The Lipoproteins and Lipid Transport in Abetalipoproteinemia *

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The disease abetalipoproteinemia results in intriguing disturbances of lipid transport (2, 3). Included among its manifestations are an inability to form chylomicrons (4) and the lowest concentrations of plasma lipids detected in any human disorder. In this familial syndrome it is possible that the elaboration of beta lipoprotein, or more strictly its beta or B apoprotein, is primarily affected by mutation. This is one of two proteins known to be constituents of human plasma lipoproteins. Abetalipoproteinemia offers unique opportunities for probing the functions of and specific requirements for the B apoprotein and for observing compensating mechanisms aroused by its deficiency.

Some explorations into these aspects of the disease have already been made (2–6). This paper adds the following studies: *1*) more intensive immunochemical analyses of the plasma lipoproteins than have heretofore been made to determine whether any detectable quantities of beta lipoprotein are present, *2*) identification of the protein present in the "low density lipoproteins" frequently reported in this disease, *3*) examination of the alpha lipoproteins and their A apoprotein in abetalipoproteinemia, and *4*) experimental determination of the effect of B apoprotein deficiency on endogenous glyceride transport.

Methods

Six patients with abetalipoproteinemia from four different kindreds were sources of samples for analyses:

L. Za., a 26-year-old male (NIH No. 00-20-77); his sister, R. Kl., a 34-year-old female (NIH No. 06-07-13); A. Co., a 23-year-old male (NIH No. 02-80-07); R. Is., a 12½-year-old male,[1] M. Sm., a 7-year-old male; and S. Sm., his 9-year-old sister.[2]

One or more of the first four of these patients have been the subject of six previous publications (3, 7–11). R. Kl. and L. Za. are the two original cases reported by Bassen and Kornzweig in 1950. Two normal volunteers, B. W., a 21-year-old white female, and G. L., a 19-year-old white male, served as controls. Unless otherwise indicated all blood samples were collected in EDTA, 1 mg per ml, after an overnight fast. Lipoprotein fractionation was usually begun immediately; occasionally plasma was stored at 4° C for periods up to 2 days before analysis.

Preparative ultracentrifugation. Lipoprotein fractions were isolated in the preparative ultracentrifuge by techniques identical to those previously described (12). The following fractions were usually prepared: D < 1.063, low density lipoprotein (LDL); D > 1.063, high density lipoprotein (HDL); and the HDL subfractions, D 1.063 to 1.1 (HDL₂) and D 1.1 to 1.21 (HDL₃). On two occasions the plasmas of patients A. Co. and L. Za. were subjected to ultracentrifugation at plasma D 1.006 for 16 hours to permit isolation of any very low density lipoprotein (VLDL) (13). Isotonic NaCl solution was usually added to the top and bottom fractions to return them to plasma concentrations before they were further utilized. All preparations were dialyzed preceding immunochemical analysis and extracted and analyzed in the same manner (12). Three-ml aliquots of the plasma samples that were centrifuged directly at D 1.063 were also precipitated with heparin and manganese in the cold as an alternative method for determination of alpha lipoprotein (14).

Paper electrophoresis. Paper electrophoresis with buffer containing albumin was used to identify lipoprotein fractions or separate plasma into four bands identified as chylomicrons, beta, pre-beta, and alpha lipoprotein (15). Barbital buffer without albumin was also used for electrophoresis of some of the isolated lipoproteins. The paper strips were dried in an oven at 100° C for 30 min-

[1] Sample kindly provided by Dr. Frank Oski, University of Pennsylvania Hospital, Philadelphia, Pa.

[2] Samples from both Sm. children kindly provided by Dr. Fred Rosen, Children's Medical Center, Boston, Mass.

utes and stained for protein with bromophenol blue or for lipid with Oil Red O (15).

Immunochemistry. The techniques employed for immunoelectrophoresis and double diffusion on plates or in microtubes with either agar or agarose were previously described (12, 13). The preparation and characteristics of the specific antisera to alpha or beta lipoproteins and the antiwhole human sera that were used were described earlier (12). Plasma or lipoprotein solutions were concentrated at 4° C by extraction of water through a dialysis membrane into Sephadex G 50. Eight- to tenfold concentration of normal beta lipoprotein by this technique produced no evidence of denaturation or altered electrophoretic behavior. When solutions initially containing beta lipoprotein in amounts less than $\frac{1}{1000}$ of that in normal plasma (0.1 mg per 100 ml) were so concentrated, beta lipoprotein could still be identified by immunoelectrophoresis.

Antisera to lipoproteins in abetalipoproteinemia. Lipoproteins from the plasma of patients A. Co. and L. Za. were used intact as antigens, together with complete Freund's adjuvant (12). The ultracentrifugal isolates of D < 1.21 (representing the high density and low density lipoproteins in the normal subject) and of D < 1.063 (normally containing all the low density lipoproteins) were used separately to challenge white New Zealand rabbits. D < 1.063 lipoproteins (representing 8 mg of protein) prepared from the top 2 ml of the supernate from 100 ml of plasma separated at D 1.063 from L. Za. were administered subcutaneously in three divided doses at 3-week intervals to one rabbit. The serum collected at the twelfth week was designated antiserum R_{11}. D < 1.21 lipoproteins, representing 30 mg of protein per rabbit and prepared from A. Co. and L. Za., were similarly used in two rabbits to prepare antisera R_{12} and R_{13}.

The precipitating antibodies in each antiserum were shown by immunoelectrophoresis to be γG-immunoglobulins (7 S γ-globulins) (12).

Dietary studies. Patients A. Co. and L. Za, and the control subjects B. W. and G. L. were studied on a metabolic ward where daily weights and caloric intakes were recorded. It was first established that on a free choice diet L. Za. and A. Co. normally ate an average of 275 to 325 g of carbohydrate, some 50 to 75 g per day more than the controls. All four subjects were then fed sequentially diets low and high in carbohydrate designed to promote maximal differences in concentrations of plasma glycerides and pre-beta lipoproteins (13). The patients were reluctant to eat fat and even during their low-carbohydrate diet period consumed 150 to 200 g of carbohydrate. Their high-carbohydrate diets contained 750 to 800 g of carbohydrate per day or about 20 g per kg, almost twice the carbohydrate load previously given to normal subjects to produce hyperlipemia (13). They ate both diets without complaint for 4 or 5 days, although each lost over 1 kg in weight on the low-carbohydrate diet. This was regained during the period of high-carbohydrate feeding. The two normal subjects each received 500 g of carbohydrate (7 to 8 g per kg) on the high-carbohydrate diet. Variations in their body weights were held to less than ± 0.3 kg by adjustment of total calories. Blood samples were obtained daily.

Chemical analysis. Cholesterol (16), phospholipid (17), triglycerides (18), and free fatty acids (FFA) (19) were measured in appropriate samples. Estimates of lipoprotein protein were made directly on nondelipidated lipoproteins by the method of Lowry, Rosebrough, Farr, and Randall (20).

Protein analysis. The lipoproteins of D < 1.063 and of D < 1.21 in the plasmas of both patients were isolated with the ultracentrifuge, washed twice with recentrifugation at the same density, and dialyzed. Upon immunophoresis with three antiwhole human sera [H_1, H_2, and H_3 as previously described (12)], only alpha lipoprotein was detectable. Samples of the lipoprotein fractions were extracted with 3:2 ethanol ether (12). The protein residues were hydrolyzed with 6 N HCl, and the amino acid compositions of hydrolysates representing 0.5 to 2 mg of protein were determined (12, 21). Some of the extracted protein from the D < 1.21 lipoproteins was also dissolved in 1% performic acid for 1 hour and lyophilized before acid hydrolysis in order to oxidize sulfhydryl groups (12, 22). D < 1.063 and D < 1.21 lipoproteins were separated and analyzed in duplicate. The results obtained agreed within 3% for each amino acid. Amino terminal acids were determined by a qualitative method (23).

Results

Ultracentrifugation. The plasma lipids in the six patients were all reduced in concentration and were in the pattern now well established for abetalipoproteinemia (Table I.) Ultracentrifugation of plasma from L. Za. and A. Co. on two occasions failed to reveal any lipid or protein of D < 1.006. Preparative ultracentrifugation at D 1.063 revealed measurable amounts of LDL in the plasma of all six patients, but of the order of only 5 to 15% of that in the plasma of the control subjects, two-thirds of whose plasma cholesterol floated at D 1.063 (Table I).

Subfractionation of HDL was carried out on samples from three patients (Table I). As has been previously described by Farquhar and Ways (2) in patients with abetalipoproteinemia, most of the cholesterol in the HDL (D 1.063 to 1.21) was found between D 1.063 and 1.12 (HDL_2) instead of between D 1.12 and 1.21 (HDL_3) as in normal subjects (Table I) (24). Data from three patients with alpha lipoprotein deficiency (Tangier disease) (25) are shown in Table I for comparison.

Precipitation. When heparin and manganese were added to the plasma of the control subjects, the usual (14) large white flocculant precipitate

TABLE I

*Plasma lipids and lipoproteins**

Subject	Age	Sex	Plasma			LDL			HDL		Heparin Mn++ supernatant		HDL₁ Chol.	HDL₂ Chol.	Reactivity of plasma to sera of	
			Chol.	PL	Trig.	Protein	Chol.	PL	Chol.	PL	Chol.	PL			Anti-αLP	Anti-βLP
Abetalipoproteinemia							*mg/100 ml*									
A. Co.†	23	M	46	76	<10	16	14	24	32	52	38	64	21	11	+	−
L. Za.†	26	M	72	96	<10	23	22	38	50	58	63	83	36	14	+	−
R. Kl.	37	F	52	83	2	17	14	26	38	57	50	80	26	12	+	−
M. Sm.	7	M	50		1		5		45						+	−
S. Sm.	9	F	54		2		13		41						+	−
R. Is.	12	M	35		2		2		32						+	−
Normals																
B. W.	21	F	160	205	70	72	108	108	52	97	52	95	18	34	+	+
G. L.	19	M	168	224	84	70	113	103	55	121	55	119	18	37	+	+
Tangier disease																
T. L.	9	F	80	131	180		77	117	3	14	3	12			±	+
E. L.	11	F	70	124	140		67	111	3	13	3	11			±	+
C. N.	46	M	120	139	531		117	128	3	11	3	11			±	+

* Abbreviations: LDL = D < 1.063, HDL = D > 1.063, chol. = cholesterol, PL = phospholipid, trig. = triglyceride, HDL₁ = D 1.063 to 1.12, HDL₂ = D 1.12 to 1.21, and LP = lipoprotein.
† Mean of 30 determinations.

appeared. Sufficient plasma was available from three patients with abetalipoproteinemia for similar treatment; it yielded only very small amounts of a gray precipitate that contained no material that could be identified as beta lipoproteins by immuno-electrophoresis. It did contain fibrinogen, albumin, γ-globulin, and alpha lipoprotein as well as trace amounts of other plasma proteins. This precipitate was dissolved in concentrated salt solutions (5 N NaCl) and the excess salt removed by dialysis. Some lipid was detected in this material by staining after electrophoresis and immunoprecipitation, but it was all bound to alpha lipoprotein.

The cholesterol concentrations in the supernatant solutions after precipitation with heparin and manganese differed from the concentrations of cholesterol in the HDL obtained by ultracentrifugation of the same plasma sample (Table I). This difference in the apparent concentration of HDL as determined by the two methods in abetalipoproteinemia was in contrast to the excellent agreement of these methods with plasma samples from other subjects (0.2 ± 1.8 mg per 100 ml, mean and SD of 46 samples) (13).

Electrophoresis. At least one plasma sample from all six patients with abetalipoproteinemia was subjected to paper electrophoresis. Numerous samples were run from patients L. Za. and A. Co. and the controls over a 2-year period, these

having been obtained at varying intervals after the subjects had eaten and while they were on various diets.

In abetalipoproteinemia only one lipoprotein band, and that having α₁ mobility, was detected by paper electrophoresis even when the strips were overloaded (see Figure 4). Only the single alpha lipoprotein band was likewise obtained by electrophoresis of isolated D < 1.063 and D < 1.21 lipoproteins, using buffer with and without albumin and lipid and protein stains.

Immunochemical findings. No material reacting with potent antihuman beta lipoprotein sera could be detected in the plasma of any of the six patients with abetalipoproteinemia (Figures 1, 2). This was true even though samples of whole plasma were concentrated four- to tenfold or isolated material of D < 1.21 at least eightfold by a combination of ultracentrifugation and Sephadex treatment. These concentrates were examined by the more sensitive Preer tube method (26) in addition to diffusion in agar plates or immunoelectrophoresis. The plasmas of both A. Co. and L. Za. were examined repeatedly over a 2-year period with consistently negative findings.

The lipoproteins of D < 1.063, which normally should have contained beta lipoproteins, were next examined. Electrophoresis on paper of this fraction from plasma of the six patients yielded a single
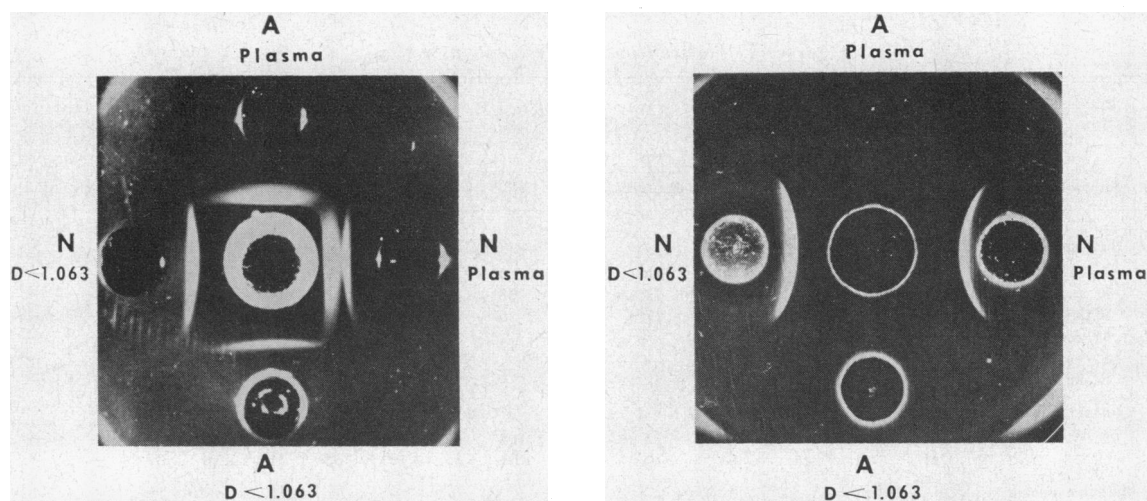
FIG. 1. IMMUNODIFFUSION PATTERNS ON AGAROSE OF THE PLASMA AND $D < 1.063$ FRACTION (LOW DENSITY LIPOPROTEINS, LDL) FROM A NORMAL SUBJECT (N) OR PATIENT WITH ABETALIPOPROTEINEMIA (A). Antiserum R_{3A} (12) (anti-alpha-beta lipoprotein sera) was employed in left center well. Antiserum R_7 (12) (anti-beta lipoprotein sera) was employed in right center well. The $D < 1.063$ and plasma of A on the right were concentrated fourfold before their use. All the precipitation lines on this and succeeding Figures are unstained. All the lines here were subsequently shown to stain for lipids.

band in the alpha$_1$ region, staining for both lipid and protein. By the Ouchterlony techniques or immunophoresis these low density lipoproteins formed only a single line with any of more than a dozen antisera of different specificities but all having in common anti-alpha lipoprotein activity. The precipitation band formed a line of identity with that produced by normal plasma alpha lipoprotein (Figures 1, 2).

Two-tenths ml of a pure anti-alpha lipoprotein serum [R_1, previously described (12)] was also mixed with 0.1 ml of concentrated LDL ($D < 1.063$) from L. Za. After 24 hours at 4°, the mixture no longer reacted with native alpha lipoprotein from a normal subject. This absorption experiment was controlled with addition of either normal LDL or saline to the antiserum. An antiserum with potent anti-beta and anti-alpha lipoprotein activity [R_{3A} (12)] was absorbed with the LDL of abetalipoproteinemia in the same manner; it then reacted with normal LDL but not normal HDL. In contrast, the addition of normal LDL appropriately removed all anti-beta activity of this antiserum, leaving the anti-alpha activity. Finally, the anti-alpha serum R_1 was shown to precipitate completely all the LDL in abetalipoproteinemia plasma; there was no detectable cholesterol in the supernate.

Nature of the high density lipoproteins. It has been shown that two antigenic forms of alpha lipoprotein or HDL may be detected in normal plasma under certain conditions (12). Consistent with these earlier findings, the D 1.063 to 1.1 lipoproteins (HDL$_2$) prepared from plasma of the two control subjects contained only the form of alpha lipoprotein called alpha LP$_A$ (12). Their D 1.1 to 1.21 lipoproteins (HDL$_3$) also contained alpha LP$_B$. Alpha LP$_B$ has slower mobility on agar and agarose than alpha LP$_A$ and contains a lower ratio of lipid to protein (12).

The immunochemical pattern of the alpha lipoproteins in each of the six patients with abetalipoproteinemia was qualitatively similar to that seen in normal subjects. Only alpha LP$_A$ was definitely detectable in fresh whole plasma or HDL$_2$ lipoproteins prepared in the ultracentrifuge. Both alpha LP$_A$ and alpha LP$_B$ were present in HDL$_3$ (Figure 3). As in normal subjects, very small amounts of alpha LP$_B$ were present in the infranate after ultracentrifugation at D 1.21 although ultracentrifugation or storage seemed to result in less alpha LP$_B$ than is usually produced by these processes in normal subjects. Those alpha lipoproteins that floated at D 1.063 in abetalipoproteinemia plasma were of the alpha LP$_A$ form (Figure 2).

Abetalipoproteinemia plasma as an antigen.
The antisera produced in rabbits by injection of lipoproteins from two of the patients are characterized in Table II. All three contained antibodies in relatively high titers to both forms of alpha lipoprotein (alpha LP_A and alpha LP_B). The lipoproteins used as antigens were not washed by repeated centrifugation as were the preparations of protein for amino acid analysis or those used previously (12) to prepare lipoprotein antibodies. Only alpha lipoprotein was detected in the concentrated lipoprotein fractions upon immunoelectrophoresis with the three antiwhole sera, H_1, H_2, and H_3.

The "impurity" of the antigens notwithstanding, the antisera harvested after their administration reacted weakly with γ-globulin, albumin, and other unidentified alpha migrating proteins. None of the antisera reacted in any way with pure preparations of normal beta lipoprotein (D 1.019 to 1.063), either before or after delipidation with ether.

Each antiserum produced a blurred lipid-staining precipitate in the beta lipoprotein zone on agar and sometimes agarose when employed in immunophoresis of fresh human plasma. Its intensity was proportional to the turbidity of the plasma
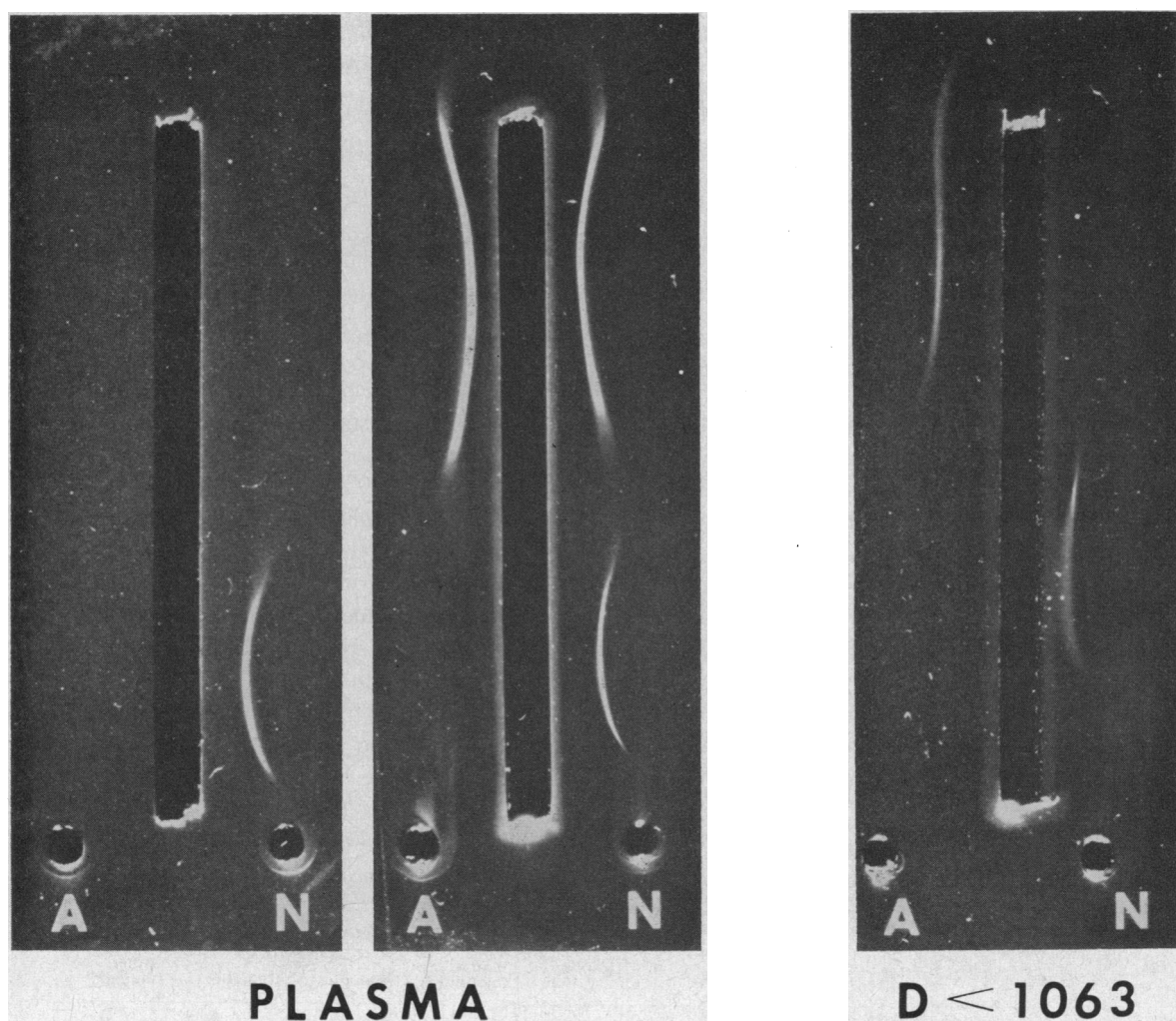

FIG. 2. IMMUNOELECTROPHORETIC PATTERNS ON AGAROSE OF THE PLASMA AND D < 1.063 FRACTION FROM A NORMAL SUBJECT (N) OR PATIENT WITH ABETALIPOPROTEINEMIA (A). Antiserum R_7 (anti-beta lipoprotein sera) was used in frame on left. Antiserum R_{3A} (anti-alpha and beta lipoprotein sera) was used in middle and right frames. All the precipitation lines seen here were subsequently shown to stain for lipid.
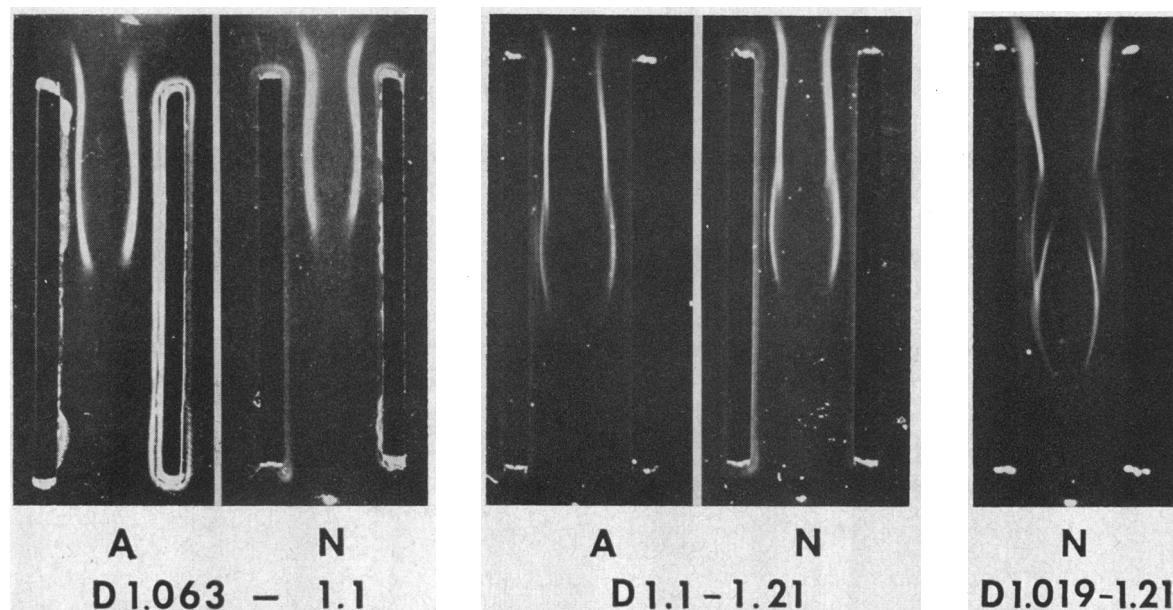
FIG. 3. IMMUNOELECTROPHORETIC PATTERNS ON AGAROSE OF TWO HIGH DENSITY LIPOPROTEIN (HDL) SUBFRACTIONS AND OF HDL + LDL (D 1.019 TO 1.21) FROM A NORMAL SUBJECT (N) OR PATIENT WITH ABETALIPOPROTEINEMIA (A). (The D 1.12 to 1.21 fraction of the patient with abetalipoproteinemia was concentrated fourfold before its use.) The left- and right-hand troughs, respectively, contained antisera R_2 and R_{3A} (12) (anti-alpha and beta lipoprotein sera). All precipitation lines stained subsequently for lipid.

used as "antigen." This phenomenon was also seen when normal human or rabbit serum was used in place of the antiserum and was considered an artifact, probably a manifestation of the known reactivity of beta lipoprotein with agar (13).

Protein analyses. The amino acid compositions of the D < 1.063 (LDL) and D < 1.21 (LDL + HDL) lipoprotein fractions from A. Co. and L. Za. were indistinguishable and corresponded to those of HDL or alpha lipoproteins from normal subjects (Table III). No attempt was made to correct for possible losses of amino acids during hydrolysis or to quantify tryptophan or amide ammonia. The proteins from both the lipoprotein

fractions contained amino-terminal aspartic acid plus trace amounts of serine and threonine. This corresponds to results of many analyses of the protein of alpha lipoproteins obtained from normal subjects (12, 27–29).

Response to induction diets. The results of extreme variation in the diet in abetalipoproteinemia are shown in Figures 4 and 5. The only changes observed were an increase in the plasma FFA and loss of weight on the low-carbohydrate diets or weight gain and return to the low normal FFA levels on a high-carbohydrate intake. The most significant observation during the high-carbohydrate diet period was the complete failure of this diet to

TABLE II

Characterization of antisera made from abetalipoproteinemia plasma fractions

		Reactivity				
Rabbit	Sensitizing antigen	Alpha lipoprotein	Beta lipoprotein	Albumin	Globulin	Other proteins
R_{11}	D < 1.063* LDL	+	−	+	−	+
R_{12}	D < 1.21† HDL + LDL	+	−	+	+	+
R_{13}	D < 1.21† HDL + LDL	+	−	+	−	+

* D < 1.063 fraction obtained from plasma of L. Za.
† D < 1.21 fraction obtained from pooled plasmas of L. Za. and A. Co.

TABLE III

*Amino acid compositions of the plasma lipoproteins**

	Normal LDL	Abeta D <1.063	Abeta D <1.21	Normal HDL
Aspartic	100	100	100	100
Threonine	56	60	59	59
Serine	74	81	82	82
Glutamic	118	240	242	242
Proline	40	57	58	58
Glycine	45	53	53	52
Alanine	59	92	94	93
Cystine†			6+	7
Valine	53	65	66	66
Methionine	13	11	11	11
Isoleucine	53	7	8	7
Leucine	108	162	166	161
Tyrosine	33	38	38	39
Phenylalanine	49	42	41	41
Lysine		129	130	131
Arginine		66	67	66
Histidine		23	22	22

* All values are expressed as moles per cent relative to aspartic acid taken as 100. LDL = D 1.019 to 1.063; HDL = D 1.063 to 1.21 (obtained from normal volunteers) (12). Abeta = abetalipoproteinemia lipoprotein fraction.
† Cystine determined as cysteic acid after pretreatment of the protein with performic acid.

produce any changes in plasma triglyceride concentrations. These remained less than 20 mg per 100 ml throughout (Figure 5). The plasma cholesterol and phospholipid concentrations also remained unchanged. Only alpha lipoproteins could be demonstrated immunochemically, and neither a chylomicron band nor a pre-beta lipoprotein band appeared (Figure 4). There was no change in alpha lipoprotein titer on the different diets.

As demonstrated in at least ten other subjects (1, 13), alpha lipoprotein concentrations in the two control subjects on similar diets varied inversely with those of plasma triglycerides and pre-beta lipoproteins.

Discussion

Both absence and deficiency of beta lipoproteins have been described in patients with abetalipoproteinemia (2–5, 9, 11, 30, 31). Some of the examinations have been limited to ultracentrifugal separations, which do not distinguish specific proteins, or to relatively insensitive electrophoretic techniques. When immunochemical methods have been used, both absence of beta lipoproteins (4, 5, 9, 11, 28) and the presence of small amounts (3) have been described. Special efforts to detect possible very low titers of beta lipoprotein after concentration of plasma have not been reported before. A more extensive immunochemical examination

was the first endeavor of the present study. No trace of beta lipoprotein could be detected in any of the six patients by using multiple antisera in three different systems and against some samples that were more concentrated than plasma by tenfold. This was true in patient R. Is. even though he had received a transfusion of normal plasma 6 weeks earlier. Admittedly, the failure of the plasma lipoproteins from two patients to provoke antibodies in animals to beta lipoproteins is imperfect proof that the antigen is missing. However, these findings should be interpreted in the light of experience indicating that beta lipoproteins are antigens of high potency, more antigenic than alpha lipoproteins (12). We have found that rabbits frequently may produce good titers of antibody to beta lipoprotein after receiving only a single challenge of 3 to 6 mg of protein in complete Freund's adjuvant. On at least two occasions we have also had alpha lipoprotein preparations that were apparently immunochemically pure induce high anti-beta lipoprotein titers without stimulating any measurable amounts of antibody to alpha lipoprotein (12).

It cannot be stated unequivocally that absence of detectable beta lipoproteins means absence of B apoprotein. Delipidation of alpha lipoproteins by solvent treatment leaves in solution a phospholipid protein complex or A apoprotein that still reacts with most anti-alpha lipoprotein sera. Partially delipidated beta lipoproteins react with antibeta lipoprotein sera (13, 32), but more complete delipidation usually denatures the protein residue (13, 32, 33), making immunochemical analyses quite difficult. Proof is therefore lacking that apoprotein B would always be recognized by antisera that react with its lipid-laden form. The likelihood is great, however, that no B apoprotein is released into plasma in abetalipoproteinemia; it may never be synthesized at all.

These inferences and the experimental evidence from which they are derived form an interesting contrast to the analogous lipoprotein deficiency state, Tangier disease. Plasma from patients with this disease usually contains no detectable alpha lipoprotein line after immunophoresis with appropriate antisera. In concentrated plasma, however, especially when the semimicro Preer technique is utilized, some alpha lipoprotein can be demonstrated (34). This appears to be antigeni-
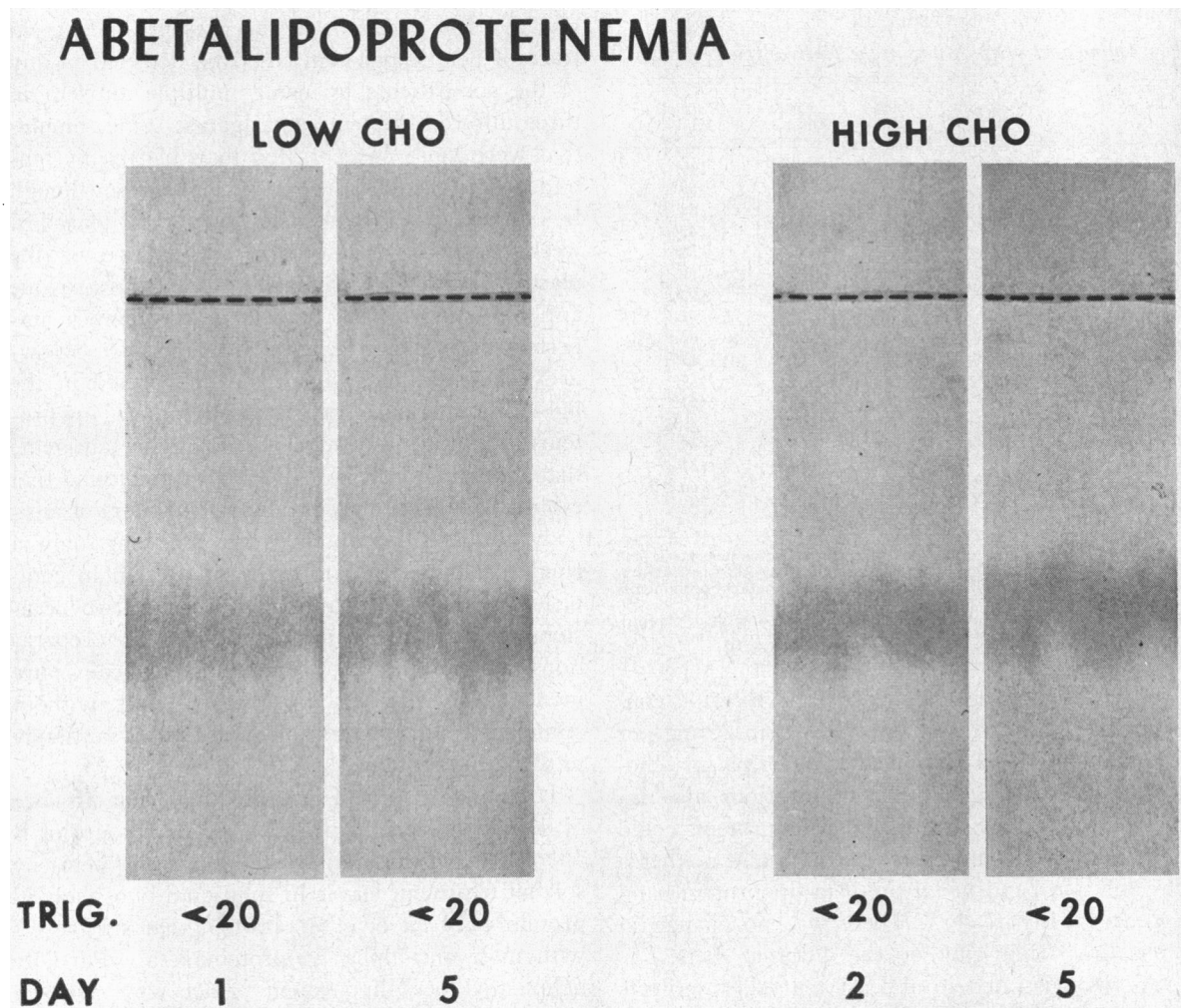
ABETALIPOPROTEINEMIA

FIG. 4. PAPER ELECTROPHORESIS OF PLASMA SAMPLES OBTAINED FROM PATIENT L. ZA. WHILE HE WAS CONSUMING TWO DIFFERENT DIETS. Strips were loaded with 40 μg of plasma rather than the 20 μg of plasma usually employed. Strips are stained with Oil Red O. CHO = carbohydrate.

cally different from the normal alpha lipoprotein.

Others have already observed that the plasma may contain low density lipoproteins (D < 1.063) in abetalipoproteinemia (2, 3, 9, 11, 31). Some have surmised that these did not represent normal beta lipoproteins (2), although their protein content had not been characterized. Evidence is here presented that this protein is the A apoprotein, identical to that found in normal alpha lipoproteins.

Alpha lipoprotein, or the A apoprotein, is not usually detectable in native lipoproteins of D 1.006 to 1.063. It is present in the very low density or pre-beta lipoproteins of D < 1.006, but can be identified only after delipidation (13). There is much evidence to suggest that alpha lipoproteins may

also be a normal constituent of chylomicrons (35). In these lipoproteins or particles, however, there are abundant glyceride concentrations, quite different from abetalipoproteinemia, in which plasma glyceride concentrations are abnormally low. In this disease alpha lipoproteins appear in abnormal quantities in lipoproteins of D between 1.006 and 1.063 and are also abnormally distributed within the conventional density spectrum for high density lipoproteins between 1.063 and 1.21. Our findings that the concentrations of HDL_2 (D 1.063 to 1.1) were increased and those of HDL_3 (1.1 to 1.2) decreased below normal are in confirmation of a previous report (2).

Perhaps the best explanation for these phe-

A.C. 23 y-o. W ♂ ABETALIPOPROTEINEMIA
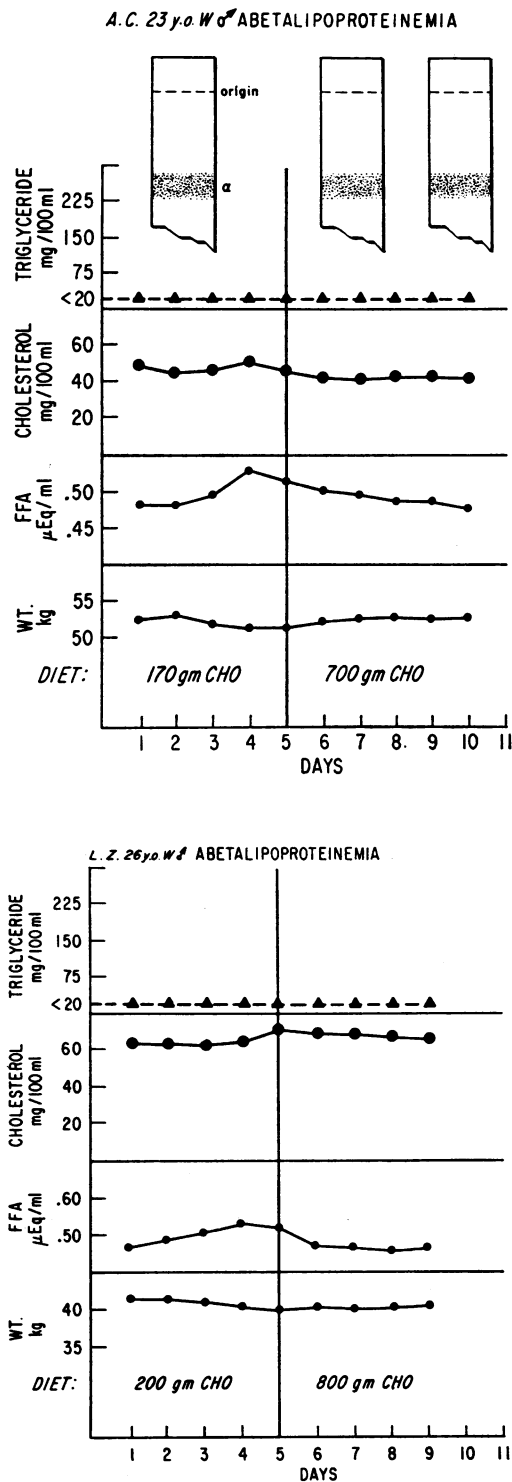

L. Z. 26 y.o. W ♀ ABETALIPOPROTEINEMIA

FIG. 5. DIETARY STUDY IN TWO PATIENTS. In both patients A. C. and L. Z. (see text) the serum cholesterol is equivalent to the alpha lipoprotein cholesterol.

nomena is an increase in the lipid carried by some of the available A apoprotein in abetalipoproteinemia. This could account for a reduction in the average density of the alpha lipoproteins and hence their unusual flotation pattern.

The available data do not prove "over-lipidation" of the apoprotein A, but indirect support was obtained in examination of the patients' lipoproteins after centrifugation. Less alpha LP$_B$ was produced by this procedure than is observed with normal plasma (12). The transformation of the native alpha LP$_A$ to the immunochemically different alpha LP$_B$ form has previously been shown to be accompanied by loss of lipid (12). When alpha LP$_A$ from plasma of patients with abetalipoproteinemia was delipidated by the more drastic treatment with organic solvents, it was all converted to alpha LP$_B$.

When plasma concentrations of endogenous glyceride are increased by feeding high-carbohydrate diets ("carbohydrate induction"), the plasma concentration of "free" alpha lipoproteins decreases as that of pre-beta lipoproteins rises (13). Under these conditions, patients with Tangier disease, who have only a tiny complement of alpha lipoproteins, cannot form pre-beta lipoproteins, but their plasma glyceride concentrations increase (13).

Two of the patients with abetalipoproteinemia were fed a diet that contained three times the amount of carbohydrate causing glycerides to rise within 3 days in normal subjects (36). Their extremely low plasma glyceride concentrations did not change, and no trace of pre-beta lipoproteins appeared. Isselbacher, Scheig, Plotkin, and Caulfield earlier biopsied the liver of one of the patients included in this paper, R. Is. (Table I). This organ contained what appeared to be excessive amounts of glyceride even while the patient was eating a relatively low-fat diet (3).

It has been repeatedly emphasized that patients with abetalipoproteinemia take up fed fat in their intestinal mucosal cells, but fail to release it in the normal fashion (2–4, 9, 10, 31). An interesting study by Sabesin, Drummey, Budz, and Isselbacher (37) has suggested that abolition of protein synthesis is accompanied by disturbance in fat assimilation analogous to abetalipoproteinemia. It is not yet known whether the chylomicrons that normally transport fat from the intestinal cells

must contain protein. If they do, any essential place of the B apoprotein in such a protein complement also remains unproved. Despite the lack of such key information, all of the evidence available does tempt one to conclude that beta lipoprotein (or B apoprotein) must have some critical function in transporting out of cells glycerides of either exogenous or endogenous origin.

Finally, it should be noted that another apoprotein, a "C peptide," has been described in glyceride-rich plasma lipoproteins by Gustafson, Alaupovic, and Furman (38). In our studies none of the 30 different antisera, which were prepared to antigens consisting of many different human lipoprotein fractions, detected such a third protein in normal VLDL (13). The only lipid-staining precipitin lines produced by them with abetalipoproteinemia sera were clearly attributable to alpha lipoprotein. All of the plasma lipid having D < 1.063 was also precipitated with anti-alpha lipoprotein sera and the amino acid composition of all the lipoprotein protein that could be isolated was not detectably different from that of the normal A apoprotein. This failure to find evidence of a C peptide in abetalipoproteinemia will be of further interest and easier to interpret when more is learned about this possible new addition to the fat-transporting proteins.

Summary

1. The lipoproteins in the plasma of six patients with abetalipoproteinemia have been studied. Antisera were employed in either double diffusion or immunophoresis on agar and agarose to characterize the lipoproteins of D < 1.21. Lipoproteins from two patients were also used to provoke antibody formation in rabbits, an additional test for quantities of lipoprotein antigens too small to detect by other means. The relative amino acid content and amino-terminal amino acid composition of the high and low density lipoprotein proteins were also determined.

2. In plasma lipoproteins concentrated greater than eightfold, no trace of material having the antigenic properties of beta lipoprotein was detected in any of the six patients. Examination was extended to lipoprotein concentrates raised up to tenfold higher than the plasma concentration.

3. Alpha lipoproteins were the only lipoproteins present. Five to 25% of these floated at D <

1.063, and more than one-half were isolated between D 1.063 and 1.1. The protein portions of all these lipoproteins were immunochemically identical and had amino acid patterns indistinguishable from those in normal alpha lipoproteins. The abnormal shifts in density were considered likely due to excessive lipid loading of normal alpha lipoproteins.

4. The abnormally low plasma glycerides of these patients were unchanged, and no pre-beta (very low density) lipoproteins were produced by diets that regularly induce hyperglyceridemia in normal subjects and patients with alpha lipoprotein deficiency.

5. These findings are offered as further support to the concept that abetalipoproteinemia is due to an inability to synthesize or release the specific apoprotein of plasma beta lipoproteins. The beta protein probably has a function in transport of both exogenous and endogenous glyceride from cells that cannot be adequately assumed by alpha lipoprotein.

Addendum

Through the generous collaboration of Dr. Fred Rosen, the responses of the Sm. children to high carbohydrate feeding have since been determined. Although they consumed more than 20 g of carbohydrate per kg for 7 days, their plasma triglyceride concentrations remained below 4 mg per 100 ml, and only alpha lipoproteins were detected in the plasma. Thus, in all of four patients with abetalipoproteinemia, there appeared to be failure to mobilize endogenous triglycerides.

Acknowledgments

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