl esters (6). The glycerides were then eluted with chloroform (8). This fraction was evaporated and the solvent replaced by ethanolic ether 3:1 v/v. The glycerides were then determined by dosing the esterified fatty acids according to Morgan and Kingsbury (7), and the results are expressed as triolein. Plasma total cholesterol was determined with the method of Zlatkis (9). Lipid phosphorus was determined with the method of Fiske and Subbarow (10), and the results expressed as lecithin.

Functional hepatectomy was performed on rabbits under ether anesthesia. The operation included the ligature of the arteria coeliaca, arteria mesenterica cranialis and caudalis, and the vena portae hepatis. It was found useful to ligate also the larger vessels of the mesenterium in order to prevent any intestinal congestion. Immediately after the operation the animals received an intravenous injection of 5 ml of a solution containing 5 per cent glucose, 0.05 mU/ml of insulin, and 0.01 mg/ml of norepinephrine. This injection was repeated every hour, and proved to control the hypoglycemia. Sham-operated animals received the same treatment.

Triton WR 1339 (Winthrop, New York) is an octyl phenol polyether.

The cholesterol diet consisted of 1 per cent cholesterol (Merck, Darmstadt, West Germany), 3 per cent arachis oil, and 96 per cent commercial rabbit chow. This diet had been given for 8 months at the time of injection of fat emulsion.

EXPERIMENTS

I. Uptake of Fat Emulsions in the Liver and Spleen.—

A. Control rabbits: Twenty normal rabbits were injected intravenously with coconut oil emulsion as described above. Three animals were sacrificed after 15 minutes, five after 2 hours, three after 6 hours, and three after 12 hours. Blood samples were obtained, immediately after the injection, after 1 hour, and before death. The quantities of lauric and myristic acids found in plasma, liver, and spleen are shown in Fig. 1 A. Each point in this figure represents the average result of the number of experiments indicated.

The injected fat rapidly disappeared from the blood stream. After 4 hours the plasma was clear and the analyses showed only traces of lauric and myristic acids remaining. These results are compatible with the data of the literature (2, 3, 11).

The spleen rapidly and actively accumulated the fat particles. The presence of fat-laden blood in this organ could not account for the large quantity of lauric and myristic acids, since the values obtained were much higher than those in the plasma. This uptake is probably due to phagocytosis of fat particles in the reticuloendothelial cells. After 2 hours the concentration had risen to
Fig. 1. Lauric + myristic acid levels in plasma, liver, and spleen of rabbits injected with coconut oil (A), and with triton and coconut oil (B).
almost 8 per cent of the weight of the organ. After this time a rapid decrease was seen, the spleen again being free of lauric and myristic acids after 6 to 12 hours. A comparison of the concentrations found in the spleen and in the plasma, gives the impression that the spleen rapidly reacts to changes of the plasma fat concentration in either direction.

The liver showed a quite different response to the injected fat. Initially much less fat was taken up per gram of tissue than in the spleen, but the fatty acids of the emulsion could still be demonstrated when the spleen was again free of them. If the response of the spleen is typical of that of the reticuloendothelial cells, then it must be assumed that in the liver the activity of the Kupffer cells is masked by a second fat-concentrating mechanism, which may be the uptake of fat in the parenchymal cells.

B. Rabbits injected with triton: Fifteen rabbits were injected intravenously with 200 mg triton per kg body weight; 10 minutes later the animals were injected with coconut oil emulsion, the remainder of the experiment being conducted as in the controls. The rabbits were sacrificed in groups of three after 15 minutes, and 2, 4, 6, and 12 hours.

The results are represented in Fig. 1 B. The points in this figure represent the averages obtained in each group of three rabbits, and the curves are the calculated lines best corresponding with these points.

It is clearly shown that a preceding injection of triton alters the reaction of the organism to the glycerides administered. The plasma concentrations remained very high, showing hardly any decrease even after 12 hours. As in the control animals, the spleen took up high concentrations but it retained them much longer. The liver after 15 minutes had taken up a low but significant concentration of lauric and myristic acids, and this remained nearly constant throughout the experiment.

The uptake of fat particles by the spleen therefore does not seem to be disturbed by triton, but the release of these triglycerides is inhibited. If, as is suggested by the results obtained in the control animals, the splenic concentrations are a function of those of the plasma, then the persistence of high fat concentrations in the spleen in triton-treated rabbits can be explained by the high plasma concentrations. These results, consequently, do not warrant the conclusion that triton has a direct effect on the activity of the spleen.

The influence of triton on the liver is seen by the fact that at no time does this organ show so marked a concentration of lauric and myristic acids as in the control rabbits. In view of the persisting high levels of injected fat in the plasma and spleen, the low levels found in the liver show that the mechanism of uptake in this organ differs from that of the spleen, and that it is probably due to removal of fat by the parenchymal cells. The specific inhibition of the latter mechanism by triton does not implicate a direct action of this detergent on the liver itself. Triton, which is known to alter the plasma lipoproteins (12), might also change the fat emulsion so that it cannot be removed by the liver cells.
C. Hypercholesterolemic rabbits: Coconut oil emulsion was injected into four hypercholesterolemic rabbits fed a high cholesterol diet during 8 months. Blood samples were taken before the injection and 5 minutes and 1, 2, 3, and 4 hours afterwards, at which time the animals were sacrificed. The same was done with three normal and three triton-injected rabbits. Plasma total esterified fatty acids were determined in each sample and the results are represented in Fig. 2. It is seen that the hypercholesterolemia did not interfere with the disappearance of the fat emulsion from the blood.

![Graph showing esterified fatty acids in plasma of cholesterol-fed, triton-injected, and control rabbits after injection of coconut oil.](image)

**Fig. 2. Left part:** Total esterified fatty acids in plasma of cholesterol-fed, triton-injected, and control rabbits after an injection of coconut oil. **Right part:** Amount of lauric and myristic acids (expressed as percentage of the injected dose) recovered from plasma, liver, and spleen 4 hours after the injection of fat emulsion.

**Distribution of the injected fat:** The absolute amounts of lauric and myristic acids present in the plasma, liver, and spleen at the end of each experiment were calculated as described above, and compared with the total quantity injected. In Fig. 3 the quantities recovered are expressed as percentages of the injected dose.

In the control rabbits the most remarkable findings were that after 2 hours more than half of the fat recovered was found in the liver, and that after 4 hours this organ contained nearly all of the fat recovered. Despite the very high concentration of lauric and myristic acids in the spleen (Fig. 1) this organ never contained a quantitatively important fraction of the emulsion injected.

The total amount of lauric and myristic acids recovered decreased steadily;
after 12 hours only 14 per cent of the injected dose was still present in liver, spleen, and plasma. No attempt was made to trace the injected fat in other organs, so that it is impossible to draw any definite conclusion about the fate of the remaining glycerides.

In the rabbits pretreated with triton, the distribution and disappearance of the fat were changed. After 12 hours a total of 71 per cent of the dose administered was still present, and the bulk of it was found in the plasma. Triton, therefore, prevents the organism from rapidly coping with the foreign triglycerides. This is partly owing to a decreased removal of circulating triglycerides by the liver, since only 7.7 per cent of laurie and myristic acids are found in the liver of the triton-injected rabbits after 4 hours instead of 38.8 per cent as in the controls.

The cholesterol-fed rabbits, finally, show that after 4 hours the amounts recovered in plasma, liver, and spleen, and also the distribution of the fat
between these organs, correspond well with the values found in the control rabbits (Fig. 2). Although the livers of the hypercholesterolemic rabbits were enlarged, somewhat fibrous, and very fatty (Table I), their capacity to handle emulsified fat did not seem to be disturbed.

II. Reappearance in the Plasma of Injected Fat Taken Up by the Liver.—It was stated in the above described experiments that triton slows the uptake of foreign triglycerides by the liver. By the following experiments we investigated whether the exogenous fat can reappear in the plasma after its disappearance from it.

For this purpose, ten rabbits were injected with coconut oil emulsion as described above, and after 6 hours 400 mg/kg of triton was administered intravenously. The gas-liquid chromatography of the total lipids of blood samples taken at 0, 6, and 18 hours after the injection of triton showed significant quantities of lauric and myristic acids reappearing in the plasma (Fig. 4 A). The increase in other plasma fatty acids (from C16 to C18:3) is shown in Fig. 4 B. Controls that did not receive triton showed a further diminution of plasma lauric and myristic acids. Two other controls receiving triton without fat emulsion showed no significant increase of lauric and myristic acids, although the endogenous fatty acids increased at a rate comparable with that observed in the rabbits injected with both fat emulsion and triton. This indicates that there exists no pool of lauric and myristic acids in normal rabbits which can be mobilized by triton, and that the reappearing lauric and myristic acids in the experimental animals result from their previous injection.

The role of the liver in this phenomenon was investigated by injecting triton into rabbits subjected to functional hepatectomy 6 hours after the administration of fat emulsion. This experiment was based on a recent report of Byers and Friedman (13), who found that hepatectomy prevented the appearance of the triton-induced hyperglyceridemia.

A first series of experiments was performed to see whether functional hepatectomy influences the post-triton increase of plasma cholesterol and phospholipids, as well as that of glycerides. For this purpose nine rabbits were subjected

| TABLE I |  |
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| Average Body Weight, Liver Weight, and Total Fatty Acid Concentration in the Liver of Control and Cholesterol-Fed Rabbits, Sacrificed 4 Hours After Injection of Coconut Oil |  |
| No. | Body weight | Liver weight | Liver total fatty acids |
| Controls | 3 | 2,650 | 91 | 4.06 |
| Cholesterol-fed rabbits | 4 | 3,675 | 166 | 6.06 |
| Ratio, cholesterol-fed/controls | 1.39 | 1.82 | 1.49 |
to functional hepatectomy and injected with 400 mg/kg of triton immediately afterwards. Five sham-operated controls were treated in the same way. Blood samples were taken at 0, 3, 4, 5, or 6 hours after the injection of triton, and the amounts of glycerides, total cholesterol, and phospholipids determined. The results are represented in Fig. 5. It is seen that the operated animals did not show a significant increase of any of the lipid fractions, but rather a slight decrease which may, however, be due to non-specific factors. The sham-operated rabbits, on the other hand, invariably showed an increase of plasma lipids, most pronounced in the glycerides and least in cholesterol. These results confirm those of Byers and Friedman (13), and of Hirsch and Kellner who described the influence of hepatectomy on the triton-induced hypercholesterolemia (14).

Functional hepatectomy was then performed on seven animals injected with coconut oil emulsion 6 hours beforehand. The animals were then injected with 400 mg/kg of triton, and gas-liquid chromatography was performed on total lipid extracts of plasma samples taken after 0 and 6 hours. The results are included in Fig. 4. It is seen that no lauric and myristic acids reappear in the circulation of these animals; the other fatty acids also fail to increase.

The conclusion can be drawn that the liver plays an essential role in the accumulation of fatty acids in the plasma after injection of triton, and the
foreign fatty acids (taken up after the injection of fat prior to the administration of triton) behave in the same way as the proper body fatty acids.

This phenomenon may be explained by an active mobilization of fat from the liver by triton. But it is even more probable that the liver lipids are in dynamic equilibrium with the plasma lipids, and that triton causes an accumulation of the latter by blocking their passage to the liver. This blocking could possibly be caused by the alteration of the physical properties of the fat particles by triton. Such alterations of plasma lipoproteins by this detergent are described by Scanu and Oriente (12). They concluded that this may possibly hamper the exit of lipoproteins from the circulation, which would explain the increase of plasma lipids after injection of triton. This is in agreement with other workers results and conclusions (15, 18). Our results indicate that the liver is the source of the glycerides retained in the plasma after injection of triton.

**DISCUSSION**

Our first series of experiments indicates that liver and spleen react in a different way towards injected artificial fat emulsions. The findings are com-

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**Fig. 5.** Plasma levels of glycerides, phospholipids, and cholesterol after injection of triton into rabbits subjected to functional hepatectomy and sham operation. ---, sham-operated rabbits; ---, hepatectomized rabbits.
compatible with the hypothesis that the liver parenchymal cells take up part of the injected fat, and that this mechanism can be disturbed by triton, whereas the uptake of fat in the reticuloendothelial cells of the spleen is not susceptible to this effect of triton. The small amount of injected fat still found in the liver after administration of triton may be present in the Kupffer cells, which are part of the reticuloendothelial system.

The rapid disappearance of the emulsion from the blood stream in control rabbits may be partly ascribed to the capacity of the liver to take up considerable amounts of the injected fat. The spleen, on the other hand, despite the high concentration of fat found in this organ, never contained a substantial fraction of the injected dose. Unless an extremely rapid turnover of triglycerides exists in the spleen, the uptake of fat particles by this organ may be considered as of secondary importance for the clearance of lipids from the blood.

The sum of the quantities of fat recovered in the plasma, liver, and spleen decreases rapidly. This may be explained by continuous utilization of fat in the liver and spleen; but almost certainly the other organs also take up fat particles. The present results do not exclude the possibility that part of the injected fat particles have left the circulation by intravascular hydrolysis by the clearing factor (lipoprotein lipase). Triton inhibits the clearing factor (15, 16) as well as the uptake of fat by the liver. In a previous study, however, we demonstrated that there is no correlation between the clearing activity of post-heparin plasma *in vivo* or *in vitro*, and the disappearance rate of injected artificial fat emulsions (17).

Exogenous triglycerides were cleared from the plasma of hypercholesterolemic rabbits at the same rate as in control rabbits. Despite the fact that these animals displayed enlarged and fatty livers, the quantities of fat taken up by this organ were also comparable with those of the controls. These findings emphasize the differences in handling of triglycerides and cholesterol.

Part of the foreign triglycerides removed from the plasma can be released again into it, as appears from the increase of plasma lauric and myristic acids following an injection of triton 6 hours after administration of fat emulsion. Since such increase is not seen in hepatectomized rabbits, the liver appears to be the sole source of these fatty acids, as well as of endogenous plasma triglycerides (13).

**SUMMARY**

The uptake of intravenously injected coconut oil emulsion by the liver and spleen was investigated by gas-liquid chromatography in normal, triton-injected, and hypercholesterolemic rabbits. The lauric and myristic acids from this emulsion, almost absent in the tissues of normal animals, were used as marked acids.

In normal rabbits only the liver took up a quantitatively important fraction
of the injected fat. The uptake by the spleen appears to be due to a different mechanism than that of the liver. In rabbits given triton the liver was incapable of taking up large quantities of fat, while the uptake in the spleen was undisturbed. 12 hours after injection the plasma, liver, and spleen of normal animals contained only 14 per cent of the dose. With animals given triton 71 per cent of the injected dose was recovered, most of it in the plasma.

Hypercholesterolemic rabbits cleared an injected fat emulsion and took fat into the liver at the same rate as the controls.

When triton is injected 6 hours after the administration of the emulsion, the fat taken up in the liver reappears in the plasma.

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