Table 1. Effect of BSA–anti-BSA supernates on smooth muscle of guinea pig.

Antigen N added per milliliter of serum (mg)	Total ppt of N (mg per ml of serum)	Tests on supernates		
		Excess antigen or antibody	Schultz-Dale (% of max.)*	Skin reaction
7.3465	0.037	Antigen	3	++
5.8772	0.037	Antigen	8	+
2.9386	0.048	Antigen	23	++
1.4693	0.048	Antigen	46	+++
0.7347	0.135	Antigen	63	+++
0.4898	0.346	Antigen	43	+++
0.3673	0.500	Antigen	19	+++
0.2939	0.663	Antigen	19	+
0.1959	0.867	Antigen	11	±
0.1469	0.982	Antigen	9	±
0.0735	0.750	Antibody	7	+
0.0368	0.515	Antibody	8	++
0.0184	0.263	Antibody	0	+
0.0092	0.140	Antibody	0	+
0.0046	0.067	Antibody	0	+
0.0023	0.037	Antibody	0	+
0.0012	0.020	Antibody	0	+
Antiserum control	0.00	Antibody	0	−
Histamine (1.0 μg)			100	++++
1% NaCl				−

* Based on the reaction to 5 μg of histamine as 100 percent. The values shown are the average for six strips of ileum.

the supernatant solutions. Control tests were also made on bovine serum albumin solutions alone and on mixtures of bovine serum albumin and normal serum. In no instance did these control systems cause any smooth-muscle contraction. The supernates were also tested for their skin reactivity by intradermal injection into the skin of normal guinea pigs which had previously received an intravenous injection of Evans blue (Table 1).

The data (Fig. 1) showed that the principal activity of the supernates occurred in the range of slight-to-moderate antigen excess. The stimulating action rapidly decreased to zero in antibody excess and nearly so in extreme antigen excess. It was noted that there was a time lag of from 30 to 90 seconds between the addition of the test solution and the contraction of the muscle. This lag was much greater than that ordinarily seen with histamine or when antigen is added to sensitized tissue, and this suggests that a different mechanism may be involved. All tissues which were allowed to react to an initial exposure of supernate, and which were then washed and exposed again, failed to contract; this is suggestive of an immune reaction. This of course is similar to typical desensitization of muscle from a sensitized animal in that the muscle becomes refractory to antigen after the primary exposure. Further studies must be made before the real significance of soluble antigen-antibody complexes in hypersensitivity reactions is understood. For example, the problem arises whether soluble complexes in excess antibody (that is, toxin-antitoxin systems) would give reactions, and whether the failure to obtain reactions in cases of extreme antigen excess is due to an inhibitory effect of free antigen or to the nature of the complexes (see Singer and Campbell, 7). The same reaction can be obtained with soluble complexes formed by dissolving carefully washed precipitates in excess antigen. However, no study has been made of solubilized precipitates formed in a decomplemented serum.

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* Postdoctoral fellow of the National Heart Institute, National Institutes of Health, U.S. Public Health Service.

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N-Terminal Amino Acid and Lipid Composition of Lipoproteins from Chyle and Plasma

One of the major theories of fat transport is that lipoproteins rich in triglycerides (S_f 20–400 and chylomicrons) are transformed into higher density lipoproteins by the successive removal of triglycerides (*1*). Two primary problems arising from this hypothesis are the relationships between two of the major classes of lipoproteins found in human plasma [*D*, 1.006–1.063 (S_f 0–20) and *D*, 1.063–1.21 (HDL)] to each other and to the lipoproteins of density less than 1.006 which appear to be the principal vehicles for triglyceride transport. If any classes of lipoproteins are interconvertible then they must contain identical protein moieties and differ only by reason of their lipid constituents. The amino acid end group analyses of Avigan *et al.* (*2*) and Shore (*3*) and studies utilizing labeled lipoproteins have demonstrated conclusively that the predominant components of two classes, *D*, 1.019–1.063 and *D*, 1.063–1.21 are not interconvertible. These results exclude the possibility that all of the lipoproteins of human plasma represent a continuous spectrum containing a common protein. However, the relationship of these lipoproteins to those having densities less than 1.006 (chylomicrons and S_f 20–400) has not been reported.

This paper contains the results of N-terminal amino acid analyses of the protein moieties of the complete spectrum of lipoproteins from the chylomicrons of chyle to the highest density lipoproteins (*D*, 1.063–1.21) in plasma.

Chylomicrons from chyle were obtained from a patient with a chylothorax associated with Hodgkins disease. Fractions I, II, and III (Fig. 1) were obtained from three separate patients with idiopathic hyperlipemia. Fractions IV, V, VI, and VII were obtained from the plasma of blood bank donors at the National Institutes of Health.

Chylomicrons from chyle and plasma (fraction I) were isolated by layering the chyle or plasma under saline at density 1.006 and centrifuging at 50,000 *g* for 30 minutes in the Spinco 21 rotor. The packed chylomicrons were then suspended in 30 percent sucrose and layered under 10 volumes of 0.055*M* phosphate, *p*H 7.0, and recentrifuged at 50,000 *g* for 30 minutes. This process was repeated up to eight times until a constant protein concentration was obtained. After removal of the chylomicrons, the plasma was centrifuged at density 1.006 for 24 hours at 80,000 to 90,000 *g*. The packed yellow material (fraction II) at the top of the tube was suspended in 30 percent sucrose and layered under 0.055*M* phos-

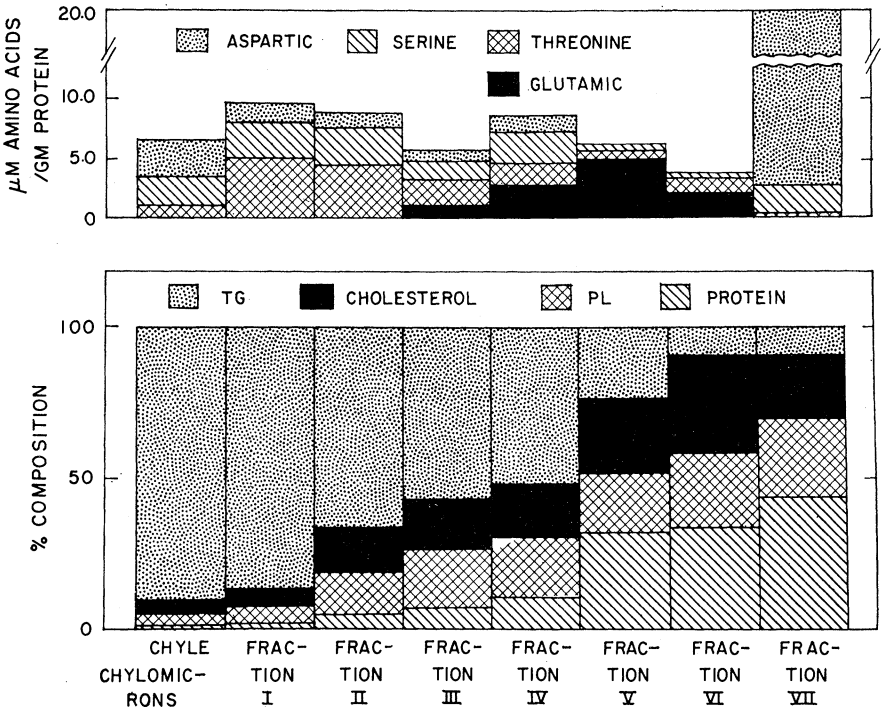

Fig. 1. N-Terminal amino acid and lipid composition of lipoproteins from chyle and plasma. Identical results were obtained from two patients in fraction III. The DNP aspartic acid recovery in fraction VII indicated a minimum molecular weight of 54,000. For dog plasma the same fraction contained only N-terminal aspartic acid with a recovery indicating a minimum molecular weight of 46,000 (*13*).

phate, *p*H 7.0, and recentrifuged. This process was repeated until constant protein concentration was achieved. After removal of fraction II, the soluble lipoproteins which floated were further washed to obtain fractions III and IV. The techniques of isolation of fractions V, VI and VII were essentially those described by Havel *et al.* (*4*) and represent lipoproteins that floated at densities 1.019, 1.063, and 1.21, respectively.

The lipid and protein content of each fraction was obtained according to the procedures used by Bragdon *et al.* (*5*) and was utilized in conjunction with data published by Oncley *et al.* (*6*) to approximate the range of S_f classes represented by each fraction. Fraction III corresponds roughly to S_f 40–100, fraction IV to S_f 20–50, fraction V to S_f 10–17, fraction VI to S_f 3–8, and fraction VII to the high density classes described by Gofman *et al.* (*7*).

The lipoproteins were freed of lipid by extraction with chloroform–methanol (2:1) and further extracted with alcohol, alcohol–acetone, and ether. The protein was reacted with dinitrofluorobenzene and then hydrolyzed. The DNP amino acids were chromatographed and measured quantitatively according to the procedure of Levy (*8*). The chromatographic procedure of Blackburn and Lowther (*9*) was used for final identification of the DNP amino acids. The correction factors used for losses during hydrolysis were those of Porter (*10*).

The results presented in Fig. 1 indicate that while N-terminal threonine and serine appear in all the fractions, they are the predominant N-terminal amino acids in fractions I to IV, the major carriers of triglyceride in plasma. N-Terminal aspartic acid appears in these fractions as well as in fraction VII, although the presence of N-terminal aspartic acid in all of these fractions, of course, does not necessarily mean that the proteins containing the aspartic acid are identical (*11*). N-Terminal glutamic acid, however, appears to be present only in fractions III to VI. It therefore is extremely unlikely that the chyle and plasma chylomicrons and the bulk of the other lipoproteins of density less than 1.006 are "convertible" to the major lipoprotein of *D* 1.006–1.063. Furthermore, the results indicate that the very low density lipoproteins, whose primary distinguishing feature is the high percentage of triglyceride, represent a group of proteins with N-terminal serine, threonine, and aspartic acid.

Since the proteins containing N-terminal serine, threonine, and aspartic acid do not appear to have any stoichiometric relationship to one another, it must be concluded that these proteins are not part of a molecular unit but are distinct entities. Obviously no functional role can be assigned to any one of these proteins until they are isolated and studied separately although it would appear that one or more of them must be intimately associated with the transport of triglyceride.

Any concept concerning lipid transport in plasma must be based on a study of the metabolism of the individual proteins as well as the lipid moieties, since each protein may have a specific role in the metabolism of lipids. It is also apparent that the methods presently used for the isolation of plasma lipoproteins are not adequate for isolating homogeneous lipoproteins. New methods for their purification are now being developed in this laboratory to facilitate further studies of the metabolic and structural properties of plasma lipoproteins (*12*).

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12. I express my appreciation to Dr. Donald S. Fredrickson for the procurement of the patients utilized in this study and to Dr. Edward Korn for his interest and stimulation in all phases of this work.
13. M. Rodbell, unpublished results.

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Yellow Acid-Cation Complexes in Lake Water

Recent studies of the yellow materials present in natural waters and commonly called humins or tannins (*1*) have shown that these materials consist of ultraviolet fluorescing dicarboxylic hydroxy aliphatic organic acids of molecular weight approximately 450. They are probably unsaturated and are apparently non-nitrogenous, containing only carbon, hydrogen, and oxygen. These acids are present in