Specific, saturable binding and uptake of rat chylomicron remnants by rat skin fibroblasts

Trevor G. Redgrave,[1] Noel H. Fidge, and Jane Yin

Department of Physiology, University of Melbourne, Parkville 3052, Victoria, and Baker Medical Research Institute, Prahran 3181, Victoria, Australia

Abstract To investigate the possible contribution of chylomicron remnants to the accumulation of cholesterol in non-hepatic tissues, rat chylomicron remnants were incubated with rat skin fibroblasts. The binding of remnants was saturable and specific. Native, undegraded chylomicrons were almost as effective as unlabeled remnants in displacing the uptake of labeled remnants. Rat low density and high density lipoproteins were relatively ineffective in displacing the uptake of labeled remnants. Accumulation of radioactive unesterified fatty acids occurred in proportion to the uptake of labeled remnants, indicating probable internalization and degradation of the particles after binding. The incorporation of added [^{14}C]acetate into cell non-saponifiable lipids was not significantly suppressed by added remnants, indicating an apparent lack of feedback regulation of cholesterol biosynthesis after remnant uptake. Our results show that the physiological mechanism underlying uptake of remnants by hepatic parenchymal cells might not be accounted for by tissue or cellular specificity, but may perhaps arise because of the lower capillary permeability of extra-hepatic sites compared with the hepatic sinusoid.—**Redgrave, T. G., N. H. Fidge, and J. Yin.** Specific, saturable binding and uptake of rat chylomicron remnants by rat skin fibroblasts. *J. Lipid Res.* 1982. **23:** 638–644.

Supplementary key words apolipoproteins • high density lipoproteins • low density lipoproteins

The usual physiological fate of the cholesteryl ester-rich core remnants that are produced during the catabolism of chylomicrons is rapid uptake by the liver (1, 2). In experimental models of hypercholesterolemia such as the cholesterol-fed rabbit (3, 4), the hypothyroid or diabetic rat (5), and also obese (6) and alcohol-fed rats (7), remnants persist in the plasma contributing to hyperlipidemia and perhaps leading to the development of atheroma (8). If it is true that remnants contribute to the development of atheroma, then a mechanism should exist for their uptake by non-hepatic tissues. Florén et al (9, 10) have recently reported the uptake of chylomicron remnants by human fibroblasts and smooth muscle cells by a saturable process in apparent competition with the uptake of LDL, and Innerarity, Pitas, and Mahley (11) showed that rat chylomicron remnants were bound with high affinity to rat fibroblasts.

In this investigation we have studied the binding and uptake of rat remnants by rat fibroblasts in culture. The specific uptake process for remnants appears to be different from the LDL uptake process described for human fibroblasts (12) and probably represents an alternate pathway for the delivery of lipids to tissues.

METHODS

Preparation of chylomicrons

The thoracic duct of male albino 250-g Wistar rats was cannulated and at the same time a cannula was secured in the stomach via a gastrostomy. Postoperative management and collection of lymph was as previously described (7). The rats were fed their usual pelletted chow and when the lymph was milky on the first postoperative day, 25 μCi of [1-^{14}C]palmitic acid (New England Nuclear, Boston, MA, USA) was given as the potassium salt dissolved in 1 ml of cow's milk. The chylomicrons present in the lymph were isolated and purified by ultracentrifugation in a discontinuous density gradient (13) to float all particles with diameter > 75 nm to the top of the centrifuge tube.

Preparation of remnants

Male albino Wistar 300-g rats were functionally hepatectomized under light ether anesthesia (1, 13). Chylomicrons were injected via a tail vein at a dose of 93 ± 4 μg of chylomicron protein per 100 g body weight. Heparin was not used. After 30 min circulation in the hepatectomized rats, blood was collected by cardiac puncture into EDTA (final concentration 5 mM) and plasma was separated by centrifugation at 1,500 *g* for 15 min. Density gradient centrifugation was again employed to

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins; BME, basal Eagle's medium; LDS, lipoprotein-deficient fetal calf serum.

[1] Present address: Biophysics Institute, Boston University Medical Center, 80 East Concord Street, Boston, MA 02118.

isolate and purify the remnant fraction from plasma. Albumin contamination was not present in chylomicrons or remnants as shown by polyacrylamide gel electrophoresis. By this procedure remnants were produced that were depleted of injected chylomicron [^{14}C]triacylglycerol label by 73.5 ± 2.75%. In control experiments 98.0 ± 2.8% of injected cholesterol radioactivity was retained in the plasma if the chylomicrons were labeled with [^{3}H]cholesterol instead of [^{14}C]palmitic acid.

Preparation of rat fibroblasts

Skin biopsies were obtained from the abdomen of male Sprague-Dawley rats weighing about 150 g. Explants measuring 1 mm^2 or less were placed in culture flasks containing reconstituted chicken embryo extract and chicken plasma. When firm clots had formed, growth medium was added. Then the flasks were left undisturbed for 7–14 days and subsequently fed three times weekly. After 3–6 weeks, cells had covered most of the growth surface.

Further serial propagation was achieved by splitting 1:5 or 1:8 every 7 or 8 days. Transfer of cells was made after treatment with 0.1% trypsin and 0.02% EDTA in Ca^{2+}- and Mg^{2+}-free balanced salt solution. Cells were studied between the second and fifth passages.

Swirls of cells characteristic of fibroblasts formed in the flasks, which were maintained in 75-cm^2 vessels in a humidified 37°C incubator under 95% air plus 5% CO_2 in BME containing 10% (v/v) fetal calf serum with final concentrations of protein, 4 mg/ml; cholesterol, 0.9 mM; $NaHCO_3$, 10 mM; glutamine, 2 mM; penicillin G, 100 units/ml; and streptomycin, 100 μg/ml; pH 7.2–7.4.

Isolation of lipoproteins

Rat lipoproteins were isolated from the serum of normal-fed male Sprague-Dawley rats (300–400 g, fasted overnight) by sequential flotation in a Beckman L5-65 ultracentrifuge using either 40 or 50 Ti rotors (Beckman Instruments Inc., Palo Alto, CA) at 210,000 *g* at 4°C for 24 hr. Rat LDL was isolated between d 1.030–1.055 g/ml and rat HDL between 1.070–1.21 g/ml. All lipoproteins were recentrifuged at the appropriate higher density, or, alternatively, were respun until each fraction was homogeneous by agarose gel electrophoresis. The isolated lipoprotein fractions were dialyzed against three changes of 100 vol of 0.15 M NaCl, pH 7.4 containing 1 mM EDTA and one final dialysis against phosphate-buffered saline without EDTA. After dialysis, the lipoproteins were sterilized by filtration through 0.45 μm Millipore filters. Human LDL (d 1.030–1.050 g/ml) and HDL_3 (1.12–1.21 g/ml) were prepared similarly.

Procedure for investigating remnant binding and uptake

Cells were grown to 70–90% confluency in 60 × 15 mm dishes 4–6 days after seeding with 0.5–1 × 10^5 cells/dish. Growth curves showed that cell numbers reached a plateau at 1.0–1.2 × 10^6 cells/dish in 6–8 days. Cell protein was 200–250 μg/dish at the time of study (about 5 days). Prior to binding experiments, the medium was replaced with 2 ml of fresh medium containing 10% (v/v) of LDS and immediately before the experiment, the medium was replaced with BME containing 10% LDS plus the radioactive remnants and other lipoproteins as described in Results.

After incubation at 37°C, the medium was removed and the cells were harvested and washed as described (14). The radioactivity released after treating the washed cells with trypsin was considered to be bound to the cell surface (binding) and the radioactivity remaining after washing the cells again was presumed to represent internalized or cell-associated radioactivity. Aliquots of medium were taken for measurement of total radioactivity and hydrolysis of triacylglycerol radioactivity to fatty acids.

Radioiodination of lipoproteins

Lipoproteins were iodinated with ^{125}I by the iodine monochloride method. After iodination, the labeled lipoproteins were dialyzed against 0.15 M NaCl, pH 7.4, and sterilized by filtration as described above. All labeled preparations contained less than 1 g atom of iodine per mole of protein. After extraction with chloroform–methanol (15), 92–95% of the ^{125}I was found attached to the protein moiety of human lipoproteins and 75–82% was bound to the protein moiety of rat lipoproteins. Specific radioactivities of each preparation varied between 200–350 cpm per ng of protein.

Chemical analysis

Protein determinations were made according to Lowry et al. (16). Lipids were extracted from lipoproteins and fibroblasts in chloroform–methanol (2:1 by volume) according to Folch, Lees, and Sloane Stanley (15). Phospholipids, cholesterol, fatty acids, triacylglycerols, and cholesteryl esters were separated by chromatography of the lipid extracts on sintered glass rods (17) in the solvent system light petroleum (bp 40–60°C)–diethyl ether–acetic acid 60:1.2:0.7 (by volume). Quantitation was by flame ionization detection in the Iatroscan TH-10 (Iatron Laboratories, Tokyo, Japan) using calibration factors calculated from standard mixtures 18-5A, B, and C obtained from Nu-Chek Prep, Inc., Elysian, MN, USA.

Radioactivity was measured by liquid scintillation

TABLE 1. Chemical composition of chylomicrons and remnants

	Chylomicrons	Remnants
	(% of total particle mass)	
Triacylglycerol	85 ± 0.6	70 ± 3.1
Cholesteryl esters	1 ± 0.1	6 ± 0.6
Cholesterol	1 ± 0.1	6 ± 0.3
Phospholipids	11 ± 0.5	12 ± 2.1
Protein	1 ± 0.1	7 ± 1.0

spectroscopy of lipids dissolved in the fluor solution, or of silica gel scrapings of lipid classes separated by thin-layer chromatography. Polyacrylamide gel electrophoresis was on 5, 10, or 15% gels containing 0.1% sodium dodecyl sulfate (18) on apoproteins delipidated with ethanol–diethyl ether 3:2 (v/v).

RESULTS

The composition of the chylomicrons and their remnants that were used in this study are given in **Table 1.**

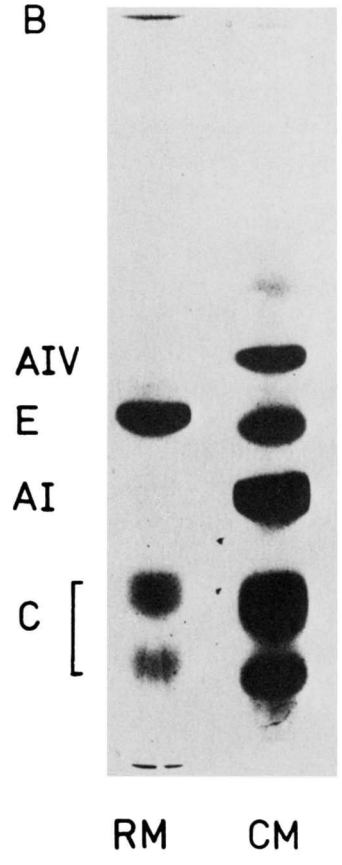

Fig. 1. Polyacrylamide gel electrophoresis of remnant (RM) and chylomicron (CM) apoproteins. About 30 μg of delipidated protein was dissolved in 5% SDS and 5% β-mercaptoethanol in 0.25 M Na_2HPO_4, pH 7.0, and applied to 5% gels containing 0.1% SDS. Note that apolipoproteins A-I and A-IV were absent from RM.

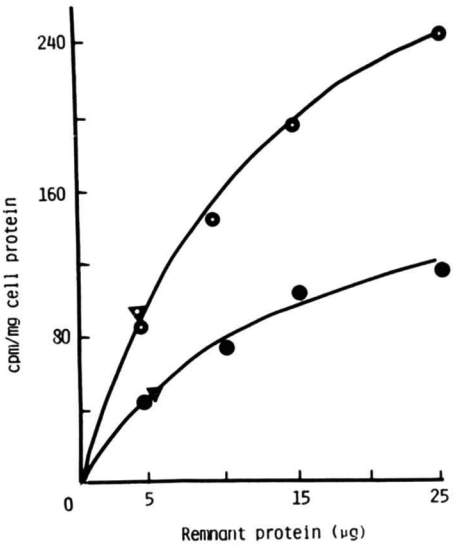

Fig. 2. Binding and internalization of [^{14}C]triacylglycerol-labeled chylomicron remnants by cultured rat skin fibroblasts. About 10^6 cells were incubated for 18 hr in 2 ml of medium containing 10% LDS. Then the medium was replaced with fresh medium containing chylomicron remnants. Cells were harvested after 3 hr at 37°C and processed as described in Methods. (●), Trypsin-releasable radioactivity (bound remnants) and (○), trypsin-resistant (internalized) radioactivity. (△), shows comparable data obtained when rat remnants were incubated with human fibroblasts. Each point represents the mean of two dishes.

Compared with their parent particles, the remnants were enriched in cholesterol, cholesteryl esters, and total protein but depleted in triacylglycerols. **Fig. 1** shows that the remnants retained apoproteins B, C, and E, but apoproteins A-I and A-IV were lost from the parent chylomicrons during their conversion to remnants.

Binding of remnants to fibroblasts began to plateau in a saturable fashion at concentrations of remnant protein between 12–15 μg/ml of incubation medium (**Fig. 2**). Sufficient excess of 'cold' remnants was added to two dishes, and the data indicated that about 20% of the binding was non-specific. This supported the data of Fig. 4 which showed that addition of unlabeled CM remnants displaced approximately 80% of labeled remnants.) Most of the cell-associated radioactivity was not released by treatment with trypsin and was probably internalized. A double-reciprocal plot of the data indicated a K_m of 12.5 μg protein/ml and half-maximal binding (v/2) occurred at 86 cpm/mg cell protein, corresponding with 12 μg lipoprotein protein/ml (**Fig. 3**). Incubations of rat remnants with human fibroblasts showed comparable binding and internalization behavior.

Unlabeled remnants displaced the binding and uptake of radioactive remnants with high specificity (**Fig. 4**). In contrast, LDL had little effect on binding or uptake; at low concentrations (10 μg of LDL protein/ml) there was some enhancement of uptake, but at higher concentra-

tions there was a slight reduction. Similarly, HDL enhanced uptake at low concentrations, but at higher concentrations there was evidence of competition for uptake of chylomicron remnants. Unlabeled chylomicrons were almost as effective as remnants in reducing the uptake of labeled remnants.

When ^{125}I-labeled LDL or HDL was incubated with rat fibroblasts, there was also evidence for specific saturable binding (**Fig. 5**), and subsequent internalization, measured in this case as radioactivity resistant to release in the presence of heparin. Confirming the results of Drevon et al. (19) and Innerarity et al. (11), the binding of rat HDL was more avid that that of rat LDL, and human LDL was poorly bound. The polyacrylamide gels of the lipoproteins used in these studies are shown in **Fig. 6.** A double-reciprocal plot (not shown) of the binding of rat LDL to rat fibroblasts indicated a K_m of 65.7 μg protein/ml and half-maximal binding (v/2) occurred at 104.3 ng protein/mg cell protein, corresponding with a lipoprotein protein concentration of 60 μg/ml. Hence, when compared on the basis of total protein concentration, remnant binding was five-fold more avid than LDL binding.

To assess whether uptake of remnants was accompanied by metabolic changes, the appearance of labeled unesterified fatty acids in the medium was studied. The data of **Fig. 7** show that during the course of the incubations there was significant hydrolysis in the absence of unlabeled remnants. When increasing amounts of unlabeled remnants were added, the hydrolysis decreased in a fashion that was consistent with the reduced uptake shown in Fig. 4. Freshly prepared remnants contained only 1.7% (SEM 0.45, n = 8) of radioactivity as free fatty acids.

At high concentrations of remnants (**Fig. 8**), incorporation of [^{14}C]acetate into fatty acids was significantly suppressed when remnants were added to the medium

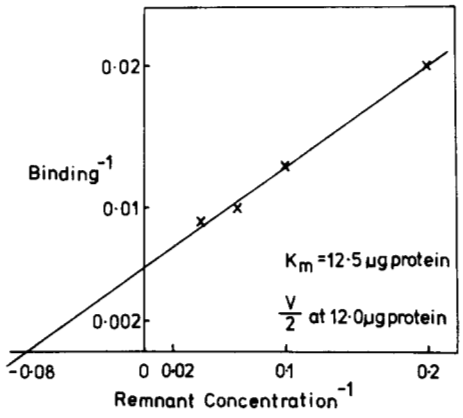

Fig. 3. Double-reciprocal plot of the binding data of Fig. 2, showing the specific binding of rat remnants to rat fibroblasts.

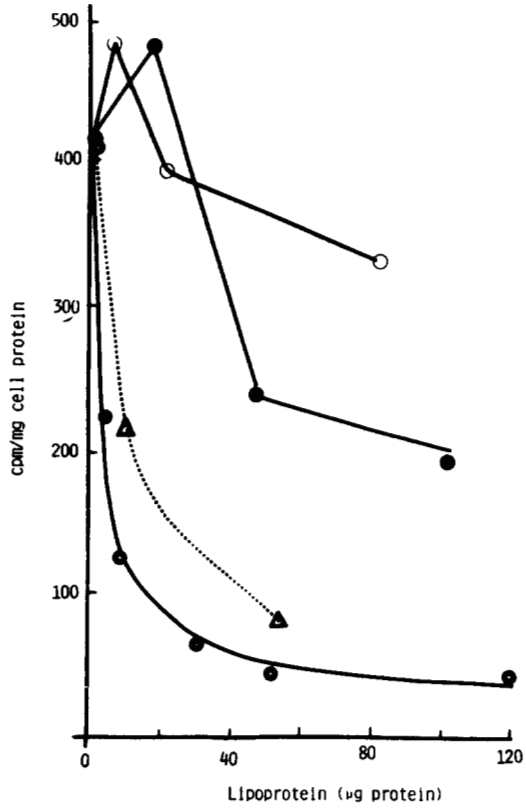

Fig. 4. Competitive displacement of [^{14}C]triacylglycerol-labeled rat chylomicron remnants by O, unlabeled rat chylomicron remnants; Δ, rat chylomicrons; ●, rat HDL, and O, rat LDL. Incubations were performed for 3 hr at 37°C as described in Fig. 2. Note that chylomicrons were almost as effective as remnants in blocking remnant binding, but HDL and LDL were relatively ineffective.

2 hr previously. Incorporation of [^{14}C]acetate into nonsaponifiable lipids was not suppressed even at high concentrations of remnants. Hence, remnants taken up by the cells influenced fatty acid biosynthesis, but there was no evidence for feedback regulation of cholesterol biosynthesis, at least within the time-span of these studies. In other studies (not shown) cholesterol biosynthesis was also not suppressed when remnants were present for 6 hr.

DISCUSSION

Our study demonstrated that the binding by rat fibroblasts of rat chylomicron remnants was saturable and showed specificity that was shared to a large extent with the parent chylomicron particles. Because LDL did not compete effectively with remnants for uptake by fibroblasts, the receptor mechanism for remnants was probably different from the LDL receptor characterized for human fibroblasts by Goldstein and Brown (12). If a protein component of the remnant was recognized and bound by the receptor site, then that protein was also

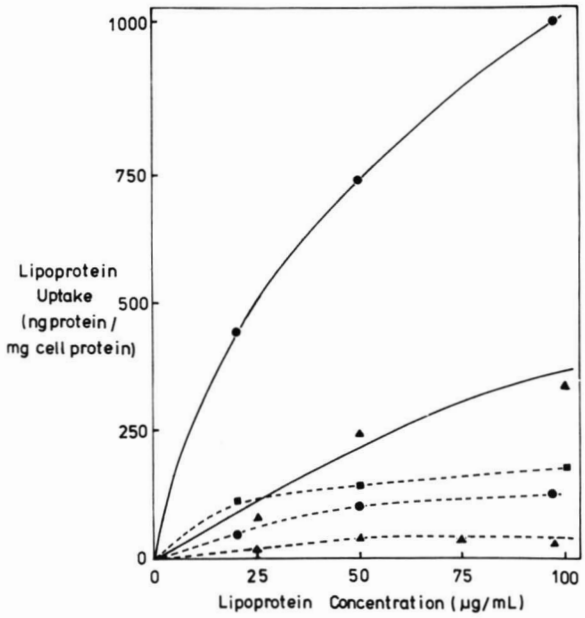

Fig. 5. Binding and internalization of human and rat lipoproteins by cultured rat skin fibroblasts. ▲, Human LDL; ●, rat LDL; ■, rat HDL. Internalization (heparin-resistant) is shown by the solid lines, and binding (heparin-releasable) by the broken lines, except for rat HDL, which is the sum of binding and internalization. Rat fibroblasts were relatively inefficient in binding or internalizing human LDL, compared with rat LDL.

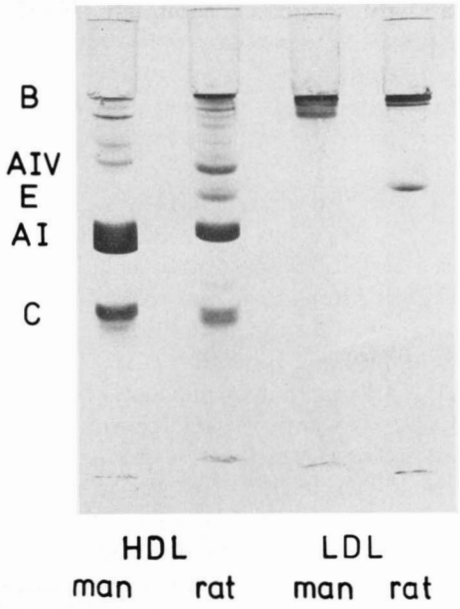

Fig. 6. Polyacrylamide gel electrophoresis of human and rat lipoprotein, used to study the competitive displacement of remnants and for binding studies. The delipidated samples were dissolved in 0.05 M Tris HCl buffer and applied to 15% gels containing 0.1% SDS. Note the presence of apolipoprotein E in rat LDL, and also in rat HDL.

present in the parent chylomicron, rather than being gained during the metabolic conversion of chylomicrons to remnants, because the parent chylomicron competed with remnants for binding. Because both rat HDL and rat LDL showed some competition at high concentrations, such a recognition protein must be common to all rat lipoproteins. Only apolipoprotein E was present on all rat lipoproteins (Fig. 1 and Fig. 6), so our results are consistent with the observations of Innerarity et al. (11), who showed that rat fibroblasts contain a receptor for apolipoprotein E. The remnant-receptor of rat fibroblasts thus is similar to the receptor of adult dog liver membranes in binding of apolipoprotein E and not B (20).

Our results obtained with rat fibroblasts differ in some respects from those of Florén et al. (9, 10) obtained with human fibroblasts. These workers (9, 10) observed that LDL competed effectively with remnants for uptake. They concluded that remnants were taken up by the LDL receptor pathway. One possible explanation for differences would be that there are indeed different mechanisms operating in rats and humans. However, we have calculated from their data (Fig. 4, Ref. 9) that the binding characteristics are remarkably similar in rats and human fibroblasts (**Table 2**), which suggests but does not prove that similar mechanisms exist in the two species. Alternatively, remnants prepared by Florén et al. (9, 10) were possibly contaminated with plasma VLDL (21) because they were prepared from post-prandial plasma "chylomicrons". If so, contamination with apolipoprotein B from plasma VLDL could have accounted for the apparent competition for uptake with LDL. Evidence for such heterogeneity in humans can be expected from the findings of Elovson et al. (22) in the rat. Firm conclusions on this important question must await further experiments in humans in which this source of contamination of remnant apoprotein B has been eliminated. Our findings are consistent with those of Innerarity et al. (11) who have also reported that rat fibroblasts bind rat chylomicron remnants with greater affinity than LDL, although they gave insufficient data for calculation

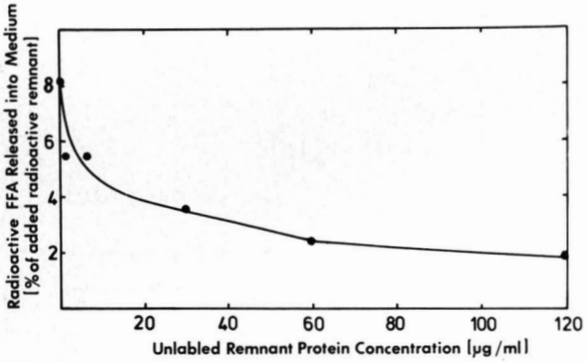

Fig. 7. FFA released into medium after incubating [^{14}C]triacylglycerol-labeled chylomicron remnants with rat fibroblasts.

of the K_m for remnant binding. Inspection of the data from various laboratories indicates that LDL seems to bind more avidly than remnants (Table 2) if compared by protein content, but when compared on a molar basis, remnants are bound more avidly than LDL. At an assumed remnant molecular weight of 50×10^6, the K_m is about 4 nmol/l.

The uptake of remnants by the liver is clearly the major catabolic pathway, and the role of apoprotein E in the binding and/or remnant uptake by the hepatocyte is well-established (23–25). Sherrill, Innerarity, and Mahley (26) calculated the K_m for remnant uptake by perfused liver to be about 10 nmol/l, similar in magnitude to our estimate for fibroblasts. Under normal physiological circumstances, the uptake of remnants by fibroblasts and smooth muscle cells is a minor pathway, so the lack of remnant uptake by extrahepatic tissues is not because of lesser cellular affinity, but must be a result of the greater permeability of the hepatic sinusoidal endothelium compared with peripheral capillaries (27).

Taken in conjunction, the data of Figs. 7 and 8 showed that the remnant triacylglycerols were hydrolyzed and metabolized after uptake by the fibroblasts. The resultant appearance of radioactive fatty acids in the incubation medium presumably followed the intracellular hydrolysis of the remnant triacylglycerols. Our data do not deliberately exclude possible extracellular hydrolysis, but this mechanism is unlikely to explain our results because of the close similarities in the uptake, internalization, and degradation of remnant triacylglycerols, cholesteryl esters, and proteins (9). In parallel with the reduced uptake

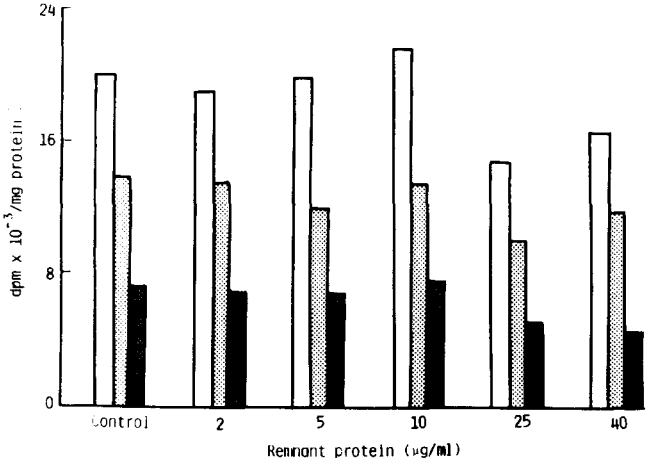

Fig. 8. Effect of chylomicron remnants on incorporation of [^{14}C]acetate into nonsaponifiable (cholesterol) and saponifiable (fatty acids) fractions of rat skin fibroblasts. Cells were incubated for 18 hr in 10% LDS and then with remnants for 2 hr prior to a pulse addition of [^{14}C]acetate (2 μCi). ▫, ^{14}C in whole lipids; □, non-saponifiable; and ▨, saponifiable radioactivity. Even at the highest remnant concentration, radioactivity in nonsaponifiable lipids (cholesterol) was not significantly reduced.

TABLE 2. Calculated Km for lipoprotein binding by fibroblasts[a]

Species	Lipoprotein	K_m µg protein/ml	K_m µg lipoprotein/ml	K_m mmol/l	Ref.
Man	LDL	9	45	18	(29)
Rat	LDL	3	15	6	(11)
Man	Remnant	13	186	4	(9)
Rat	Remnant	12.5	179	4	(this work)

[a] Assuming LDL = 20% protein, remnants = 7% protein, LDL $M_r = 2.5 \times 10^6$, remnant $M_r = 50 \times 10^6$.

of labeled remnants in the presence of unlabeled remnants (Fig. 7), increasing amounts of unlabeled remnants progressively decreased the amount of radioactive fatty acids released into the medium. Furthermore, new synthesis of fatty acids from [^{14}C]acetate was suppressed when the hydrolyzed fatty acids were available for cellular metabolic requirements after remnant uptake, again in proportion to the added amount of remnants. In contrast, the new synthesis of nonsaponifiable lipids from [^{14}C]acetate was not suppressed by remnants, which suggests that remnant cholesterol did not inhibit cellular cholesterol biosynthesis at times less than 6 hr. This finding has implications for the development of atheroma or cholesterol deposition in tissues, because remnant cholesterol would increase cellular cholesterol content unless remnant uptake suppressed endogenous sterol biosynthesis sufficiently to preserve cellular cholesterol homeostasis. However, exposure to remnants for 24 hr did suppress both receptor activity and cholesterol biosynthesis (9).

The flux of cholesterol through the plasma compartment in the form of remnants is large in terms of the plasma pool of cholesterol but, under normal physiological circumstances, the steady-state plasma content of remnants is maintained low because of their rapid uptake by the liver. The process demonstrated in these experiments may have pathological implications in situations where the normal uptake of remnants by the liver is deficient. Our experiments show a mechanism for cellular uptake of remnants that could contribute to the accumulation of cholesterol in tissues and possibly lead to atherosclerosis (28).

This work was carried out with the assistance of grants from the National Heart Foundation of Australia and the National Health and Medical Research Council.

Manuscript received 5 May 1981 and in revised form 8 January 1982.

REFERENCES

1. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.

2. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoprotein of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
3. Redgrave, T. G., K. B. Dunne, D. C. K. Roberts, and C. E. West. 1976. Chylomicron metabolism in rabbits fed diets with or without added cholesterol. *Atherosclerosis.* **24:** 501–508.
4. Ross, A. C., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
5. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism.* **26:** 493–503.
6. Redgrave, T. G. 1977. Catabolism of chylomicron triacylglycerol and cholesteryl ester in genetically obese rats. *J. Lipid Res.* **18:** 604–612.
7. Redgrave, T. G., and G. Martin. 1977. Effects of chronic ethanol consumption on the catabolism of chylomicron triacylglycerol and cholesteryl ester in the rat. *Atherosclerosis.* **28:** 69–80.
8. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60:** 473–485.
9. Florén, C-H., J. J. Albers, B. J. Kudchodkar, and E. L. Bierman. 1981. Receptor-dependent uptake of human chylomicron remnants by cultured skin fibroblasts. *J. Biol. Chem.* **256:** 425–433.
10. Florén, C-H., J. J. Albers, and E. L. Bierman. 1981. Uptake of chylomicron remnants causes cholesterol accumulation in cultured human arterial smooth muscle cells. *Biochim. Biophys. Acta.* **663:** 336–349.
11. Innerarity, T. L., R. E. Pitas, and R. W. Mahley. 1980. Disparities in the interaction of rat and human lipoproteins with cultured rat fibroblasts and smooth muscle cells. *J. Biol. Chem.* **255:** 11163–11172.
12. Goldstein, J. L., and M. S. Brown. 1977. The low density lipoprotein pathway and its relation to atherosclerosis. *Ann. Rev. Biochem.* **46:** 897–930.
13. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
14. Miller, N. E., D. B. Weinstein, and D. Steinberg. 1977. Binding, internalization, and degradation of high density lipoproteins by cultured normal human fibroblasts. *J. Lipid Res.* **18:** 438–450.
15. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
17. Vandamme D., V. Blaton and H. Peeters. 1978. Screening of plasma lipids by thin-layer chromatography with flame ionization detection on chromarods. *J. Chromatogr.* **145:** 151–154.
18. Weber, K., and M. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
19. Drevon, C. A., A. D. Attie, S. H. Pangborn, and D. Steinberg. 1981. Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts. *J. Lipid Res.* **22:** 37–46.
20. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes. *J. Biol. Chem.* **256:** 5646–5655.
21. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1981. Heterogenity of apolipoprotein B. Isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
22. Elovson, J., Y. Huang, N. Baker, and R. Kannan. 1981. Apolipoprotein B is structurally and metabolically heterogeneous in the rat. *Proc. Natl. Acad. Sci. USA.* **78:** 157–161.
23. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652–658.
24. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255:** 5475–5480.
25. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. *J. Biol. Chem.* **255:** 8303–8307.
26. Sherrill, B. C., T. L. Innerarity, and R. W. Mahley. 1980. Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor. *J. Biol. Chem.* **255:** 1804–1807.
27. Mayerson, H. S., C. H. Wolfram, H. H. Shirley, and K. Wasserman. 1960. Regional differences in capillary permeability. *Am. J. Physiol.* **198:** 155–160.
28. Small, D. M. 1977. Cellular mechanisms for lipid deposition in atherosclerosis. *N. Engl. J. Med.* **297:** 873–877; 924–929.
29. Brown, M. S., and J. L. Goldstein. 1976. Receptor-mediated control of cholesterol metabolism. *Science.* **191:** 150–154.