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ApoHDL contained two major apoproteins designated apoLp-Gln-I and apoLp-Gln-II; together they comprised 85-90% of the total protein content. Both of the major HDL apoproteins were present in apoHDLt; but apoLp-Gln-I was disproportionately decreased with respect to apoLp-Gln-II, the ratio of their concentrations being 1: 12 in apoHDLt as compared with 3: 1 in apoHDL. Several minor apoprotein components which together comprise 5-15% of apoHDL were present in approximately normal proportions in apoHDLt. In the HDL of Tangier patients it was estimated that, compared with normal individuals, the concentration of apoLp-Gln-I was decreased about 600-fold and the concentration of apoLp-Gln-II about 17-fold. The decrease in these apoproteins was not […]

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ISOLATION AND CHARACTERIZATION OF AN ABNORMAL HIGH DENSITY LIPOPROTEIN

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ABSTRACT High density lipoproteins (d 1.063–1.210 g/ml) were isolated from the plasma of normal individuals (HDL) and seven homozygous patients with Tangier disease (HDL$_T$). In Tangier patients, the concentration of protein in the high density region (HDL$_T$) was only 0.5–4.5% of normal. Immunochemical studies, including mixing experiments conducted in vivo and in vitro, indicated that HDL$_T$ was different from HDL. HDL$_T$ was the only high density lipoprotein detectable in the plasma of Tangier homozygotes. In heterozygotes both HDL and HDL$_T$ were present. HDL$_T$ was not detected in the plasma of over 300 normal persons and 10 patients with secondary high density lipoprotein deficiency and appeared to be a unique marker for Tangier disease.

ApoHDL contained two major apoproteins designated apoLp-Gln-I and apoLp-Gln-II; together they comprised 85–90% of the total protein content. Both of the major HDL apoproteins were present in apoHDL$_T$; but apoLp-Gln-I was disproportionately decreased with respect to apoLp-Gln-II, the ratio of their concentrations being 1:12 in apoHDL$_T$ as compared with 3:1 in apoHDL. Several minor apoprotein components which together comprise 5–15% of apoHDL were present in approximately normal proportions in apoHDL$_T$. In the HDL of Tangier patients it was estimated that, compared with normal individuals, the concentration of apoLp-Gln-I was decreased about 600-fold and the con-

centration of apoLp-Gln-II about 17-fold. The decrease in these apoproteins was not due to preferential segregation with the lipoprotein fractions of d < 1.063 g/ml or with the plasma proteins of d > 1.21 g/ml. Tangier apoLp-Gln-I and apoLp-Gln-II appeared to be immunochemically identical with their normal counterparts, and no differences between the two sets of apoproteins were detected on polyacrylamide gel electrophoresis at pH 9.4 or 2.9. These results are most compatible with the hypothesis that the hereditary defect in Tangier disease is a mutation in an allele-regulating synthesis of apoLp-Gln-I.

INTRODUCTION

Tangier disease is a rare autosomal recessive disorder characterized by the near absence of plasma high density lipoprotein and the storage of cholesteryl esters in foam cells in many tissues (1). Prominent clinical features include splenomegaly, enlarged, orange-colored tonsils, and a relapsing sensory-motor neuropathy which may be quite disabling (1, 2).

Recent observations (3–6) indicate that HDL[1] contains two major apoproteins (apoLp-Gln-I and apoLp-

[1] *Abbreviations used in this paper*: HDL, high density lipoproteins, isolated from normal plasma between d 1.063–1.210 g/ml; HDL$_T$, high density lipoproteins, isolated from plasma of patients with Tangier disease between d 1.063-1.210 g/ml; LDL, low density lipoproteins, isolated from normal plasma between d 1.019–1.063 g/ml (S$_f$ 0–12); VLDL, very low density lipoproteins, isolated from fasting normal plasma at d < 1.006 g/ml (S$_f$ > 20); apoHDL, protein moiety of HDL; apoHDL$_T$, protein moiety of HDL$_T$; HSA, human serum albumin. The apoprotein components of HDL are designated by the term "apoLp-" followed by the carboxyl-terminal amino acid. (*continued on page 2506*)

Gln-II) and a group of minor apoproteins, including three which are also important apoprotein constituents of very low density lipoproteins (apoLp-Ser, apoLp-Glu, and apoLp-Ala) (7–9). These apoproteins can be purified by chromatography in dissociating agents such as urea (3, 4) or acetic acid (5) and have been characterized in several laboratories (3–19 and footnote 2). Complete amino acid sequences of apoLp-Ala (12) and apoLp-Gln-II (13) are known as well as a portion of the sequence of apoLp-Gln-I (19). These apoproteins are now believed to have a variety of functions in lipid metabolism in addition to their ability to solubilize and transport lipids. ApoLp-Glu has been shown to stimulate lipoprotein triglyceride lipase activity (20–22). ApoLp-Gln-I and apoLp-Gln-II stimulate and inhibit, respectively, lecithin-cholesterol acyltransferase, the plasma enzyme which synthesizes cholesterol esters by the transfer of a fatty acid from lecithin to unesterified cholesterol (23). ApoLp-Gln-II can also substitute for liver squalene-sterol carrier protein in facilitation of cholesterol biosynthesis (24). Finally, apoLp-Ser, apoLp-Glu, and apoLp-Ala are known to cycle between very low density lipoprotein (VLDL) and HDL in the course of metabolism of VLDL (25). Tangier disease provides a unique opportunity to investigate the consequences of the near absence of some of these apoproteins and alteration in the distribution of others on lipid metabolism.

Preliminary experiments disclosed the presence of traces of a HDL (d 1.063–1.21), designated HDLт, in Tangier plasma and suggested that HDLт was immunochemically distinguishable from normal HDL (26). In the present study, the immunochemical properties of the Tangier high density lipoprotein were further examined, and techniques for HDL protein fractionation

were employed to investigate the composition of HDLт. In particular, we were interested in the relative amounts of apoLp-Gln-I and apoLp-Gln-II in the Tangier HDL and in comparison of the isolated Tangier apoproteins with their normal counterparts.

METHODS

Patients. Seven patients homozygous for Tangier disease (Pe. Lo., Pa. Lo., T. La., E. La., J. Mi., C. Mi., and C. No.) who came from four unrelated kindreds and two sets of heterozygous parents (E. Lo., M. Lo., G. La., and P. La.) were donors of the Tangier plasma used in this report. All of these patients have been described in detail in previous reports from this laboratory (1, 27–30). Also sampled were 10 patients with secondary deficiency of HDL due to severe liver disease (31, 32), a number of patients with various types of hyperlipoproteinemia, and over 300 normal individuals with normal lipoprotein levels and electrophoretic patterns.

Dietary studies. The details of the dietary protocol for "carbohydrate induction" and its effects on the plasma lipids and lipoprotein fractions in four of the Tangier homozygotes, two sets of parents and normal subjects have been previously described (32). In all of these subjects, the intake of an isocaloric diet containing greater than 7 g carbohydrate/kg body weight caused the concentration of plasma triglycerides to rise and the concentration of HDL to fall. These effects were apparent within 2 days and appeared to be maximal at about 7 days (32).

Transfusion. One patient homozygous for Tangier disease, Pe. Lo., underwent open heart surgery for correction of pulmonic stenosis. Samples of her blood were obtained before the operation, and a large volume (2 liters) was removed as the patient was about to go on cardiopulmonary bypass. During and immediately after surgery, the patient received 10 U of normal whole blood in addition to that used to prime and maintain the blood level in the oxygenator. Blood samples were obtained at the conclusion of surgery, every 4 hr during the 1st postoperative day, twice daily for 3 more days, and then daily for 10 days. During the first 4 days after the operation, the patient was febrile and received most nourishment intravenously. She lost some fluid by drainage from her thoracotomy wound, but no additional blood or plasma was administered in the postoperative period.

Isolation of lipoproteins. Plasma, obtained by venopuncture or plasmapheresis of patients or normal subjects who had fasted overnight, was collected in 0.01% EDTA and stored at 4°C. Fractionations were begun within 48 hr of collection. Tangier and normal plasmas were carried through all of the subsequent physical and chemical separations in parallel.

Initially, HDL was separated by the method of Havel, Eder, and Bragdon (33). Subsequently, a modification of this procedure was used. In the modified procedure, the plasma was raised to a density of 1.063 by the addition of solid KBr. The plasma was then centrifuged at 15°C in a Spinco model L2 preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 60,000 rpm, using a 60 Ti rotor, for 16 hr (2.44×10^8 g-min). The tubes were sliced 25 mm below the tops, the infranatant fraction adjusted to d 1.210 with additional KBr, and recentrifuged at 60,000 rpm in the 60 Ti rotor for 24 hr (3.66×10^8 g-min). The d 1.120 supernatant fraction, obtained by slicing the tubes 19 mm below the tops, was raised to d 1.215 and washed twice by layering under a d 1.210

Designation in Other Systems of Nomenclature

Apoprotein	Carboxyl-terminal amino acid	Kostner and Alaupovic (6, 7)	Shore and Shore (3)	Brown et al. (8, 9)	Scanu et al. (4)	Rudman et al. (5)
apoLp-Gln-I	Threonine (3) Glutamine (6)	A-I	R-Thr	—	III	II, C
apoLp-Gln-II	Glutamine (3,6)	A-II	R-Gln	—	IV	III, D
apoLp-Ser	Serine (10,11)	C-I	—	D1 ⎫		
apoLp-Glu	Glutamic Acid (9–11)	C-II	—	D2 ⎬ V		—
apoLp-Ala	Alanine (8,11)	C-III	R-Ala	D3, D4 ⎭		—

Although previously considered to be threonine, the carboxyl-terminal residue of apoLp-Gln-I has recently been shown to be glutamine (6), a finding that we have confirmed.[2] ApoLp-Ser (10) is identical to the previously misnamed apoLp-Val (8). In several instances where microheterogeneity of a particular apoprotein exists the different forms of the protein are designated by a subscript. Thus, apoLp-Ala contains a mixture of apoLp-Ala$_1$ and apoLp-Ala$_2$.

[2] Lux, S. E., and K. M. John. 1972. Further characterization of the polymorphic forms of a human high density apolipoprotein, apo-Gln-I (apoA-I). Submitted for publication.

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KBr solution and centrifuging at 40,000 rpm in a 40 rotor for 48 hr (3.04×10^8 g-min). Appropriate blanks of identical salt composition and density were included in each rotor and used to determine the salt density of the supernatant and infranatant fractions after each centrifugation. The yield of unwashed HDL agreed with the yield predicted from determination of HDL cholesterol in the starting plasma (34), and published values for the per cent cholesterol in HDL (35).

For quantitation of plasma HDL in small samples of plasma, the plasma density was raised to 1.063 by the addition of NaCl and KBr (33) and the plasma centrifuged in a 40.3 rotor for 16 hr. The top and bottom fractions were obtained by tube slicing and brought to the concentration of the starting plasma by addition of 0.9% NaCl solution. HDL concentration was defined in terms of the cholesterol in the bottom fraction.

Measurement of HDL by precipitation of LDL and VLDL. 0.15 ml of 0.1 M manganese chloride and 6 mg of sodium heparin were added to 3-ml portions of plasma (36). A precipitate was allowed to form for 15 min at 4°C, and then the plasma was centrifuged for 15 min at 4°C. HDL is not precipitated by this procedure. The heparin-manganese supernates were always checked for contamination with VLDL and LDL by use of immunoelectrophoresis with antisera to LDL; none was found.

Delipidation and solubilization. Lipoproteins were delipidated with ethanol-diethyl ether (1:3 v/v) at 4°C as previously described (38). They contained less than 0.4% phospholipid by weight (16). The apoproteins were completely soluble in aqueous buffers above pH 7.0.

Fractionation of HDL apoproteins. HDL apoproteins for use as purified standards were isolated by a combination of the published techniques of gel filtration and ion-exchange chromatography (3, 4). Delipidated apoproteins were completely dissolved at a concentration of 8–12 mg/ml in 0.2 M Tris-HCl buffer containing 0.01% EDTA and 6 M urea at pH 8.2. 10–50 mg were fractionated by Sephadex G-200 chromatography in a manner similar to that described by Scanu, Toth, Edelstein, Koga, and Stiller (4), with the minor modification that the column was equilibrated and eluted with 0.2 M Tris-HCl containing 0.01% EDTA and 6 M urea at pH 8.2. However, because it was difficult to obtain apoLP-Gln-II completely free of apoLP-Gln-I by this method and because the apoLP-Gln-I and "minor protein" (fraction V of Scanu et al. [(4)] peaks could be fractionated further by ion-exchange chromatography, the Sephadex fractions were often further purified by chromatography on diethylaminoethyl (DEAE)-cellulose in 6 M urea, using a modification[3] of the procedure described by Shore and Shore (3). With this procedure, apoLp-Gln-II eluted earlier than apoLp-Gln-I and was judged to be pure by polyacrylamide gel electrophoresis and its unique amino acid composition (i.e., absence of histidine, arginine, and tryptophan).[3] The "minor-protein" fraction was resolved into four principal components by DEAE-cellulose chromatography. These corresponded to the four proteins (apoLp-Ser, apoLp-Glu, apoLp-Ala₁, and apoLp-Ala₂) that are also major components of VLDL (8–10). Several additional minor components representing < 1% of the total HDL protein remained and were not identified.

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[3] Lux, S. E., K. M. John, and H. B. Brewer, Jr. 1972. The amino acid sequence of apoLp-Gln-II, a human plasma high density apolipoprotein. I. Isolation and characterization of apoLp-Gln-II (apoA-II). Submitted for publication.

To retard carbamylation of proteins (39), urea solutions were passed over a mixed bed ion-exchange resin (Rexyn I-300, Fisher Scientific Co., Pittsburgh, Pa.); and buffers made with this de-ionized urea were stored at 4°C and replenished every 24–48 hr during experiments conducted at room temperature. With Sephadex G-200 chromatography, elution volumes were determined by weighing tared tubes and correcting the difference in weight for density of the buffer. The volumes were expressed as $K_{av} = (V_e - V_o)/(V_t - V_o)$ where V_e represents the elution volume of the substance, V_o, the void volume, and V_t, the total bed volume of the column (40). Void volumes were determined using blue dextran (Pharmacia Fine Chemicals Inc., Piscataway, N. J.). Column fractions were monitored for protein by their absorbance at 280 or 230 nm and dialyzed against 0.01% EDTA, pH 8.2, using No. 18 cellulose casing (Union Carbide Corp., New York).

Storage of apoproteins. Apoprotein solutions were stored up to 30 days at 4°C and for longer periods at −20°C. It was found that apoproteins stored in solution at 4°C, particularly in the presence of urea, showed a gradual change in polyacrylamide gel electrophoresis patterns over 1–6 months (and occasionally as early as 2 wk). The earliest observed change was usually a separation of the apoLp-Gln-II into two bands; later the single, broad apo-Lp-Gln-I band split into multiple ones. These changes were considered to be artifactual since they were usually not present in freshly prepared samples of apoLp-Gln-I or apoLp-Gln-II. They were not accompanied by detectable alterations in immunochemical properties. Apoproteins stored frozen in solution at −20°C in the absence of urea or lyophilized and stored at 4 or −20°C showed none of these changes for periods up to 6 months.

Immunochemistry. Antisera prepared as described previously (37) were characterized as shown in Table I and stored frozen in small lots. Equivalence points were determined semiquantitatively using the device described by Piazzi (41). Parallel rows of five wells were created in an immunodiffusion plate, the wells in one row decreasing, those in the adjacent row increasing in size. The wells in the two rows were so arranged that each well in one row was equidistant from two wells in the opposite row. The volume of the wells were 50, 20, 10, 5, and 2.5 μl. By placing antigen in one row and antibody in the other, nine reactions were observed with antigen:antibody ratios (micrograms antigen:microliters antibody) of 2.5x:50, 2.5x:20, 5x:20, 5x:10, 10x:10, 10x:5, 20x:5, 20x:2.5, and 50x:2.5 (where x = antigen concentration). For purposes of comparison, the ratios were normalized to 1 μg of antigen, giving antigen: antibody ratios from (1:20) (x) to 1:0.05 (x). The antigen:antibody ratios were exactly reproducible if run on the same day but varied with time due to alterations in antibody titers on prolonged storage at 4°C. Equivalence points and titers were checked at the time each batch of antibody was thawed and every 4 wk thereafter.

Absorption of antibodies was carried out with sufficient antigen to produce an antigen:antibody ratio fivefold greater than the antigen:antibody ratio at equivalence. The precipitates were allowed to stand 48–72 hr at 4°C before the absorbed sera were harvested (42).

Toward the end of the study, monospecific antisera were isolated by coupling 10 mg of highly purified apoLp-Gln-I or apoLp-Gln-II to 10 ml of packed Sepharose 4B (Pharmacia Fine Chemicals, Inc.) using procedures described by Cuatrecasas (43). The Sepharose apoLp-Gln-I and Sepharose apoLp-Gln-II were washed free of unbound protein

TABLE I

Characterization of Antisera

Antibody*	Previous designation (37)	Immunizing antigen	Prepared in	Reactivity*						
				ApoLp-Gln-I	ApoLp-Gln-II	ApoLp-Ser	ApoLp-Glu	ApoLp-Ala	LDL‡	HSA‡
Anti-HDL-1	S_1	HDL	Sheep	+	+	±	0	0	+	+
Anti-HDL-2	S_2	HDL	Sheep	+	+	0	0	0	+	+
Anti-HDL-3	—	HDL§	Sheep	+	+	0	0	0	0	+
Anti-HDL-4	R_2	HDL	Rabbit	+	+	0	0	0	+	0
Anti-HDL-5	R_4	HDL	Rabbit	0	+	NT‖	NT	NT	+	+
Anti-HDL-6	—	HDL	Rabbit	+	0	NT	NT	NT	NT	NT
Anti-HDL-7	R_7	HDL	Rabbit	±	0	NT	NT	NT	+	0
Anti-HDL-8	R_3	HDL	Rabbit	+	+	NT	NT	NT	+	+
Anti-HDL-9	R_5	HDL	Rabbit	+	+	NT	NT	NT	+	+
Anti-HDL-10	R_6	HDL	Rabbit	+	0	NT	NT	NT	+	+
Anti-HDL$_T$	—	HDL$_T$¶	Rabbit	±	+	0	0	0	+	+
Anti-apoLp-Gln-I**	—	—	—	+	0	±	0	0	+	+
Anti-apoLp-Gln-II**	—	—	—	0	+	±	0	0	+	+
Anti-HSA‡‡	—	HSA	Rabbit	0	0	0	0	0	0	+
Anti-LDL	—	LDL	Sheep	0	0	0	0	0	+	0

* Antibody reactivities were determined semiquantitatively against purified antigens as described in Methods. Reactivities are expressed at + (good reactivity; maximum antigen:antibody ratio <1:20, ± (weak reactivity, maximum antigen:antibody ratio >1:20), 0 (no reaction seen).

‡ The difficulty in preparing HDL antisera free of reactivity to LDL and HSA has been noted previously (37).

§ HDL isolated from a patient with abetalipoproteinemia.

‖ NT, not tested.

¶ Tangier HDL isolated from pooled plasma of Pe. Lo. and Pa. Lo.

** Anti-apoLp-Gln-I and anti-apoLp-Gln-II were prepared by absorption of anti-HDL-1 with a fivefold excess of chromatographically purified apoLp-Gln-II (anti-apoLp-Gln-I) or apoLp-Gln-I (anti-apoLp-Gln-II).

‡‡ Purchased from Hyland Laboratories, Los Angeles, Calif.

and packed into columns. Anti-HDL-1 (15 ml) was passed over each column and the bound antibody eluted with 0.1 M acetic acid adjusted to pH 3 with NaOH. The eluate was tested by immunoelectrophoresis against antisheep whole serum and was found to contain only γ-globulin which reacted specifically with the antigen bound to the Sepharose. Antibodies were concentrated by ultrafiltration and a portion of each was again bound to Sepharose 4B (50 mg antibody/ml packed Sepharose) to produce columns of Sepharose anti-apoLp-Gln-I and Sepharose anti-apoLp-Gln-II.

Immunodiffusion was performed on $3\frac{1}{2} \times 4$-inch glass slides coated with 10 ml of 1% agarose (Seakem, Bausch & Lomb, Inc., Rochester, N. Y.) in saline. The experiments were designed to produce reactions at or near equivalence. The plates were developed at 4°C and were observed for at least 7 days. Immunoelectrophoresis in agar or agarose was carried out in veronal buffer at pH 8.2 as previously described (37).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed in 0.6 × 8.0-cm tubes. Gels containing 10% acrylamide were run in 8 M urea at pH 9.4 (44) and pH 2.9[4] and stained with 0.05% coomassie blue (Colab Laboratories, Inc., Glenwood, Ill.) as described by Chrambach, Reisfeld, Wyckoff, and Zaccari (45). Less than 0.5 μg of apoLp-Gln-I or apoLp-Gln-II would be detected

[4] The pH 2.9 system was developed and kindly supplied by Dr. Ralph Reisfeld, Scripps Clinic Research Foundation, La Jolla, Calif.

in the pH 9.4 system. The sensitivity of the pH 2.9 system was not determined.

Amino acid analysis. Amino acid analyses were performed with a Beckman-Spinco automatic amino acid analyzer (model 120B, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), adapted for high sensitivity (46) and a rapid elution schedule (47). The samples were hydrolyzed in 6 N HCl for 22 hr at 110°C in open tubes contained within a clamped, sealed desiccator. The desiccator was repeatedly evacuated and flushed with oxygen-free nitrogen before hydrolysis. By this technique both the Tangier and control samples were exposed to identical hydrolysis conditions. Cystine and tryptophan were destroyed completely by this procedure and were not determined separately. No corrections were made for possible degradation or incomplete hydrolysis of amino acid residues.

Other techniques. Protein (48), cholesterol (49), phospholipid (50), and triglyceride (51) concentrations were determined as previously described. Lipoprotein paper electrophoresis was performed in barbital buffer containing albumin (52). The strips were dried at 100°C for 10 min and stained for protein with bromphenol blue or for lipid with Oil Red-O (32).

RESULTS

Isolation of HDL$_T$. No α-lipoprotein band was obtained after paper electrophoresis of plasma from the seven patients homozygous for Tangier disease. Less

than 4 mg/100 ml of cholesterol were recovered in either the density 1.063–1.210 ultracentrifugal fraction or in the supernatant fraction after VLDL and LDL were precipitated from these plasmas with heparin and manganese. In normal subjects, ultracentrifugation or precipitation yields 35–70 mg HDL cholesterol/100 ml of plasma (34).

In every patient, however, a faint precipitin line having α mobility was obtained by reacting whole plasma with antisera made to HDL (Fig. 1B). The small amount of lipoprotein isolated from each of the Tangier patients after ultracentrifugation between 1.063 and 1.210 contained this reactivity (Fig. 1D). It was also present in the supernatant layer obtained by precipitation of the plasma fraction of < 1.210 with heparin and manganese. No precipitin line with α mobility was obtained when the plasma fractions of > 1.210 or of < 1.063 were reacted with any of the 10 different HDL antisera.

Characterization of HDLτ. The average lipid contents of HDLτ, as measured in three separate samples, were: triglycerides (13.3%), free cholesterol (9.2%), cholesteryl esters (26.1%), and phospholipids (51.2%). HDLτ had α_1 electrophoretic mobility on agar or agarose gel (Fig. 1D), or on paper. The mobility of HDLτ was slightly slower than that of HDL when they were coelectrophoresed on agar or agarose (Fig. 1E).

Preparations of HDLτ isolated from each of the homozygotes reacted with each of the 10 anti-HDL sera and appeared to be immunochemically identical with each other. All of the antisera to HDL except anti-HDL-5 revealed apparent antigenic differences between HDLτ and its normal counterpart in the form of one or more spur lines at the junction of the precipitin lines formed by HDL and HDLτ.

The antiserum made to HDLτ reacted with HDL from normal subjects and patients with Tangier disease (Fig. 1C, 1D). Absorption of the anti-HDLτ serum with either HDLτ or HDL removed all reactivity to either form of HDL. This is in contrast to the results obtained when the antisera made to HDL were absorbed with HDLτ. All, except anti-HDL-5, reacted with normal HDL after all reactivity with HDLτ was removed. These initial immunochemical experiments indicated that HDL and HDLτ were immunochemically similar but not identical. Since anti-HDL-5 was the only HDL antiserum lacking reactivity to apoLp-Gln-I, (Table I), they suggested that apoLp-Gln-I was missing from HDLτ or was present in an immunochemically altered form.

In vivo studies of HDLτ. The partial exchange transfusion occurring in patient Pe. Lo. provided an unusual opportunity to document the apparent immunochemical uniqueness of HDLτ through an in vivo
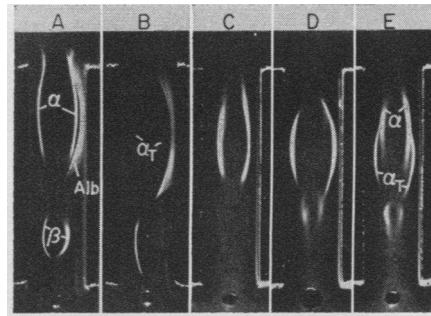

FIGURE 1 Comparisons of normal and Tangier plasma and HDL by immunoelectrophoresis in agarose. Antigens are: A, normal plasma; B, Tangier plasma; C, 1.063–1.210 g/ml fraction of normal plasma (HDL); D, 1.063–1.210 g/ml fraction of Tangier plasma (HDLτ); E, mixture of equal amounts of HDL and HDLτ. Antibodies were: A and B anti-HDL-8 (left) and anti-HDL-5 (right). C, D, and E anti-HDL-1 (left) and anti-HDLτ (right). Precipitin lines corresponding to HDL (α), LDL (β), HDLτ (α_T), and human albumin (Alb) are designated in the figure.

mixing experiment (Fig. 2). The patient had normal concentrations of HDL (36 mg/100 ml) immediately after cardiopulmonary bypass. The concentration fell to 29 mg/100 ml 8 hr later, and to 10 mg/100 ml 48 hr after surgery. These changes were accompanied by a marked fall in the titer of immunoreactive HDL. Between the 2nd and 4th postoperative days, a second α-lipoprotein precipitin line of slightly slower mobility appeared on immunoelectrophoresis (Fig. 2). The normal HDL precipitin line had almost disappeared by the 6th day.
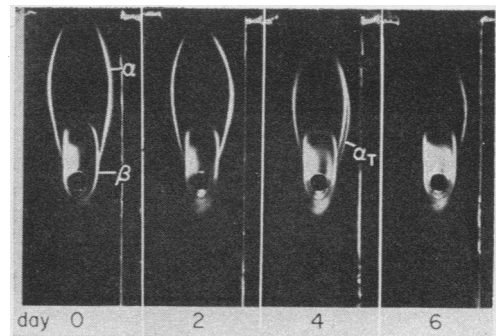

FIGURE 2 Immunoelectrophoresis in agar of serial samples of plasma from Tangier patient Pe. Lo. after a massive transfusion with normal plasma. The details of this study are presented in Methods. The antisera employed were anti-HDL-1 (right trough) and anti-HDL-2 (left trough). Both antisera were absorbed to remove anti-albumin reactivity. Immunoprecipitin lines corresponding to HDL (α) and LDL (β) are designated in the pattern produced on day 0. On day 4 a second α-migrating immunoprecipitin reaction corresponding to HDLτ (α_T) is seen. By day 6 only the α_T reaction remains. The reaction of HDLτ with anti-HDL-2 is present but is much fainter than the reaction with anti-HDL-1.
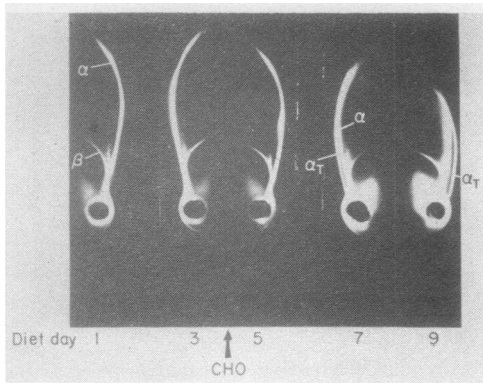
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FIGURE 3 Immunoelectrophoresis in agar of serial plasma samples from G. La., a Tangier heterozygote, before and after lowering of HDL levels by a diet high in carbohydrate (CHO). The details of this study are presented in Methods. The anti-serum employed was anti-HDL-1 which had been absorbed to remove anti-albumin reactivity. Immunoprecipitin lines corresponding to HDL (α) and LDL (β) are designated in the pattern produced on day 1. On day 7, a second α-migrating immunoprecipitin reaction corresponding to HDL$_T$ (α_T) is seen. This latter reaction is dominant in the sample procured on day 9.

HDL$_T$ in Tangier heterozygotes. On a standard American diet, the Tangier heterozygotes had HDL cholesterol concentrations between 22 and 30 mg/100 ml. Only one precipitin band was obtained by immunoelectrophoresis when their plasmas were reacted with the anti-HDL sera. Two HDL lines were obtained when the plasmas were diluted twofold; similar dilution of normal plasma produced only one line. The HDL con-
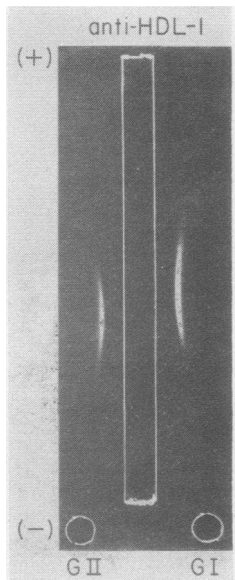

FIGURE 4 Immunoelectrophoresis in agarose of apoLp-Gln-I (GI) and apoLp-Gln-II (GII) against anti-HDL-1. Antigen concentrations were 0.1 mg/ml. Photograph at 24 hr.
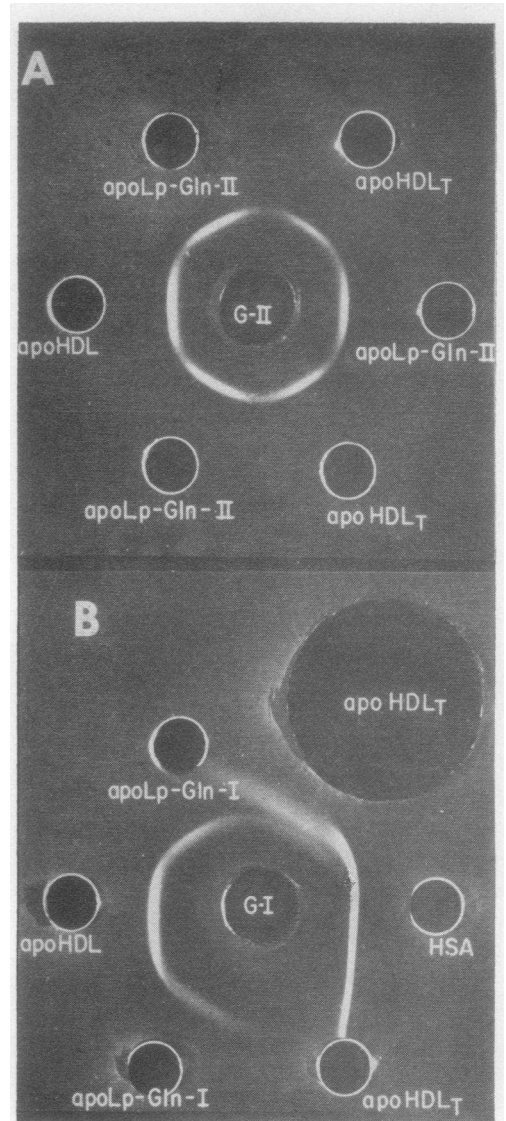

FIGURE 5 Comparison of Tangier (apoHDL$_T$) and normal (apoHDL) high density apoproteins by double diffusion in agarose against anti-apoLp-Gln-II (GII) in A and anti-apoLp-Gln-I (GI) in B. These antisera were made by absorption of anti-HDL-1 with either apoLp-Gln-I or apoLp-Gln-II. They also reacted with albumin (Table I). The apoHDL$_T$ was from Pe. Lo. Protein concentrations were identical in both experiments: HDL (0.4 mg/ml), apoLp-Gln-II (0.1 mg/ml), apoLp-Gln-I (0.1 mg/ml), and human serum albumin (HSA) (0.5 mg/ml). The *large well* in the experiment in B contained 24 times the volume of the smaller wells. Photograph at 36 hr.

centrations of each of four heterozygotes were considerably reduced by feeding a high carbohydrate diet, and a second and more slowly migrating precipitation line then became apparent (Fig. 3). When 15 normal subjects and more than 20 patients with primary hyperlipoproteinemia were similarly fed the high carbohy-

drate diet, their plasma HDL cholesterol concentrations were also reduced; in 7 of these individuals, the concentration of HDL cholesterol fell below 15 mg/100 ml. Nevertheless only one HDL precipitin line was ever observed at the nadir of the HDL concentration. Immunoelectrophoresis of whole plasma from 10 patients with hepatocellular disease (HDL cholesterol concentrations of 6–10 mg/100 ml) likewise revealed only a single HDL precipitation line. The HDL from these 10 patients was isolated by ultracentrifugation and was immunochemically identical with normal HDL and not identical with HDLτ.

Immunochemical studies of apoHDLτ. In preliminary experiments, apoLp-Gln-I and apoLp-Gln-II were shown to be immunochemically nonidentical in their reactions with the anti-HDL sera 1–4 (Table I) and with anti-HDLτ. ApoLpGln-I had a slightly faster electrophoretic mobility than apoLp-Gln-II (Fig. 4).

The earlier suggestion that apoLp-Gln-I might be missing or immunochemically abnormal in HDLτ was tested more definitively in the experiment shown in Fig. 5. Anti-HDL-1 was made specific for either apoLp-Gln-I or apoLp-Gln-II by absorption with the alternate antigen. This antiserum also reacted with albumin. As shown in Fig. 5A, both apoHDL and apoHDLτ contained an

FIGURE 6 Polyacrylamide gel electrophoresis of equal amounts of normal (apoHDL) and Tangier (apoHDLτ) high density apoproteins. The apoHDLτ was obtained from Pa. Lo. Gels containing 20 μg of protein were run at pH 9.4 in 8 M urea and stained with coomassie blue. The acrylamide concentration was 10%. The labeled bands correspond to apoLp-Gln-I and apoLp-Gln-II, the major proteins of HDL.

TABLE II

Anti-apoLp-Gln-I and anti-apoLp-Gln-II Reactivity of an Antiserum to Normal High Density Lipoprotein after Absorption with Normal or Tangier High Density Apoproteins

Antibody*	Antigen	Maximum antigen: antibody ratio‡
		μg:μl
Anti-HDL-1 + saline	apoLp-Gln-I	>1:0.5
	apoLp-Gln-II	>1:0.5
Anti-HDL-1 + apoHDLτ§	apoLp-Gln-I	1:10
	apoLp-Gln-II	<1:200
Anti-HDL-1 + apoHDL	apoLp-Gln-I	<1:200
	apoLp-Gln-II	<1:200

* Equal volumes of an antiserum to normal human high density lipoprotein (anti-HDL-1) were absorbed with equal amounts of either normal (anti-HDL-1 + apoHDL) or Tangier (anti-HDL-1 + apoHDLτ) high density apoprotein. An equal volume of saline was added to a third volume of antiserum (anti-HDL-1 + saline) as a dilutional control.
‡ Semiquantitative titers were obtained as described in Methods. The higher antigen to antibody ratio of 1:0.5 represents a greater titer than 1:10; <1:200 indicates no reaction was seen. Antigen concentrations were 0.1 mg/ml. All reactions were run on the same day.
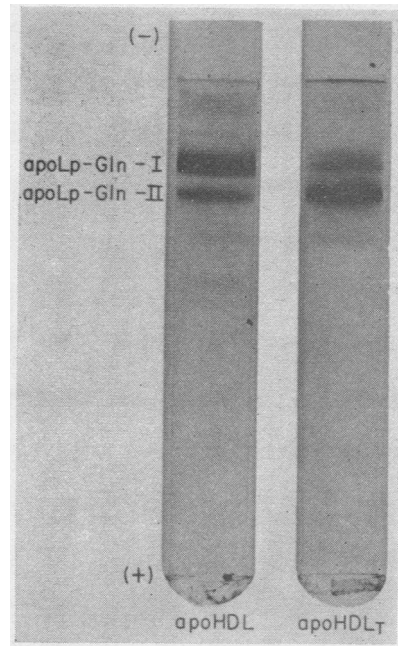§ The apoHDLτ was isolated from Pe. Lo. plasma.

antigen which was immunochemically identical with normal apoLp-Gln-II. In contrast (Fig. 5B), anti-apoLp-Gln-I demonstrated immunochemically normal apoLp-Gln-I in apoHDL but did not react with the same amount of apoHDLτ. However, if the amount of apoHDLτ was increased 12-fold (not shown) or 24-fold (large well in Fig. 5B), two immunoprecipitin lines appeared; one immunochemically identical with normal apoLp-Gln-I and the other identical with human serum albumin, a trace reactant in this preparation. Two additional observations confirmed the presence of small amounts of apoLp-Gln-I in apoHDLτ. Firstly, an antiserum to HDLτ (anti-HDLτ) reacted weakly with chromatographically purified apoLp-Gln-I (Table I, Fig. 10C). This reactivity was removed by absorption with apoLp-Gln-I but not with apoLp-Gln-II. Secondly, absorption of an antiserum to normal HDL with apoHDLτ caused a slight but definite reduction in apoLp-Gln-I reactivity (Table II). In the latter experiment, equal volumes of anti-HDL-1 were absorbed with equal amounts of either apoHDL or apoHDLτ. The apoHDL absorbed all detectable reactivity with either apoLp-Gln-I or apoLp-Gln-II (maximum antigen:antibody ratio <1:200). ApoHDLτ, on the other hand, absorbed all reactivity with apoLp-Gln-II but only decreased the titer of anti-apoLp-Gln-I reactivity from 1:0.5 to 1:10.
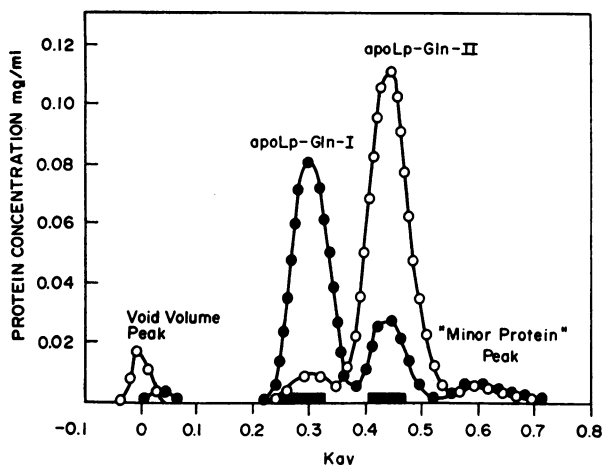
FIGURE 7 Sephadex G-200 chromatography of equal amounts of apoHDL (●——●) and apoHDLᴛ (○——○). 10 ml of each apoprotein were dissolved in 0.2 м Tris-HCl, 6 м urea, 0.01% EDTA, pH 8.2, applied to a 2.5 × 120 cm column of Sephadex G-200 and eluted with same buffer at a flow rate of 6 ml/hr. The effluent volume is expressed as Kₐᵥ (see Methods). The apoHDLᴛ was obtained from Pa. Lo. The portions of the apoLp-Gln-I and apoLp-Gln-II peaks marked by the bars were pooled and used for subsequent studies.

These immunochemical experiments indicated that both of the major HDL apoproteins were present in Tangier HDL but suggested a marked decrease in the ratio of apoLp-Gln-I to apoLp-Gln-II compared with that in normal HDL.

Polyacrylamide gel electrophoresis of apoHDLᴛ. The results of the immunochemical experiments were confirmed qualitatively by polyacrylamide gel electrophoresis (Fig. 6). At pH 9.4 apoLp-Gln-I and apoLp-Gln-II differed in their relative mobilities. In apoHDL, apoLp-Gln-I produced a broader and more intense band than apoLp-Gln-II, consistent with the approximate apoLp-Gln-I : apoLp-Gln-II ratio of 3:1 previously reported (4, 5, 17). When the same quantity of apoHDLᴛ was examined under identical conditions, there was an intensely staining band with the mobility of apoLp-Gln-II and one or two very faint bands in the region of apoLp-Gln-I. Similar results were obtained with apoHDLᴛ from four patients studied (Pe. Lo., Pa. Lo., T. La., and E. La.). Human serum albumin migrated in the same position as apoLp-Gln-I in the pH 9.4 system, and the possibility that one or both of the faint "apoLp-Gln-I" bands were attributable to albumin was considered. However, when a very large load of apoHDLᴛ (300 µg) was run at pH 9.4 and slices of the unstained gel then tested by immunodiffusion, both apoLp-Gln-I and albumin were identified in the band corresponding in mobility to apoLp-Gln-I.

Gel filtration chromatography of apoHDLᴛ. The protein components of Tangier HDL from one patient

(Pa. Lo.) were isolated and quantified by Sephadex G-200 chromatography in 6 м urea. The results are presented in Fig. 7. 10 mg of apoHDLᴛ and apoHDL were chromatographed in succession on the same column. Recoveries were 96 and 92%, respectively. The elution pattern of normal apoHDL showed the usual 3 to 1 predominance of apoLp-Gln-I (69%) over apoLp-Gln-II (23%). The Tangier apoprotein pattern was different, there being a 12 to 1 predominance of apoLp-Gln-II (83%) over apoLp-Gln-I (7%).

The percentage of the total protein eluted in the void volume was 1% for apoHDL and 5% for apoHDLᴛ. Both of these values are within the usually observed range (4, 5). The content of the two void volume peaks differed however, as determined immunochemically. The apoHDL void volume peak contained primarily apoLp-Gln-I, with only a trace of apoLp-Gln-II, while the predominant component of the apoHDLᴛ void volume peak was apoLp-Gln-II with traces of apoLp-Gln-I.

The proportion of the total protein appearing in the "minor protein" peaks was comparable for apoHDL (7%) and apoHDLᴛ (5%). A polyacrylamide gel electrophoretic comparison of the two "minor protein"
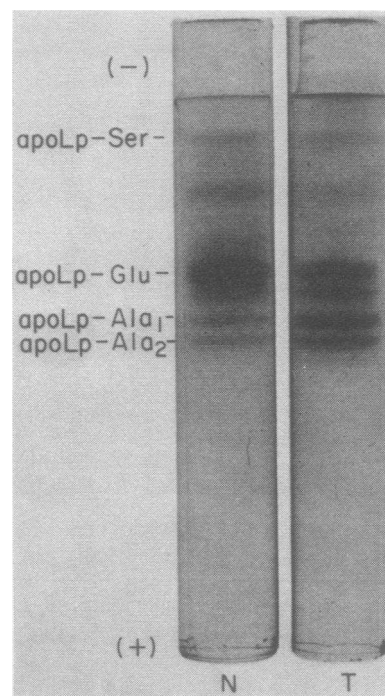

FIGURE 8 Polyacrylamide gel electrophoretic comparison of the minor protein fraction from Sephadex G-200 chromatography of normal (N) and Tangier (T) HDL. 15 µ of each fraction were applied to 10% gels, run at pH 9.4 in 8 м urea, and stained with coomassie blue. Bands corresponding to apoLp-Ser, apoLp-Glu, apoLp-Ala₁, and apoLp-Ala₂ (9) are labeled. The remaining bands represent unidentified proteins present in both apoHDL and apoHDLᴛ.
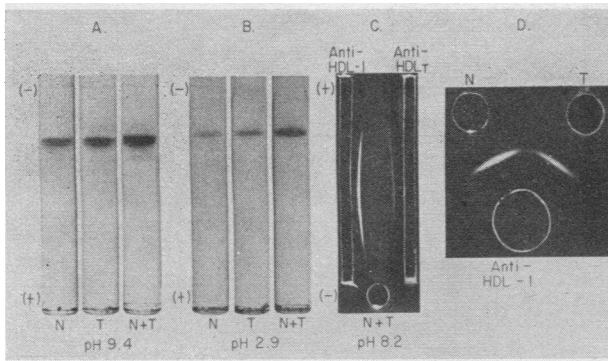
FIGURE 9 Comparison of Tangier and normal *apoLp-Gln-II*. A, polyacrylamide gels contained 7.5 μg of either Tangier (T) or normal (N) apoLp-Gln-II or 7.5 μg of each (N + T), and were run as described in Fig. 6; B, same samples run at pH 2.9; C, immunoelectrophoresis in agarose of a mixture of equal amounts of normal and Tangier apoLp-Gln-II (N + T) against anti-HDL-1 and anti-HDLτ (photograph at 24 hr); D, immunodiffusion in agarose of equal amounts of Tangier (T) and normal (N) apoLp-Gln-II against anti-HDL-1. The antigen concentration was 0.1 mg/ml. Photograph at 36 hr.

fractions is presented in Fig. 8. Bands corresponding in mobility to apoLp-Ser, apoLp-Glu, apoLp-Ala₁, and apoLp-Ala₂ were observed in both preparations. Another obvious major band appeared between apoLp-Glu and apoLp-Ala₁. Its identity is currently under investigation in this laboratory. Several other minor bands remain to be identified. All visible bands were present in both apoHDL and apoHDLτ, although, based on intensity of the stained bands, some variation in the relative proportions of the different components was apparent.

Comparison of the isolated Tangier and normal apoproteins. The apoproteins isolated from normal and Tangier HDL were further compared to see if any differences could be detected (Figs. 9 and 10). In order to avoid cross-contamination, only the fractions comprising the middle third of the apoLp-Gln-II peak and the first two-thirds of the apoLp-Gln-I peak were used. No difference in the electrophoretic mobility of Tangier and normal apoLp-Gln-II run separately or mixed was detected at either pH 9.4 or pH 2.9 (Figs. 9A and 9B). At pH 9.4, both apoLp-Gln-II bands appeared as a doublet. This was considered to be a storage artifact, as described in Methods, since only a single apoLp-Gln-II band was present when these same preparations were first examined following isolation (Fig. 6). Using immunoelectrophoresis and double immunodiffusion we could detect no difference between normal and Tangier apoLp-Gln-II with anti-HDL sera 1–4 or anti-HDLτ. Representative reactions are shown in Figs. 9C and 9D. All of the protein in both apoLp-Gln-II preparations was removed by a single passage over a column of Sepharose anti-apoLp-Gln-II. The polyacrylamide gel

patterns (pH 2.9) were unchanged after passage over a column of Sepharose anti-apoLp-Gln-I.

Similarly, no difference was detected between Tangier and normal apoLp-Gln-I following polyacrylamide gel electrophoresis at either pH 9.4 (Fig. 10A) or 2.9 (Fig. 10B). In the pH 2.9 system, both apoLp-Gln-I preparations produced doublet bands. The faster migrating band was considered to be the monomeric form of apoLp-Gln-I and the slower migrating band a polymeric form. In unlyophilized preparations of normal apoLp-Gln-I, only the faster band was seen; but, because of the low concentration of the Tangier apoLp-Gln-I sample, both the normal and Tangier apoLp-Gln-I samples were concentrated by lyophilization before electrophoresis at pH 2.9. Concentration was avoided in the experiment at pH 9.4 by using extra long stacking gels. Rudman, Garcia, and Howard (5) have previously shown that lyophilization produces polymeric forms of apoLp-Gln-I on polyacrylamide gel electrophoresis. In addition, both bands were absorbed when the Tangier and normal apoLp-Gln-I preparations were passed over a column of Sepharose anti-apoLp-Gln-I. Normal and Tangier apoLp-Gln-I were also identical when tested by immunoelectrophoresis (Fig. 10C) and double immunodiffusion (Fig. 10D) against four antisera to HDL (anti-HDL-1 to anti-HDL-4) and the antiserum to HDLτ. Immunoelectrophoresis of a concentrated portion of Tangier apoLp-Gln-I (0.5 mg/ml) produced only a single line against antihuman whole serum or anti-HDL-1 and did not react with antisera to human albumin or human LDL.

The Tangier and normal apoproteins were further compared by amino acid analysis (Table III). The com-
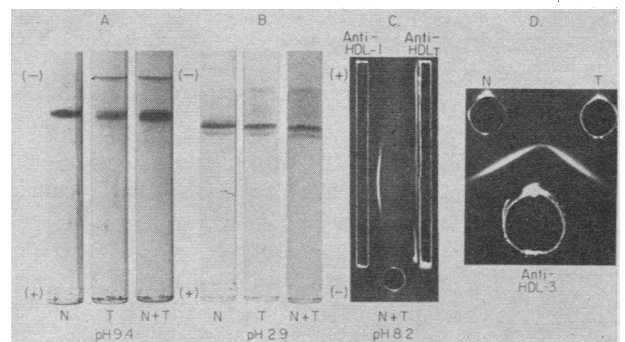

FIGURE 10 Comparison of Tangier and normal *apoLp-Gln-I*. A, polyacrylamide gels contained 7.5 μg of either Tangier (T) or normal (N) apoLp-Gln-I or 7.5 μg of each (N + T), and were run as described in Fig. 6; B, same samples run at pH 2.9; C, immunoelectrophoresis in agarose of a mixture of equal amounts of Tangier and normal apoLp-Gln-I (N + T) against anti-HDL-1 and anti-HDLτ (photograph at 24 hr); D, immunodiffusion in agarose of equal amounts of Tangier (T) and normal (N) apoLp-Gln-I against anti-HDL-3. The antigen concentration was 0.1 mg/ml. Photograph at 36 hr.

TABLE III

Amino Acid Composition of Tangier and Normal apoLp-Gln-I and apoLp-Gln-II

Amino acid‡	ApoLp-Gln-I*			ApoLp-Gln-II*		
	Tangier	Normal	Range of published values§	Tangier	Normal	Range of published values§
Aspartic acid	8.9	9.9	8.3–9.8	4.5	5.2	4.1–5.4
Threonine	5.1	4.2	3.8–4.3	7.9	7.6	7.0–8.2
Serine	7.4	5.9	5.4–6.3	7.7	7.7	7.2–8.2
Glutamic acid	18.9	19.7	16.4–19.2	21.2	20.5	19.6–20.7
Proline	4.2	4.2	3.7–5.0	5.0	4.9	3.9–5.1
Glycine	6.8	4.7	4.4–4.6	4.4	5.0	3.7–4.6
Alanine	7.8	8.1	7.3–7.6	6.6	7.0	6.4–6.9
Valine	5.3	5.6	5.1–5.8	7.6	7.5	6.9–7.7
Methionine	0.7	1.1	0.8–1.5	0.6	0.9	1.3–1.7
Isoleucine	1.3	0.2	0.0–0.2	1.1	1.7	1.5–2.0
Leucine	12.8	15.2	14.8–16.7	10.4	11.0	9.8–10.3
Tyrosine	2.5	2.3	2.3–3.3	4.8	4.0	3.9–4.1
Phenylalanine	3.3	2.3	2.3–3.1	5.0	4.9	4.0–5.0
Lysine	9.1	9.0	7.5–9.5	12.5	11.7	11.5–12.9
Histidine	1.2	1.6	1.7–2.3	0.0	0.1	0.0–0.4
Arginine	4.8	6.0	6.0–6.7	0.1	0.4	0.0–0.9
Half-cystine‖	- -	- -	0.0	—	—	2.4–3.0
Tryptophan‖	—	—	1.3–2.6	—	—	0.0

* The amino acid composition is expressed as moles/10^2 moles. The values for the Tangier and normal apoLp-Gln-II and the normal apoLp-Gln-I samples were the average of duplicate determinations. Only a single analysis of the Tangier apoLp-Gln-I sample was possible with the limited amount of material available.

‡ No correction was made for possible degradation or incomplete hydrolysis of particular residues.

§ Values are those of Shore and Shore (3), Scanu et al. (4), and Rudman et al. (5).

‖ These residues were completely destroyed under the hydrolysis conditions used (6 N HCl, 22 hr, 110°C).

positions of the normal and Tangier apoLp-Gln-II samples were identical within experimental variation. The compositions of the normal and Tangier apoLp-Gln-I samples were similar but not identical. The percentages of threonine, serine, glycine, isoleucine, and phenylalanine were higher and the percentages of leucine and arginine lower in the Tangier apoLp-Gln-I sample compared with normal apoLp-Gln-I.

Amounts of apoLp-Gln-I and apoLp-Gln-II in the HDL fraction of normal and Tangier plasma. The concentrations of apoLp-Gln-I, apoLp-Gln-II, and the "minor proteins" in the high density lipoproteins of normal and Tangier plasma were estimated from the quantity of HDL or HDLT protein isolated per 100 ml of plasma and the percentage composition obtained from Sephadex G-200 chromatography on the same preparations. The results are expressed in Table IV. Both apoLp-Gln-I and apoLp-Gln-II were decreased in the d 1.063–1.210 g/ml fraction of Tangier plasma, apoLp-Gln-II to 6.0%, and apoLp-Gln-I to 0.16% of the concentration in the normal subject. Thus, apoLp-Gln-I

was disproportionately decreased 36 times more than apoLp-Gln-II. The proportion of the minor proteins to the total protein was approximately the same in HDLT as in HDL.

Total content of apoLp-Gln-I and apoLp-Gln-II in normal and Tangier plasma. Additional experiments were performed to determine whether there was an absolute decrease in the amount of apoLp-Gln-I and apoLp-Gln-II in Tangier plasma, or whether the decrease in these apoproteins in the HDL region could be due to their preferential segregation with the other lipoprotein fractions of d < 1.063 g/ml or with the plasma proteins of d > 1.210 g/ml. The total amount of apoLp-Gln-I and apoLp-Gln-II in Tangier and normal plasmas was estimated by determining the anti-apoLp-Gln-I and anti-apoLp-Gln-II reactivity remaining after absorption of 1.0 ml of anti-HDL-1 with either 0.3 or 1.0 ml of Tangier or normal plasma (Table V). Plasma from four Tangier patients (Pe. Lo., Pa. Lo., T. La., and E. La.) and three normal subjects was tested. All of the anti-apoLp-Gln-I and anti-apoLp-Gln-II reactivity was re-

moved by 0.2 ml of normal plasma, but the same amount of Tangier plasma decreased the anti-apoLp-Gln-I and anti-apoLp-Gln-II reactivities only slightly. The range of antigen: antibody ratios after absorption with 0.2 ml of any one of the four Tangier plasmas was $> 1:0.5$ to $1:2.5$ (apoLp-Gln-I), $>1:0.5$ to $1:10$ (apoLp-Gln-II) and $>1:0.5$ (saline control). However, after absorption of 1.0 ml of anti-HDL-1 with 1.0 ml of the Tangier plasmas, all of the anti-apoLp-Gln-II reactivity was removed while only a small additional increment of anti-apoLp-Gln-I reactivity was removed (range of antigen: antibody ratios $-1:2.5$ to $1:10$, saline control $= 1:2.5$).

Hence, there is an absolute decrease in the amount of immunochemically recognizable apoLp-Gln-I and apoLp-Gln-II in Tangier plasma, and the disproportionate decrease in apoLp-Gln-I exists in the whole plasma as well as in the HDL fraction. Normally HDL apoproteins are detectable immunochemically in the lipoproteins of d < 1.063 g/ml (32, 53). Small amounts of both apoLp-Gln-I and apoLp-Gln-II have been identified.[5] In addition, apoLp-Gln-I is easily dissociated from HDL by storage or ultracentrifugation and is subsequently recovered in the fraction of d > 1.210 g/ml (37, 54). In order to exclude preferential segregation of the Tangier apoproteins with these "non-HDL" fractions, the concentrations of apoLp-Gln-I and apoLp-Gln-II were also estimated in the fractions of d < 1.063 g/ml and d > 1.210 g/ml from Tangier and normal plasma and in the infranatant fractions obtained when HDL and HDL$_T$ were washed by reflotation at d > 1.210 g/ml. Each Tangier fraction contained much less apoLp-Gln-I and apoLp-Gln-II than its normal counterpart and relatively more apoLp-Gln-II than apoLp-Gln-I.

DISCUSSION

An understanding of the protein defect in Tangier disease is gradually evolving. In the initial reports of this entity, trace amounts of HDL of uncertain identification were detected (27–29). The data in the present report provide unequivocal evidence that patients with Tangier disease have small amounts of HDL in their plasma that is similar but not identical with the normal lipoproteins of this density class. The Tangier HDL, designated as HDL$_T$, is present in the usual density range of 1.063–1.210, is not precipitated by heparin and manganese, and contains cholesterol, cholesteryl esters, and phospholipids in roughly the same proportion as normal HDL. The triglyceride content may be slightly higher than normal, a possible abnormality that has been earlier emphasized by Kocen, Lloyd, Lascelles, Fosbrooke, and Williams (2).

[5] Bilheimer, D., and S. Lux. Unpublished observations.

TABLE IV

Comparison of the Amounts of apoLp-Gln-I and apoLp-Gln-II in the d 1.063–1.210 Fraction of Normal and Tangier Plasma

	Concentration in d 1.063–1.210 fraction of normal control	Concentration in d 1.063–1.210 fraction of Tangier patient (Pa. Lo.)	% of Tangier patient compared with control
	mg/100 ml	mg/100 ml	%
HDL or HDL$_T$*	87.0	1.6	1.8
apoLp-Gln-I‡	60.0	0.1	0.16
apoLp-Gln-II‡	20.0	1.3	6.0
Minor proteins‡	6.1	0.008	1.3

* Concentrations are low because of material lost in washings.
‡ Calculated from HDL concentrations and per cent of apoLp-Gln-I, apoLp-Gln-II, and minor proteins as obtained by Sephadex G-200 chromatography of these same preparations. Concentrations of the three fractions do not add up to the concentrations of HDL or HDL$_T$ since the material in the void volume peak is not included.

These data on the lipid composition of HDL$_T$ must be considered only approximate, however, since they are not corrected for any possible contamination of "sinking prebeta lipoprotein" (Lp(a) antigen) which is also found in the fraction of d > 1.063 (55). The existence of such a contaminant in HDL$_T$ is suggested by

TABLE V

Immunochemical Comparison of the apoLp-Gln-I and apoLp-Gln-II Content of Tangier and Normal Plasma

Anti-HDL-1 absorbed with*	Amount of absorbent	Antigen	Range of antigen: antibody ratios
	ml		µg:µl
Saline	0.2	apoLp-Gln-II	$>1:0.5$
	1.0	apoLp-Gln-II	$>1:0.5$
Tangier plasmas (4)	0.2	apoLp-Gln-II	$>1:0.5–1:10$
	1.0	apoLp-Gln-II	$<1:200$
Normal plasmas (3)	0.2	apoLp-Gln-II	$<1:200$
	1.0	apoLp-Gln-II	$<1:200$
Saline	0.2	apoLp-Gln-I	$>1:0.5$
	1.0	apoLp-Gln-I	$1:2.5$
Tangier plasmas (4)	0.2	apoLp-Gln-I	$>1:0.5–1:2.5$
	1.0	apoLp-Gln-I	$1:2.5–1:10$
Normal plasmas (3)	0.2	apoLp-Gln-I	$<1:200$
	1.0	apoLp-Gln-I	$<1:200$

* 1 ml of anti-HDL-1 was absorbed with 0.2 or 1.0 ml of normal or Tangier plasma or with saline. The supernates were harvested, and the anti-apoLp-Gln-I and anti-apoLp-Gln-II activity *remaining* was determined as described in Methods. An antigen:antibody ratio of 1:0.5 represents a greater antibody titer than 1:2.5 or 1:10. An antigen:antibody ratio $<1:200$ indicates no reaction was seen. All reactions were run on the same day. Antigen concentrations were 0.1 mg/ml. The numbers in parentheses indicate the number of subjects studied.

the presence of a band that reacts with LDL antisera and migrates with prebeta mobility on immunoelectrophoresis (Fig. 1D) and by unpublished observations (in collaboration with Dr. T. Forte, Berkeley, Calif.) in which particles compatible with Lp(a) antigen (56) were observed on electron microscopy of HDLt. The contribution of such a contaminant to the lipid composition of the high density fraction, while normally small, could be significant in Tangier disease due to the 50-to-100-fold reduction in the amount of HDL. Further detailed examination of the lipid composition and physical properties of purified HDLt and of the other lipoproteins in Tangier plasma is still needed.

HDLt cross-reacts with antisera to HDL, but is distinguished from normal HDL by slightly slower electrophoretic mobility. The observation of both HDL and HDLt in the plasma of Tangier heterozygotes and in a homozygous individual following infusion of HDL during surgery confirms the difference in electrophoretic mobility of the isolated lipoproteins and eliminates the possibility that HDLt is produced artifactually during the preparation of Tangier HDL.

The studies of the composition of HDLt suggests a basis for this immunochemical discrimination. HDLt contains proportionately much less apoLp-Gln-I and more apoLp-Gln-II than normal HDL, the ratio of apoLp-Gln-I to apoLp-Gln-II being about 1:12 instead of the usual 3:1. ApoLp-Gln-II has a slower mobility on immunoelectrophoresis than apoLp-Gln-I (Fig. 4); and HDLt might be expected, on the basis of its higher content of apoLp-Gln-II to have a slower mobility than HDL. This does not exclude the possibility that the differences in lipid composition or that still undetected differences in carbohydrate composition might also contribute to the different electrophoretic mobilities of HDL and HDLt.

Despite this new insight into the apoprotein composition of HDLt, the nature of the genetic alteration that underlies Tangier disease remains speculative. If we assume the defect is limited to a single allele, the disproportionate decrease in apoLp-Gln-I suggests this apoprotein is the product of the defective gene. Because the Tangier HDL apoproteins have no detectable differences from their normal counterparts in size, charge, or immunochemical reactivity, it is tempting to infer that a mutation in an allele-regulating synthesis rather than structure of apoLp-Gln-I is involved.

Such an hypothesis is always of considerable interest, since existence of a mutation analogous to the regulatory gene mutations discovered in microorganisms by Jacob and Monod (57) has yet to be proved in man. Nevertheless, the possibility of a structural gene abnormality in Tangier disease has not been excluded. As in the recent example of the Hektoen variant of

glucose-6-phosphate dehydrogenase (58, 59), postulated regulatory mutations have often yielded to eventual discovery of a structural alteration in the protein in question.

At the present time, the only evidence suggesting a structural mutation in either of the Tangier aproproteins is the observation of minor differences in the amino acid composition between the Tangier and normal apoLp-Gln-I samples. Since the observed differences involved several amino acids the most plausible explanation is that the apoprotein samples included small amounts of other proteins or amino acids, contamination that would have been considerably amplified by the marked decrease in the quantity of Tangier apoLp-Gln-I compared with normal apoLp-Gln-I. Mutations resulting in the addition or deletion of multiple amino acids have been described (60–62). However, such extensive changes in the composition of the Tangier apoLp-Gln-I would likely have altered the electrophoretic or immunochemical properties of the mutant protein. In either case, additional studies, including peptide mapping and possibly even determination of the amnio acid sequence of the HDLt apoproteins, will be required to substantiate or refute the presence of a regulatory mutation.

A corollary of the hypothesis that the mutation is limited to a single allele-regulating apoLp-Gln-I synthesis is that the synthesis of other HDL apoproteins should be normal. As shown in Table IV and V, however, the concentration of both apoLp-Gln-I and apoLp-Gln-II is decreased in Tangier plasma. There are several possible explanations for this apparent paradox. We believe it likely that the mutant HDLt complex containing essentially only apoLp-Gln-II is unstable and is rapidly removed from the circulation. Alternatively, it is possible that apoLp-Gln-II, though synthesized normally, is not easily released from the liver cell (63) in the absence of apoLp-Gln-I. A third possibility is that the synthesis of apoLp-Gln-II is also decreased but to a lesser extent than apoLp-Gln-I. This could occur if the structural genes for apoLp-Gln-I and apoLp-Gln-II were controlled by a single mutant genome or if Tangier patients were doubly heterozygous for mutant alleles affecting both apoLp-Gln-I and apoLp-Gln-II production. Quantitation of apoLp-Gln-I and apo-Gln-II in HDL from Tangier heterozygotes and radioactive turnover studies of HDLt should allow discrimination between some of these possibilities.

Insofar as could be determined from examination of plasma from over 300 subjects with normal lipoprotein concentrations or other types of dyslipoproteinemia, HDLt appears to be a unique marker for Tangier disease. From samples generously provided by their physicians, we have been able to demonstrate HDLt in the

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plasmas of single patients with Tangier disease from Britain, Germany, and New Zealand (1) in addition to the seven described here. Thus, HDLᴛ is a feature common to all patients with Tangier disease whose plasma has been appropriately examined.

From experiments employing immunoelectrophoresis, it appears that the obligatory heterozygote for Tangier disease also has circulating HDLᴛ. It has not yet been possible to confirm this by isolation of the abnormal lipoprotein or to obtain meaningful estimates of the relative amounts of HDL and HDLᴛ in the plasma of heterozygotes. The demonstration of HDLᴛ in the heterozygote offers an opportunity to use a specific determinant of the carrier state for this rare disease, a simple decrease in HDL concentration having been previously recognized as a nonspecific test for the heterozygous phenotype (29). The need for prior reduction of HDL concentration in the heterozygote by high carbohydrate feeding, however, makes the immunochemical test for HDLᴛ impractical. The simpler expedient of diluting the plasma to enhance detection of HDLᴛ has not yet been tested in an adequate number of subjects to prove its infallibility.

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