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Uptake of phospholipid-depleted chylomicrons by the perfused rat liver

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1. Rat lymph chylomicrons were depleted of their surface phospholipids by treatment with pure phospholipase A_2 from *Crotalus adamanteus* venom. 2. About 80% of the phospholipids could be removed from the chylomicrons without any apparent effect on their size, neutral lipid composition or qualitative profile of their tetramethylurea-soluble apoproteins. 3. Phospholipid-depleted chylomicrons were rapidly taken up whole by liver cells when perfused through isolated rat liver preparations. The rate of uptake was dependent on the extent of phospholipid depletion and reached a maximum (4–6.5-fold greater than control chylomicrons) when 80% of the phospholipids had been removed. 4. It is speculated that the hepatic uptake of phospholipid-depleted chylomicrons occurs by a mechanism similar to that of chylomicron-remnants uptake.

Chylomicrons are lipoproteins formed in the intestine and secreted into the plasma as spherical particles made up of a core of triacylglycerol and cholesteryl esters surrounded by a surface layer of proteins, phospholipids and unesterified cholesterol (Smith *et al.*, 1978). In the plasma, chylomicrons have a relatively short half-life. They are removed from circulation in two distinct phases: (*a*) extrahepatic; (*b*) hepatic. In the extrahepatic phase, the triacylglycerol in the core of the chylomicrons, and, to a lesser extent, some of the surface phospholipids, are hydrolysed by lipoprotein lipase located on the endothelial surface of the capillaries of adipose tissue, skeletal muscle and heart (Borensztajn, 1979). As a result of lipoprotein lipase action, the chylomicrons are decreased in size and lose some of the surface phospholipids and apoproteins, which are transferred to other plasma lipoproteins (Mjøs *et al.*, 1975; Tall *et al.*, 1979). The resulting particles, the chylomicron remnants, which are relatively enriched in cholesterol, are then transported to the liver where they are taken up as a unit, apparently both by parenchymal and non-parenchymal cells (Redgrave, 1970; Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Van Berkel & Van Tol, 1979).

Studies with intact animals, isolated hepatocytes and with the isolated perfused liver have shown that chylomicron remnants, but not intact chylomicrons, are readily taken up by the liver cells (Redgrave, 1970; Felts *et al.*, 1975; Noel *et al.*, 1975; Floren & Nilsson, 1977; Gardner & Mayes, 1978; Cooper & Yu, 1978; Sherrill & Dietschy, 1978). It has been suggested that the uptake of remnants requires the binding of these particles to specific receptors on the surface of the liver cell (Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Carrella & Cooper, 1979). However, the nature of the binding components on the remnant surface and how they become accessible to the putative receptor have not yet been elucidated. Two general possibilities can be considered. (*a*) In the course of their degradation by lipoprotein lipase, chylomicrons acquire surface components that could then bind to the liver cell receptor. (*b*) Chylomicrons possess the binding components, which are, however, inaccessible to the liver cell receptors. As a lipoprotein is degraded by lipoprotein lipase, the loss of some surface apoproteins and/or phospholipids results in the surface binding components becoming accessible to the receptor. If this latter hypothesis is correct, the selective removal of apoproteins and/or phospholipids from the chylomicron surface, without degradation of the triacylglycerol core of the particle by lipoprotein lipase, might 'expose' the components that bind to the liver cell receptor. In this paper we report results of experiments that are consistent with this interpretation. Chylomicrons that were depleted of surface phospholipids by phospholipase A_2 and that retained their original size as well as their apoprotein and neutral lipid composition could be readily taken up by the isolated rat liver preparation.

Materials and methods

Animals

Male Sprague–Dawley rats (180–250g) were maintained on laboratory chow and kept in alternat-

ing 12-h periods of light and darkness. They were starved overnight before use in the perfusion experiments.

Chylomicron preparation

The thoracic ducts of fed rats were 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, triacylglycerol fatty acids and phospholipid moieties 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), 125μCi of [1-^{14}C]palmitic acid (sp. radioactivity 58Ci/mol) or 500μCi of [^{32}P]P_i (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. Chylomicrons were then washed by layering 20ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH7.4, over 20ml of the chyle/serum mixture in centrifuge tubes and spun in an SW27 rotor at 23500rev./min for 45min at 16°C in a Beckman model L5-75 preparative ultracentrifuge. The floating chylomicron layer was harvested and re-dispersed by passage through a 21 gauge needle and incubated with phospholipase A_2.

Phospholipase treatment

Homogeneous phospholipase A_2 (α form) was purified from *Crotalus adamanteus* venom by the method of Wells & Hanahan (1969). The purified enzyme was a generous gift from Dr. Ferenc Kezdy, Department of Biochemistry, University of Chicago, U.S.A. The incubation medium included, in addition to chylomicrons, phospholipase A_2 in 0.1M-Tris/HCl buffer, pH8.1 (0.48μg of protein/mg of chylomicron phospholipid), and bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) defatted by the method of Chen (1967) in 0.1M-Tris/HCl buffer, pH8.1, to a final concentration of 1.2mol of albumin/mol of phosphatidylcholine. The mixture was incubated at 37°C in a shaking water bath. At different time intervals the enzyme reaction was stopped by the addition of EGTA (Sigma Chemical Co.) in 0.1M-Tris/HCl buffer, pH8.1, to a final concentration of 0.01M. For the control chylomicron preparation EGTA was added before the enzyme. After enzyme treatment, the chylomicrons were concentrated by layering the assay mixture under 0.9% (w/v) NaCl and centrifuging as described above. The floating chylomicrons were harvested, re-dispersed as described above and chromatographed in columns (1.4cm × 50cm) of 2% Agarose (Bio-Gel A-50m, 50–100mesh; Bio-Rad Laboratories, Richmond, VA, U.S.A.) as previously described (Kotlar & Borensztajn, 1979).

Liver perfusion

The technique for the liver perfusion was essentially that described by Hems *et al.* (1966), except the medium was not re-circulated. The basic perfusion medium was Krebs–Ringer bicarbonate buffer, pH7.4, continuously gassed with O_2/CO_2 (19:1, v/v) containing 3% bovine serum albumin (Sigma Chemical Co.) and 1mg of glucose/ml. Chylomicrons were added to this medium as indicated. Livers were first perfused for 3min with the basic medium to remove blood from the preparation and then with the medium containing chylomicrons for various time intervals as indicated. This was followed by a 2-min perfusion with the basic medium to wash out chylomicrons that might have been trapped in the sinusoidal spaces. After the washing, 1g samples of the liver were taken for analysis. All livers were perfused at a flow rate of 14ml/min.

Preparation of isolated liver cells

When indicated, the washing period was followed by perfusion of the liver with 100ml of calcium-free Krebs–Ringer bicarbonate buffer containing 100mg of glucose and 50mg of collagenase (Worthington Biochemical Corp., Freehold, NJ, U.S.A.). This medium was re-circulated through the liver at 37°C for 15min. At the end of this period, the liver cells were combed off the stroma and re-suspended in Krebs–Ringer bicarbonate buffer, pH7.4, at room temperature. The cell suspension was sequentially filtered through layers of coarse and then fine nylon mesh. The filtered cell suspension was transferred to a 50ml plastic centrifuge tube and centrifuged at 20*g* for 2min at room temperature. The supernatant was discarded, the cells re-suspended in the buffer and the washing was repeated twice more. The final pellet was re-suspended in the buffer and portions were taken for cell counts and radioactivity determination. Cells were counted in a Neubauer haemocytometer.

Liver extraction

For the determination of radioactivity incorporated into the perfused liver, 1g samples of tissue were homogenized in 10ml of methanol with a Duall ground-glass grinder (Kontes Glass Co., Chicago, IL, U.S.A.). Chloroform (20ml) was added and after shaking the mixture was allowed to stand overnight at room temperature. This extract was filtered through filter paper (Whatman no. 1) and portions of the filtrate were dried, re-dissolved in scintillation fluid (PCS; Amersham Corp., Arlington Heights, IL, U.S.A.) and radioactivity was measured in a Beckman LS-9000 scintillation counter (Beckman

Instruments Inc., Palo Alto, CA, U.S.A.). For the determination of radioactivity incorporated into isolated liver cells, portions of the cell suspension were centrifuged for 5min at 20*g* at room temperature, and the pellet was homogenized, extracted and counted for radioactivity as described above.

T.l.c.

Chylomicron lipids were extracted and washed by the procedure of Folch *et al.* (1957). Separation of the phospholipids was carried out in precoated silica-gel G thin-layer plates (250μm) (Analtech Inc., Newark, DE, U.S.A.), with chloroform/methanol (97:3, v/v) followed by chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) as solvent systems (Skipski *et al.*, 1964). The spots were detected by I_2 vapour, scraped off the plate into scintillation vials containing scintillation fluid and counted for radioactivity as described above. The recovery of the label from the plates was greater than 97%.

Microscopy

Chylomicrons were negatively stained with a 2% solution of phosphotungstic acid, detected in a Hitachi HU-12 electron microscope (Hitachi, Tokyo, Japan), photographed and enlarged to 64000 magnifications. The diameter of the particles was then measured directly.

Other procedures

Electrophoresis of chylomicron tetramethylurea-soluble apoproteins was as described by Kane (1973). Triacylglycerol was determined by the method of Fletcher (1968), phospholipid phosphorus by the method of Bartlett (1959) and cholesterol by the method of Abell *et al.* (1951). Statistical significance of the difference between means was analysed by Student's *t* test.

Results

Hydrolysis of chylomicron phospholipids by phospholipase A_2

Fig. 1 illustrates an experiment in which chylomicrons that had been labelled with ^{32}P in their phospholipid moiety were incubated with phospholipase A_2 for increasing time periods. Under the conditions used, there was a rapid removal of ^{32}P phospholipids from the chylomicrons with maximal depletion (80%) observed within 60min. Most of this phospholipid depletion was accounted for by the hydrolysis and removal of phosphatidylcholine and to a lesser extent phosphatidylethanolamine from the chylomicron surface. The depletion of phosphatidylserine from the chylomicrons accounted for the loss of only 3% of the total phospholipid radioactivity. The radioactivity of the sphingomyelin fraction could not be meaningfully measured. Of the lysophospholipids formed as a result of phospholipase A_2 action and retained on the chylomicron surface, only ^{32}P-labelled lysophosphatidylcholine was measured. In the intact particle, this phospholipid accounted for 0.6% of the total radioactivity, but at the end of 120min of incubation, it represented 6.4% of the total initial radioactivity. The extent of removal of the phospholipids from the chylomicron surface was dependent on the ratio albumin/phospholipid in the incubation medium. At ratios below those used in the present study (1.2mol

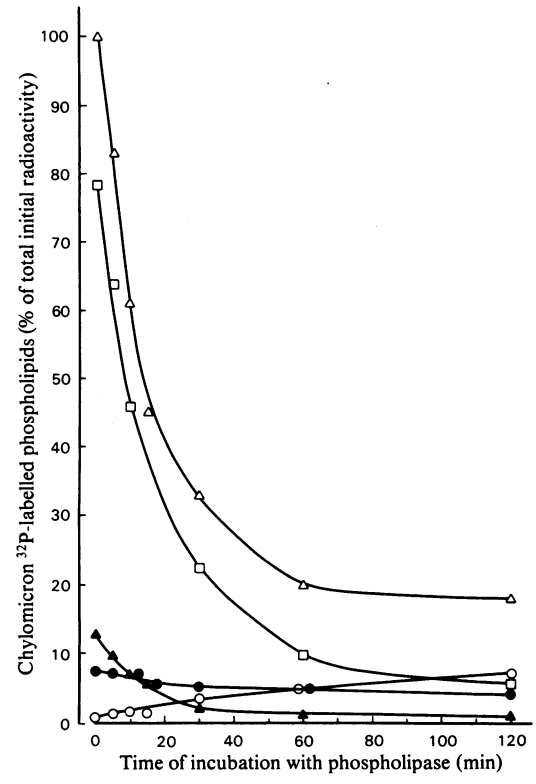

Fig. 1. *Effect of phospholipase A_2 on chylomicron phospholipids*

Chylomicrons labeled with ^{32}P in their phospholipid moiety were incubated with phospholipase A_2 as described in the Materials and methods section. At the time intervals indicated, portions were taken for lipid extraction, phospholipids were separated and identified by t.l.c. and their radioactivity was measured as described in the Materials and methods section. Each point represents the average of duplicate extractions. The results are expressed as a percentage of the total initial radioactivity. Symbols: △, total phospholipids; □, phosphatidylcholine; ○, lysophosphatidylcholine; ▲, phosphatidylethanolamine; ●, phosphatidylserine.

of albumin/mol of phosphatidylcholine) hydrolysis of the various phospholipids still occurred, but most of the lysophospholipids formed remained bound to the chylomicrons (T. J. Kotlar & J. Borensztajn, unpublished work).

Chylomicrons that were depleted of about 80% of their surface phospholipids maintained their spherical shape. No major differences could be detected in the size distribution of untreated and phospholipase-treated chylomicrons (Fig. 2), nor were there any changes in triacylglycerol and cholesterol concentrations (results not shown). The apoprotein pattern of the two preparations obtained by tetramethylurea/polyacrylamide-gel electrophoresis is shown in Fig. 3. It is apparent that the overall apoprotein composition was not altered after the phospholipid depletion.

Uptake of phospholipid-depleted chylomicrons by the perfused liver

Fig. 4 illustrates the results of an experiment in which livers were perfused with [^{3}H]cholesterol-labelled intact chylomicrons or chylomicrons that had been depleted of 80% of their phospholipids by phospholipase A_2 at identical cholesterol concentrations. The uptake of ^{3}H-labelled cholesterol by the livers perfused with both types of particles was linear for at least 20 min of perfusion. It is apparent, however, that the rate of cholesterol uptake was considerably greater (6–8-fold) in the livers perfused with phospholipid-depleted chylomicrons. To rule out the possibility that the results described above were due to selective uptake of the chylomicron cholesterol moiety, we perfused livers with control and phospholipase-treated chylomicrons that had been double-labelled with [^{3}H]cholesterol and [^{14}C]palmitic acid. In these chylomicron pre-

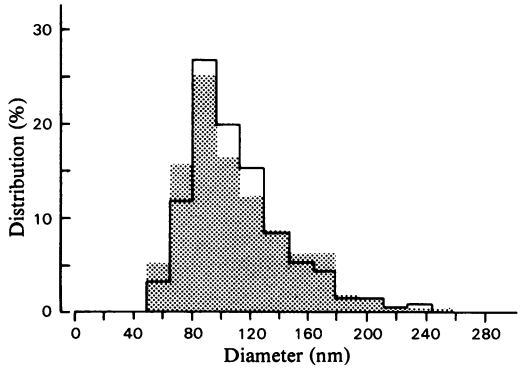

Fig. 2. *Size distribution of chylomicrons*
Control chylomicrons (light area) and chylomicrons that had been depleted of 80% of their phospholipids (dark area) were negatively stained with 2% phosphotungstic acid, photographed and their diameters were measured as described in the Materials and methods section; 450 random particles were measured in each group.

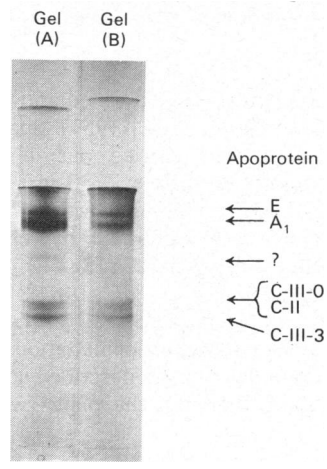

Fig. 3. *Tetramethylurea/polyacrylamide-gel electrophoresis of chylomicron apoproteins*
Control chylomicrons (gel A) and chylomicrons depleted of 80% of their phospholipids (gel B) were delipidated and the material obtained was applied to the gels. The material applied to gels (A) and (B) corresponded to the delipidation of respectively 10 and 5 mg of chylomicrons, as determined from the triacylglycerol concentration.

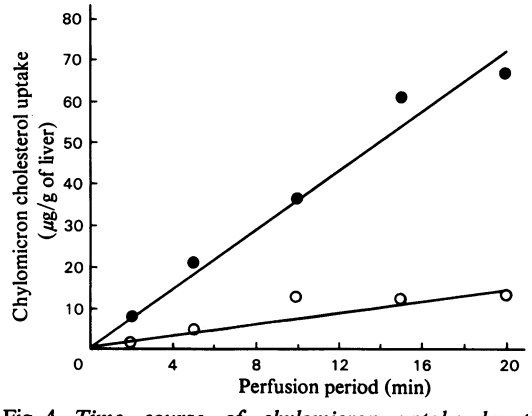

Fig. 4. *Time course of chylomicron uptake by the perfused liver*
Livers were perfused with [^{3}H]cholesterol-labelled control chylomicrons (○) and chylomicrons depleted of 80% of their phospholipids (●) at a concentration of 4.3 μg of cholesterol/ml of perfusate. At the times indicated, samples of the perfused livers were taken for the determination of the radioactivity in their lipid extracts as described in the Materials and methods section. Each point represents the average of duplicate determination from two separate livers.

parations over 90% of the ^{14}C label was in the triacylglycerol moiety. Three livers were perfused for 5 min each with intact chylomicrons and three livers for 5 min each with chylomicrons that had been depleted of 80% of their phospholipids. For both groups, the perfusion concentration was 5.8 μg of cholesterol/ml of perfusate. After perfusion, lipid extractions of liver samples were performed in duplicate and radioactivity was measured as described in the Materials and methods section. In both groups the $^{3}H/^{14}C$ ratio in the livers was found to be similar to that of the perfusate. When the ratio of [^{3}H]cholesterol to ^{14}C-labelled fatty acids in the perfusate was divided by the ratio of [^{3}H]cholesterol to ^{14}C-labelled fatty acid in the livers, the following results were obtained: for intact chylomicrons, 0.89 ± 0.07 (mean $\pm$ S.D.); for phospholipid-depleted chylomicrons, 1.14 ± 0.04 (mean $\pm$ S.D.) ($n = 6$). The nearness of these ratios to 1.0 strongly suggests that the chylomicrons were taken up whole by the livers. To rule out the possibility that these results could be accounted for by the entrapment of phospholipid-depleted chylomicrons in the sinusoidal spaces of the perfused liver, we measured the radioactivity incorporated into cells isolated from livers that had been perfused with ^{14}C-labelled chylomicron triacylglycerol fatty acids. Fig. 5 shows the results of this experiment. The effect of phospholipase treatment on uptake of labelled chylomicrons was similar for isolated cells and homogenized samples of whole liver.

Fig. 6 shows the results obtained when livers were perfused with an increasing concentration of [^{3}H]cholesterol-labelled intact chylomicrons and chylomicrons that had been depleted of 80% of their phospholipids. The rate of uptake of these latter particles increased rapidly as a function of the concentration in the perfusate and seemed to reach a plateau when the perfusate concentration was 14 μg of chylomicron cholesterol/ml. The uptake of intact chylomicrons occurred at much lower rates and appeared to reach saturation at lower concentrations of chylomicron cholesterol in the perfusate. In Fig. 7 are shown results of an experiment in which the livers were perfused with chylomicrons treated with phospholipase A_2 for increasing time periods. The progressive phospholipid depletion agrees well with the results obtained with ^{32}P-labelled chylomicrons (Fig. 1), i.e. 40% loss within 5 min, 80% within 30–60 min and no further losses thereafter. In agreement with the results shown in Figs. 4, 5 and 6, and those reported by others (Noel *et al.*, 1975; Felts *et al.*, 1975; Cooper & Yu, 1978; Sherrill & Dietschy, 1978; Gardner & Mayes, 1978), intact chylomicrons were taken up by the liver at low rates (3 μg of cholesterol/g of liver per 5 min of perfusion). The progressive loss of phospho-

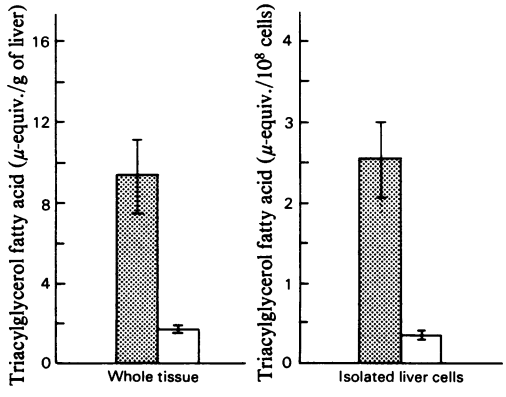

Fig. 5. *Uptake of chylomicrons by the perfused liver: comparison between whole tissue and isolated cells*

Livers were perfused with ^{14}C-labelled control chylomicrons (light areas), and chylomicrons depleted of 80% of the phospholipids (dark areas) for 5 min at a concentration of 5.8 μg of cholesterol/ml. At the end of the perfusion, radioactivity was measured in lipid extracts of samples of the whole liver or from cells isolated by collagenase treatment as described in the Materials and methods section. For each group three livers were perfused and radioactivity determinations were carried out in duplicate. The results are expressed as means $\pm$S.D.

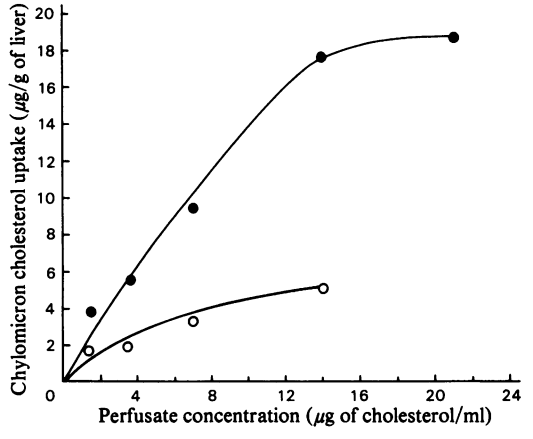

Fig. 6. *Concentration course of chylomicron uptake by the perfused liver*

^{3}H-labelled control chylomicrons (○) and chylomicrons depleted on 80% of their phospholipids (●) were perfused through livers at increasing concentrations for 5 min. At the end of the perfusion, samples of the livers were taken for the measurement of radioactivity in their lipid extracts as described in the Materials and methods section. Each point represents the average of duplicate determinations from two separate livers.

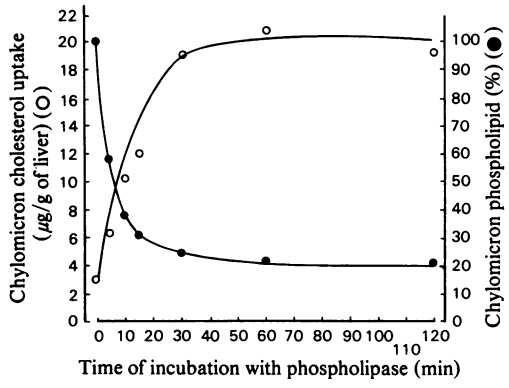

Fig. 7. *Effect of phospholipid depletion on the uptake of chylomicrons by the perfused liver*

[^{3}H]Cholesterol-labelled chylomicrons were incubated with phospholipase A_2 as described in the Materials and methods section. At the time intervals indicated, the enzyme reaction was stopped and portions were taken for phospholipid phosphorus determination and for liver perfusion. At each point, two livers were perfused for 5 min with a medium containing 5.7 μg of chylomicron cholesterol/ml. At the end of the perfusion the radioactivity incorporated in their lipid extracts was measured as described in the Materials and methods section. Each point represents the average of determinations carried out in duplicate.

lipids from the particles was accompanied by a gradual increase in the rate of uptake by the perfused liver. When the chylomicrons were depleted of 80% of their phospholipids, the uptake was 20 μg of cholesterol/g of liver per 5 min of perfusion.

Discussion

Phospholipase A_2 has been used as an important tool in the study of the surface structure of plasma lipoproteins. Pattnaik *et al.* (1976) and Aggerbeck *et al.* (1976) showed that the phospholipids of human serum low-density lipoprotein and high-density lipoprotein are accessible to, and readily hydrolysed by, phospholipase A_2. Further, they showed that most of the product of the enzyme hydrolysis, i.e. unesterified fatty acids and lysophospholipids, could be removed by albumin from the lipoprotein surface without any remarkable alteration in the structure of the particle. Thus, in spite of extensive losses of phospholipids from their surface, the lipoproteins did not collapse or coalesce, but retained their overall size and composition. Indirect evidence that chylomicrons can also lose surface phospholipids without having their integrity destroyed has been provided by Scow & Egelrud (1976). They reported that although 90% of phosphatidylcholine, the major chylomicron phospholipid, could be hydrolysed by phospholipase A_2, the particles could be concentrated by flotation in the ultracentrifuge in a manner similar to untreated chylomicrons. In the present paper, we confirmed and extended the observations by Scow & Egelrud (1976). The hydrolysis and removal of 80% of rat lymph chylomicron phospholipid by phospholipase A_2 did not affect the size (Fig. 2), qualitative apoprotein profile (Fig. 3) or neutral lipid composition of the lipoprotein. Taken together, these studies show that the structural integrity of chylomicrons, like low-density lipoproteins and high-density lipoproteins, is not dependent on the presence of phosphatidylcholine on their surface. After phospholipase treatment, the residual surface phospholipids or a combination of phospholipids and unesterified fatty acids are apparently sufficient to maintain the structure of the lipoprotein (Pattnaik *et al.*, 1976).

The results of the present investigation demonstrate that the depletion of phospholipids from the surface of chylomicrons yields particles that can be readily taken up by liver cells. The mechanism by which this uptake occurs remains to be determined. One possibility is that removal of phospholipids by phospholipase A_2 alters the surface charge of the chylomicrons leading to their non-specific uptake by Kupffer cells. Enhanced uptake of plasma low-density lipoproteins by Kupffer cells has been shown to occur when the negative charge of these particles is increased (Mahley *et al.*, 1979).

Alternatively, it can be speculated that the mechanism of hepatic uptake of phospholipid-depleted chylomicrons is similar to that of chylomicron remnants. This latter mechanism is generally assumed to involve the binding of one or more remnant-surface apoproteins to specific liver cell receptors. How the liver distinguishes remnant apoprotein (or, as in the present investigation, apoproteins of phospholipid-depleted chylomicrons) from those of intact chylomicrons remains to be explained. A possible explanation is that the binding apoprotein(s), although present on the intact chylomicron surface, are not accessible to the receptor. It is tempting to speculate that the removal of phospholipid from the chylomicron surface, during its transformation to a remnant or by the action of phospholipase A_2, as in the present study, increases the accessibility of these proteins to the receptors. One might envisage, for example, that the removal of phospholipids from the lipoprotein surface causes clustering of the apoproteins or changes in their conformation that would facilitate their binding.

Chylomicron remnants are not readily available and whether produced *in vivo* or *in vitro*, constitute a mixed population of lipoprotein particles at various stages of degradation. In contrast, phospholipid-

depleted chylomicrons can be harvested in large amounts and separated by particle size into homogeneous populations of known lipid and apoprotein composition. If our overall hypothesis is correct, i.e. that the mechanism of hepatic uptake of remnants is the same as that of phospholipid-depleted chylomicrons, the latter particles may be advantageously used to study the process of hepatic remnant recognition.

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References

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

Aggerbeck, L. P., Kezdy, F. J. & Scanu, A. M. (1976) *J. Biol. Chem.* **251**, 3823–3830

Bartlett, G. R. (1959) *J. Biol. Chem.* **243**, 466–468

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

Borensztajn, J. (1979) in *The Biochemistry of Atherosclerosis* (Scanu, A. M., Wissler, R. W. & Getz, G. S., eds.), pp. 231–245, Marcel Dekker, New York and Basel

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

Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181

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

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

Floren, C.-H. & Nilsson, A. (1977) *Biochem. J.* **168**, 483–494

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509

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

Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284–292

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

Mahley, R. W., Weisgraber, K. H., Innerarity, T. L. & Windmueller, H. G. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1746–1750

Mjøs, O. D., Faergeman, O., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 603–615

Noel, S. P., Dolphin, P. J. & Rubinstein, D. (1975) *Biochem. Biophys. Res. Commun.* **63**, 764–772

Pattnaik, N. M., Kezdy, F. J. & Scanu, A. M. (1976) *J. Biol. Chem.* **251**, 1984–1990

Redgrave, T. G. (1970) *J. Clin. Invest.* **49**, 465–471

Scow, R. O. & Egelrud, T. (1976) *Biochim. Biophys. Acta* **431**, 538–549

Sherrill, B. C. & Dietschy, J. M. (1978) *J. Biol. Chem.* **253**, 1859–1867

Skipski, V. P., Peterson, R. F. & Barclay, M. (1964) *Biochem. J.* **90**, 374–378

Smith, L. C., Pownall, H. J. & Gotto, A. M., Jr. (1978) *Annu. Rev. Biochem.* **47**, 751–777

Tall, A. R., Green, P. H. R., Glickman, R. M. & Riley, J. W. (1979) *J. Clin. Invest.* **64**, 977–989

Van Berkel, T. J. C. & Van Tol, A. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1097–1101

Wells, M. A. & Hanahan, D. J. (1969) *Biochemistry* **8**, 414–424