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Characterization and Quantitation of the Apolipoproteins from Human Chyle Chylomicrons*

G. Kostner and A. Holasek

ABSTRACT: The composition of human chyle chylomicrons was studied. For this purpose, thoracic duct chylomicrons from a female subject were isolated, delipidized, and the lipid and protein part investigated separately. For the characterization of the protein part, polyacrylamide gel electrophoresis, immunoelectrophoresis, and immunodiffusion were performed. Pure peptides isolated from human serum lipoproteins were used as reference substances. Monospecific antibodies were used to check the identity of chylomicron peptides. In order to quantitate the amount of different constituents, a densitometric scan after separation in polyacrylamide gels and staining with Amido-Schwarz and coomassie blue were performed. In other experiments, the peptides were isolated using gel permeation and ion-exchange chromatography and amino acid analysis of the fractions. The quantitation of the peptides in these experiments was performed gravimetrically. The results indicate that all the peptides of human serum very low density lipoproteins were present in chyle chylomicrons too. The major part consists of apolipoprotein C (R-Val, R-Glu, R-Ala_{1+2}). About 20% apolipoprotein B were found and the amount of apolipoprotein A peptides was about 15%. From the presence of apolipoprotein AI and apolipoprotein AII in almost equal concentration it was concluded, that the apolipoprotein A content is not due to an adsorption of lipoprotein A on to the chylomicron particle from the lipoproteins of the blood.

Chylomicrons are triglyceride-rich lipoproteins of diameter >800 Å, found in intestinal lymph after absorption of dietary fat (Havel, 1970). There is evidence that all classes of lipoproteins can be synthesized in the mucosal cells of the small intestine (Alaupovic *et al.*, 1967; Hatch, 1966; Roheim *et al.*, 1966). Little is known of the structure and composition of human lymph lipoproteins with the exception of the chylomicrons. Since chylomicrons contain a small and variable amount of protein (less than 2%), investigations have concentrated upon the lipid composition. There is not complete agreement about amount, kind, and function of the protein

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part of chylomicrons. The amount of protein found in washed chylomicrons depended upon whether serum or lymph was used for the isolation (Lossow *et al.*, 1967). The values for serum chylomicrons were higher because of the adsorption of serum proteins. Further variation results from the average hydrated density of the fraction itself (Gustafson *et al.*, 1965).

Paper electrophoresis and N-terminal studies led to the conclusion that three different proteins constitute the protein moiety from chylomicrons (Rodbell and Fredrickson, 1959). One of these proteins was found to be identical with apo-HDL:[1] another, apo-LP-B, was identified in chylomicrons using immunochemical methods (Lees, 1967).

The most recent and detailed investigation concerning the structure of chyle chylomicrons was performed by Alaupovic *et al.* (1968), who found evidence of apolipoproteins A and B present in chylomicrons with $S_f > 5000$. They also identified in postprandial lymph, the third constituent as apo-LP-C. Since then a great deal of investigation has been done, to characterize different peptides of human serum lipoproteins. Two major peptides were found in HDL (Shore and Shore, 1968; Scanu *et al.*, 1969) and in VLDL besides LP-A and LP-B peptides, three others could be identified (Brown *et al.*, 1969, 1970).

With respect to the above findings, a reinvestigation of the peptide content and distribution of human chyle chylomicrons seemed to be necessary. The peptides were characterized by polyacrylamide gel electrophoresis, immunochemical methods, and amino acid composition and an attempt was made to quantitate the weight distribution.

Material and Methods

Isolation of Human Chyle Chylomicrons. Human thoracic duct chyle was obtained by cannulation with a polyethylene tube, of the thoracic duct *via* the pleural cavity of a 48-year-old female subject. In Agarose gel electrophoresis, a completely normal pattern of serum lipoproteins was found and the values for serum lipids of the patient too were within the range of normals for this age and sex. Samples were withdrawn between 3 and 8 hr after a fat-rich meal and allowed to clot at 4°. The supernatant was removed by decantation and 0.1 mg of Na_2EDTA and 1 mg of sodium azide were added per ml. To reduce the volume, chyle from several drawings was mixed and dialyzed in Visking tubing against solid polyethylene glycol (mol wt 20,000) until a fourfold concentration was reached. Chylomicrons were isolated in a Beckman L4 preparative ultracentrifuge using the 50.1 fixed-angle rotor at a speed of 25,000 rpm for 900,000g × min. The upper creamy layer was removed with a spatula, resuspended in the original volume of 0.15 M NaCl solution adjusted to pH of 7.4 with NaOH, and recentrifuged at 900,000g × min. The wash procedure was repeated five to six times to yield a chylomicron fraction free of immunochemically detectable albumin or other serum proteins, with a flotation rate $S_f > 400$.

Delipidation of Chylomicrons. After the final wash, the chylomicrons were resuspended in distilled water, dialyzed for 2 days against several changes of distilled water at 4°, and finally lyophilized. The lipids were extracted in several steps with diethyl ether, followed by extractions with ethanol–ether (3:2, v/v), and finally again with diethyl ether. The temperature was not allowed to rise above 4°. The amount of solvent necessary for the total delipidization of 1 g of chylomicrons was about 1 l. of diethyl ether and 300 ml of ethanol–ether (3:2, v/v). The progress of the delipidization was checked by thin-layer chromatography of the remaining material. The phospholipid content of the final material was less than 0.2%. The protein part isolated as described was completely soluble in 2 M acetic acid or in phosphate buffer (pH 7.2) containing 8 M urea. Occasionally in one or the other preparation, a varying amount of material remained insoluble. These batches were not used for quantitation of the peptide distribution.

Analytical Methods. For the lipid analysis a separate sample of lyophilized chylomicrons was extracted according to Folch *et al.* (1957). The free and total cholesterol was determined according to Michaelis *et al.* (1958) and the triglycerides according to Eggstein and Kreutz (1966). For the analysis of different phospholipids thin-layer chromatography was employed using the solvent system chloroform–methanol–acetic acid–water (50:30:8:4, v/v). Phosphorus was assayed by the method of Gerlach and Deuticke (1963). The lipid:protein ratio was determined gravimetrically after extraction of a separate sample according to Folch *et al.* (1957).

Polyacrylamide gel electrophoresis was performed in glass tubes, 5-mm i.d. and 8 cm long. Tubes were filled to 6 cm with a 10% acrylamide solution (pH 9.2) and 8 M with respect to urea (Ornstein, 1964). The stacking gel (0.8 cm) was added and 100–300 μg of protein in 0.5-ml loading gel was applied on each tube. Electrophoresis was carried out at a current of 4 mA/tube for 60–80 min and was stopped when the reference dye (bromophenol blue) reached the bottom of the tube. Gels were stained in 0.5% (w/v) Amido-Schwarz 10B in 7% (v/v) acetic acid for 0.5 hr and destained by shaking the gels in several changes of 7% acetic acid at room temperature. In control experiments, gels were fixed in 10% trichloroacetic acid and stained with coomassie blue (Chrambach *et al.*, 1967). Densitometric recording of stained gels was performed with a Beckman Analytrol, modified in our own laboratory.

Immunoelectrophoretic and immunodiffusion experiments as well as agar gel electrophoresis were performed in 1% agar or Agarose gel in barbital buffer (pH 8.2), ionic strength 0.05. Pure peptides, isolated from human serum lipoproteins, were used as standards. Monospecific and polyvalent antibodies were prepared by immunizing rabbits, sheeps, and goats. The exact procedure for the isolation of pure peptides and preparation of antisera is described in a separate publication (G. Kostner and P. Alaupovic, in preparation). For the separation of the peptides from the delipidized chylomicrons, a combined column chromatography using Sephadex G-100 followed by DEAE-cellulose was carried out. In the first step, 50 mg of material in 5 ml of 2 M acetic acid was applied on a LKB column (2.5 × 100 cm) filled with Sephadex G-100 in 2 M acetic acid. The flow rate was about 40 ml/hr. Fractions of 4 ml were collected and their absorbance monitored at 280 mμ. Fractions belonging to each peak were combined and lyophilized. For further separation of these fractions, ion-exchange chromatography was carried out. DEAE-cellulose (Cellex D Bio-Rad, 0.39-mequiv capacity) was washed sequentially with 1 M HCl–1 M NaOH and distilled water. Finally the material was equilibrated with 0.005 M sodium phosphate buffer (pH 7.2), containing 8 M

[1] Abbreviations used are: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo-LP-A, protein part of lipoprotein A; apo-LP-B, protein part of lipoprotein B; apo-LP-C, protein part of lipoprotein C.

TABLE I: Chemical Composition of Human Lymph Cyhlomicrons: Mean Values and Standard Deviation from Duplicates of Two Different Chylomicron Preparations from the Same Subject.

Protein	Glycerides	Free Cholesterol	Cholesterol Ester	Phospholipids
% ±Std Dev	% ±Std Dev	% ±Std Dev	% ±Std Dev	% ±Std Dev
1.5 ± 0.2	86.4 ± 2.4	1.6 ± 0.2	1.4 ± 0.1	8.6 ± 1.1

Lysolecithin	2.5 ± 0.4
Sphinogmyelin	11.9 ± 2.2
Lecithin	69.1 ± 3.1
Phosphatidylethanolamine	13.8 ± 2.0
Phosphatidylserine + inositol	1.3 ± 0.3

urea (starting buffer), and packed in a column of 1.4 × 30 cm. Protein (20–40 mg) was solubilized in starting buffer and applied on the column. To elute the sample, a salt gradient was prepared by pumping 500 ml of starting buffer containing 2.4 g of NaCl into a mixing reservior filled with 400 ml of starting buffer and connected to the column. The fractions from the column were treated in the same way as the eluate from the Sephadex column except that exhaustive dialysis against 0.05 M ammonium carbonate preceded the lyophilization.

Amino Acid Analysis. Samples obtained from bands in polyacrylamide gel and column chromatographic separations were rechromatographed and hydrolyzed in constant boiling HCl under vacuum at 110° for 24 and 72 hr. Norleucine was used as internal standard. The amino acid loss was corrected after extrapolation to zero time. Cysteine was determined as cysteic acid after performic acid oxidation prior to hydrolysis. The analysis was performed in a Bio-Cal BC 200 amino acid analyzer with automatic sample injector. The resin Aminex A-G from Bio-Rad was used in a one column program described in the instruction manual. Tryptophan was determined by the method of Spies and Chambers (1949). Analytical ultracentrifugations were carried out in a Beckman–Spinco Model E.

Results

The purity of isolated chylomicrons was checked by immunoelectrophoresis, agar gel electrophoresis, and analytical ultracentrifugation under conditions which would have detected more than 1% contaminations of other chyle proteins or lipoproteins. In immunoelectrophoresis no precipitine line could be detected using anti-human serum; when antisera to LP-B or LP-A were used, no precipitin arc in the α or β region was visible. By performing lipoprotein electrophoresis in Agarose gel, the whole material remained at the start and no band was present in the pre-β-region, indicating the absence of VLDL. In the analytical ultracentrifuge at a solution density of 1.0630, all the material was floated and accumulated at the top of the cell at zero time after reaching the speed of 52,000 rpm and no sedimenting peak could be detected during a 1-hr run. From this experiment it was concluded that no lipoprotein with a S_f lower than 400 was present in the preparation (Jensen *et al.*, 1969).

Table I shows the chemical composition of the human chyle chylomicrons. The values are in fair agreement with those found by other investigators (Wood *et al.*, 1964; Gustafson *et al.*, 1965) except that a relative high concentration of phosphatidylethanolamine was present.

After delipidation with ethanol–ether and evaporation of the combined solvent extracts, the lipid part of the chylomicrons was resolubilized in chloroform–methanol (2:1, v/v) and several washes according to Folch *et al.* (1957) were performed. The aqueous phases were dialyzed against 0.05 M ammonium carbonate and lyophilized. This material represented up to 4% (by weight) of the total chylomicron protein. These values are not included in Table I because of the contamination of an unknown amount of phospholipids (found on thin-layer chromatography). The Folch extracts were tested by polyacrylamide gel electrophoresis and a quantitation was made by scanning the gels. It was found that the kind and the distribution of peptides was essentially the same as in the protein residue after ethanol ether delipidation except that no apo-LP-B and very little apo-AI was present.

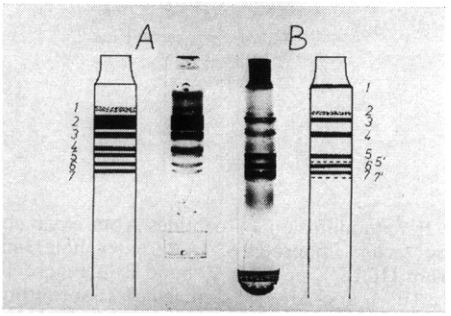

FIGURE 1: Gel electrophoresis in 10% polyacrylamide, containing 8 M urea. (A) Apo-HDL_3 from human serum. (B) Protein part of lymph chylomicrons; anode on bottom; staining with coomassie blue.

TABLE II: Peptide Content and Position in 10% Polyacrylamide Gel of Human Serum HDL.

Band No.[a]	Peptide	Reference
1	R-Val	Brown *et al.* (1969).
2	Apo-AI	Kostner and Alaupovic (1971)
3	Apo-AII	Kostner and Alaupovic (1971)
4	R-X	
5	R-Glu	Brown *et al.* (1969)
6	$R\text{-}Ala_1$	Brown *et al.* (1969)
7	$R\text{-}Ala_2$	Brown *et al.* (1969)

[a] In Figure 1A.

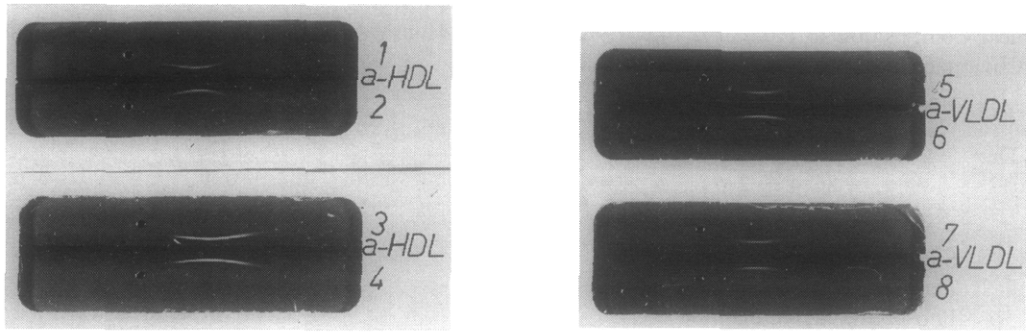

FIGURE 2: Immunoelectrophoretic pattern of apolipoproteins from chyle chylomicrons (material from polyacrylamide gel) compared with peptides isolated from human serum VLDL and HDL by column chromatography, in 1% Agarose gels. a-HDL, antiserum to human serum HDL from rabbit. a-VLDL, antiserum to human serum VLDL from rabbit. 1, apo-AI; 2, band 3 in Figure 1B; 3, apo-AII; 4, band 4 in Figure 1B; 5, R-Ala$_1$; 6; band 6 in Figure 1B; 7, R-Glu, 8, band 5 in Figure 1B,

In Figure 1, the pattern of the protein part of chylomicrons is compared with apo-HDL from human serum. In order to get also faint bands on the picture, a much higher concentration of solubilized protein was applied to the gels used for photography than to gels used for scanning. Figure 1B therefore shows material in the spacer gel stainable with coomassie blue. In gels used for scanning, only a minor staining of the spacer gels was observed. This material proved to be predominantly apo-LpB by amino acid composition (a few per cent of the amount found by densitometry). In previous experiments (G. Kostner and P. Alaupovic, 1971) the peptides of apo-HDL were isolated and characterized by amino acid composition and determination of N- and C-terminal amino acid. Together with the results of other investigators (Shore and Shore, 1968; Scanu *et al.*, 1969; Brown *et al.*, 1970), the following correlations between bands and peptides were found (Table II).

To characterize the protein part of chylomicrons, a separation in polyacrylamide gel was carried out, the bands were cut out, and immunoelectrophoresis and immunodiffusion

TABLE III: Amino Acid Composition[a] (moles/10^5 g) of Polypeptides Isolated from Human Lymph Chylomicrons.[b]

Amino Acid	Bands in Polyacrylamide Gel				
	1	2	3	4	6 + 7
Lys	45	141	54	84	73
His	14	Trace	14	0	11
Arg	18	39	41	0	26
Asp	81	74	63	39	74
Thr	58	43	30	58	52
Ser	69	111	46	54	108
Glu	110	151	145	175	111
Pro	35	12	37	48	28
Gly	41	17	32	53	38
Ala	52	43	61	54	110
$^1/_2$-Cys	5	0	0	11	0
Val	36	25	38	57	61
Met	14	10	8	11	22
Ile	37	35	0	12	0
Leu	83	88	107	87	52
Tyr	22	0	21	36	19
Phe	35	48	19	38	42
Trp[c]	4	16	16	0	31

[a] Values for Ser, Thr, and Tyr were obtained by linear extrapolation of recoveries from 24- and 72-hr hydrolysis. [b] Bands represent the position in 10% polyacrylamide gel containing 8 M urea as shown in Figure 1B. Values are means of two analyses. Amino acid content of band 5 was not determined. [c] Determined by the method of Spies and Chambers (1949).

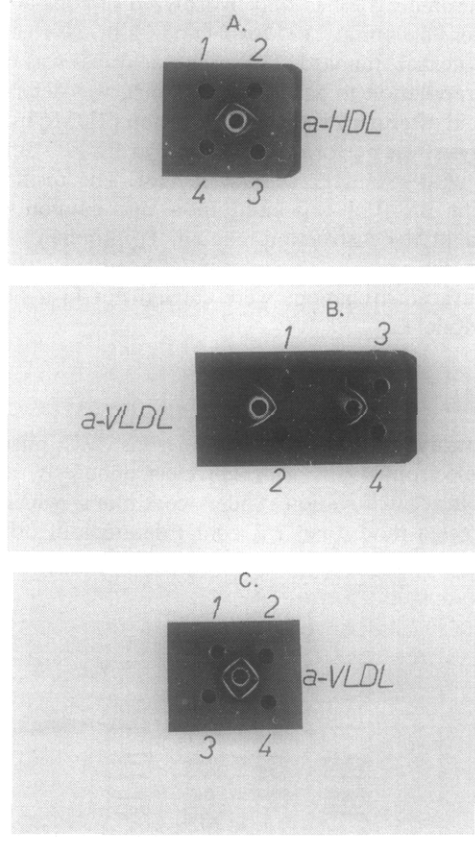

FIGURE 3: Immunodiffusion of peptides from chyle chylomicrons and human serum lipoproteins. (A) Center hole: antiserum to human serum HDL. 1, apo-AI; 2, band 3 in Figure 1B; 3, band 4 in Figure 1B, 4, apo-AII. (B) Center holes: antiserum to human serum VLDL from rabbit. 1; R-Val from serum VLDL; 2, material from peak 1 in Figure 5; 3, R-Glu from serum VLDL; 4, band 5 in Figure 1B. (C) Center hole: antiserum to serum VLDL from rabbit. 1, R-Ala$_1$ from serum VLDL; 2, R-Ala$_2$ from serum VLDL; 3, band 7 in Figure 1B; 4, band 6 in Figure 1B.

TABLE IV: Peptide Content of Human Chyle Chylomicrons Calculated from Scan of Polyacrylamide Gels and Weight Distribution after Separation by Column Chromatography on Sephadex G-100 and DEAE-cellulose.

Band No.[a]	Peptide	Scan of Gels[b] (% ±Std Dev)	Separation[c] on Sephadex (%)	Separation[c,d] on DEAE-cellulose (%)
1	Apo-LP-B	18.9 ± 1.3	22.5	
2	R-Val	11.3 ± 0.9		15.2
3	Apo-AI	6.8 ± 0.7	7.4	
4	Apo-AII	4.9 ± 0.6	4.2	
5	R-Glu	14.6 ± 0.9		15.1
5′	Nd	2.0 ± 0.4		
6	R-Ala_1	19.9 ± 1.2		20.2
7	R-Ala_2	21.0 ± 1.3		16.9
7′	Nd	2.0 ± 0.2		
2,5,5′,6,7,7′,	Apo LP-C	70.8 ± 4.6	65.9	

[a] In Figure 1B. [b] Mean plus and minus standard deviation from six different scans. [c] Mean of two separations. [d] These values were calculated assuming 68% of the chylomicron protein consists of apo-LP-C.

experiments using pure peptide standards and monospecific and polyvalent antisera were performed. The position of the bands was found after soaking the gels for a few minutes in 5% trichloroacetic acid and illuminating with diffuse light. Figures 2 and 3 shows the immunochemical identity of different peptides from chyle chylomicrons with peptides isolated from human serum lipoproteins by gel permeation and ion-exchange chromatography. In control experiments the whole gels were soaked in a few milliliters of monospecific antiserum and shaken until a ring of precipitate was detected. Since apo-LP-B gave no immunochemical reaction after total delipidization, the bands between stacking and separating gel from several separations were cut out and amino acid analysis performed after elution with 0.1 N NaOH and dialysis against distilled water. From immunochemical identity reactions with pure peptides, positive immunoprecipitation with monospecific antisera, comparison of the position in the PAG with that of pure standards, and finally the amino acid composition shown in Table III, the following identity of lymph chylomicron peptides with those found in VLDL and HDL of the human serum was concluded. The results are summarized in Table IV. With the exception of band 4 (Figure 1A) all peptides from apo-HDL_3 were present in chylomicrons too.

For the determination of the relative concentration of the different chylomicron peptides, the gels were scanned in a densitometer after staining with Amido-Schwarz 10B and coomassie blue. The values in Table IV are mean values from six different runs and two different preparations. Band 5′ and 7′ have not been isolated in pure form or described in the literature so far.

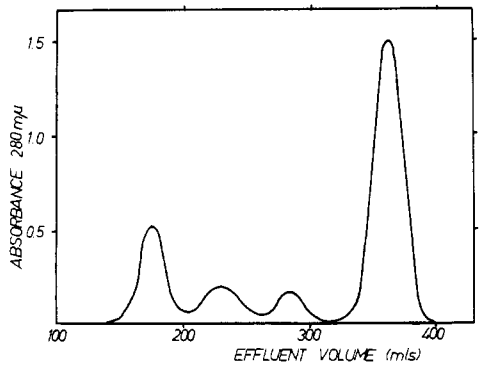

FIGURE 4: Chromatographic separation of 50 mg of lipid-free protein of lymph chylomicrons on a Sephadex G-100 column (2.5 × 100 cm) in 2 M acetic acid at room temperature. Flow rate 40 ml/hr.

In another experiment, a preparative separation of the peptides was carried out. The elution pattern of 50 mg of chylomicron protein from the 1-m Sephadex G-100 column can be seen in Figure 4. After lyophilization of the material from peaks 1–4, the weight distribution was determined gravimetrically. The identification of the components from each peak was made in the same way as described above by disc electrophoresis, immunochemical methods, and determination of the amino acid composition. The results are summarized in Table IV. On Sephadex, a separation of the apo LP-C peptides (R-Val, R-Glu, R-Ala_1, and R-Ala_2) cannot be obtained and R-Val does not give a sharp single band on polyacrylamide gel electrophoresis, therefore a subfractionation of peak 4 from the Sephadex experiment was performed on a DEAE-cellulose column. The elution pattern is shown in Figure 5. After dialysis and lyophilization of the material belonging to one peak, each fraction was passed through a small column (2 × 40 cm) filled with Sephadex G-25 in 0.05 M ammonium carbonate for the removal of salt impurities, and finally lyophilized. The evaluation of the weight distribution was made as before (see Table IV for results). Peak one represented pure R-Val while R-Glu, R-Ala_1, and R-Ala_2 from peak 2, 3, and 4, respectively, could not be received completely immunochemically pure after one chromatographic step. From pattern in polyacrylamide gel electrophoresis the impurities were estimated as 5–10%. In Figure 6, the material from peak one of the DEAE-cellulose experiment is compared with peak four (apo-LP-C) from Sephadex column.

Discussion

Most if not all circulating chylomicrons are synthesized by the small intestine. In addition to chylomicrons, Fredrickson *et al.* (1967) found particles with an S_f 400 in the intestinal and thoracic duct lymph. The VLDL in the serum represent a mixture of exogenous VLDL derived from the lymph, remnants of

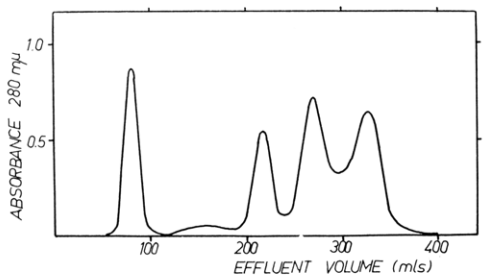

FIGURE 5: Chromatographic separation of 60 mg of apo-LP-C (material from peak 4 in Figure 4) on a DEAE-cellulose column (1.4 × 30 cm) equilibrated with 0.005 M sodium phosphate buffer (pH 7.2) in 8 M urea. Elution was performed with a linear salt gradient.

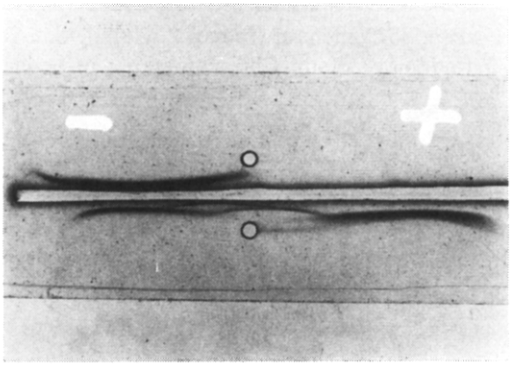

FIGURE 6: Immunoelectrophoresis of R-Val (material from peak 1 in Figure 5) compared with apo-LP-C (material from peak 4 in Figure 4); 1% agar gel in 0.05 M barbital buffer (pH 8.2); staining, Amido-Schwarz 10B; top hole, R-Val; bottom hole, apo-LP-C; anti-LP-C from rabbit.

chylomicrons after the action of lipoprotein lipase, and endogenous VLDL synthesized in the liver. Because of the small concentration of VLDL in the normal fasting plasma, most of the studies dealing with the characterization of the protein moiety of VLDL or chylomicrons have been carried out with postprandial serum or serum from patients with hyperlipoproteinemia. Hence a mixture of primary and secondary particles was always investigated. Since the cannulation of the ductus intestinalis in humans is a rather complicated procedure and of no therapeutical interest, thoracic duct lymph, a mixture of lymph derived from the intestine and the liver, was obtained for the investigation. Since it has been reported, that the migration rate of lymph VLDL changes in Agarose gel electrophoresis when mixed with serum from the same animal (Ockner *et al.*, 1969), it was of interest to evaluate a possible difference in the peptide composition of lymph chylomicrons and serum VLDL.

For a long time there was a considerable disagreement about origin and function of the protein part of VLDL and chylomicrons. Some authors showed, that isolated washed chylomicrons were able to adsorb proteins during incubation with human serum (Lossow *et al.*, 1967). The ideas concerning the function of the peptides are still speculatory. One part of the protein moiety certainly is necessary for the structure and stability of the chylomicron particle. Another part of the protein moiety serves as a mediator for the action of lipases, transferases, and phospholipases (Schumaker, 1970). Most recent studies suggest, that some of the apo-LP-C peptides isolated from human serum lipoproteins were responsible for the activation of artificial triglyceride emulsions making them accessible for the action of lipoprotein lipase (Havel *et al.*, 1970; La Rosa *et al.*, 1970).

The densitometric quantitation of a protein mixture after electrophoretic separation depends upon several parameters. One of the major source of error is the staining procedure. Since the dye uptake varies from protein to protein, no absolute values can be obtained and the determination remains semiquantitative. This is the reason why a preparative separation of the chylomicron peptides was performed. It is clear that a complete separation of a complex mixture of peptides cannot be achieved in one chromatographic step. In the Sephadex experiment a good separation of apo-LP-A, apo-LP-B, and apo-LP-C could be obtained, but for further subfractionation of apo-LP-C peptides, a second chromatography on DEAE-cellulose was necessary. This gave an excellent separation of R-Val from all the other peptides, while an overlapping of the peaks containing R-Glu and R-Ala was observed. A separation of these peptides from the small amount, of as yet unidentified constituents (band 5′ and 7′ in Figure 1), was also impossible. Since this minor band together with R-Glu and R-Ala_{1+2} are very well separated on polyacrylamide gel electrophoresis, the values from this experiment seem to be more reliable. The R-Val, on the other hand, shows at least after separation on DEAE-cellulose a broad, not uniform band. The gravimetric determinatin of this peptide is therefore preferable.

Results of this investigation indicate, that the two apo-LP-A peptides apo-AI and apo-AII are present in chylomicrons in almost the same amount while in apo-HDL_2 a molar ratio of 3:1 and in apo-HDL_3 of 2:1 was found (Kostner and Alaupovic, 1971). Unfortunately, comparable values for human serum VLDL have not been published. Taking these values into account, it seems unlikely that the apo-LP-A content of the chylomicrons is a consequence of an adsorption of LP-A on the chylomicron particle proposed by other investigators.

In addition to the known peptides of the human serum apolipoproteins, at least two peptides were found in lymph chylomicrons (band 5′ and 7′). Band 5′ may be identical with the apolipoprotein Ala_0 isolated by Albers and Scanu (1971) using isoelectric focusing. The presence of these peptides is not characteristic of lymph chylomicrons since they could be detected in VLDL and HDL from human serum in comparable concentrations.

The identity of the chylomicron peptides seems to be well established by the methods used in this study. Since apo-LP-B did not react with anti-β-lipoprotein antisera, the identity was concluded by comparing the amino acid content with serum LP-B. A further support for the presence of LP-B peptides in chylomicrons is the finding, that two rabbits showed a strong titer of anti-LP-B antibodies after short time immunization with intact chylomicrons.

Most of the preparations of chylomicron protein were completely soluble in the buffers used for the separation of peptides. After column chromatography on Sephadex G-100 and lyophilization, the material from the first peak became insoluble in 2 M acetic acid or in urea-containing phosphate buffer. It seems therefore likely that the relative high concentration of of apo-LP-C peptides in the unfractionated preparation is responsible for the complete solubility of apo-LP-B without using detergents, which cannot be observed with apo-LP-B isolated from human serum LDL.

It has been reported from other investigators that a certain amount of protein remains in the organic phase during the delipidation (Scanu, 1966). This is mainly the case if

delipidation is made with lipoprotein solutions instead of lyophilized material (unpublished observation). Therefore an extraction according to Folch of the chylomicron lipids were performed. A small amount of protein was found with essentially the same peptide distribution than in the ethanol–ether residue. However it cannot be excluded that a small amount of nonextractable peptides remains in the lipid part even after this procedure and escapes the detection. The finding, that chyle chylomicrons carry the same peptides as the lipoproteins from serum establishes, that the protein part of the chylomicrons is not due to a nonspecific adsorption of proteins on entering the blood stream. The data of this and other investigations indicate, that lymph chylomicrons possess a distinct and characteristic protein moiety consisting of apo-LP-A, apo-LP-B, and apo-LP-C peptides.

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