AN IN VITRO MODEL FOR THE CATABOLISM OF RAT CHYLOMICRONS

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SUMMARY: An in vitro model for the study of the catabolism of chylomicrons is suggested and characterized. The model utilizes the ability of perfused rat hearts to hydrolyze triglycerides of chylomicrons obtained from rat thoracic ducts. The resulting remnants were re-isolated and perfused through rat livers where the remnant lipid and protein was rapidly removed. In contrast intact chylomicrons were taken up by perfused liver to a limited extent. The remnants produced by cardiac perfusion contained a decreased percent of triglycerides and apoproteins C-2 and C-3, with a relative increase primarily in diglycerides and, to a lesser extent, monoglycerides and cholesterol. Most of the ^{125}I-labelled remnant protein lost during hepatic perfusion was recovered in the tissue. The model thus simulated many of the known characteristics of chylomicron catabolism in vivo.

Chylomicrons[1] are rapidly cleared from the circulation(1) by a mechanism which involves several steps. Although the CM TG are taken up slightly more rapidly than CM cholesterol in vivo, this difference is very much greater following hepatectomy(2). Redgrave(3) isolated CM remnants from the serum of hepatectomized rats and found that they were rapidly cleared upon re-injection into normal rats. On the basis of splanchnic flow studies in intact animals Bergman et al(4) suggested that CM TG are primarily removed by extra-hepatic tissue, while the liver, which removes intact CM relatively slowly(5), takes up the cholesterol of the remnants. Analogous particles in the form of intermediate density lipoproteins have been obtained from VLDL in vivo (6,7) and in vitro (8).

The observation that CM and VLDL are converted to remnant particles prior to their removal by the liver raises a number of important questions. These include: To what extent is hydrolysis of TG by extra-hepatic tissue necessary before the CM becomes a remnant which can be rapidly removed by the liver?

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[1] Abbreviations: CM: chylomicrons; VLDL: very low density lipoproteins; TG: triglycerides; DG: diglycerides; MG: monoglycerides; LPL: lipoprotein lipase; LCAT: lecithin-cholesterol acyltransferase; PAGE: polyacrylamide gel electrophoresis; I/P ratio: iodine to protein molar ratio.

How does the liver distinguish between intact CM (which vary in size from 750 to 4,500 Å(9)) and remnants? What is the role and level of the various apoproteins of CM and VLDL during formation of their remnants and removal of the latter by the liver? In order to answer these and other questions, we are proposing and characterizing a two-step *in vitro* model for the study of CM catabolism, using perfused hearts to generate remnants and perfused liver to catabolize them.

MATERIALS AND METHODS Male hooded rats were used in all experiments. CM were obtained from cannulated thoracic ducts of fed rats after stomach intubation with corn oil. During collection of the CM the rats were maintained on a diet of chow saturated with corn oil. The CM were isolated by three successive 1 h centrifugations at 100,000 xg in NaCl solution (d: 1.006) with 0.01% EDTA, pH 7.2. The re-suspended CM were dialyzed against 0.9% NaCl and iodinated by a modification of the method of MacFarlane(10) at pH 10. After dialysis to remove free ^{125}I the iodinated CM were isolated twice on NaCl gradients to separate the S_f >3200 particles(9), added to carrier CM of S_f >3200 and dialyzed against 0.9% NaCl to remove the EDTA present in the gradient.

TABLE 1: The Radioiodination of Thoracic Duct CM by the ICl Method

ICl/Protein Molar Ratio	Labelling Efficiency (%)	^{125}I Distribution		I/P Ratio
		Lipid (%)	Protein (%)	
25	53	97	3	0.36
60	51	87	13	4.0
75	42	58	42	13.3
150	39	43	57	33.2
250	34	37	63	53.7

The molecular weight of the CM apo-protein is assumed to be 100,000. 9-10 nmoles of protein were iodinated in a total volume of 5.1 ml.

There have been several studies of conditions for the iodination of VLDL (11,12) but none for CM. A brief study of these conditions for CM was therefore undertaken. From the resulting data, shown in Table 1, it was decided that 60 nmoles ICl/nmole protein represented a reasonable compromise between a low I/P ratio and significant labelling of the protein moiety and was therefore used for subsequent studies.

Hearts were perfused by the method of Bleehen and Fisher(13) with the perfusate oxygenated and recycled in a modification of the apparatus of Miller et al(14). All glassware was siliconized prior to use. The perfusate was Krebs-Ringer bicarbonate buffer, pH 7.4 containing 0.22M Ca^{++} and 375 mg% glucose, gased with 95% O_2 and 5% CO_2. Rat hearts, which were changed every 30 min, were washed with the Ringer solution prior to perfusion to remove residual blood. The hearts beat steadily during the perfusion (>175 beats/min). A more rapid and regular heart rate was found when albumin was omitted from the perfusate. Omission of albumin had no effect on the extent of hydrolysis of CM TG labelled with [^{3}H]palmitate. However free [^{3}H]palmitate did not accumulate in the perfusate as it did in the presence of albumin. The CM and remnants were isolated after perfusions by centrifugation for 1 h at 100,000xg in saline of d:1.006 (S_f > 400) before being added to the liver perfusate. They were then circulated for 30 min in the apparatus before perfusion through the liver. Liver perfusions were carried out by the method of Miller et al(14) using a plasma-free perfusate previously described(15). Lipoproteins were delipidated and analyzed by PAGE in 7M urea(16). Lipids for analysis were extracted from CM and remnants by the method of Folch et al(17) and neutral lipids, prepared by silicic acid column chromatography, were separated by thin layer chromatography(18). Colorimetric procedures were used to determine the glyceride-glycerol(19), cholesterol(20) and protein(21). Radioactivity in CM protein was determined after delipidation by ethanol-ether (3:1) at -10° on filter paper discs(22) and in liver protein by the method of Dolphin et al(23).

RESULTS AND DISCUSSION The changes in CM resulting from the heart perfusion

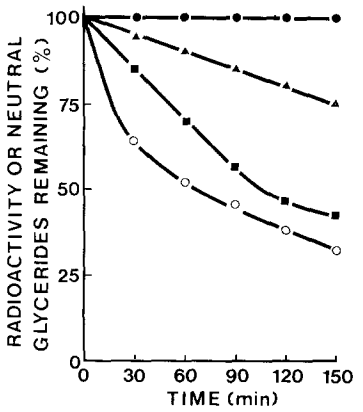

Fig. 1. Metabolism of ^{125}I-labelled thoracic duct CM (S_f > 3200) by isolated perfused rat hearts. CM containing 550 mg neutral glycerides was added to 130 ml perfusate. (●): Total perfusate ^{125}I-labelled protein; (▲): S_f > 400 ^{125}I-labelled protein; (■): neutral glyceride-glycerol in S_f > 400 lipoproteins; (○): Total ^{125}I in S_f > 400 lipoproteins. Perfusion of ^{125}I-labelled S_f > 3200 CM though the apparatus for 150 min in the absence of hearts resulted in a 15% loss of ^{125}I radioactivity from the S_f > 400 fraction but losses from all other parameters were negligible.

were studied first. During a 2 1/2 h perfusion, shown in Fig. 1, there was no loss of ^{125}I-labelled protein from the perfusate, although that associated with the CM diminished. The radioactivity and quantity of neutral glycerides in the CM decreased by at least 50%. The greater decrease of CM-bound ^{125}I than that observed with neutral glycerides is attributable to the measurement of glyceride-glycerol which includes partially hydrolysed MG and DG. For the purpose of characterizing the model system we have arbitrarily designated as remnants the lipoproteins of S_f > 400 isolated after a 2 h heart perfusion. Inspection of these remnants by electron microscopy after negative staining with phosphotungstic acid revealed structures similar to the remnants observed by Blanchette-Mackie and Scow(24).

Distribution of ^{125}I among the CM and remnant peptides separated by PAGE is shown in Table 2. The A, B and C apoproteins are designated on the basis of their mobility on PAGE as described by Herbert et al(25) for purified rat apoproteins and the D apoprotein by analogy with human VLDL(26). It will be noted that there is a marked decrease in the percentage of apo C-2 and C-3 in remnants.

TABLE 2: Distribution of ^{125}I in CM and remnant peptides

Apoprotein	Distribution (%)	
	Chylomicrons	Remnants
B	44	60
A	13	21
D	1	3
C-II and C-III-0	9	2
C-III-3	27	4
	Total Radioactivity (cpm x 10^3)	
	206	15

The loss of these rapidly migrating peptides following hydrolysis of the CM TG resembles that reported for VLDL remnants following treatment with post-heparin plasma(8). Since some of these peptides activate LPL(27,28), the possibility that their loss limits the hydrolysis of remnant TG must be considered.

The rate of removal of CM and remnant TG when equal quantities of TG are perfused through the liver are compared in Fig. 2. Within 30 min of perfusion most of the remnant TG was removed, corresponding to the rapid removal of remnants *in vivo* reported by Redgrave(3), while only about 25% of the CM TG was taken up. The relatively small uptake of intact CM by perfused liver agrees with previously reported observations(5). As a result of the rapid hepatic removal of remnants,a 30 min perfusion period was routinely used in our model.

The lipid composition of the CM and remnants before and after liver perfusion is shown in Table 3. The difference between the CM and remnant lipid composition before liver perfusion represents the effect of the heart perfusion. The latter produces a decrease in the percentage of TG with concommitant increases in DG and, to a lesser degree, in MG and cholesterol. The increased proportion of

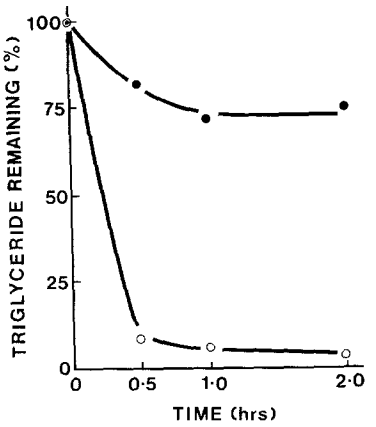

Fig. 2. Removal of TG from CM and remnant fractions (S_f > 400) by isolated perfused rat livers. 32 mg of CM or remnant neutral glycerides was added to 100 ml. of perfusate. (●): CM perfusion; (○) remnant perfusion.

TABLE 3: Lipid Composition of thoracic duct CM and their remnants before and after liver perfusion

	Distribution (%)			
	Chylomicrons		Remnants	
	before	after	before	after
Triglycerides	82	83	64	44
Diglycerides	5	4	18	20
Monoglycerides	2	1	7	7
Cholesterol	2	2	4	5
Cholesterol esters	1	2	2	5
Phospholipids	7	8	5	19

Analyses were performed on CM and remnants of S_f > 400

DG rather than MG, although surprising in view of the known specificity of LPL(29), was consistently observed. The relatively small quantities of cholesterol and cholesterol esters are probably due to the use of corn oil to generate the CM and the absence of LCAT in our model. The hepatic perfusion had little effect on

TABLE 4: Distribution of ^{125}I-labelled CM and remnant proteins before and after liver perfusion

	Distribution (%)			
	chylomicrons		remnants	
	before	after	before	after
S_f > 400	97	63	96	17
Remaining perfusate	3	18	4	20
Liver	-	19	-	63
	Total Radioactivity (cpm x 10^4)			
	124	125	80	70

the relative distribution of CM lipids, indicating that hepatic uptake of CM may be due to trapping, as has been suggested in vivo(30). In contrast, after hepatic perfusion there was a further decrease in the proportion of TG in the remnant with a concommittant increase in the percentage of phospholipid, although the amount of lipid was small (c.f. Fig. 2). In several experiments the perfusate containing the CM was circulated and oxygenated in the perfusion apparatus for 2 h at 37° in the absence of the heart. This resulted in a slight decrease in the percentage of TG with an increase in DG. Slightly more of these CM than the uncirculated CM were subsequently removed by the liver. This effect may be due to the presence of LPL in the lymph(31) which may bind to the isolated CM.

The fate of the CM and remnant ^{125}I-labelled protein after liver perfusion is shown in Table 4. In these experiments the CM used for the control were suspended in the heart perfusate, circulated and oxygenated for 2 h in the absence of the heart and then re-isolated at S_f > 400. Most of the CM protein radioactivity remained in the S_f > 400 fraction after hepatic perfusion, with some in the denser fractions of the perfusate. The low level of protein radioactivity found in the liver reflects the relatively small uptake of CM lipids. In contrast, most of

the labelled remnant protein appeared in the liver. The uptake of the remnant protein by the perfused liver is analagous to the deposition of ^{125}I-labelled VLDL protein in the liver in vivo(32).

The successive 2 h heart and 30 min liver perfusions appears to be a suitable model for investigation of CM and probably VLDL catabolism. It seems to simulate the in vivo situation of extra-hepatic formation of remnants and their rapid removal by the liver and obviates some of the complications such as exchange(16) or acquisition(33) of peptides when hepatectomized animals or post-heparin plasma are used to form remnants. In addition various purified components such as LCAT and high density lipoproteins may be added to the system under controlled conditions.

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