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Hepatic uptake of phospholipid-depleted chylomicrons *in vivo*

Comparison with the uptake of chylomicron remnants

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1. Rats pretreated with Triton WR-1339 to prevent the formation of remnants were injected with [^{3}H]cholesterol-labelled remnants, intact chylomicrons or chylomicrons depleted of most of their surface phospholipids by treatment with phospholipase A_2. Within 5 min about 80% of the injected label of remnants and phospholipid-depleted chylomicrons was incorporated into the livers compared with less than 10% of the injected radioactivity of intact chylomicrons. A similar rapid hepatic uptake of radioactivity occurred when rats not pretreated with Triton were injected with [^{3}H]cholesterol-labelled phospholipid-depleted chylomicrons. This rapid hepatic uptake of phospholipid-depleted chylomicrons occurred apparently without any alteration in the apoprotein composition of the particles. 2. The participation of hepatocytes in the uptake of remnants and phospholipid-depleted chylomicrons was examined. Both types of particles were taken up by the hepatocytes. However, small chylomicrons (Sf < 400) were taken up more efficiently than were large chylomicrons (Sf > 400), but neither was taken up as efficiently as the remnants. 3. The results of this study lend support to the hypothesis that phospholipid-depleted chylomicrons and chylomicron remnants are taken up by the liver by a similar mechanism, which depends on the loss of surface phospholipids.

Chylomicron remnants are formed in the vascular space of extrahepatic tissues as a result of the partial degradation of chylomicrons by lipoprotein lipase (Redgrave, 1970). Compared with intact chylomicrons, remnants are smaller particles with a greatly reduced content of triacylglycerol, apoproteins C, A-I and A-IV and phospholipids, but relatively unchanged concentrations of cholesterol and apoproteins B and E (Mjøs *et al.*, 1975; Tall *et al.*, 1979). Studies with intact animals, hepatocyte monolayers and isolated perfused livers have shown that liver cells remove chylomicron remnants, but not intact chylomicrons from circulation at a rapid rate (Redgrave, 1970; Felts *et al.*, 1975; Noel *et al.*, 1975; Gardner & Mayes, 1978; Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Nilsson *et al.*, 1981). The uptake of remnants occurs by a saturable high-affinity process suggesting the existence of receptors on the surface of the liver cells capable of specifically binding these particles (Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Carella & Cooper, 1979). Several reports have indicated that binding of remnants to liver cell receptors is mediated by apoprotein E (Sherrill *et al.*, 1980; Shelburne *et al.*, 1980; Windler *et al.*, 1980) or by the apoprotein B of intestinal origin (Krishnaiah *et al.*, 1980; Elovson *et al.*, 1981; Sparks & Marsh, 1981) present on the surface of the particles. However, apoproteins E and B are also present on the surface of intact chylomicrons, but these lipoproteins are not taken up by the liver cells at a rapid rate. How the liver recognizes apoproteins E and/or B on the surface of remnants, but not on the surface of intact chylomicrons, has been the subject of speculation. We have suggested that in intact chylomicrons binding of the apoproteins to the liver cell receptor is inhibited by the lipoprotein phospholipids (Borensztajn *et al.*, 1980). According to this hypothesis, as the chylomicrons lose phospholipids during their transformation into remnants, the binding apoproteins become more accessible to the receptors. This suggestion was made on the basis of observations showing that removal of phospholipids from the surface of intact chylomicrons by phospholipase A_2 treatment resulted in particles that could be readily taken up by the isolated perfused liver. In the present study, we compared the hepatic

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein; Sf, sedimentation factor (Svedberg units).

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uptake of phospholipid-depleted chylomicrons with that of chylomicron remnants *in vivo*. The results obtained support the hypothesis that the process of hepatic recognition and uptake of chylomicron remnants is dependent on the removal of phospholipids from the chylomicron surface.

Methods

Animals

Male Sprague–Dawley rats (180–250g) were maintained on laboratory chow and kept in alternating 12-h periods of light and darkness. They were starved overnight before use in the experiments. Unless otherwise indicated all rats were given intravenous injections of a 10% (v/v) solution of Triton WR1339 (Ruger Chemical Co., Irvington, NJ, U.S.A.) in 0.9% (w/v) NaCl 20min before injection with lipoproteins.

Chylomicron preparation

The thoracic duct of fed rats was cannulated by the technique of Bollman *et al.* (1948) and the animals were kept in restraining cages with free access to food and water. Radioactive labelling of the chylomicron cholesterol and triacylglycerol fatty acids was accomplished by feeding the animals by stomach tube 2ml of corn oil containing 125μCi of [1,2(n)-^{3}H]cholesterol (sp. radioactivity 50Ci/mol) or 125μCi of [1-^{14}C]palmitic acid (sp. radioactivity 58Ci/mol) (Amersham, Arlington Heights, IL, U.S.A.). The chyle was collected overnight at room temperature in the absence of preservatives. After defibrination with applicators the chyle was incubated with rat serum (9:1, v/v) for 30min at room temperature. Large (Sf >400) and small (Sf <400) chylomicrons were separated from the chyle. This was done by layering 20ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH7.4, over 20ml of chyle/serum mixture in centrifuge tubes and spun in an SW 27 rotor at 23500rev./min for 45min at 16°C in a Beckman model L5-75 preparative ultracentrifuge. Chylomicrons of Sf >400 were harvested from the floating layer. The chylomicrons of Sf <400 were separated from the infranatant solution by centrifuging at 23500rev./min for 19h and the floating layer collected. The harvested fractions were redispersed by passage through a 23-gauge needle and incubated with or without phospholipase A_2 as previously described (Borensztajn *et al.*, 1980) before being injected into rats.

VLD-lipoprotein-free serum was obtained by centrifuging rat serum at 23500rev./min for 19h, as described above, and removing the VLD-lipoprotein-containing floating layer. When indicated control and phospholipid-depleted chylomicrons were incubated with VLD-lipoprotein-free serum (1mg of triacylglycerol/10μl of VLD-lipoprotein-free serum) for 20min at room temperature. The chylomicrons were then concentrated by layering the mixtures under 0.9% (w/v) NaCl and centrifuging for 45min as described above. The floating chylomicrons were harvested, redispersed and chromatographed in columns (1.4cm × 50cm) of 2% agarose (Bio-Gel A-50m, 50–100 mesh; Bio-Rad Laboratories, Richmond, CA, U.S.A.), as previously described (Kotlar & Borensztajn, 1979).

Preparation of remnants

Chylomicron remnants were prepared in rats (300–400g) that had been hepatectomized and eviscerated under diethyl ether anaesthesia essentially as described by Redgrave (1970). After the rats had recovered from the anaesthesia they were injected through a tail vein with [^{3}H]cholesterol-labelled chylomicrons (Sf >400) containing approx. 400μg of cholesterol. The animals were maintained in restraining cages for 1h and during this time were infused with a solution of 5% (w/v) dextrose in water containing porcine insulin (0.04unit/ml). The rate of infusion was 0.5ml/h. At the end of 1h, the animals were bled through the aorta, the blood allowed to clot at room temperature and the serum separated by centrifugation. This remnant-containing serum, which was in all cases free of turbidity, was used for the experiments.

Isolation of hepatocytes

Liver cells were separated as described previously (Borensztajn *et al.*, 1980) and the hepatocytes were isolated by a modification of the method described by Nilsson & Berg (1977). The liver cell mixture was centrifuged in 50ml plastic tubes at 20***g*** for 30s at room temperature. The supernatant was discarded, the cells resuspended in Krebs–Ringer bicarbonate buffer, pH7.4, and the procedure was repeated three more times. The final pellet was resuspended in Krebs–Ringer bicarbonate buffer, pH7.4, and portions were taken for radioactivity determination.

Other procedures

The determination of radioactivity incorporated into the liver or isolated hepatocytes was as previously described (Borensztajn *et al.*, 1980). Chylomicron triacylglycerol was determined by the method of Fletcher (1968) and cholesterol by the method of Abell *et al.* (1951). Electrophoresis of tetramethylurea-soluble apoproteins was performed by the method of Kane (1973) and scanning of the gels was done with a Beckman R-112 densitometer.

Statistical significance of the difference between means was analysed by Student's *t* test.

Results

Hepatic uptake of phospholipid-depleted chylomicrons in vivo

The first experiment was designed to establish whether, as in the perfused rat liver, phospholipid-depleted chylomicrons are taken up by the liver *in vivo* more rapidly than control chylomicrons. Table 1 shows the results of one experiment in which rats were injected intravenously with [^{3}H]cholesterol-labelled control chylomicrons and chylomicrons depleted of 80% of their phospholipids and the radioactivity incorporated into the livers measured at 2 and 5 min after the injection. In the animals given phospholipid-depleted chylomicrons, 73% of the injected dose was recovered in the liver within 2 min of injection, compared with only 16% of the injected dose of control chylomicrons ($P < 0.001$). By 5 min the recovery of the injected radioactivity in the livers was 95% and 28% respectively for the phospholipid-depleted and control chylomicrons. The more rapid uptake of phospholipid-depleted chylomicrons is in agreement with our previous observations using the isolated perfused liver (Borensztajn *et al.*, 1980).

The rapid hepatic uptake of injected phospholipid-depleted chylomicrons occurred apparently without any alteration in the particle's apoprotein composition. This conclusion is based on the observation that incubation of phospholipid-depleted chylomicrons with VLD-lipoprotein-free serum produced no changes in the apoprotein profile of the particles, when compared with control chylomicrons (Fig. 1). In addition, this incubation did not result in the re-acquisition of phospholipids by the phospholipid-depleted particles (results not shown).

From the results shown in Table 1, it was not possible to determine whether or not phospholipid-depleted chylomicrons were degraded to remnants before the hepatic uptake of their cholesterol moiety. Because phospholipid-depleted chylomicrons can serve as substrate for lipoprotein lipase (Scow & Egelrud, 1976), the possibility could not be ruled out that these particles were transformed into remnants more rapidly than the control chylomicrons and that the uptake of the newly formed remnants accounted for the higher radioactivity in the livers. We therefore repeated the experiments shown in Table 1 using rats that had been pretreated with Triton WR-1339. This non-ionic detergent inhibits the lipolytic action of lipoprotein lipase (Schotz *et al.*, 1957; Borensztajn *et al.*, 1976), thus preventing the formation of remnants. The results of this experiment, in which we also compared the hepatic uptake of chylomicron remnants with that of phospholipid-depleted chylomicrons, are shown in Fig. 2. When Triton-treated rats were injected intravenously with [^{3}H]cholesterol-labelled phospholipid-depleted chylomicrons, about 70% of the radioactivity was recovered in the livers within 2 min of injection. In the case of control chylomicrons, on the other hand, not more than 9% of the injected radioactivity was present in the livers 10 min after injection. When Triton-treated rats were injected with serum containing [^{3}H]cholesterol-labelled remnants, the radioactivity recovered in the livers at 2, 5 and 10 min was comparable with that observed in rats injected with phospholipid-depleted chylomicrons (Fig. 2). The rapid hepatic uptake of remnants has been previously reported in experiments with rats not treated with Triton (Redgrave, 1970; Kris-Etherton & Cooper, 1980). Although the [^{3}H]cholesterol-labelled remnants present in the injected serum were not quantified, the uptake of about 70% of the injected radioactivity within 2 min indicates that the amounts administered did not saturate the liver cell receptor sites. Taken together, the results described above (Table 1 and Fig. 2) clearly show that phospholipid-depleted chylomicrons can be taken up by the liver as efficiently as remnants without undergoing partial degradation by lipoprotein lipase.

Fig. 3 shows the results obtained when Triton-treated rats were injected with increasing concentrations of [^{14}C]triacylglycerol-labelled intact or phospholipid-depleted chylomicrons and the radioactivity incorporated into the livers measured 2 min after the injection. It is apparent that the capacity of

Table 1. *Hepatic uptake of control and phospholipid-depleted chylomicrons*

Rats were injected intravenously with 1 ml of [^{3}H]cholesterol-labelled control chylomicrons or chylomicrons that had been depleted of 80% of their surface phospholipids as described in the Methods section. Each injection corresponded to a dose of 38 μg of cholesterol. After the times indicated, the livers were perfused for 1 min with Krebs–Ringer bicarbonate buffer, as described in the Methods section, to wash out chylomicrons that might have been trapped in the sinusoidal spaces. At the end of the perfusion, the livers were weighed and two separate samples taken for the measurement of radioactivity in their lipid extracts as described in the Methods section. For each group three rats were injected and radioactivity determinations were carried out in duplicate. Results are expressed as means ± S.D.

	Proportion of injected radioactivity/liver (%)	
Time (min)	Control chylomicrons	Phospholipid-depleted chylomicrons
2	16.2 ± 1.9	72.9 ± 7.9
5	27.9 ± 5.7	95.0 ± 9.2

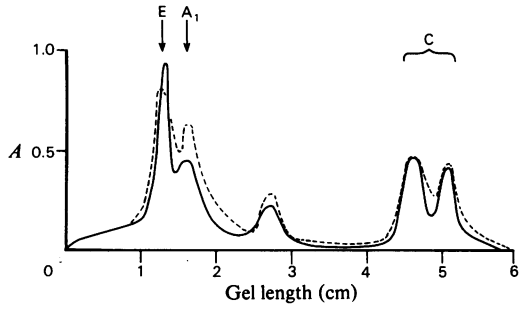

Fig. 1. *Densitometric scanning of control and phospholipid-depleted chylomicron apoproteins separated by tetramethylurea/polyacrylamide-gel electrophoresis*
Control and phospholipid-depleted chylomicrons were incubated with VLD-lipoprotein-free serum and re-isolated as described in the Methods section. The protein equivalent of 5mg of chylomicron triacylglycerol was applied to each gel. Photodensitometric scanning was done at 600nm. ——, Control; ----, phospholipid-depleted. The letters above the curves refer to apoprotein type.

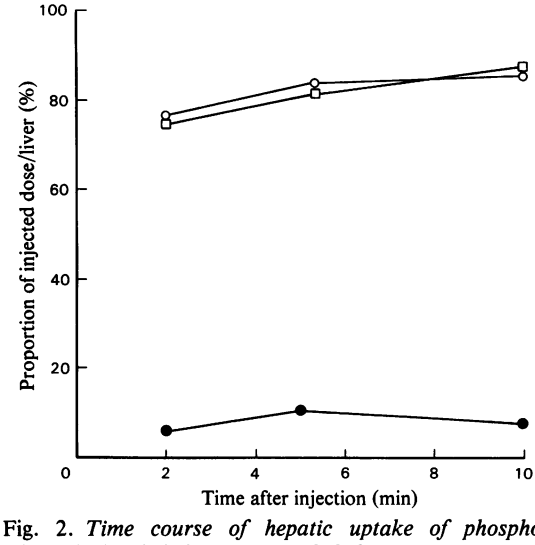

Fig. 2. *Time course of hepatic uptake of phospholipid-depleted chylomicrons and chylomicron remnants*
Rats were injected with 0.7ml of a 10% (v/v) solution of Triton WR1339 and 20min later with [^{3}H]cholesterol-labelled control chylomicrons (●) (50μg of cholesterol), chylomicrons depleted of 80% of their phospholipid (○) (50μg of cholesterol) or serum containing [^{3}H]cholesterol-labelled remnants (□). At the times indicated, the livers were perfused for 1min with Krebs–Ringer bicarbonate buffer as described in the Methods section to wash out chylomicrons that might have been trapped in the sinusoidal spaces. At the end of the perfusion, the livers were weighed and samples taken for the measurement of radioactivity in their lipid extracts as described in the Methods section. Each point represents the average of duplicate determinations from two separate livers.

the livers to take up phospholipid-depleted chylomicrons was not saturated by the amounts injected. For all concentrations used, an average of 70% of the injected radioactivity was taken up by the livers, whereas in the case of control chylomicrons the hepatic uptake was not more than 5% of the injected dose. These results are comparable with those obtained in the experiments using [^{3}H]cholesterol-labelled chylomicrons (Table 1 and Fig. 2).

Cellular uptake of phospholipid-depleted chylomicrons

As shown in the above results, phospholipid-depleted chylomicrons in the amounts injected were taken up by the liver as rapidly as remnants. A possible interpretation of these results is that treatment of chylomicrons by phospholipase resulted in a modification of the lipoproteins such that they were recognized and taken up by Kupffer cells as foreign particles. Accordingly we set out to ascertain if the same liver cell type is responsible for the uptake of remnants and phospholipid-depleted chylomicrons. Specifically, we examined the participation of hepatocytes in this process. For this purpose, Triton-treated rats were injected simul-

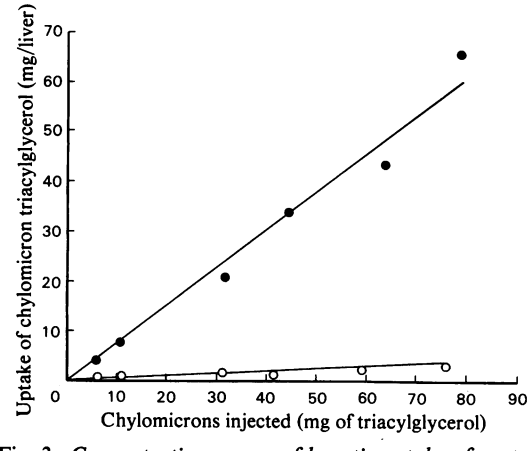

Fig. 3. *Concentration course of hepatic uptake of control and phospholipid-depleted chylomicrons*
Rats were injected intravenously with 0.7ml of a 10% (v/v) solution of Triton WR-1339 and 20min later with 0.8ml of a [^{14}C]triacylglycerol-labelled control chylomicrons (○) and chylomicrons that had been depleted of 80% of the phospholipids (●) at increasing concentrations. After 2min the livers were perfused for 1min to wash out particles that might have been entrapped in the sinusoidal spaces, weighed and samples taken for the measurement of radioactivity in their lipid extracts as described in the Methods section. Each point represents the average of duplicate determinations from two separate livers.

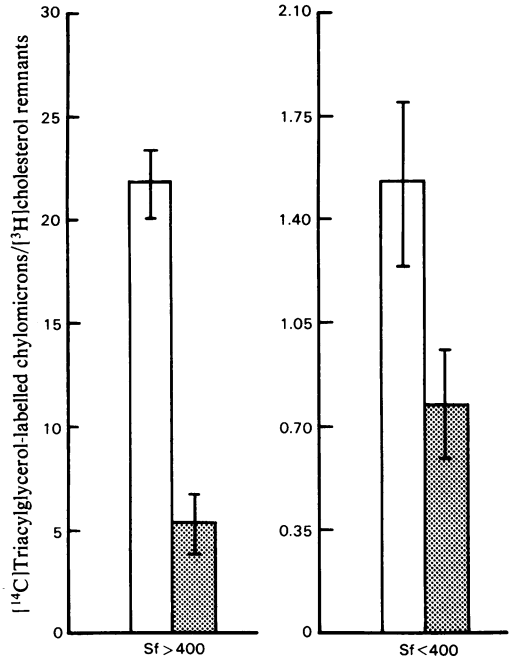

Fig. 4. *Liver uptake of phospholipid-depleted chylomicrons and chylomicron remnants: comparison between whole tissue and hepatocytes*

Rats were injected with 0.7ml of a 10% Triton solution and 20min later with 1ml of rat serum containing [^{3}H]cholesterol-labelled remnants to which ^{14}C-labelled phospholipid-depleted chylomicrons (50–60μg of cholesterol) of Sf >400 or Sf <400 were added. At 5min after injection, the livers were perfused for 1min with Krebs–Ringer bicarbonate buffer to wash out particles that might have been entrapped in the sinusoids and then perfused for 15min with collagenase to isolate the hepatocytes as described in the Methods section. For each group three rats were injected and two separate samples of the liver (open columns) and duplicate portions of the isolated hepatocytes (shaded columns) were taken for determination of the double-labelled radioactivity in their lipid extracts as described in the Methods section. The results are expressed as means ± S.D. (indicated by the bars).

taneously with [^{14}C]triacylglycerol-labelled phospholipid-depleted chylomicrons and [^{3}H]cholesterol-labelled remnants, and the ^{14}C/^{3}H ratio recovered in the whole liver compared with that of isolated hepatocytes. Because remnants are smaller than chylomicrons their ability to penetrate the spaces of Disse to gain access to the hepatocytes is presumably greater (Wisse & Knooke, 1979). In this experiment, therefore, the effect of particle size on hepatocyte uptake had to be considered. Accordingly, we used [^{14}C]triacylglycerol-labelled large chylomicrons (Sf >400), as in the previous experiments, as well as small chylomicrons (Sf <400). The results obtained are shown in Fig. 4. For chylomicrons of Sf >400, the ^{14}C/^{3}H ratio in the hepatocytes was 24.3 ± 6.7% of this ratio in the whole liver, whereas for chylomicrons of Sf <400, the ^{14}C/^{3}H ratio in the hepatocytes was 50.1 ± 7.7% of this ratio in whole liver ($n = 6$, $P < 0.01$). These results clearly show that smaller chylomicrons were taken up by the hepatocytes more efficiently than the larger chylomicrons but that neither population of particles was taken up by these cells to the same extent as remnants.

Discussion

The results of the present study carried out *in vivo* confirm our previous observations with the isolated perfused liver (Borensztajn *et al.*, 1980) that chylomicrons depleted of their surface phospholipids are readily removed from circulation by the liver. The results of this study also support the hypothesis that the mechanism of hepatic uptake of phospholipid-depleted chylomicrons is similar to that of chylomicron remnants. First, the extent of hepatic uptake of phospholipid-depleted chylomicrons and chylomicron remnants within 2min was essentially identical (Fig. 2), and second phospholipid-depleted chylomicrons, like chylomicron remnants, were recognized and internalized by the liver parenchymal cells (Fig. 4). The finding that phospholipid-depleted chylomicrons were taken up by hepatocytes argues against the possibility that they were treated as foreign bodies. The observation that remnants were taken up by hepatocytes more efficiently than phospholipid-depleted chylomicrons may be explained by the differences in the size of the particles. In order for a particle to be taken up by the hepatocyte it must first penetrate the spaces of Disse through endothelial fenestrae. Only particles of less than 0.1μm in diameter can readily pass through these fenestrae (Wisse & Knooke, 1979). Although we did not measure the particles used in the present study, in previous work (Borensztajn *et al.*, 1980) this was done and we found that only a small proportion of the chylomicrons of Sf >400 were sufficiently small to freely pass the liver endothelial fenestrae. The small chylomicrons (Sf <400) used in the present study presumably had a higher proportion of particles with a diameter less than 0.1μm, and in fact they were taken up by the hepatocytes to a significantly greater extent than the large chylomicrons (Fig. 4). The remnants produced in eviscerated rats almost certainly had an even greater number of particles smaller than 0.1μm in diameter (Redgrave & Small, 1979), explaining their more efficient uptake by the hepatocytes.

Electron micrographs from livers of rats injected with large phospholipid-depleted chylomicrons showed that Kupffer cells were actively involved in the uptake of the particles (J. Borensztajn & T. J. Kotlar, unpublished work). Kupffer cells have been suggested to play an important role in the removal of remnants from circulation (Van Tol & Van Berkel, 1980). The present results indicate that the extent to which Kupffer cells participate in this process may depend on the size of the remnant particle. The finding that small phospholipid-depleted chylomicrons were taken up by the hepatocytes more efficiently than the large chylomicrons (Fig. 4) suggests that only the particles that do not penetrate the spaces of Disse are taken up by the Kupffer cells. In that respect it would be of interest to investigate the cellular distribution of small and large remnants taken up by the liver.

On the basis of experiments with intact chylomicrons and triacylglycerol emulsions, Shelburne *et al.* (1980) and Windler *et al.* (1980) suggested that liver cells distinguish chylomicrons from chylomicron remnants because of differences in the composition of their surface apoproteins. According to these authors the apoprotein C/E ratio on the surface of the lipoprotein determines whether a particle is recognized by the liver cell. Particles with a high apoprotein C/E ratio (e.g. large chylomicrons) would not be recognized by the liver, whereas particles with a low apoprotein C/E ratio (e.g. remnants) would be readily recognized. It is noteworthy, however, that the removal of most of the phospholipids from the surface of chylomicrons by phospholipase A_2 treatment causes no changes in the apoprotein composition of the particles (Eisenberg, 1977; Borensztajn *et al.*, 1980). Even after exposure to serum, the apoprotein C/E ratio of the phospholipid-depleted chylomicrons is not different from that of control particles (Fig. 1). Yet, when perfused through isolated livers (Borensztajn *et al.*, 1980) or, as in the present study, injected intravenously, these particles are readily recognized and taken up by the liver. We did not examine whether changes occurred in the apoprotein composition of the phospholipid-depleted chylomicrons exposed to serum of Triton-treated rats. Ishikawa & Fidge (1979) reported that after Triton administration to rats there is a rapid change in the apoprotein composition of various plasma lipoproteins. The apoprotein C/E ratio of VLD lipoproteins, for example, is markedly elevated within a few minutes of Triton injection. It is conceivable that some alterations occurred in the apoprotein composition of the phospholipid-depleted chylomicrons injected into Triton-treated rats. If this was the case, however, such changes did not inhibit the uptake of these particles by the liver.

From the results of the present study carried out *in vivo* and from our previous studies with the isolated perfused liver (Borensztajn *et al.*, 1980), it is clear that the loss of phospholipids alone from the surface of the chylomicrons results in particles that, like remnants, can be readily taken up by the liver. Because one of the major alterations that occurs during the transformation of chylomicrons into remnants is the loss of surface phospholipids (Tall *et al.*, 1979) we have suggested that the hepatic uptake of these two types of particles occurs by a similar mechanism. The nature of this mechanism can presently only be speculated on. The phospholipid composition on the surface of the chylomicrons treated with phospholipase A_2, and presumably on the surface of remnants, is quite different from that of the intact particle (Borensztajn *et al.*, 1980). Such an alteration in the phospholipid composition might be accompanied by rearrangement of the apoproteins on the surface of the particles making them (e.g. apoprotein E) more easily accessible for binding to the liver cell receptors.

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References

Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1951) *J. Biol. Chem.* **195**, 357–366

Bollman, J. L., Cain, J. C. & Grindlay, J. H. (1948) *J. Lab. Clin. Med.* **33**, 1349–1352

Borensztajn, J., Rone, M. S. & Kotlar, T. J. (1976) *Biochem. J.* **156**, 539–543

Borensztajn, J., Kotlar, T. J. & McNeill, B. J. (1980) *Biochem. J.* **192**, 845–851

Carrella, M. & Cooper, A. D. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 338–342

Cooper, A. D. & Yu, P. Y. S. (1978) *J. Lipid Res.* **19**, 635–643

Eisenberg, S. (1977) *Biochim. Biophys. Acta* **489**, 337–342

Elovson, J., Huang, Y. O., Baker, N. & Kannan, R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 157–161

Felts, J. M., Itakura, H. & Crane, R. T. (1975) *Biochem. Biophys. Res. Commun.* **66**, 1467–1475

Fletcher, M. J. (1968) *Clin. Chim. Acta* **22**, 393–397

Gardner, R. S. & Mayes, P. A. (1978) *Biochem. J.* **170**, 47–55

Ishikawa, T. & Fidge, N. (1979) *J. Lipid Res.* **20**, 254–264

Kane, J. P. (1973) *Anal. Biochem.* **53**, 350–364

Kotlar, T. J. & Borensztajn, J. (1979) *Biochem. J.* **183**, 171–174

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36

Kris-Etherton, P. M. & Cooper, A. D. (1980) *J. Lipid Res.* **21**, 435–442

Krishnaiah, K. V., Walker, L. F., Borensztajn, J., Schonfeld, G. & Getz, G. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3806–3810

Mjøs, O. D., Faergeman, O., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 603–615
Nilsson, A., Ehnholm, C. & Floren, C. H. (1981) *Biochim. Biophys. Acta* **663**, 408–420
Nilsson, M. & Berg, T. (1977) *Biochim. Biophys. Acta* **497**, 171–182
Noel, S. P., Dolphin, P. J. & Rubinstein, D. (1975) *Biochem. Biophys. Res. Commun.* **63**, 764–772
Redgrave, T. G. (1970) *J. Clin. Invest.* **49**, 465–471
Redgrave, T. G. & Small, D. M. (1979) *J. Clin. Invest.* **64**, 162–171
Schotz, M. C., Scanu, A. & Page, I. H. (1957) *Am. J. Physiol.* **188**, 399–402
Scow, R. O. & Egelrud, T. (1976) *Biochim. Biophys. Acta* **431**, 538–549
Shelburne, F., Hanks, J., Meyers, W. & Quarfordt, S. (1980) *J. Clin. Invest.* **65**, 652–658
Sherrill, B. C. & Dietschy, J. M. (1978) *J. Biol. Chem.* **253**, 1859–1867
Sherrill, B. C., Innerarity, T. L. & Mahley, R. M. (1980) *J. Biol. Chem.* **255**, 1804–1807
Sparks, C. E. & Marsh, J. B. (1981) *J. Lipid Res.* **22**, 519–527
Tall, A. R., Green, P. H. R., Glickman, R. M. & Riley, J. W. (1979) *J. Clin. Invest.* **64**, 977–989
Van Tol, A. & Van Berkel, J. C. (1980) *Biochim. Biophys. Acta* **619**, 156–166
Windler, E., Chao, Y. & Havel, R. J. (1980) *J. Biol. Chem.* **255**, 8303–8307
Wisse, E. & Knooke, D. L. (1979) in *Progress in Liver Disease* (Popper, H. & Schaffner, F., eds.), vol. 6, pp. 153–171, Grune and Stratton, New York