

# Apolipoprotein B-100 Deficiency

## Intestinal Steatosis Despite Apolipoprotein B-48 Synthesis

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### Abstract

We describe a child, the issue of phenotypically normal parents, who had fat malabsorption, both intestinal and hepatic steatosis, and serum cholesterol and triglyceride concentrations of 38 and 63 mg/dl, respectively. Lipoprotein electrophoresis, Ouchterlony double diffusion, and electron microscopy demonstrated that normal low density lipoproteins (LDL: 1.006  $< \rho < 1.063$  g/ml) were absent. Lipoprotein particles in the  $\rho < 1.006$  g/ml fraction were triglyceride rich, very large ( $93.2 \pm 35.1$  nm), and contained the B-48 but not the B-100 apoprotein; both species of apolipoprotein (apo) B were found in the parents' lipoproteins. These chylomicrons and chylomircron remnants were present even in the patient's fasting plasma, which suggested prolonged dietary fat absorption. Plasma levels of high density lipoprotein lipids and proteins were low, and the phosphatidylcholine/sphingomyelin ratio was reduced as in typical abetalipoproteinemia. The monosialylated form of apo C-III was not identified on polyacrylamide gel electrophoresis, which suggested that this protein was elaborated only with very low density lipoproteins (VLDL).

A radioimmunoassay for apo B employing a polyclonal antisera to plasma LDL gave apparent plasma apo B levels of 0.6, 66, and 57 mg/dl in the patient and his father and mother, respectively. The displacement curve generated by the parents' VLDL and LDL did not differ from control lipoproteins. The patient's chylomircron-chylomircron remnant fraction displaced normal LDL over the entire radioimmunoassay range, but the efficiency of displacement was strikingly less than with B-100 containing lipoproteins. If the patient's B-48 protein is not qualitatively abnormal, these results confirm very limited immunochemical cross-reactivity between at least one major epitope on B-100 and the epitopes expressed on B-48.

The apo B defect in this patient appears to be recessive. It abolishes B-100 production and may additionally limit the formation of B-48.

### Introduction

Two genetically distinct disorders manifested by the absence from plasma of chylomicrons, very low density lipoproteins

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(VLDL), and low density lipoproteins (LDL) have been defined (1, 2). Classical abetalipoproteinemia is an autosomal recessive disorder in which obligate heterozygotes are phenotypically normal. In hypobetalipoproteinemia, homozygotes have clinical and biochemical findings like recessive abetalipoproteinemia; heterozygotes, however, have hypocholesterolemia secondary to low LDL concentrations. The molecular basis of the deficiency of apolipoprotein B (apo B)<sup>1</sup>-containing lipoproteins has not been identified in either disorder.

It was formerly believed that one species of apo B existed and that a common structural or regulatory defect in abetalipoproteinemia accounted for the failure of the intestine and liver, respectively, to secrete chylomicrons and VLDL. In 1980, apo B heterogeneity was defined in man (3) and in the rat (4). One apo B species of 370,000–550,000 mol wt, designated B-100 in a centile nomenclature system (3), is the predominant species in VLDL and LDL. A smaller protein with apparent molecular weight of 260,000 (B-48) and different amino acid composition (3) is the major form of apo B in mesenteric lymph chylomicrons (5). The two forms of apo B have limited immunologic cross-reactivity (4, 6–9) and different metabolic properties (10–13).

While these observations suggest that the B-100 and B-48 proteins are products of different genes, both proteins are absent from plasma in abetalipoproteinemia and homozygous hypobetalipoproteinemia. Recently, Malloy and co-workers (14) described a patient whose plasma triglyceride-rich lipoproteins contained the B-48 but no B-100 protein. Moreover, an intestinal biopsy showed no lipid accumulation. B-100 and B-48 synthesis appeared to be dissociated in this case.

We describe in this report a child who also lacks B-100-containing lipoproteins and whose triglyceride-rich lipoproteins contain a protein of the size of B-48. In contrast to the patient of Malloy et al., however, lipid accumulation was found in intestinal epithelial cells. The genetic defect in this disorder appears to abolish B-100 production and may additionally limit the formation of B-48.

### Methods

**Case report.** The patient weighed 3.2 kg at birth after a full-term pregnancy. He was breast fed for the first month of life and was subsequently fed a cow-milk formula. Stools were noted to be loose, but initial weight gain was considered satisfactory. Weight gain slowed at 5 mo of age, and his stools became more frequent and foul smelling.

He was hospitalized at 10 mo because of persistent diarrhea and failure to thrive. Weight (7.4 kg) and length (67 cm) were both below the third percentile. Other physical findings included wasted extremities,

1. Abbreviations used in this paper: apo, apolipoprotein; apolipoproteins A-I, A-II, B, C-I, C-II, and C-III, apolipoproteins A-I, A-II, B, C-I, C-II, and C-III, respectively.

abdominal distention, and mild truncal hypotonia. Retinitis pigmentosa was not observed. Laboratory examinations showed hematocrit, 37%; white blood count of 10,900 with 23% polymorphonuclear leukocytes and 69% lymphocytes; platelet count of 300,000; prothrombin time of 10 s; aspartate aminotransferase, 223 IU/liter; alanine aminotransferase, 122 IU/liter; lactic dehydrogenase, 179 IU/liter; alkaline phosphatase, 209 IU/liter; total bilirubin, 0.3 mg/dl; glucose, 73 mg/dl; blood urea nitrogen, 16 mg/dl; creatinine, 0.4 mg/dl, and albumin, 4.0 g/dl. Alpha-1-antitrypsin was 263 mg/dl, and the protease inhibitor type was MM. Serologies for hepatitis A and B, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex viruses were all negative. Multiple stool examinations for enteric pathogens and parasites were negative, and a sweat chloride determination was normal. A 72-h fecal fat collection while the patient was consuming 5 g/kg per d of dietary fat demonstrated a coefficient of absorption of 0.85.

At 1 yr of age, hepatomegaly was noted, but coagulation tests, liver function tests, and serum albumin were normal. The hematocrit was 43%, and acanthocytes accounted for 5–10% of erythrocytes. After a 12-h fast, total cholesterol was 30 mg/dl and total triglycerides 75 mg/dl. Serum vitamin E was 1.8  $\mu$ g/ml (normal, 5–20  $\mu$ g/ml).

16 h after a fatty meal, small bowel biopsy was performed with a Crosby capsule positioned fluoroscopically at the ligament of Treitz. The duodenal mucosa had intact and well-developed villi, a normal crypt/villous ratio, and normal lamina propria. There were diffuse microvesicular droplets in the apical portions of the villi (Fig. 1 A). Electronmicroscopic evaluation confirmed the presence of intraepithelial lipid droplets. A percutaneous liver biopsy revealed well-preserved architecture, but hepatocytes contained macrovesicular lipid droplets (Fig. 1 B). Electronmicroscopic examination confirmed the lipid accumulation and also revealed a dilated endoplasmic reticulum. A tentative diagnosis of abetalipoproteinemia was established as a result of these biopsies, and the patient was started on a low fat diet with supplemental vitamins A, D, and E. Over the next 7 mo he gained 1.2 kg and grew 7 cm. His motor and language development were normal.

The patient's parents were not blood relatives. The mother was of German-Czechoslovakian extraction and the father of French-Canadian ancestry. The patient had one brother who was 2 yr older and enjoyed good health.

**Blood sampling.** Plasma and serum samples were obtained from the proband and his parents at intervals over a 2-yr period. These were immediately refrigerated but not frozen, and analyses were begun within 3 d of venipuncture. On one occasion, blood was drawn directly into syringes containing EDTA (0.017 M, final concentration), benzamidine (2 mM), and aprotinin (250 kallikrein inhibitory U/ml).

**Analytical methods.** Cholesterol (15) and triglycerides (16) were quantified by enzymatic methods on a Gilford Impact 400 computer-directed analyzer. Free cholesterol was determined by omitting cholesterol esterase from the reaction mixture, and esterified cholesterol was calculated as the difference between total and free cholesterol multiplied by 1.69 (molecular weight cholesterol oleate/molecular weight cholesterol). Phospholipids were measured by the method of Ibel and Lands (17). Phospholipid class separation was performed by the method of Parker and Peterson (18). Phosphatidylcholine and sphingomyelin were also quantified using enzymatic methods (19).

High density lipoprotein (HDL)-cholesterol was estimated (20) using heparin-Mn<sup>2+</sup> precipitation. Lipoprotein electrophoretograms in 1% agarose (Corning Universal Electrophoresis Film, Corning Medical, Palo Alto, CA) were stained with fat red 7B stain (Corning Medical). Apo A-I (21) and A-II (22) were quantified using previously described radioimmunoassays (RIAs). Apo B was similarly quantified using a solution-phase double antibody RIA. The first antibody was prepared in a rabbit and the second in a goat. LDL used for immunization, standards, and tracer were isolated between densities 1.019 and 1.063 g/ml, and re-centrifuged once at the higher density. This LDL did not react on Ouchterlony double diffusion with antisera prepared against apo A-I, A-II, C-I, C-II, C-III, and human serum albumin. The protein content of LDL preparations was estimated by the method of

Lowry (23) using a bovine serum albumin (BSA) standard. The protein mass of the serum albumin standard was estimated by triplicate quantitative amino acid analyses (24). LDL was radioiodinated using the iodine monochloride method (25), and tracer specific activity ranged between 2 and 5  $\mu$ Ci/ $\mu$ g LDL protein. Assuming a B-100 molecular weight of 500,000, the tracer contained 150–200 mol of iodine per mole of protein. 83–95% of the radioiodine was associated with LDL protein, and the tracer was suitable for RIA for at least 4 mo. Test samples, standard, and tracer were diluted in buffer containing 1% BSA and 0.05 M barbital, pH 8.6. First and second antibody were diluted in this buffer containing 0.1 M sodium cholate and 2% polyethylene glycol. The diluted first antibody also contained 0.5% non-immune rabbit serum. The first antibody was diluted so that the 90 ng standard gave a B/B<sub>0</sub> of 0.5.

Test samples and the first antibody were incubated at 37°C for 75 min before addition of tracer. After an additional incubation at 4°C for 16–18 h, second antibody was added, and the samples were held for 3 h at 4°C. Immunoprecipitates were sedimented by low-speed centrifugation, the supernatant solution discarded, and radioiodine in the precipitates assayed. Apo B concentrations in test samples were calculated by the log-logit method (26). Appropriate dilutions of plasma and serum generated displacement curves that were parallel to the LDL standard. Diluted standards and tracers generated identical displacement curves after storage at 4°C for at least 4 mo.

Plasma from the patient and a control were also adsorbed with an antisera to apo B. The antisera was rendered free of endogenous lipoproteins by ultracentrifugation at 1.21 g/ml, and was then dialyzed against 0.15 M NaCl. 1 vol of the patient and control plasma was mixed with 2 vol of antiserum and incubated for 30 min at 37°C. After addition of 75  $\mu$ g of activated and hydrated protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ), the samples were left for 16 h at 4°C. The immunoprecipitate protein A-Sepharose was removed by low-speed centrifugation, and the supernatant solution examined by lipoprotein electrophoresis.

Anionic polyacrylamide gel electrophoresis was performed as described by Reisfeld and Small (27), except that all buffers contained 8 M urea. Electrophoresis in 3% polyacrylamide gels containing SDS was performed as described (3). HDL were fractionated by gradient gel electrophoresis (28) using 4–30% slab gradient gels (Pharmacia Fine Chemicals). Samples (20–40  $\mu$ g protein) and a standard protein mixture (HMW Calibration Kit, Pharmacia Fine Chemicals) were electrophoresed in buffer (pH 8.3) for 24 h at 125 V constant voltage and at 10°C. Gels were fixed in 10% sulfosalicylic acid for 1 h, stained for 1.5 h in 0.04% Coomassie Brilliant Blue G-250 in 3.5% perchloric acid, and destained in 5% acetic acid. Gels were scanned with a model RFT densitometer (Transidyne Corp., Ann Arbor, MI), and scan data were processed on a PDP 8/e minicomputer (Digital Equipment Corp., Maynard, MA) equipped with a display terminal and hard copier (Tektronix, Inc., Beaverton, OR). All lipoprotein classes were examined by negative stain electronmicroscopy as detailed elsewhere (29).

Lipoproteins of  $\rho$  < 1.006 g/ml from the proband and his parents were also radioiodinated by the iodine monochloride method (25). Aliquots of the <sup>125</sup>I-chylomicron/VLDL fractions were delipidated with cold diethyl ether/methanol (3:1), and protein dried under a stream of N<sub>2</sub> was resolubilized in 1% SDS. Samples were heated at 100°C for 5 min and analyzed by SDS polyacrylamide gel electrophoresis (3). Gels were sliced as indicated and radioassayed.

## Results

**Plasma lipids and lipoproteins.** Over the 2 yr in which these studies were conducted, the patient's total cholesterol ranged from 27 to 38 mg/dl and triglycerides from 63 to 119 mg/dl. Triglycerides did not fall below 60 mg/dl even after a 12-h fast (Table I). Unesterified cholesterol was quantified on two occasions and accounted for 37 and 48% of the patient's total plasma cholesterol. Only 26% of the total cholesterol in the parents' plasma was unesterified.

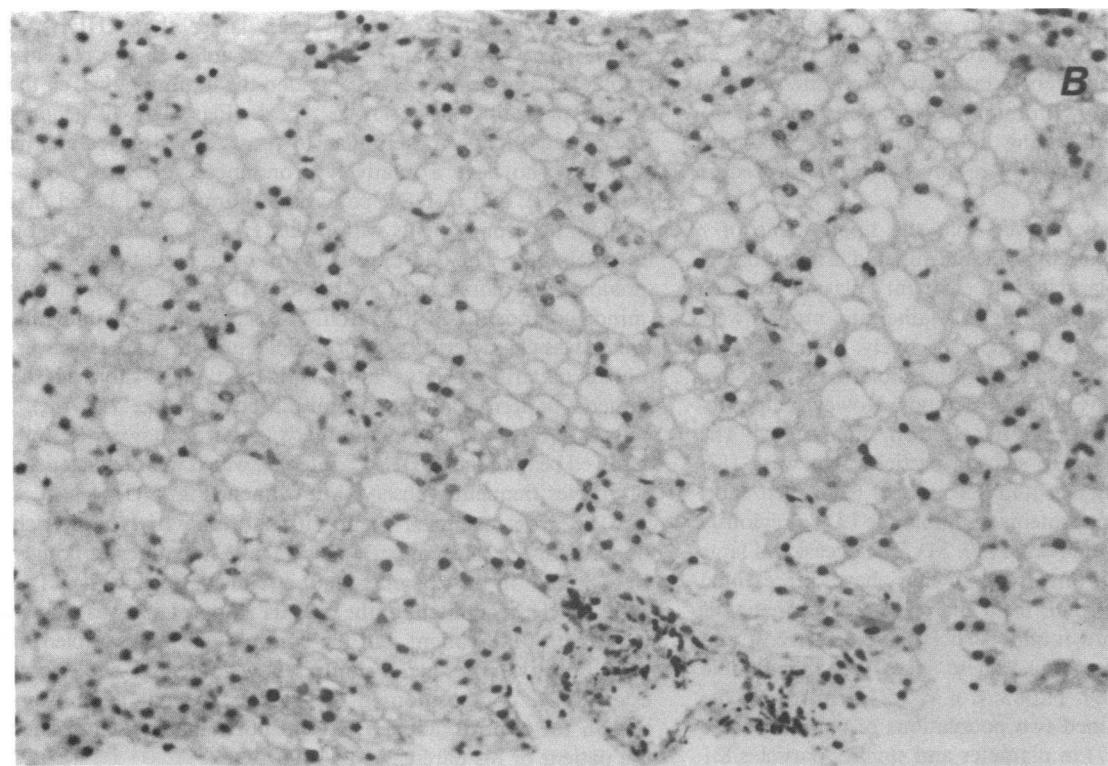
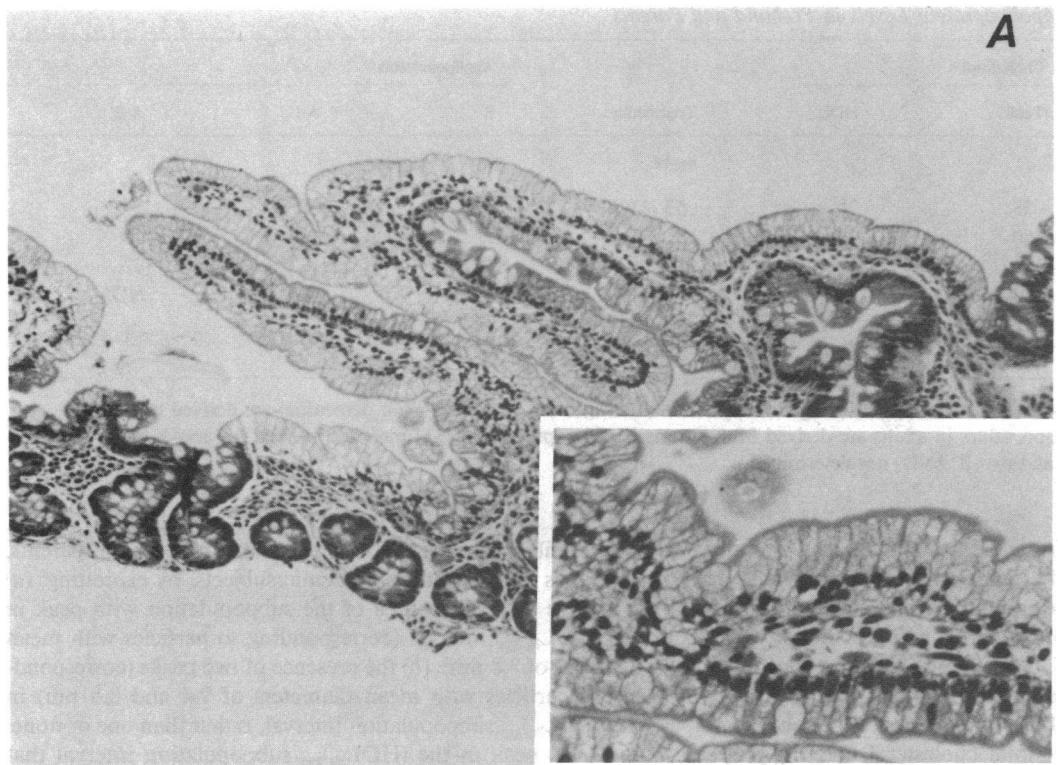


Figure 1. Small intestine and liver biopsies from proband. (A) Jejunal biopsy with normal villous architecture and (inset) marked apical cytoplasmic vacuolization of absorptive epithelial cells (hematoxylin and eosin;  $\times 80$ ;  $\times 460$ , inset). (B) Liver biopsy with diffuse fatty cytoplasmic vacuolization (hematoxylin and eosin;  $\times 200$ ).

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A distinct band of  $\beta$ -mobility was never visualized when the patient's plasma was examined by lipoprotein electrophoresis. However, a lightly staining zone of mobility intermediate

between beta and prebeta was observed (Fig. 2 A). Preparative ultracentrifugation demonstrated that these lipoproteins had hydrated densities  $< 1.006$  g/ml, and they had lower electro-

Table I. Serum Lipid\* and Apolipoprotein Levels in Proband and Parents

	Cholesterol		Apolipoproteins			
	Total	HDL	Triglycerides	B	A-I	A-II
<i>mg/dl</i>						
Proband	38	28	63	0.6	48	9
Mother	150	40	61	57	134	16
Father	196	42	113	66	123	22
Children	80-250	39-74	31-153	ND‡	ND‡	ND‡
Adult men	142-286	33-67	36-304	47-146	60-225	13-42
Adult women	122-274	40-70	39-159			

\* Lipid and HDL-cholesterol values in normal children aged 0-9 yr and adults aged 20-24 yr (5th-95th percentile) are derived from Lipid Research Clinic data (41-43); apo values in adults are derived from an unselected population of 337 men and women for apo A-I and 1,030 men and women for apo A-II and apo B. ‡ND, not determined.

phoretic mobility than normal VLDL (Fig. 2 B). This does not appear due to artifactual degradation, since plasma collected in the presence of protease inhibitors generated an identical pattern. No lipoproteins of beta or prebeta mobility were observed when fractions of  $\rho < 1.006$  g/ml were concentrated 5-10-fold by ultracentrifugation.

Both parents had slightly low plasma HDL-cholesterol concentrations and normal total cholesterol and triglyceride levels (Table I). Distinct beta and prebeta bands as well as alpha lipoproteins were visualized in the parents' plasma (Fig. 2 A). When a polyclonal antiserum against normal LDL was added to the plasma of the patient and his mother, all lipoproteins of beta and prebeta mobility were removed from the mother's plasma by immunoprecipitation (Fig. 2 C). However, the slowly migrating lipoproteins in the patient's plasma were not quantitatively removed by this procedure (Fig. 2 C).

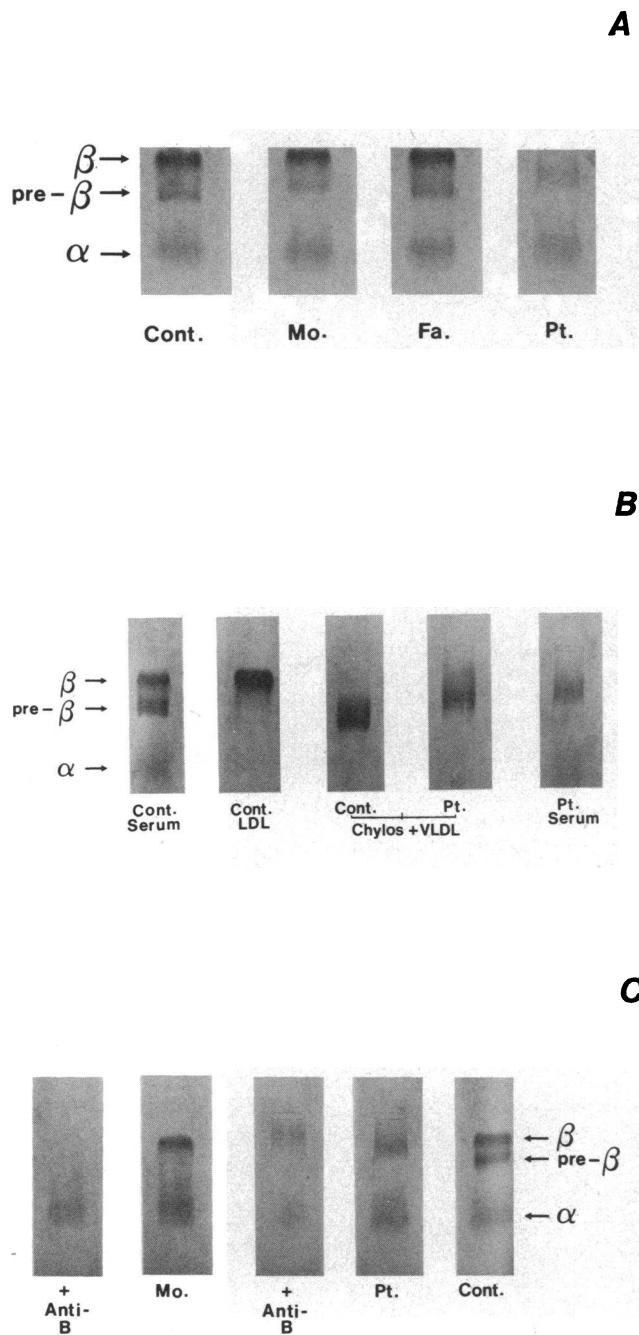
Electron microscopy showed that the lipoproteins of  $\rho < 1.006$  g/ml in the patient's plasma differed from those in his parents (Fig. 3 a). Particles in the patient's plasma averaged 93.2 nm ( $\pm 35.1$ ), while those from his father and mother were 45.3 ( $\pm 13.0$ ) and 44.3 ( $\pm 10.4$ ) nm, respectively. In the patient's LDL range ( $1.006 < \rho < 1.063$  g/ml), no cholesterol or protein were detected using conventional methods. However, lipoprotein particles were visualized by electron microscopy. The predominant structures were stacked particles that were 18.3 nm ( $\pm 4.2$ ) in their long axis and 8.3 nm ( $\pm 1.6$ ) in their short axis (Fig. 3 b). These lipoproteins resemble the LDL previously reported in abetalipoproteinemia (30, 31). The occasional large particles contained in this fraction may have represented chylomicron remnants. LDL from the plasma of the patient's father and mother were round particles of 26.1 ( $\pm 2.9$ ) and 24.2 ( $\pm 2.7$ ) nm, respectively. These were indistinguishable from control LDL. HDL ( $1.063 < \rho < 1.21$  g/ml) from the patient's plasma contained two populations of particles: large particles 10.6 nm ( $\pm 1.7$ ) in diameter and smaller particles 8.1 nm ( $\pm 1.3$ ) in diameter. HDL in the father appeared more homogeneous and had a mean diameter of 8.7 nm ( $\pm 1.5$ ).

Detailed information on the particle size distribution of plasma HDL of the patient and parents was obtained by gradient gel electrophoresis. The patient's HDL profile showed peaks with maxima within each of the major HDL subpopulation intervals, except in the  $(HDL_{3c})_{ggc}$  interval (Fig. 4 A).

The profile of the patient differed from those of the parents, as well as those of normolipidemic subjects, by exhibiting: (a) a relative increase in area of the subpopulation with peak in the  $(HDL_{3b})_{ggc}$  interval (corresponding to particles with mean diameter of 7.8 nm); (b) the presence of two peaks (corresponding to particles with mean diameters of 9.4 and 8.8 nm) in the  $(HDL_{2a})_{ggc}$  subpopulation interval, rather than one or none; and (c) a peak in the  $(HDL_{2b})_{ggc}$  subpopulation interval that corresponded to a component larger (mean diameter 11.6 nm) than ordinarily observed (mean diameter 10.6 nm) in this interval. Lipoprotein material even larger than  $(HDL_{2b})_{ggc}$  species (i.e., larger than 12.9 nm) was noted in the patient's profile and appeared as area under the trailing edge of the profile preceding the  $(HDL_{2b})_{ggc}$  peak (Fig. 4 A). In general, the patient's pattern is similar to those hypertriglyceridemic subjects who have a relatively pronounced  $(HDL_{3b})_{ggc}$  component (32), and to HDL patterns of human cord blood that contain two components within the  $(HDL_{2a})_{ggc}$  subpopulation interval (33). The HDL profiles of both parents showed a predominant component in the  $(HDL_{3a})_{ggc}$  and a relatively minor component in the  $(HDL_{3b})_{ggc}$  subpopulation intervals, which was characteristic of normolipidemic subjects. However, unlike patterns of most normolipidemic male subjects, the father's pattern showed a resolved peak in the  $(HDL_{2a})_{ggc}$  interval (Fig. 4 B). The mother's pattern showed a well-resolved peak in the  $(HDL_{2b})_{ggc}$  interval, which was characteristic of profiles frequently observed in women (Fig. 4 C).

The gross chemical composition of the triglyceride-rich lipoproteins in the patient's plasma did not differ strikingly from those in his parents and a control (Table II). The cholestryler ester content of the parents' lipoproteins was greater than that of the patient, but the control, who was sampled in the absorptive state, had a similarly low content of cholestryler esters. LDL composition in the parents did not differ from that in the control, and HDL were not remarkable in either the patient or his parents (Table II).

*Erythrocytes and lipoprotein phospholipids.* In classical abetalipoproteinemia, both plasma lipoproteins and erythrocytes are proportionately enriched in sphingomyelin and poor in phosphatidylcholine. There was a moderate reduction in the phosphatidylcholine/sphingomyelin ratio in the erythrocyte lipids of our patient, but the abnormality was not as marked as in typical abetalipoproteinemia (Table III). The phosphati-



**Figure 2.** Agarose gel electrophoretograms stained with fat red 7B. (A) Sera from a control (Cont.), the patient (Pt.), and his mother (Mo.) and father (Fa.). (B) Serum from the patient and a control, control LDL, and the  $\rho < 1.006$ -g/ml lipoprotein fraction (chylos and VLDL) from the patient and a control. (C) Sera from the patient, his mother, and a control, and after adsorption of the mother's (far left) and patient's (third from left) sera with antisera to normal LDL.

dylcholine/sphingomyelin ratio in the HDL fraction of our patient was 3.6, while corresponding values in the control and parents were 5.7, 6.2, and 6.9, respectively.

**Apolipoproteins.** The patient's undiluted plasma generated no precipitin lines when examined by Ouchterlony double diffusion against two polyclonal antisera to normal LDL. A radial immunodiffusion assay with a sensitivity of at least 1.4

mg LDL protein/dl also gave no reaction, whereas the patient's parents' plasma had apparent concentrations of 59 and 72 mg/dl, respectively. However, when undiluted plasma of the patient were examined in an RIA, apparent apo B concentrations of 0.6–0.9 mg/dl were observed. This led to more extensive evaluation of the  $\beta$ -apoproteins in the patient and his parents.

The quantity of protein in the patient's LDL fraction ( $1.006 < \rho < 1.063$  g/ml) was too low to confidently quantify by conventional methods, and this fraction was not reactive in the RIA. LDL from both parents and from a simultaneously prepared control sample reacted in parallel with the LDL standard (Fig. 5 A). By this immunochemical criterion, therefore, the LDL of the parents were not qualitatively different from normal.

Lipoproteins of  $\rho < 1.006$  g/ml were also tested in the RIA. The slope of the displacement curve of normal VLDL in this assay was slightly but significantly less than LDL (data not shown). We compared the displacement curves of triglyceride-rich lipoproteins from the patient with those of his parents and with a nonfasting control whose plasma contained chylomicrons (Fig. 5 B). Chylomicron enrichment of the control lipoproteins shifted the curve to the right, but the slope did not differ from the parents. In contrast, the displacement curve generated by the patient's lipoproteins differed strikingly and very significantly from the parents and control (Table IV; Fig. 5 B).

Electrophoresis in 3% polyacrylamide, which was performed in both Providence and Berkeley, demonstrated no material as large as the B-100 protein in the patient's VLDL/chylomicron fraction, although B-100 was readily demonstrated in the VLDL and LDL of both parents (Fig. 6). A small amount of protein with mobility identical to B-48 was consistently identified in the patient's triglyceride-rich lipoprotein fraction (Fig. 6). This protein co-migrated with the major high molecular weight protein in cynomolgus monkey thoracic duct lymph chylomicrons, and the comparable protein from normal human plasma chylomicrons, extracted from SDS gels, had an amino acid composition similar to that described by Kane et al. (3) for B-48 (not shown). We could not isolate sufficient B-48 from the child's plasma for amino acid analysis.

It was important to exclude proteolysis as the source of the B-48 or the explanation for the apparent B-100 deficiency in our patient. Lipoproteins from the proband and his parents were isolated from plasma collected in EDTA, benzamidine, and aprotinin. The VLDL/chylomicron and LDL fractions from the parents contained no B-76 or B-24, indicating that B-100 degradation was inhibited (not shown). The chylomicron remnant fraction of the proband again displaced abnormally in the LDL radioimmunoassay, which indicated that proteolysis was not responsible for the different immunoreactivity.

The VLDL/chylomicron fractions from the proband and his parents were radioiodinated and the labeled proteins separated by SDS polyacrylamide gel electrophoresis (Fig. 7). Radioactivity in the gel-zone corresponding to B-100 was barely above background in the patient's lipoproteins, whereas the B-48 region contained 15% of the total radioactivity recovered. The C-protein fraction accounted for more than half of the proband's radiolabeled protein. The mother's  $\rho < 1.006$ -g/ml fraction contained primarily VLDL, and the B-100 zone contained 49% of the total radioactivity. The father

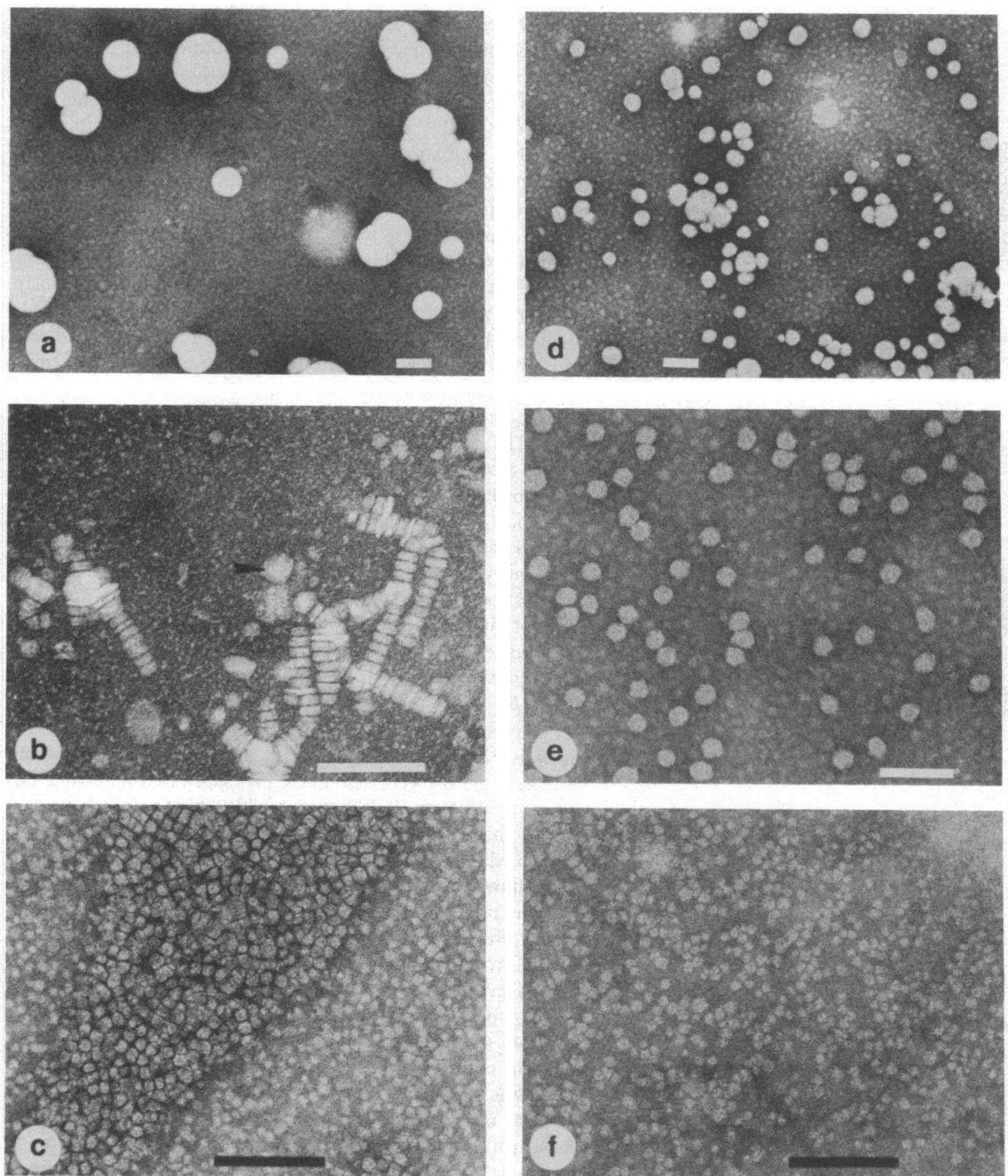


Figure 3. Electron micrographs of negatively stained lipoproteins from the patient (a-c) and his father (d-f). (a)  $\rho < 1.006\text{-g/ml}$  fraction of the patient. Note large particles present even in the fasting state. (b)  $\rho 1.006\text{-}1.063\text{-g/ml}$  fraction from patient. The characteristic particles of this fraction form rouleaux and have a long and short axis of 18.3 and 8.3 nm, respectively. Arrow indicates large, round structure, which may be a chylomicron remnant. (c) Patient's  $\rho$

$1.063\text{-}1.21\text{-g/ml}$  fraction. Two distinct populations of particles are visible. On the left side of the micrograph, the particles are large and aggregated. To the right, freestanding particles with small diameters are visible. (d)  $\rho < 1.006\text{-g/ml}$  fraction from the father. (e)  $\rho 1.006\text{-}1.063\text{-g/ml}$  fraction from the father. (f)  $\rho 1.063\text{-}1.21\text{-g/ml}$  fraction from the father. Bars represent 100 nm.

was sampled in the absorptive state, and his VLDL/chylomicron fraction contained moderate amounts of both B-100 and B-48. Unidentified proteins in zone 5 were prominent in both the proband and his father; these may be plasma proteins adsorbed to chylomicrons.

Analysis of the patient's plasma lipoprotein fraction of  $P < 1.006\text{ g/ml}$  and his HDL by alkaline-urea polyacrylamide

gel electrophoresis failed to demonstrate the monosialylated form of apo C-III, while disialylated apo C-III was present in both VLDL and HDL (Fig. 8). Both species of apo C-III were apparent in the parents' HDL and VLDL. A distinct apo E band was not visualized in the patient's triglyceride-rich lipoproteins using this gel system (Fig. 8), but a protein identical to apo E in size was easily identified by SDS electrophoresis

Table II. Lipoprotein Composition\* in Proband, Parents, and a Control

	UC	CE	TG	PL	PRO
% dry weight					
<b>VLDL/chylols</b>					
Proband	5	2	62	26	5
Mother	6	10	47	27	9
Father	7	9	53	23	8
Control	4	1	53	32	9
<b>LDL</b>					
Proband	—‡	—	—	—	—
Mother	8	43	7	23	20
Father	6	44	7	23	19
Control	8	43	6	24	19
<b>HDL</b>					
Proband	2	16	5	24	53
Mother	2	15	5	26	52
Father	2	15	4	25	54
Control	3	19	2	29	47

Abbreviations: UC, unesterified cholesterol; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids; and PRO, protein.

\* VLDL/chylols, LDL, and HDL were isolated by sequential ultracentrifugation at densities 1.006 g/ml, 1.063 g/ml and 1.21 g/ml. Lipoproteins were floated through a salt solution of the same density to minimize contamination but were not subjected to recentrifugation. The control was a normolipidemic man sampled in the absorptive state.

‡ No measurable protein or cholesterol.

in 8.5% polyacrylamide (not shown). RIA demonstrated low levels of apo A-I and A-II in the patient, and normal levels in his parents (Table I). These results are consistent with previous observations in adults with abetalipoproteinemia in whom plasma apo A-I and A-II levels are, respectively, 50–70% and 30–40% lower than normal (34, 35).

## Discussion

The lipoprotein deficiency state described in this report appears to differ from that disorder designated "normotriglyceridemic

Table III. Erythrocyte Phospholipids

	LPC	SPH	PC	PS	PE
% total					
Proband	4	31	25	14	26
Mother	5	25	29	15	27
Father	5	23	29	14	29
Control	4	25	29	14	28
Literature (32)					
Abetalipoproteinemia	ND	34	22	15	29
Normals	ND	25	33	14	28

Abbreviations: LPC, lysophosphatidylcholine; SPH, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidyl-ethanolamine.

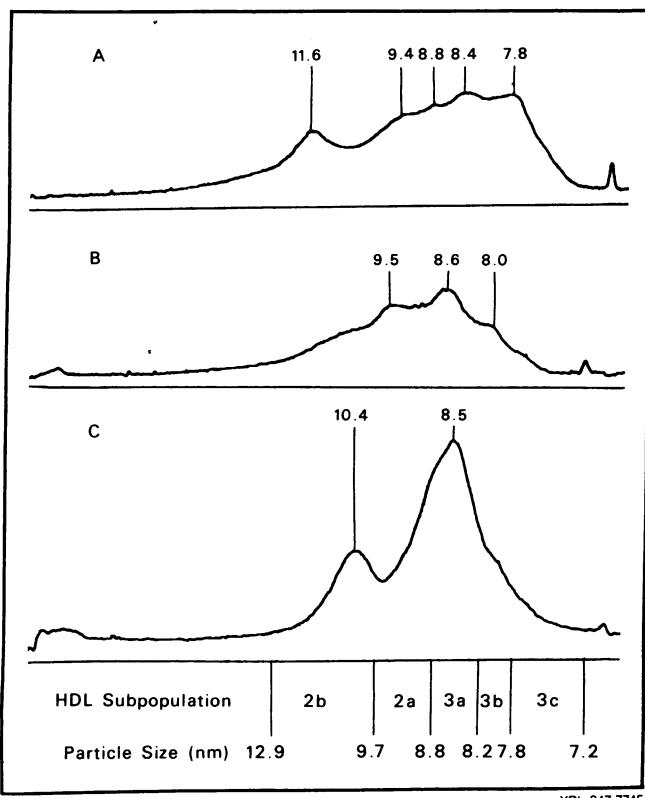


Figure 4. Gradient gel electrophoresis patterns of HDL ( $\rho$  1.063–1.21 g/ml) isolated from plasma of (a) patient, (b) father, and (c) mother. Particle size intervals of major HDL subpopulations [ $(HDL_{2b})_{apo}$ ,  $(HDL_{2a})_{apo}$ ,  $(HDL_{3a})_{apo}$ ,  $(HDL_{3b})_{apo}$ , and  $(HDL_{3c})_{apo}$ ] are indicated. Conditions of electrophoresis and calibration of densitometric scans are described in Methods.

abetalipoproteinemia" by Malloy and co-workers (14). Small intestinal biopsy in Malloy's patient (14) showed that normal morphology and chylomicron production was probably normal. Since this manuscript was submitted for publication, a second case thought to represent isolated B-100 deficiency has been reported (36). Intestinal biopsy was not performed, but malabsorption was documented, and this was attributed to the B-100 deficiency. This latter case is more likely identical to that reported here. The diarrhea, mild steatorrhea, and intestinal mucosal fat accumulation in our patient all suggest limited capacity for chylomicron production despite synthesis of the B-48 protein.

Our patient had no retinal or neuromuscular abnormalities, but this would be unusual at this age even in classical abetalipoproteinemia (34); and the presence of hepatic steatosis is typical of all forms of abetalipoproteinemia (1). Hepatic fat accumulation is presumably due to inability to elaborate VLDL, as reflected in the absence of the B-100 protein from plasma. Liver biopsy was not performed, and hepatomegaly was not reported in Malloy's patient (14), but gross hepatic enlargement is not typical even in recessive abetalipoproteinemia (34). Nevertheless, it remains uncertain whether Malloy's patient (14) was incapable of any VLDL synthesis, since she responded to a fat-free, high carbohydrate diet with an increase in plasma triglycerides. It is conceivable that B-48 containing VLDL may be elaborated by the liver in man, as

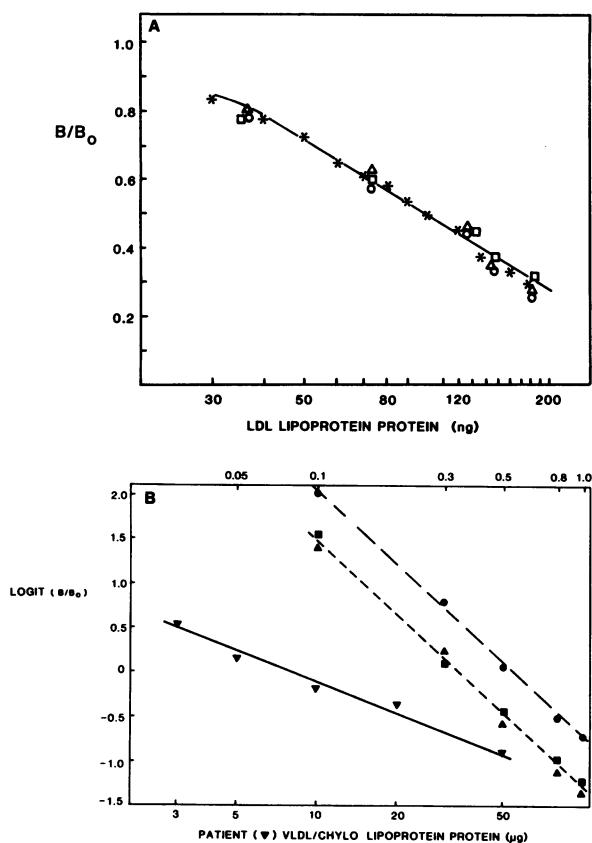


Figure 5. Apo B RIA analysis of lipoproteins from the patient and his parents. (A) Displacement curves generated by LDL from a control (○) and the parents are parallel to those produced by the LDL standard (●). □ Father; △, mother. (B) Logit ( $B/B_0$ ) curves produced by the  $\rho < 1.006\text{-g/ml}$  (VLDL/chylo) fraction obtained from the patient and his parents while fasting and from a control subject in the absorptive state. Control (●), parents (▲, ■) VLDL/chylo lipoprotein protein ( $\mu$ g).

has been documented in the rat (10, 11), although a human hepatoma cell line was found to secrete lipoproteins containing only B-100 and no B-48 (37).

Our patient's  $\rho < 1.006\text{-g/ml}$  lipoproteins were similar in size to those described in normotriglyceridemic abetalipoproteinemia (14). The particles in both disorders are extremely large, even in the fasting state. Lipoproteins in the LDL fraction of our patient are morphologically similar to those

Table IV. Parameters\* of the Displacement Curves Generated by VLDL/Chylomicron Fraction in the LDL Radioimmunoassay

VLDL/chylomicron fraction	a	b	Probability
Control	2.7	-0.35	—
Proband	1.9	-0.88	0.004
Mother	2.5	-0.36	0.3
Father	2.5	-0.36	0.4

\* Displacement curves are described by the equation:  $\text{Log}(\text{total protein}) = a + b \text{ logit}(B/B_0)$ , where  $B_0$  is the fraction of tracer bound when no standard LDL is added and  $B$  that fraction bound in the presence of standards or test samples.

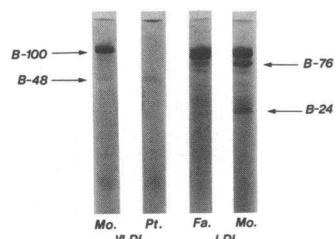


Figure 6. SDS polyacrylamide gel electrophoresograms of  $\rho < 1.006\text{-g/ml}$  lipoproteins (VLDL) from the patient (Pt) and his mother (Mo) and the  $\rho 1.006\text{-}1.063\text{-g/ml}$  fraction (LDL) from the parents. Identification of the B-48 protein is based on mobility identical to monkey lymph chylomicron B-48. Lipoproteins were isolated from serum collected without protease inhibitors; B-76 and B-24 are tentatively identified on the basis of published (3) electrophoretic patterns.

described for autosomal recessive abetalipoproteinemia (30), but differ from that described in normotriglyceridemic abetalipoproteinemia, in that we observed few large 30-nm particles. HDL also differed in those two cases. The normotriglyceridemic abetalipoproteinemic patient of Malloy et al. (14) had only HDL<sub>3</sub> particles; in contrast, we identified both HDL<sub>2</sub> and HDL<sub>3</sub> components in our patient, as described earlier in classical abetalipoproteinemia (31, 34). In our patient, the large particles with mean diameters of 10.6 nm were consistent with the presence of the distinct (HDL<sub>2b</sub>)<sub>gg</sub> peak seen on gradient gel electrophoresis. The smaller particles visualized by electron microscopy probably correspond to the multi-peaked region designated (HDL<sub>2a-3c</sub>)<sub>gg</sub> on gradient gel electrophoresis.

An additional interesting contrast between our patient and Molly's normotriglyceridemic abetalipoproteinemia patient (14) is the striking deficiency of monosialylated apo C-III in our patient, whereas this protein was present in reduced proportion in the child reported by Malloy and co-workers (14). Apo C-III-1 is completely absent or greatly reduced in recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia (31, 35), and we have postulated that this species of apo C-III is elaborated together with VLDL while the disialylated apo C-III-2 is secreted independently (38). Apo C-III-1 was also not identified in the unusual case of hypobetalipopro-

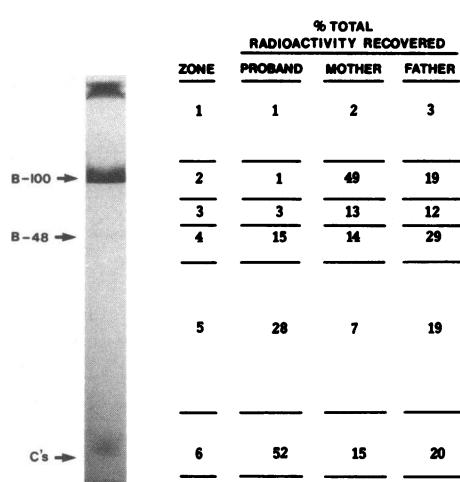


Figure 7. Distribution of radioiodinated VLDL/chylomicron apolipoproteins from the proband and his parents. The representative gel containing B-100, B-48, and the C-proteins is from the father. Gels were sliced for radioassay as indicated.

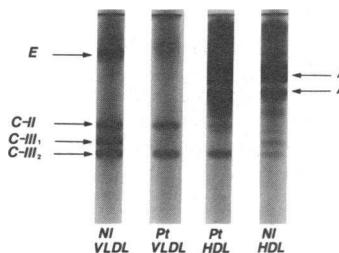


Figure 8. Alkaline-urea polyacrylamide gel electrophoretograms of apoproteins from  $\rho < 1.006$  g/ml fraction (VLDL) and  $\rho 1.063-1.21$  (HDL) from the patient (Pt) and a normal (nl) man.

teinemia reported by Steinberg et al. (39). The latter patient responded to a high carbohydrate diet with a fall in plasma triglycerides and, like our patient, had mild steatorrhea and persistent chylomicronemia after an overnight fast. The absorptive state may continue for many hours postprandially in these patients if mucosal cells efficiently absorb dietary fat but are incapable of normal chylomicron synthesis and secretion.

Since secretion of B-48 and B-100 are both abolished in homozygous hypobetalipoproteinemia and recessive abetalipoproteinemia, it appears likely that the alleles for the two proteins are either structurally linked or similarly regulated. Immunologic cross-reactivity between the B-48 and B-100 proteins suggest that they possess regions of homology or identity. This might be due to derivation from a common ancestral gene, as suggested for a number of the apolipoproteins (40), to the presence of common subunits in the two proteins, or to structural duplication within B-100.

Some monoclonal antibodies produced after immunization with human VLDL and LDL react with both the B-48 and B-100 proteins (6, 8, 9). Studies of binding affinity, however, have been limited because methods such as ultracentrifugation do not completely separate B-100 and B-48 containing triglyceride-rich lipoproteins. Since the patient described here had no B-100 containing lipoproteins, the displacement curves presented in Fig. 5 B may provide information on the immunochemical relatedness of the B-100 and B-48 proteins. This obviously is true only if the patient's B-48 protein is of normal structure. Two points are noteworthy. First, the slope of the displacement curve generated by the patient's triglyceride-rich lipoproteins is very different from that of his parents and the control (Table IV), which indicates a much lower affinity for the antibodies used in this RIA. This suggests that the epitopes on B-100 and B-48 reacting in our immunoassay are homologous but not identical. Studies in the rat have demonstrated similarly divergent displacement curves for mesenteric lymph VLDL and plasma LDL (4).

Secondly, our results are not necessarily inconsistent with the postulate that the structure of B-48 is contained within B-100 (8). Since our polyclonal antisera was employed at very high dilution (1:40,000), the immunoassay may be functionally monoclonal or oligoclonal. Antibodies with high affinity for B-48, if contained in this antisera, may have been reduced to negligible quantities by dilution.

The variant form of hypobetalipoproteinemia observed in our patient is a recessive disorder, since his parents are phenotypically normal. The parents of Malloy's patient (14) were not available for testing. In the kindred reported by Steinberg and co-workers (39), the disorder segregated like an autosomal dominant trait with highly variable penetrance. Affected children of the proband and several of his hypocholesterolemic siblings had subnormal plasma triglycerides (39),

which suggested reduced VLDL synthesis. Comparison with our patient may explain the apparent paradox of normal triglyceride levels in the proband. It is possible that the proband was the only family member in which the synthesis of B-48 as well as B-100 was limited, and the absorptive state may have been very long. Intestinal biopsy was not performed but, as already noted, this patient had mild fat malabsorption and avoided pastries, candies, and sweets.

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