Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver

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Abstract Like rat C apolipoproteins, each of the C apolipoproteins from human blood plasma (C-I, C-II, C-III-1, and C-III-2) bound to small chylomicrons from mesenteric lymph of estradiol-treated rats and inhibited their uptake by the isolated perfused rat liver. This inhibitory effect of the C apolipoproteins was independent of apolipoprotein E, which is present only in trace amounts in these chylomicrons. Addition of rat apolipoprotein E to small chylomicrons from mesenteric lymph of normal rats did not displace C apolipoproteins and had no effect on the uptake of these particles by the perfused liver, indicating that an increased ratio of E apolipoproteins to C apolipoproteins on chylomicron particles, unaccompanied by depletion of the latter, may not promote recognition by the chylomicron remnant receptor. The hepatic uptake of remnants of rat hepatic very low density lipoproteins (VLDL) and small chylomicrons, which had been produced in functionally eviscerated rats, was also inhibited by addition of C apolipoproteins. These observations are consistent with the hypothesis that the addition of all of the C apolipoproteins to newly secreted chylomicrons and VLDL inhibits premature uptake of these particles by the liver and that depletion of all of these apolipoproteins from remnant particles facilitates their hepatic uptake. Remnants of chylomicrons and VLDL incubated with rat C apolipoproteins efficiently took up C-III apolipoproteins, but not apolipoprotein C-II (the activator protein for lipoprotein lipase). Preferential loss of apolipoprotein C-II during remnant formation may regulate the termination of triglyceride hydrolysis prior to complete removal of triglycerides from chylomicrons and VLDL. —**Windler, E., and R. J. Havel.** Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J. Lipid Res.* 1985. **26:** 556–565.

Supplementary key words apoE • chylomicrons • VLDL • apoB

The liver, as the major site from which cholesterol and its degradation products, the bile acids, are excreted from the body, is of central importance in the regulation of plasma cholesterol metabolism. The hepatic uptake of cholesterol in plasma lipoproteins has been studied extensively and it is now recognized that all of the lipoproteins that contain apolipoprotein (apo) B, apoE, or both of these proteins are taken up into hepatic parenchymal cells by saturable processes of high affinity (1). Preparations of membranes from liver cells have been found to exhibit saturable binding of these lipoprotein species, but the binding affinity is substantially greater for those species that contain apoE, as compared with low density lipoproteins (LDL), which contain solely apoB (2). The affinity of binding of triglyceride-rich lipoproteins to liver membranes is reduced by addition of C apolipoproteins to these lipoproteins. These findings are consistent with our observations of lipoprotein uptake by the isolated, perfused rat liver (3). We found that small chylomicrons from mesenteric lymph and very low density lipoproteins (VLDL) from liver perfusates were readily taken up by perfused livers. When these lipoproteins were incubated with rat serum, their content of C apoproteins increased, as occurs normally when these lipoproteins enter the blood. Hepatic uptake of the modified chylomicrons and VLDL was substantially reduced. Remnant particles, produced when the chylomicrons or VLDL were injected into functionally eviscerated rats, were depleted of the C apoproteins, but not of apoE and apoB. These remnants were removed with high efficiency from perfusates of isolated livers. These observations, which suggested that the C apoproteins and apoE have opposing effects upon the hepatic uptake of triglyceride-rich lipoproteins, were confirmed by experiments in which small chylomicrons from mesenteric lymph of rats treated with pharmacological amounts of 17-α-ethinyl estradiol were used (4). These chylomicrons contain apolipoprotein B-48 but little C apoproteins and only trace amounts of apoE. The apo-

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Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein(s); TG, triglycerides; CE, cholesteryl esters.
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protein composition of these chylomicrons was modified by incubating them with purified rat apoproteins without alteration of their size or the composition of core lipids. Addition of apoE resulted in rapid hepatic uptake of the particles, whereas addition of individual C apoproteins (C-II, C-III-0, and C-III-3) all caused similar retardation of hepatic uptake (4). These results are consistent with the hypothesis that the changes in the apoprotein composition of triglyceride-rich lipoproteins that occur as they are secreted from the intestine or liver and as they are subsequently metabolized can explain both the low rate of initial hepatic uptake and the rapid uptake of their remnants.

In the current research we have examined the effect of adding individual human C apoproteins (C-I, C-II, C-III-1, and C-III-2) to chylomicrons from estradiol-treated rats on the uptake of these particles by perfused rat livers. We have also evaluated further the opposing effects of apoE and the C apoproteins on hepatic uptake by enriching chylomicrons from normal rats (which contain appreciable C apoproteins) with apoE. Finally, we have evaluated the effects of enriching remnants of both chylomicrons and hepatic VLDL (which contain substantial amounts of apoE) with C apoproteins. A preliminary report of this work has appeared (5).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats, weighing 275–350 g, maintained on standard Purina rat chow (Ralston Purina Co., St. Louis, MO) and tap water were used except for preparation of erythrocytes and isolated rat apoproteins, for which blood plasma was obtained from retired breeders (3). Estradiol-treated rats, used to obtain intestinal lymph, had received daily injections of 17-α-ethinyl estradiol (5 mg/kg body weight) (6).

Preparation of apolipoproteins

Rat apoproteins and human C apoproteins were isolated from plasma VLDL, prepared by centrifuging plasma for 10^8 g_{av}-min at a density of 1.006 g/ml and purified by recentrifugation under the same conditions. VLDL were delipidated with ethanol–ether 3:1 and ether (7) and the precipitated apoproteins were dissolved in 0.015 M Tris-HCl containing 6 M urea, pH 8.2. The soluble proteins were separated by gel permeation chromatography (Sephadex G-200 or G-150, Pharmacia, Piscataway, NJ) in the same buffer (8). Fractions containing apoE or C apoproteins were pooled, concentrated by vacuum dialysis, and then dialyzed against Krebs-Henseleit buffer, containing no glucose or Ca^{2+}, pH 7.4 (9). Purity of these apoproteins was evaluated by isoelectric focusing poly-

acrylamide gel electrophoresis in the absence of reducing agents (10). Rat C apoproteins contained no detectable albumin or apoA-I, apoA-IV, or apoE (refer to Fig. 7). Preparations of human C apoproteins also appeared to be devoid of these proteins, except for a faint band that focused with human apoE3. Rat apoE contained no detectable albumin, apoA-I, or C apoproteins (**Fig. 1**).

Individual human C apoproteins (apoC-I, apoC-II, apoC-III-1, apoC-III-2) were isolated from unfractionated C apoproteins, prepared as described above, by ion-exchange chromatography on columns (1.2 × 18 cm) of DEAE cellulose (DE-52, Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent, England) (11). Elution was carried out with a gradient of Tris-HCl, 0.015–0.15 M containing 6 M urea, pH 8.2. Fractions containing individual C apoproteins were pooled, concentrated by vacuum dialysis, and dialyzed against Krebs-Henseleit buffer without glucose or Ca^{2+}, pH 7.4. ApoC-I was repurified by chromatography on a column (1.2 × 8 cm) of CM-cellulose (CM-52, Whatman Chemicals). The void-volume fraction from the column of DEAE-cellulose was dialyzed against 0.02 M acetate buffer, pH 5.4, containing 6 M urea, and then applied to a column of CM-cellulose. Elution was accomplished with a linear gradient of acetate
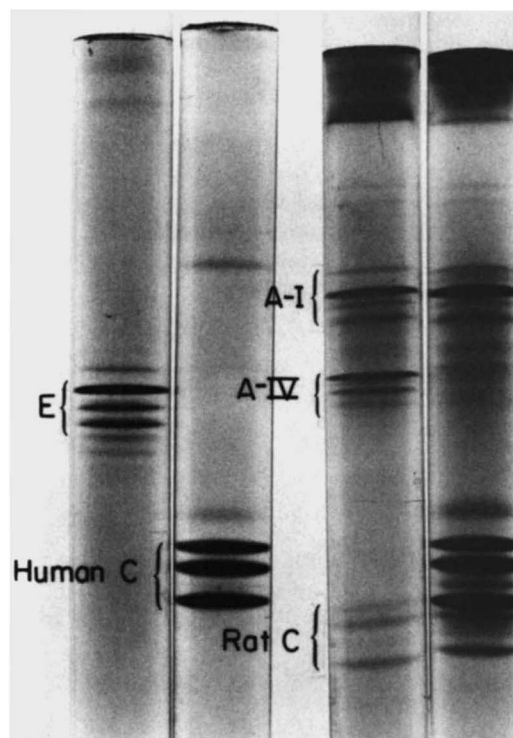

Fig. 1. Isoelectric focusing polyacrylamide gel electrophoretograms of apoproteins. Left pair, isolated rat apoprotein E and human C apoproteins. Right pair, small chylomicrons from estradiol-treated rats and these chylomicrons after incubation with human C apoproteins. Proteins applied to each gel on right were derived from chylomicrons containing the same amount of [^{3}H]CE.

(0.02–0.13 M), pH 5.4, containing 6 M urea (12). Purity of apoC-II, apoC-III-1, and apoC-III-2 was evaluated by analytical isoelectric focusing polyacrylamide gel electrophoresis (10) and amino acid analysis (13). Purity of apoC-I was evaluated by polyacrylamide gel electrophoresis in urea (14) and by amino acid analysis. None of the C apoproteins contained detectable amounts of albumin, apoA-I, apoA-IV, or apoE. The amino acid analyses resembled closely those reported (15).

Lipoproteins enriched in C apoproteins and apoE

Intestinal lymph containing [^{3}H]cholesteryl esters (CE) and [^{14}C]triglycerides (TG) was obtained from normal or estradiol-treated rats during intraduodenal infusion of 10% glucose in 0.15 M NaCl (4). Lymph serum was layered under Krebs-Henseleit buffer, without glucose or Ca^{2+}, pH 7.4 (ϱ = 1.007 g/ml) and small chylomicrons were separated by centrifugation for 3 × 10^7 g_{av}-min, followed by recentrifugation under the same conditions. To obtain small chylomicrons enriched in an apoprotein, chylomicrons, obtained by a single centrifugation from lymph, were incubated with the isolated apoprotein or with 0.15 M NaCl. The mixtures were gently shaken for 1 hr at room temperature, layered under Krebs-Henseleit buffer without glucose or Ca^{2+}, pH 7.4, and then submitted to a second centrifugation as described above. Small chylomicrons from estradiol-treated rats were incubated with 25 μg of human C apoproteins per mg of TG or with individual human C apoproteins (25 and 50 μg per mg of TG). Small chylomicrons from normal rats were incubated with 50 μg of rat apoE per mg of TG. Isotopically labeled remnants of hepatic VLDL and lymphatic small chylomicrons were prepared as described (3), but were separated by centrifugation for 5 × 10^7 g_{av}-min. The remnants were incubated as described for small chylomicrons with 0.15 M NaCl or unfractionated rat C apoproteins, 100 μg of protein per mg of TG (calculated as that amount present in the native particles). This amount of TG was calculated with the assumption that the ^{3}H-labeled CE content of the remnants was unchanged:

$$\frac{\text{(TG in parent lipoproteins)} \times (^3\text{H-labeled CE in remnants})}{^3\text{H-labeled CE in parent lipoproteins}}.$$

The incubated remnants were layered under Krebs-Henseleit buffer without glucose or Ca^{2+}, pH 7.4, and repurified by flotation for 5 × 10^7 g_{av}-min.

Uptake of lipoproteins by perfused livers

Liver perfusions, addition of labeled lipoproteins, and measurement of ^{3}H-labeled CE and ^{14}C-labeled TG in perfusates were performed exactly as described (3). Lipoproteins were used within 24 hr of preparation. Samples that were aggregated, detected by an increase in absor-bance at 550 nm per mg of TG, were discarded.

Analyses

Protein was determined by a modified procedure of Lowry with bovine serum albumin as standard (16). ApoE was measured by specific radioimmunoassay (8). TG in lipoproteins were estimated by an automated procedure (17) and lipid composition of lipoproteins was determined by standard methods (18).

Materials

[1, 2-^{3}H]Cholesterol, 40–60 Ci/mmol and [1-^{14}C]palmitic acid, 40–60 mCi/mmol, were from New England Nuclear, Boston, MA.

RESULTS

Hepatic uptake of small chylomicrons enriched in C apoproteins and apoE

As reported previously (4), small chylomicrons from estradiol-treated rats contained much less C apoproteins and apoE than small chylomicrons from normal rats. Chylomicrons from estradiol-treated rats that had been incubated with human C apoproteins were enriched in these proteins and contained less apoA-IV (Fig. 1). Content of protein increased from 30 to 55 μg/mg TG. The rate of removal of ^{3}H-labeled CE in the small chylomicrons enriched in C apoproteins was substantially lower than that of the control chylomicrons (**Fig. 2**). Small chylomicrons from normal rats incubated with rat apoE became enriched in this protein but apparently lost no C apoproteins (**Fig. 3**). The protein content increased from 108 to 144 μg/mg TG. ^{3}H-Labeled CE in these incubated chylomicrons was removed from the perfusate of isolated livers at the same rate as control chylomicrons (**Fig. 4**).

Hepatic uptake of small chylomicrons enriched in individual human C apoproteins

Small chylomicrons from estradiol-treated rats that had been incubated with individual human C apoproteins (apoC-I, apoC-II, apoC-III-1, or apoC-III-2) were enriched in the added protein and depleted of apoA-I and apoA-IV (**Fig. 5**). The content of protein increased substantially only when 50 μg of apoC-I per mg of TG was added, from 47 ± 17 (n = 3) to 79 ± 16 (n = 3) μg/mg TG ($P < 0.07$). The rate of removal from the perfusate of isolated livers of ^{3}H-labeled CE in small chylomicrons enriched in individual human C apoproteins was lower than that of control small chylomicrons (**Fig. 6**). Incubation with 50 μg of protein/mg of TG generally had a greater effect than incubation with 25 μg/mg of TG. This dose-dependency was most pronounced with apoC-I.

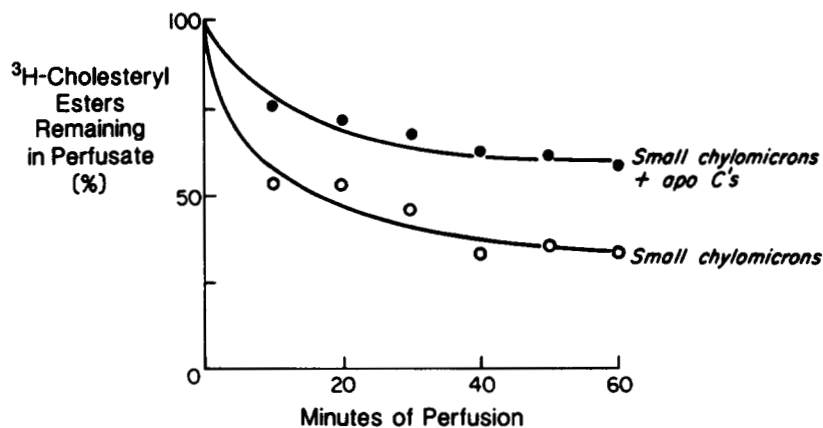**Fig. 2.** Removal from the medium of perfused rat livers of [³H]CE of small chylomicrons from estradiol-treated rats (1.4 mg of chylomicron triglyceride added to perfusate) and of these chylomicrons after incubation with human C apoproteins (1.0 mg of chylomicron triglyceride added to perfusate).

Hepatic uptake of remnants enriched in rat C apoproteins

Circulation of double-labeled hepatic VLDL (15 mg of TG) in functionally eviscerated rats for 15 and 30 min yielded remnants in which the ratio ^{14}C-labeled TG:^{3}H-labeled CE had decreased by 41 and 69%. Circulation of double-labeled small chylomicrons (20 mg of TG) for 30 and 60 min similarly resulted in a decrease of the ratio by 41 and 85%. Isoelectric focusing polyacrylamide gel electrophoretograms of the apoproteins showed that VLDL remnants had lost essentially all of the resolved apoproteins of the parent lipoproteins except for apoE (**Fig. 7**). The ratio of apoE:^{3}H-labeled CE increased 2.6- and 5.6-fold and the mass of apoE increased from a normal value of 14% in hepatic VLDL (3) to 32% and 45% of the total protein in remnants obtained 15 and 30 min, respectively, after injection of hepatic VLDL. Remnants that had been incubated with unfractionated rat C apoproteins were enriched in apoC-III-0 and C-III-3, but not in C-II and had lost apoE, as judged from isoelectric focusing polyacrylamide gel electrophoretograms (Fig. 7). As compared with the unincubated remnants, the ratio of apoE:^{3}H-labeled CE in the incubated remnants had decreased 29- and 30-fold. Small chylomicrons from normal rats also gained apoE during the formation of remnants and they contained virtually no apoA-I or apoA-IV; apoE comprised 17% and 45% of the total protein in remnants obtained 30 and 60 min after injection, respectively. Incubation of the remnants with C apoproteins again resulted in an uptake only of apoC-III and a loss of apoE (Fig. 7). ApoE was not detectable by radioimmunoassay (<0.1% of the protein). ^{3}H-labeled CE in remnants of VLDL and chylomicrons was rapidly removed from the medium of perfused livers (**Fig. 8**). Incubation of the remnants with rat C apoproteins invariably reduced the rate of hepatic uptake; inhibition of uptake was more pronounced for VLDL remnants than for chylomicron remnants (Fig. 8).

DISCUSSION

The results of this investigation show that each of the C apoproteins from human blood plasma inhibits the hepatic uptake of triglyceride-rich lipoproteins in the rat. These findings match those obtained with rat apoC-II, C-III-0, and C-III-3 (4) and strongly support the concept that remnant uptake in humans, as in rats, is facilitated by the loss of C apoproteins from the parent particles as triglycerides are hydrolyzed. It should, however, be kept in mind that, in humans, chylomicron remnants are probably taken up efficiently by the liver whereas, in contrast to the rat, a large fraction of the VLDL, which are smaller in humans than in rats, is converted to LDL (19, 20).

We observed no substantial differences among the human C apoproteins on the retardation of chylomicron uptake. The effect, which was concentration-dependent with each protein, was greatest with apoC-I, but this probably reflects a higher content of C-I per particle, as reflected by the increased protein:TG ratio after incubation. In all cases, the effects on chylomicron uptake were most pronounced during the first few minutes of perfusion. The subsequent lessening of inhibition may be the result of loss of apoproteins to newly synthesized VLDL secreted into the perfusates.

Our results for human apoC-III-1 are in agreement with those reported by Shelburne et al. (21), who found that this protein inhibited the apoE-induced hepatic uptake of large rat chylomicrons. These investigators did not find an inhibitory effect of human apoC-II but, in a more recent study from the same laboratory, human apoC-I, C-II, and C-III-2 were found to inhibit the uptake of phos-
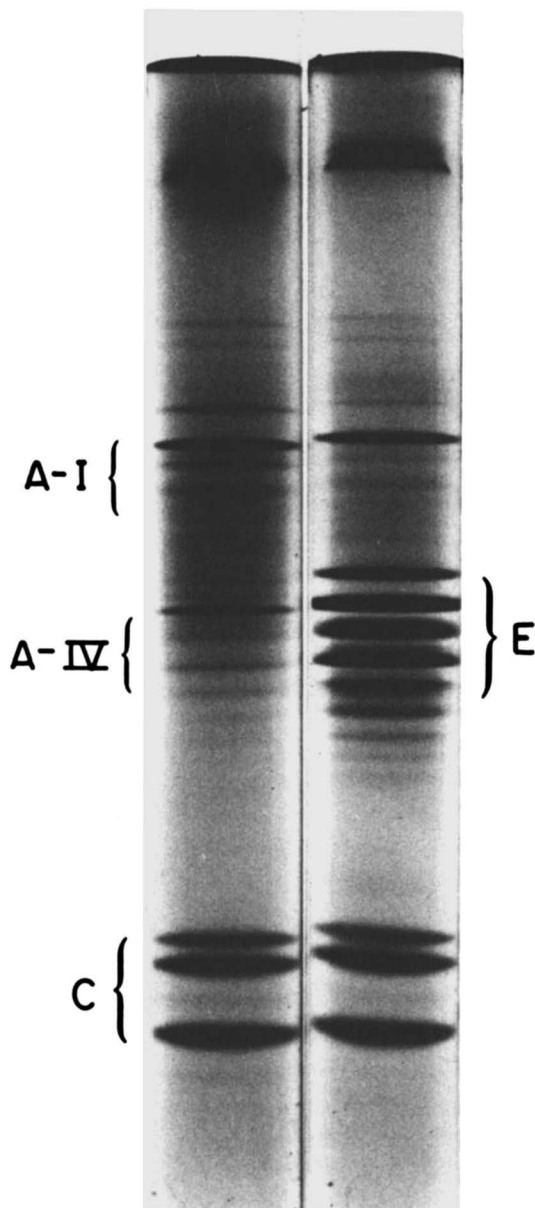
Fig. 3. Isoelectric focusing polyacrylamide gel electrophoretograms of apoproteins of small chylomicrons from normal rats (left) and of small chylomicrons that had been incubated with apoprotein E (right). Proteins applied to each gel were derived from chylomicrons containing the same amount of [³H]CE.

emulsion particles by hepatocyte monolayers is in agreement with our findings. Furthermore, their observation of an inhibition by all human C apoproteins is in accordance with the conclusion drawn from our experiments with individual rat C apoproteins that neither sialic acid nor other components are crucial to their inhibitory action (4).

The promotion of the hepatic uptake of lipoproteins by apoE is well established for the rat (2–4, 21). The clearest demonstration of this is the great enhancement produced by addition of apoE to small chylomicrons from estradiol-treated rats, which contain very little of this protein (4). Indirect evidence for a role of apoE in the hepatic uptake and conversion to LDL of human triglyceride-rich lipoproteins is provided by the disorder of familial dysbeta-lipoproteinemia, which is caused by point mutations in the structural gene for apoE (23). In affected individuals, remnant-like chylomicrons and VLDL accumulate in the blood that are neither rapidly removed from the blood nor converted to LDL (20, 24). Unlike normal apoE, the structurally abnormal protein does not promote the hepatic uptake of phospholipid complexes by perfused rat livers and binds poorly to lipoprotein receptors (25, 26).

We observed appreciable variability in the rate of hepatic uptake in different preparations of small chylomicrons from estradiol-treated rats. Small variations of their low content of apoE (which averages about 1/3 molecule per particle (4)) may have influenced the rate. Also, we found hepatic uptake of remnants to be less rapid than we had observed previously (3), possibly because in the current experiments the remnants were isolated by two centrifugations rather than one. This could have led to greater loss of apoE owing to centrifugal dissociation (27).

Several of our earlier results suggested that the inhibition of hepatic lipoprotein uptake by C apoproteins does not depend on displacement of apoE from the lipoprotein particles. First, uptake of VLDL was retarded after incubation of newly secreted VLDL with blood plasma, which leads to an enrichment of C apoproteins, but does not displace any apoE (3). Second, uptake of VLDL that had been incubated with the individual C apoproteins was consistently decreased, despite variable displacement of apoE (4). Third, lymph chylomicrons incubated in plasma not only acquired C apoproteins but apoE as well, yet their hepatic uptake was inhibited (3). The current finding that addition of apoE to chylomicrons from normal rats (which contain substantial C apoproteins) does not lead to enhanced hepatic uptake, despite an increased ratio of apoE to apoC, contrasts with the strong promoting effect of apoE added to small chylomicrons from estradiol-treated rats (which contain much less apoprotein C) (4). This substantiates our earlier observations and argues not only for an independent effect of the C apoproteins but also for a major role for them in the regulation of the
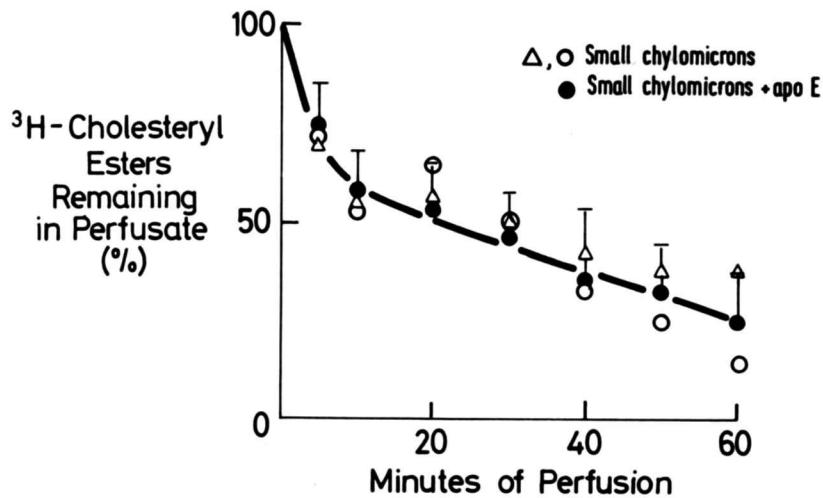
pholipid-stabilized triglyceride emulsions by perfused rat livers and hepatocyte monolayers (22). How these observations relate to those with lipoproteins is not yet clear, as these emulsions are readily taken up in the absence of any added apoproteins. Moreover, the C apoproteins were found to interfere with such nonspecific interactions as the association of the emulsions with red blood cells (22). However, their observation that the various human C apoproteins inhibited the apoE-induced uptake of the

Fig. 4. Removal from the medium of perfused livers of [^{3}H]CE of small chylomicrons from normal rats (○, △; n = 2) and small chylomicrons that had been incubated with apoprotein E (●; n = 3, mean and SD). The amount of chylomicron triglyceride added to the perfusate was 0.9 and 2.2 mg for small chylomicrons, and 0.8, 3.1, and 5.9 mg for chylomicrons incubated with apoprotein E.
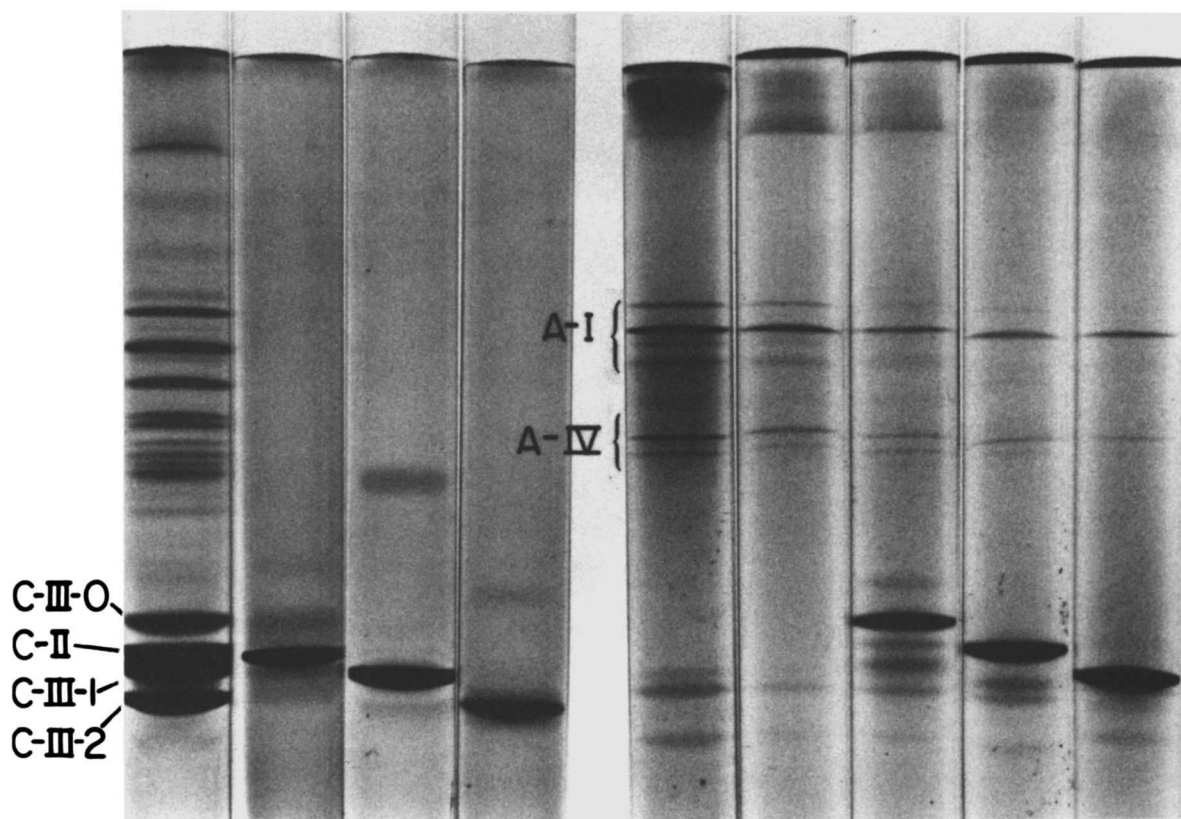

Fig. 5. Isoelectric focusing polyacrylamide gel electrophoretograms of apoproteins of small chylomicrons from estradiol-treated rats. Left group, individual human C apoproteins and the apoproteins of the VLDL from which they were isolated. Right group (from left), apoproteins of small chylomicrons from estradiol-treated rats (two gels) and these small chylomicrons after incubation with human apoprotein C-II, C-III-1, and C-III-2 (50 μg/mg of TG). Proteins applied to gels in the group on the right were derived from chylomicrons containing equal amounts of [^{3}H]CE, except for the first gel, to which twice that amount was applied.
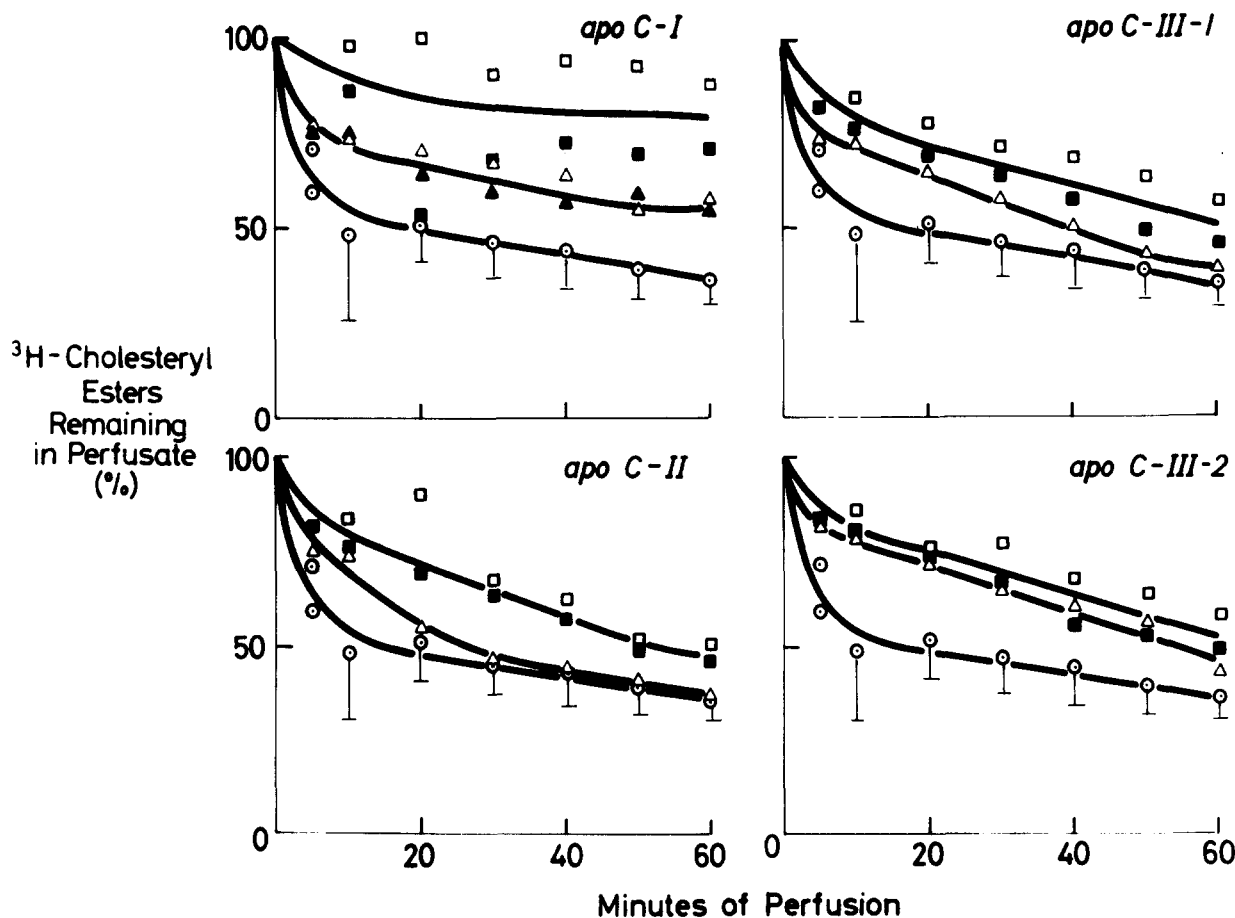
Fig. 6. Removal from the medium of perfused rat livers of [³H]CE of small chylomicrons from estradiol-treated rats (⊙; n = 3, mean and SD), or of these chylomicrons after incubation with 25 µg/mg of TG (△, ▲) of human apoproteins C-I (n = 2), C-II (n = 1), C-III-1 (n = 1), C-III-2 (n = 1), and after incubation with 50 µg/mg of TG (□, ■) of the indicated human C apoproteins (n = 2 each). The amount of chylomicron triglyceride added to the perfusates ranged from 1.1 to 2.5 mg.

hepatic uptake by inhibiting the uptake-promoting property of apoE. In the current experiments, the uptake of small chylomicrons from normal rats by the perfused liver was more rapid than we had observed previously (3). However, a stimulating effect of apoE should still have been evident. Addition of apoE to chylomicrons from estradiol-treated rats led to an uptake of 50% of the particles in 5 min, whereas comparable uptake of chylomicrons from control rats, incubated with apoE or not, required about 20 min (Fig. 4).

How C apoproteins prevent the uptake-promoting effect of apoE is unknown. Three possibilities can be considered. First, C apoproteins may interact directly with a recognition site on apoE for lipoprotein receptors. Second, they may produce an altered conformation of apoE, in which the recognition site is obscured. Third, C apoproteins on the particles may themselves bind to receptors, but in a manner that does not lead to endocytosis. It is likely that apoE must be in a suitable conformation to be recognized by lipoprotein receptors. Thus, complexes of

lecithin and apoE are taken up by perfused rat livers at a much lower rate than the canine HDL$_c$ (6, 28), even though apoE is the sole protein associated with each. Van't Hooft and Havel (29) found that ultracentrifugation of rat HDL labeled with apoE promotes uptake of the labeled protein by the liver in vivo, even though the ultracentrifuged HDL contains less apoE. Also, Hui, Innerarity, and Mahley (30) have shown that chemical modification of mutant apoE that binds poorly to apoprotein receptors increases binding substantially.

If our hypothesis is correct, that remnants can only be taken up rapidly by the liver after most of the C apoproteins are lost by transfer to HDL, this uptake should be reversible by the addition of C apoproteins to remnants. We found this to be true for remnants of both chylomicrons and VLDL and at different extents of TG-hydrolysis. Uptake of VLDL remnants was inhibited more than that of chylomicron remnants. This difference could reflect a higher affinity to lipoprotein receptors of chylomicrons that contain apoB-48 than that of VLDL, which also con-

Fig. 7. Isoelectric focusing polyacrylamide gel electrophoretograms of apoproteins. Left, isolated rat C apoproteins. Middle group (from left), rat VLDL, VLDL remnants, and these remnants incubated with C apoproteins. Right group (from left), lymph small chylomicrons, small chylomicron remnants, and these remnants incubated with C apoproteins. In each group apoproteins derived from lipoproteins containing the same amount of [³H]CE were applied to the gels. Note that the uppermost of the C apoproteins (C-II) is almost lacking in the remnants incubated with C apoproteins and that virtually no apoE remains.

tains apoB-100. We found previously that chylomicrons from estradiol-treated rats are taken up more rapidly by rat livers than rat LDL, even though neither lipoprotein contains appreciable apoE.

B apoproteins from the intestine (B-48) and liver (B-100) have been shown to be distinct not only structurally and genetically (31, 32), but also to differ metabolically. Whereas VLDL containing B-100 may be converted to LDL, B-48 in chylomicrons is not (20, 33). Moreover, B-100, the sole protein of human LDL, binds to LDL receptors, whereas binding to LDL receptors of B-48 in chylomicrons from estrogen-treated rats that contain hardly any apoE has not been demonstrated (29, 34). This observation is consistent with data obtained in Watanabe heritable hyperlipidemic rabbits which virtually lack LDL-receptors and consequently have elevated levels of the apoB-100-containing lipoproteins, VLDL, IDL, and LDL (35). Removal of chylomicrons from blood to liver is normal in these animals (36), indicating a distinct mechanism for the hepatic uptake of B-48-containing lipoproteins. Experiments with chylomicrons that were

freed of apoB-48 by treatment with pronase strongly suggest that apoB-48 itself is not essential for the hepatic catabolism of chylomicron remnants (37). In the case of triglyceride emulsions, it appears that no apoproteins are required for cellular uptake of component lipids (21). It is therefore possible that C apoproteins interfere with the binding of lipids to liver cells, whether associated with B-48 or no apoproteins at all. A role for phospholipids in hepatic uptake of chylomicron remnants has been suggested by Borensztajn and associates (38, 39) who have found that depletion of phospholipids from chylomicrons by treatment with phospholipase A₂ promotes uptake of the particles into hepatocytes. At the present time, the relative importance of the several determinants of uptake of chylomicron remnants into hepatocytes remains to be established, although participation of apoE is strongly indicated (4).

An unexpected observation in the current studies was the almost complete lack of uptake of apoC-II by remnants of chylomicrons and VLDL, incubated with unfractionated C apoproteins. This suggests that the affinity of
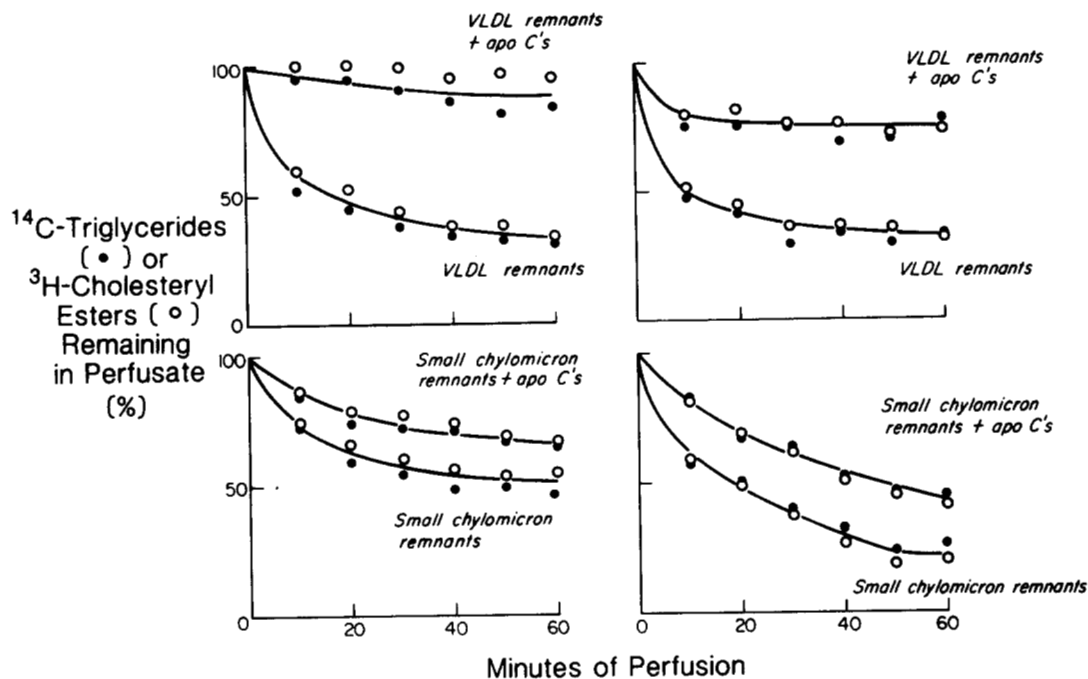
Fig. 8. Removal of [^{14}C]TG and [^{3}H]CE of remnants and remnants that had been incubated with unfractionated rat C apoproteins. Upper left, VLDL remnants (41% TG hydrolysis). Upper right, VLDL remnants (69% hydrolysis). Lower left, chylomicron remnants (41% hydrolysis). Lower right, chylomicron remnants (85% hydrolysis). The amount of remnant protein added to perfusates ranged from 0.11 to 0.26 mg, but was similar for each pair of preparations (control remnants and incubated remnants).

apoC-II for the particle surface is reduced preferentially during hydrolysis of component triglycerides by lipoprotein lipase. The binding of the individual C apoproteins may be determined by different surface components, alterations of which independently regulate the termination of TG-hydrolysis. Loss of apoC-II before hydrolysis is complete could be responsible for the uptake of appreciable TG by the liver with the remnant particles. Continuing research has related these changes in the affinity of remnants for different C apoproteins to changes in phospholipid composition (Windler, E. E. T., S. Preyer, and H. Greten, unpublished observations). ∎

REFERENCES

1. Havel, R. J. 1985. Role of the liver in lipoprotein catabolism. *Methods Enzymol.* In press.
2. Windler, E. E. T., P. T. Kovanen, Y-s. Chao, M. S. Brown, R. J. Havel, and J. L. Goldstein. 1980. The estradiol-stimulated lipoprotein receptor of rat liver: a binding site that mediates the uptake of rat lipoproteins containing apoproteins B and E. *J. Biol. Chem.* **255:** 10464–10471.
3. 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.
4. 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.
5. Windler, E., and R. J. Havel. 1981. Einfluss von apoprotein E und C-apoproteinen auf die aufnahme triglyzerideicher lipoproteine und deren remnants durch die rattenleber. *Verh. Dtsch. Ges. Inn. Med.* **87:** 893–895.
6. Chao, Y-s., E. Windler, G. C. Chen, and R. J. Havel. 1979. Hepatic catabolism of rat and human lipoproteins in rats treated with 17α-ethinyl estradiol. *J. Biol. Chem.* **254:** 11360–11366.
7. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244:** 5687–5694.
8. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Radioimmunoassay of arginine-rich apolipoprotein of rat serum. *Biochim. Biophys. Acta.* **490:** 144–155.
9. Krebs, H. A., and K. Henseleit. 1932. Untersuchungen uber die Harnstoffbildung im Tierkorper. *Hoppe-Seyler's Z. Physiol. Chem.* **210:** 33–66.
10. Pagnan, A., R. J. Havel, J. P. Kane, and L. Kotite. 1977. Characterization of human very low density lipoproteins containing two electrophoretic populations: double pre-beta lipoproteinemia and primary dysbetalipoproteinemia. *J. Lipid Res.* **18:** 613–622.
11. Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry.* **12:** 502–507.
12. Polz, E., L. Kotite, R. J. Havel, J. P. Kane, and T. Sata. 1980. Human apolipoprotein C-I: concentration in blood

serum and lipoproteins. *Biochem. Med.* **24:** 225–237.

13. Spackman, D. H. 1967. Accelerated methods. *Methods Enzymol.* **11:** 3–15.

14. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins in human serum. *J. Clin. Invest.* **56:** 1622–1634.

15. Kane, J. P. 1977. Serum lipoproteins: structure and metabolism. *In* Lipid Metabolism in Mammals. F. Snyder, editor. Plenum Press, New York. 209–257.

16. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.

17. Rush, R., L. Leon, and J. Turrell. 1970. Automated simultaneous cholesterol and triglyceride determinations on the AutoAnalyzer II Instrument. Advances in Automated Analysis—Technicon International Congress. **1:** 503–507.

18. Faergeman, O., and R. J. Havel. 1975. Metabolism of cholesterol esters of rat very low density lipoproteins. *J. Clin. Invest.* **55:** 1210–1218.

19. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. *J. Clin. Invest.* **56:** 1481–1490.

20. Stalenhoef, A. F. H., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.

21. 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.

22. Quarfordt, S. H., G. Michalopoulos, and B. Schirmer. 1982. The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. *J. Biol. Chem.* **257:** 14642–14647.

23. Rall, S. C., K. H. Weisgraber, T. L. Innerarity, and R. W. Mahley. 1982. Structural bases for receptor binding heterogeneity of apolipoprotein E from type III hyperlipoproteinemic subjects. *Proc. Natl. Acad. Sci. USA.* **79:** 4696–4700.

24. Havel, R. J. 1982. Familial dysbetalipoproteinemia: new aspects of pathogenesis and diagnosis. *Med. Clin. N. Am.* **66:** 441–454.

25. Havel, R. J., Y-s. Chao, E. E. Windler, L. Kotite, and L. S. S. Guo. 1980. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **77:** 4349–4353.

26. Schneider, W. J., P. T. Kovanen, M. S. Brown, J. L. Goldstein, G. Utermann, W. Weber, R. J. Havel, L. Kotite, J. P. Kane, T. L. Innerarity, and R. W. Mahley. 1981. Familial dysbetalipoproteinemia. Abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits, and cows. *J. Clin. Invest.* **68:** 1075–1085.

27. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. *Biochem. Med.* **17:** 347–355.

28. 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.

29. van't Hooft, F., and R. J. Havel. 1982. Metabolism of apolipoprotein E in plasma high density lipoproteins from normal and cholesterol-fed rats. *J. Biol. Chem.* **257:** 10996–11001.

30. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1984. Defective hepatic lipoprotein receptor binding of beta-very low density lipoproteins from type III hyperlipoproteinemic patients. Importance of apolipoprotein E. *J. Biol. Chem.* **259:** 860–869.

31. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2468.

32. Malloy, M. J., J. P. Kane, D. A. Hardman, and R. L. Hamilton. 1981. Normotriglyceridemic abetalipoproteinemia. *J. Clin. Invest.* **67:** 1441–1450.

33. van't Hooft, F., and R. J. Havel. 1982. Apolipoprotein B (B-48) of rat chylomicrons is not a precursor of the apolipoprotein of low density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **79:** 179–182.

34. Havel, R. J. 1983. Metabolism of triglyceride-rich lipoproteins. *In* Proceedings of the VI International Symposium on Atherosclerosis. G. Schettler and G. Middelhoff, editors. Springer Verlag, Berlin. 480–488.

35. Havel, R. J., T. Kita, L. Kotite, J. P. Kane, R. L. Hamilton, J. L. Goldstein, and M. S. Brown. 1982. Concentration and composition of lipoproteins in blood plasma of the WHHL rabbit, an animal model of human familial hypercholesterolemia. *Arteriosclerosis.* **2:** 467–474.

36. Kita, T., J. L. Goldstein, M. S. Brown, Y. Watanabe, C. A. Hornick, and R. J. Havel. 1982. Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **79:** 3623–3627.

37. Borensztajn, J., G. S. Getz, R. J. Padley, and T. J. Kotlar. 1982. The apoprotein B-independent hepatic uptake of chylomicron remnants. *Biochem. J.* **204:** 609–612.

38. Borensztajn, J., T. J. Kotlar, and B. J. McNeill. 1980. Uptake of phospholipid-depleted chylomicrons by the perfused rat liver. *Biochem. J.* **192:** 845–851.

39. Borensztajn, J., and T. J. Kotlar. 1981. Hepatic uptake of phospholipid-depleted chylomicrons *in vivo. Biochem. J.* **200:** 547–553.