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The apoprotein B-independent hepatic uptake of chylomicron remnants

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Rat lymph chylomicrons were treated with Pronase resulting in particles completely devoid of surface apoproteins. On re-incubation with serum, the Pronase-treated chylomicrons re-acquired, by transfer from other lipoproteins, all apoproteins except apoprotein B, which is water-insoluble and non-transferable. When two groups of rats were injected with [^{3}H]cholesterol-labelled control or Pronase-treated chylomicrons, radioactivity was incorporated into the livers of both groups at similar rates. It is concluded that the remnants of the control and Pronase-treated chylomicrons formed in the vascular space were recognized and taken up by liver cells by a process that does not require apoprotein B.

Soon after chylomicrons are released into the circulation from the intestine, they are partially degraded by lipoprotein lipase present on the endothelial surface of the skeletal muscle and adipose tissue (Borensztajn, 1979). This partial degradation results in the formation of remnants that, compared with the intact chylomicrons, are smaller particles with a much-reduced content of triacylglycerol, phospholipids and apoproteins C and A. Much of the cholesteryl ester and apoprotein E, and all the apoprotein B, is retained (Mjøs *et al.*, 1975; Tall *et al.*, 1979). Remnants are rapidly removed from circulation by the liver by a process that involves the binding of the particle to receptors on the liver cell surface, followed by internalization of the whole remnant by the cell (Sherrill & Dietschy, 1978; Cooper & Yu, 1978). The identity of the components on the surface of remnant particles that are recognized by, and bind to, the cell receptors has been the subject of several recent investigations. Liver cells have been shown to possess receptors capable of binding apoprotein E-carrying lipoproteins (Windler *et al.*, 1980), and convincing evidence has been presented showing that apoprotein E on the surface of remnants participates in the hepatic recognition and uptake of these particles (Sherrill *et al.*, 1980). Liver cells possess, in addition, receptors capable of binding apoprotein B-carrying lipoproteins (Kovanen *et al.*, 1979; Windler *et al.*, 1980) and on the basis of indirect evidence the suggestion has been made that the apoprotein B on the surface of chylomicron remnants may also participate in the uptake of these particles by the liver (Elovson *et al.*, 1981; Wu & Windmueller, 1981; Sparks & Marsh, 1981).

The purpose of the present study was to investigate whether the uptake of chylomicron remnants by the liver requires the mediation by apoprotein B. To this end we prepared [^{3}H]cholesterol-labelled rat lymph chylomicrons devoid of apoprotein B. These particles as well as control chylomicrons were injected into rats and the rate of hepatic uptake of their remnants formed in the vascular space was measured. The results obtained indicate that apoprotein B is not required for the hepatic uptake of remnants.

Abbreviations used: VLD lipoprotein, very-low-density lipoproteins; HD lipoprotein, high-density lipoproteins; LD lipoprotein, low-density liproteins.

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Materials and methods

Preparation of chylomicrons

The thoracic ducts of rats were cannulated by the technique of Bollman *et al.* (1948) and the animals were then fed 2ml of corn oil containing 250μCi of [1,2(n)-^{3}H]cholesterol (sp. radioactivity 50Ci/mol; Amersham, Arlington Heights, IL, U.S.A.). The lymph was collected during the following 12h at room temperature in the absence of preservatives. After defibrination with applicators, the labelled chyle was incubated with rat serum (9:1, v/v) for 30min at room temperature, and the chylomicrons were then separated by layering 20ml of Krebs–Ringer bicarbonate buffer, 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

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ultracentrifuge. The floating chylomicron layer was harvested and redispersed by passage through a 21 gauge needle.

Proteolytic digestion

Pronase obtained from *Streptococcus griseus* (Calbiochem, La Jolla, CA, U.S.A.) was dissolved in 0.05M-barbitol buffer, pH8.1, and mixed with the washed chyle (1:2, v/v) to a concentration of 1mg of Pronase/mg of chyle protein. The mixture was incubated in a shaking water bath at 37°C for 2h. After the incubation, the mixture was applied to chromatography columns (50 cm × 1.4 cm) of Sepharose 2B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) and eluted with 0.2M-NaCl, pH7.0. Control samples were treated identically, except that Pronase was omitted from the incubation mixture. Where indicated, the column eluates were incubated with chylomicron-free serum (10μl/mg of chylomicron triacylglycerol) for 15min at 37°C in a shaking water bath. After incubation the chylomicrons were repurified by centrifugation and chromatography as described above. The chylomicron-free serum used was obtained by centrifuging sera from 18-h-starved rats in an SW50.1 rotor at 35000 rev./min for 60min at 16°C in a Beckman L5-75 preparative ultracentrifuge. The floating chylomicrons were removed by aspiration and discarded.

Electrophoresis

After purification by chromatography, control chylomicrons, Pronase-treated chylomicrons and Pronase-treated chylomicrons that were re-incubated with chylomicron-free serum were dialysed against water for 18h at 4°C, and portions containing 15–45mg of triacylglycerol were freeze-dried. The freeze-dried samples were then partially delipidated with 10ml of diethyl ether to which 200μl of water had been added. Extraction was performed with mixing for 30min at 4°C. After centrifugation at 3000 rev./min in a Sorvall GCL-2 centrifuge (Dupont Co., Wilmington, DE, U.S.A.) for 10min the diethyl ether phase was removed by aspiration. Portions of the water phase were electrophoresed on 7.5% sodium dodecyl sulphate/polyacrylamide disc gels by the method of Weber & Osborn (1969). The gels were stained with Coomasie Blue.

Hepatic uptake of remnants

Male Sprague–Dawley rats (180–220g) were starved overnight before use in the experiments. They were lightly anaesthetized with diethyl ether and injected through a tail vein with 1ml of chyle containing 200μg of radioactive cholesterol. To obtain liver samples, rats were re-anaesthetized at specified times, and the livers were perfused through the portal vein with Krebs–Ringer bicarbonate buffer, pH7.4, at 37°C for 1min, to remove chylomicrons that might have been trapped in the sinusoidal spaces. Liver samples were extracted with chloroform/methanol (2:1, v/v) for the determination of radioactivity as previously described (Borensztajn *et al.*, 1980).

Other procedures

Chylomicron protein was determined by the method of Lowry *et al.* (1951), triacylglycerols by the method of Fletcher (1968) and cholesterol by the method of Abell *et al.* (1951).

Results

Preparation of chylomicrons devoid of apoprotein B

One of the characteristics of apoprotein B, not shared with any other apoprotein, is its inability to transfer among lipoproteins (Smith *et al.*, 1978). We took advantage of this property to prepare rat lymph chylomicrons specifically devoid of apoprotein B. To this end we employed a previously described procedure (Lukens & Borensztajn, 1978). Chylomicrons are first stripped of their surface apoproteins by proteolysis and then, on incubation with HD lipoprotein or serum, they re-acquire their full complement of transferable apoproteins. In our original report (Lukens & Borensztajn, 1978) the apoprotein profile of the chylomicrons was determined by tetramethylurea/polyacrylamide-gel electrophoresis. Since apoprotein B, unlike all other chylomicron apoproteins, is not soluble in tetramethylurea, its fate, after exposure to a proteolytic enzyme, was not ascertained. In the present study we used sodium dodecyl sulphate/polyacrylamide-gel electrophoresis to separate the chylomicron apoproteins. The results obtained are shown in Fig. 1. Intact rat lymph chylomicrons (gel *a*) were exposed to Pronase, which completely degraded the tetramethylurea-soluble apoproteins (apoproteins A, E and C) as well as apoprotein B (gel *b*). When chylomicrons stripped of their apoproteins were incubated with serum, they re-acquired all the transferable apoproteins but not apoprotein B (gel *c*).

The effectiveness of the Pronase treatment in the removal of apoprotein B from the chylomicrons and the absence of apoprotein B or apoprotein B fragments on the reconstituted chylomicrons was further confirmed by immunological techniques using affinity-enriched rabbit anti-apoprotein B antibody (R. J. Padley, unpublished work).

Hepatic uptake of remnants from chylomicrons devoid of apoprotein B

Experiments with the isolated perfused rat heart have shown that chylomicrons that have been stripped of their apoproteins by proteolytic digestion

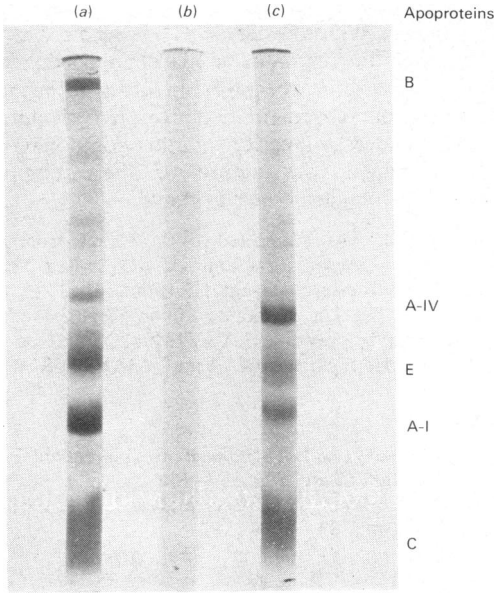

Fig. 1. *Apoprotein patterns on polyacrylamide-gel electrophoresis*

Control chylomicrons (gel *a*), Pronase-treated chylomicrons (gel *b*) and Pronase-treated chylomicrons incubated with chylomicron-free serum (gel *c*) were purified as described in the Materials and methods section. The protein equivalent of 30 mg of chylomicron triacylglycerol was applied to each gel. Electrophoresis was on 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulphate.

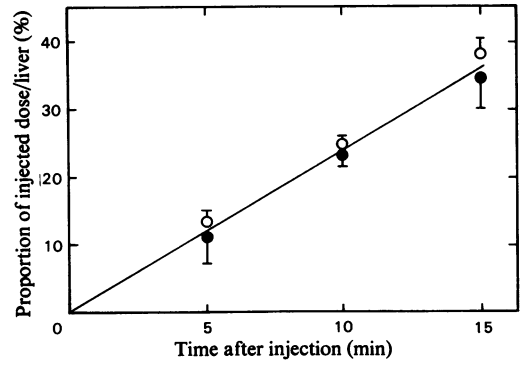

Fig. 2. *Time course of hepatic uptake of control and Pronase-treated chylomicrons*

[^{3}H]Cholesterol-labelled control (○) and Pronase-treated chylomicrons (●) purified by centrifugation and chromatography, as described in the Materials and methods section, were injected intravenously into rats (200 μg of cholesterol/rat). At the times indicated the livers were perfused for 1 min with Krebs–Ringer bicarbonate buffer 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 measurement of radioactivity in their lipid extracts. Results are expressed as means ± S.D. and each point represents the average of duplicate samples taken from three separate livers.

are poor substrates for lipoprotein lipase (Lukens & Borensztajn, 1978). When these apoprotein-stripped chylomicrons re-acquire apoprotein C-II, together with the other tetramethylurea-soluble apoproteins by transfer from serum lipoproteins, their capacity to act as substrate for lipoprotein lipase is completely restored (Lukens & Borensztajn, 1978). Thus by exposing chylomicrons devoid of apoprotein B to lipoprotein lipase, remnants devoid of apoprotein B can be formed and the rate of their uptake by the liver can be compared with that of remnants from control chylomicrons. Fig. 2 shows the results of an experiment in which [^{3}H]cholesterol-labelled control chylomicrons and Pronase-treated chylomicrons were injected into rats. The hepatic uptake of label, derived mainly from the remnants formed by the action of lipoprotein lipase in the extrahepatic tissues (Borensztajn & Kotlar, 1981), was measured at 5, 10 and 15 min. It is apparent that at all three time intervals the radioactivity incorporated into the livers of both groups of animals was similar.

Discussion

The results of the present study extend previous findings that chylomicrons can be stripped of all their surface apoproteins by means of proteolytic digestion (Lukens & Borensztajn, 1978). These apoprotein-free chylomicrons can readily re-acquire their full complement of apoproteins, except, as shown in the present study, apoprotein B (Fig. 1). The reconstituted apoprotein B-free chylomicrons cannot be differentiated from control chylomicrons with respect to their capacity to act as substrate for lipoprotein lipase, solubilized or endothelium-bound (Lukens & Borensztajn, 1978). It is reasonable to assume, therefore, that when apoprotein-free chylomicrons are injected intravenously into rats, they acquire the soluble apoproteins and that apoprotein B-free remnants are formed at rates similar to those of control chylomicrons. As remnants are rapidly removed from circulation by the liver, the present findings showing the similarity in the rate of label incorporation into the livers of rats injected with [^{3}H]cholesterol-labelled control and Pronase-treated chylomicrons (Fig. 2) support this assumption. Further, these results indicate that the hepatic recognition and uptake of remnants formed in the vascular space does not require the presence of apoprotein B on the particle surface.

Two immunologically distinct variants of apo-

protein B, with apparent molecular weights of 240000 (B_1) and 335000 (B_h) occur in the plasma of rat (Krishnaiah *et al.*, 1980). The apoprotein B_1 variant is synthesized in the intestine as well as in the liver, whereas the B_h variant is synthesized mainly, if not exclusively, in the liver (Wu & Windmueller, 1981). Thus, VLD lipoprotein of hepatic origin may carry either variant of apoprotein B, whereas VLD lipoprotein of intestinal origin and chylomicrons carry only the B_1 variant (Wu & Windmueller, 1981). Studies with rats injected with protein-labelled triacylglycerol-rich lipoproteins carrying both apoprotein B variants have shown that the apoprotein B_1 has a significantly shorter half-life in the plasma than the B_h variant (Wu & Windmueller, 1981; Sparks & Marsh, 1981). This shorter plasma half-life of apoprotein B_1 was correlated with a more rapid hepatic uptake of this apoprotein. On the assumption that all the injected triacylglycerol-rich lipoprotein had identical composition except for the type of apoprotein B, it was suggested that receptors on the surface of liver cells preferentially recognize and bind the apoprotein B_1 variant leading to the rapid internalization of lipoprotein remnants containing this apoprotein.

The results of the present study showing that the liver does not discriminate between chylomicron remnants devoid of apoprotein B_1 and those containing apoprotein B_1 does not support this hypothesis. Thus, provided that the chylomicron remnant possesses tetramethylurea-soluble apoproteins, the particles are recognized and taken up by the liver. Liver cell membranes possess two receptors capable of binding lipoprotein-carrying apoprotein E and/or apoprotein B (Hui *et al.*, 1981). One of these receptors, thought to be the chylomicron receptor, recognizes only apoprotein E. The other receptor, which shares many properties with the peripheral LD-lipoprotein receptor, recognizes both apoproteins B and E. Our results are consistent with the evidence showing that recognition of chylomicron remnants by their receptor requires only apoprotein E. To the extent that the liver distinguishes between particles containing one or other apoprotein B variant (Sparks & Marsh, 1981), our results suggest that this distinction must be at the level of the B/E receptor or a hitherto uncharacterized receptor.

Although the presence of apoprotein B_1 on chylomicron remnants is not required for their uptake by the liver, the present results do not rule out a role for the apoprotein B_h variant modulating the efficiency with which apoprotein E binds to its receptors. Such a role could explain the more extended half-life in the circulation of lipoproteins containing the apoprotein B_h variant. It is noteworthy, however, that the residence time in the circulation of apoprotein B_1 of intestinal origin is shorter than that of the apoprotein B_1 of hepatic origin (Wu & Windmueller, 1981). It seems that the interaction of the remnants of VLD lipoproteins with hepatic cell-surface receptors involves a primary recognition of apoprotein E and/or B with uptake being modulated by a variety of other components of these remnants. The nature of these complex modulations remains to be elucidated.

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References

Abell, L. I., 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. (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

Borensztajn, J. & Kotlar, T. J. (1981) *Biochem. J.* **200**, 547–553

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

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

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

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

Hui, D. Y., Innerarity, T. L. & Mahley, R. W. (1981) *J. Biol. Chem.* **256**, 5646–5655

Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) *J. Biol. Chem.* **254**, 11367–11373

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

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Lukens, T. W. & Borensztajn, J. (1978) *Biochem. J.* **175**, 53–61

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

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

Sherrill, B. C., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1804–1807

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

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

Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412

Windler, E. E. T., Kovanen, P. T., Chao, Y. S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 10464–10471

Wu, A.-L. & Windmueller, H. G. (1981) *J. Biol. Chem.* **256**, 3615–3616