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Evaluation of Electroimmunoassay of Human Plasma Apolipoprotein B

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SUMMARY The purpose of the present study was to develop and evaluate electroimmunoassay (EIA) for human plasma apolipoprotein (Apo) B and quantify Apo B concentrations in plasma and lipoprotein density fractions from Japanese control healthy subjects, cord blood and pregnant women. LDL of narrow density range (LDL_2: $1.030<d<1.050$g/ml) was isolated by ultracentrifugation. Antiserum against lipoprotein B was obtained by injecting LDL_2 intraperitoneally into rabbits. Double immunodiffusion and immunoelectrophoresis revealed this antiserum is monospecific to lipoprotein B. Electroimmunoassay of Apo B was performed essentially according to the method of Laurell. Concentration of antigen and antibody and time for electrophoresis were investigated. Linear relationship between height of rocket-shaped immunoprecipitates and antigen concentraton from 20 to 160μg/ml was observed utilizing 550-fold dilution of antiserum and electrophoresis for 5 hours with 10 volts/cm. The accuracy of the method was the highest with a peak height of 2 to 5cm. On the basis of 10 analyses of single sample, the standard error was found to be 2.0%. Apo B concentrations in plasma and lipoprotein density fractions from healthy control subjects, cord blood and pregnant women, were investigated. Apo B concentrations in plasma, VLDL, LDL and HDL from control subjects were 128±40, 11±4, 103±7 and 13±5mg/100 ml, respectively. Percent distribution of Apo B in VLDL, LDL and HDL were 9.0±3.2, 81.0 ±5.8 and 10.0±3.8%, respectively. Plasma Apo B concentration of the mothers (n=15) was 263±37mg/100ml, which was approximately two times of plasma Apo B concentration of control healthy subjects. However, there was no difference in Apo B distribution in lipoprotein density classes between pregnant women and control. In cord blood (n=15), plasma Apo B concentration (48±16mg/100ml) was less than one half of control. Percent Apo B distribution in VLDL, LDL and HDL were 8.4, 87.7 and 4.0%, respectively. Percent Apo B concentration in LDL and HDL were higher and lower, respectively, than those in LDL and HDL from control healthy subjects.

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Introduction

The close association between low density lipoprotein or its major lipoprotein, lipoprotein B (LP-B) and atherosclerosis has been suggested epidemiologically and by the presence of apolipoprotein (Apo) B in the atherosclerotic legion[1)~4)]. Therefore, it seems to be very important to quantify the plasma lipoprotein or apolipoprotein B in order to investigate the metabolic abnormalities in atherosclerotic cardiovascular diseases, diabetes mellitus and endocrine diseases. Several investigators have described electroimmunoassay (EIA)[5)~8)], radioimmunoassay (RIA)[9)~14)], radial immunodiffusion assay (RID)[15)16)], enzyme immunoassay[17)] or immunonephelometry[18)] for plasma Apo B quantification. Curry, M. D. reported the advantages of EIA over RIA and RID[5)]. Even though quantification by radial immunodiffusion is a technically simple procedure, there are at least three aspects of electroimmunoassay that make it the method of choice. Firstly, quantification by electroimmunoassay can be accomplished with polyvalent antisera. Secondly, the electroimmunoassay can be completed in 5—8 hours, whereas radial immunodiffusion requires at least 3 days. Finally, the rocket-shaped immunoprecipitates often provide important clues about the structure of lipoproteins. These arguments are also applicable to a comparison of electroimmunoassay and radioimmunoassay. More importantly, electroimmunoassay does not require some of the burdensome tasks of radioimmunoassay such as frequent preparations of radioactively-labeled antigens with retained immunoactivity, rechromatography of the labeled antigen prior to each assay and evaluation of all reagents for non-specific binding. The purpose of the present study was to develop and evaluate EIA for human plasma Apo B and quantify Apo B concentrations in plasma and lipoprotein density fractions from Japanese control healthy subjects, cord blood and pregnant women.

Materials and Methods

Isolation of the Low-Density Lipoproteins (LDL) Subfraction, LDL_2

Blood was obtained from normal control subjects who had been fasted for 14 hours. Blood was placed in centrifuge tube containing disodium ethylenediamine tetraacetate (EDTA : Sigma Chemical Co., St. Louis, Mo.) 1mg per m*l* of blood. One part of 0.25M EDTA in 0.5M phosphate buffer, pH 7.5 was added to 49 parts of serum to give a serum EDTA concentration of 0.005M and a phosphate buffer concentration of 0.01M. To inhibit bacterial growth, NaN_3 was added to give a concentration of 0.1mg in each milliliter of plasma. The solvent density of plasma was adjusted to d=1.030g per m*l* with solid KBr (Wako Biochemicals, Osaka, Japan) and centrifuged in a Type 40.3 rotor on the L5-50 preparative ultracentrifuge (Beckman Instruments Inc., Palo Alto, Calif.) for 44 hours at 105,000xg. After 44 hours, the top 2m*l* was removed by the tube-slicing technique. LDL_2 ($1.030<d<1.050$g/m*l*) was isolated by adjusting the solvent density of the infranatant solution with solid KBr to d=1.050g/m*l* and centrifuging at 105,000xg for 44 hours. LDL_2 was washed at d=1.050g/m*l* three times to remove contaminating serum albumin (checked immunochemically using anti-albumin serum) and was dialyzed exhaustively against 0.154M NaCl, pH 7.5. The rationale for using narrow range of LDL (LDL_2 : $1.030<d<1.050$g/m*l*) was that the

majority of the Apo B (77%) and its corresponding lipoprotein family, LP-B is confined to this density fraction[19].

Preparation of Antibodies

Equal volumes of antigen solution, LDL_2 (approximately 3.0mg/m*l*) and Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) were mixed and then injected intraperitoneally into rabbits weighing 2Kg. Several additional injections through the same site were made at intervals for 7 days. Four weeks after initial injection, approximately 40m*l* of blood were obtained weekly by cardiac puncture. The antiserum was examined by immunodiffusion and immunoelectrophoresis with various antigens. The antisera were stored at −80℃ with sodium azide (1mg/m*l* of antisera) as preservative.

Double Immunodiffusion and Immunoelectrophoresis

Double immunodiffusion in gel was performed at room temperature in a high humidity chamber, using glass slides (25 ×75mm) prepared with 1 per cent agarose (Bio-Rad Laboratories, Richmond, Calif.); veronal buffer, pH 8.6, ionic strength0.10; and 0.25 percent sodium azide. Immunoelectrophoresis was performed in the same veronal buffer using voltage of 120V and constant current of 70mA for 70min. Development of precipitin lines was observed for four days, although the process was usually completed within 24 hours. Precipitin bands were recorded by direct photography of the plates.

Polyacrylamide Gel Electrophoresis (PAGE)

Analytical discontinous PAGE was performed essentially as described by Davis B. J.[20] A separating gel of 7 per cent polyacrylamide was used for the separation of apolipoproteins as described by Alaupovic P. et al.[21], employing a Tris-glycine buffer (pH 8.3). The electrophoretic separation of polypeptides was usually carried out with 8M urea in the separating gel and 4M in the stacking and sample gels, with a constant current of 5.0mA per tube. The electrophoresis was terminated when the tracking dye (Bromophenol Blue) had migrated 4.0cm into the separating gel. The gels were fixed and stained simultaneously by placing them in a solution of 0.12 per cent Coomassie Brilliant Blue R (Sigma) in 10 per cent trichloroacetic acid (TCA) (1.0/10.0, v/v).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in 1 per cent agarose (Bio-Rad Lab.) under the same conditions as described for immunoelectrophoresis. The sample was mixed with an equal volume of 1 per cent agarose and poured into a trough (1.5mm by 13mm) previously cut in the gel, until the trough was completely filled. After completion of the electrophoresis, we fixed the slides in 10 per cent TCA for 1 hour, washed them in distilled water for 2 hours, and dried them overnight. After drying, the slices were stained in freshly prepared Fat Red 7B in absolute methanol for appromixately 10 min at 37℃ and rinsed for 30 seconds with 75% ethanol. Plates were then

immediately rinsed in 20% glycerol for thirty seconds and dried.

Isolation of Lipoprotein B

Concanavalin (Con) A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) was equilibrated with 0.02M Tris (pH 7.2), 1.0M NaCl, 1mM $MgCl_2$, 1mM $CaCl_2$ buffer (Con A buffer I) and poured into a column (2×30cm) with a glass filter. LDL of narrow density range (1.030—1.050g/m*l*) was isolated by ultracentrifugation, dialyzed against Con A buffer I and applied to a Con A-Sepharose column. Retained lipoproteins were eluted with equilibration buffer which contained 0.2M α-D-methyl-glucoside (Con A buffer II)[22]. The elution of fractions from columns was monitored by absorption at 280nm.

Electroimmunoassay of Apolipoprotein B

In 1966, Laurell C. B. published an immunochemical quantitative method which has since been termed electroimmunoassay (EIA) or "rocket" immunoelectrophoresis[23]. We utilized the principles of electroimmunoassay to develop and evaluate quantitative methods for human plasma Apo B. Optimal assay condition was investigated. In the present adaptation of Laurell's electroimmunoassay, the supporting media were prepared by melting 2.5% agarose (w/v) in electrophoresis buffer (Veronal buffer, pH 8.6, ionic strength 0.10) on a boiling water-bath with continuous stirring. Curry M. D. reported that he obtained the best results when the supporting gels were firm and the water content was at a minimum[5]. The percentage of agarose, 2.5% to be put into solution was considered maximal when the solution began to congeal above 50℃. It has been reported that the antibody-containing gels could be sealed and stored in the cold (4℃) moist chamber for up to 4 weeks. Usually stored gels were used within one week. A monospecific antiserum against LP-B was thoroughly mixed with 30m*l* of the agarose mixture which had been allowed to cool to 55℃. Laurell C. B. pointed out that in quantitative work the antibodies in the gel must be evenly distributed and the gel must be of uniform thickness along the migration route[23]. For this purpose, we made the gel in a mold constructed of U-frame and two glass plates (Farbwerke Hoechst, AG, Frankfurt, West Germany : 205×110×1.5mm). The gels containing antiserum were stored in a humid chamber at 4℃ for one to two days before use, for maximum gel stability. Eighteen antigen wells of 4mm diameter were punched out with center-to-center distances of 10 mm using punching template and gel punch (Farbwerke Hoechst AG). Ten microliters of standard or sample, properly diluted with buffer containing 0.25M sucrose, 0.154M NaCl and 2.7mM NaN_3 (Normal plasma samples required a 15-to 40-fold dilution), were applied with a Drummond microdispenser (Drummond Scientific Co., Broomall, Pa., USA). Electrophoresis was performed with a constant voltage of 10 V/cm. Water circulating through the electrophoresis platform (CIS-1, Kayagaki Irika Kogyo, Tokyo, Japan) was maintained at 10—15℃ by water circulating system (C-P30B, Hitachi Kaden, Tokyo, Japan). After electrophoresis, the assay plate was put into a bath containing 0.9 % (w/v) NaCl for 12 hours at 4℃, blotted with Whatman No. 2 filter paper and dried with cold air. Immunoprecipitates were stained with a Coomassie Brilliant Blue R (CBBR) solution (0.12% CBBR-water-acetic acid-95% ethanol, 2 : 2 : 2 : 1, v/v)

for 10min and destained to a clear background in a solution of acetic acid-95% ethanol-water (1 : 3 : 5, v/v). The height in millimeters (center of antigen well to apex of immunoprecipitate) was measured using a ruler over fluorescent lighting. Measurements were generally made with a ruler to the nearest 0.5mm.

Protein Analysis

Protein in lipoprotein fractions was determined by the metod of Lowry O. H.[24] and co-workers using bovine serum albumin (Sigma Chemical Co. : crystallized and lyophilized) as standard.

Results

Characterization of LDL_2 ($1.030<d<1.050g/ml$)

On agarose gel electrophoresis, LDL_2 showed only one lipoprotein band. When LDL_2 was analyzed in 7 per cent PAGE with 8M urea and stained for protein, not only a Apo B band at the junction between stacking gel and separating gel but also several other minor bands were observed, indicating that LDL_2 contains at least 3 or 4 protein moieties suggestive of Apo B, Apo C, Apo E and Apo A.

LDL_2 on Concanavalin A-Sepharose

McConathy W. J. and Alaupovic P. suggested a selective binding of concanavalin A to lipoprotein B[22]. Elution profile of LDL_2 applied to concanavalin A-Sepharose 4B column is shown in Fig. 1. Retained fraction reacted positively with anti-LP-B. PAGE of unretained fraction showed several minor bands (Fig. 2). However, only very faint bands were visible on PAGE of retained fraction.

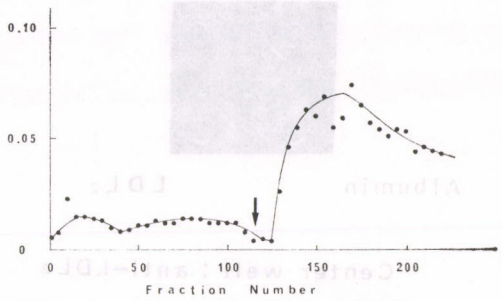

Fig. 1 Elution profile of Concanavalin A-Sepharose 4B affinity chromatography of LDL_2 ($1.030< d <1.050g/ml$). An arrow indicates the application of Con A buffer II containing 0.2M α-D-methyl-glucoside.

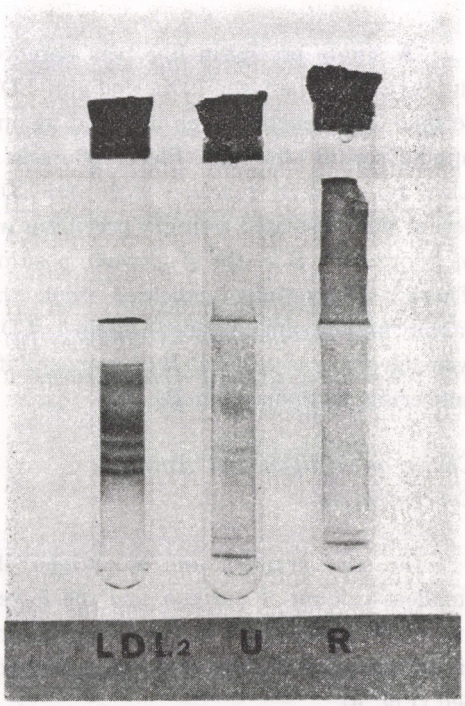

Fig. 2 Pattern of polyacrylamide gel electrophoresis of LDL_2, unretained (U) and retained (R) fracton from Concanavalin A-Sepharose 4B affinity chromatography.

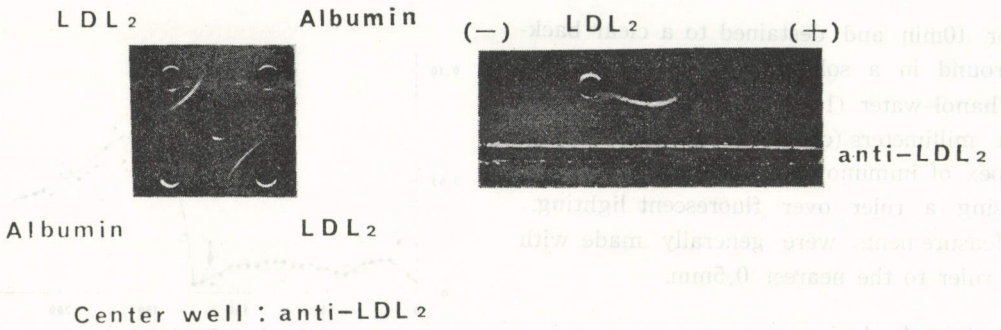

Fig. 3 Left : Double immunodiffusion of LDL_2 and human albumin (Sigma) against anti-LDL_2 rabbit serum.
Right : Immunoelectrophoresis of LDL_2 with anti-LDL_2 rabbit serum.

Characterization of Anti-LDL_2 Rabbit Serum (Fig. 3)

A single precipitin line was observed in the reaction between LDL_2 and anti-LDL_2 serum. No reaction was detected against human serum albumin. Electrophoresis of LDL_2 followed by reaction with anti-LDL_2 serum demonstrated a single precipitin arc of lipoprotein B in the β-globulin position. These observations indicated that this rabbit anti-serum induced against LDL_2 was the same as anti-LP-B or monospecific antiserum to lipoprotein B.

Choice of Antigen and Antibody Concentration

To save antisera and/or antigen, the smallest amount of antigen and the lowest antiserum concentration giving a well precipitation peak 2—5cm high were determined (Fig. 4).

The gels contained electrophoresis buffer and an increasing amount of antiserum (300-fold dilution to 700-fold dilution). During these tests the electrophoresis was run for 5 hours with 10 volts/cm. The antigen concentrations tested were from 20 to 160μg/m*l*. Immunoprecipitates were considered adequate when they had definite apexes and their side lines extended to the antigen well. The amount of

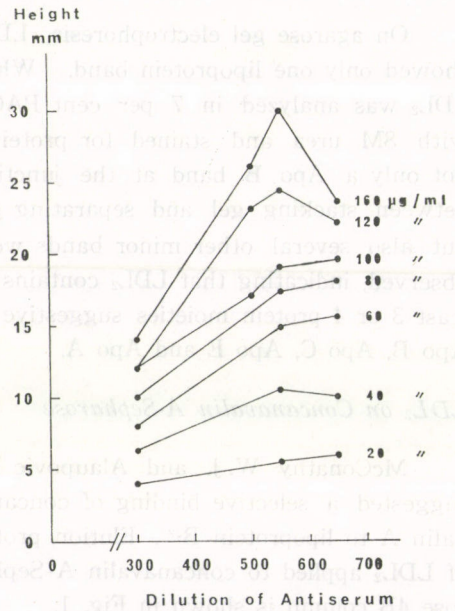

Fig. 4 Relationship between rocket height and dilution of antiserum (300-fold dilution to 700-fold dilution) utilizing antigen concentration from 20 to 160μg/m*l*.

antigen that would give a thin but distinct precipitation line with the lowest useful concentration of antiserum in the gel was estimated from the appearance of the precipitation pattern on the slides. When dilution of antiserum was over 600 times, the appearances of the rocket were not clear. Five hundreds and fifty fold dilution of the anti-serum used for the present study was considered to be appropriate.

Selection of Time for Electrophoresis

Fig. 5 and 6 show the height of the precipitin lines obtained for an increasing time utilizing the increasing concentrations of antigen. The amount of protein of LDL_2 applied in the wells varied from 20 to 160μg/m*l* and the antiserum concentration in the agarose was 550-fold dilution. The duration of electrophoresis was established by studying the relationship between rocket heights and antigen concentrations with time utilizing field strength of 10V/cm. The precipitation frontiers migrated rapidly during the early period of electrophoresis but within 2 hours those in the gel with the smallest amount of antigen

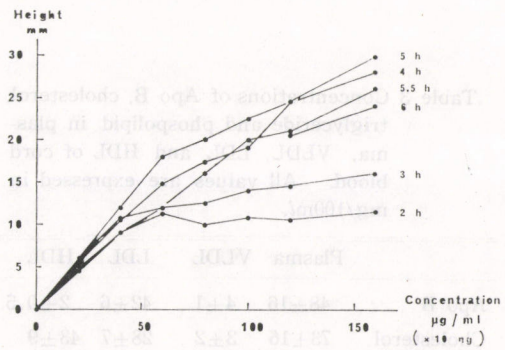

Fig. 5 Relationship between rocket height and concentration of antigen utilizing various duration of electrophoresis.

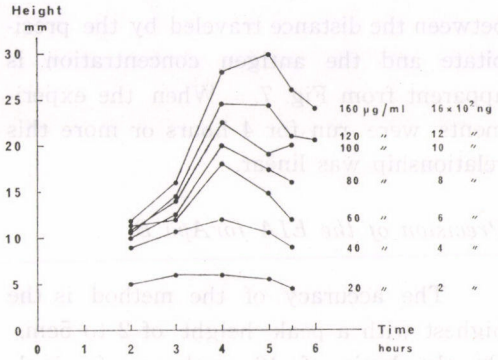

Fig. 6 Relationship between rocket height and duration of electrophoresis utilizing antigen concentration from 20 to 160 μg/m*l*.

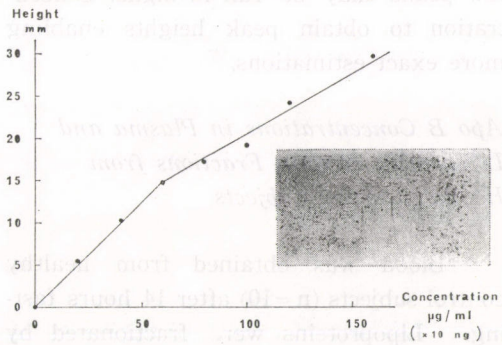

Fig. 7 Relationship between rocket height and antigen concentration. Antiserum concentration in the agarose and duration of electrophoresis were 550-fold dilution and 5 hours with 10 volts/cm, respectively. The inset shows the appearance of the rockets.

ceased to move at a measurable speed. In gels with the largest amount of antigen, the precipitation front continued to migrate for 4—6 hours. The results of a series of model experiments are shown in Fig. 7 to show how the migration rate of the precipitation frontiers changes with the antigen concentration at constant antibody concentrations. The variation of the ratio

between the distance traveled by the precipitate and the antigen concentration is apparent from Fig. 7. When the experiments were run for 4 hours or more this relationship was linear.

Precision of the EIA forApo B

The accuracy of the method is the highest with a peak height of 2 to 5cm. On the basis of 10 analyses of single sample, the standard error was found to be 2.0%. The peak height used was 2.5 cm. The relative error was roughly constant at peak heights between 2 to 5cm and increases below 2cm. Samples giving low peaks may be run in higher concentration to obtain peak heights enabling more exact estimations.

Apo B Concentrations in Plasma and Lipoprotein Density Fractions from Healthy Control Subjects

Blood was obtained from healthy control subjects (n=10) after 14 hours fasting. Lipoproteins were fractionated by sequential ultracentrifugation at 1.006g/ml and 1.063g/ml. Apo B concentrations in plasma, VLDL, LDL and HDL were 128±40, 11±4, 103±7 and 13±5mg/100ml, respectively. (Table 1). Percent distribution of Apo B in VLDL, LDL and HDL were 9.0±3.2, 81.0±5.8 and 10.0±3.8%, respectively.

Apo B Concentrations in Plasma and Lipoprotein Density Fractions from Cord Blood and Pregnant Women

Cord blood were obtained from the 15 unselected apparently normal neonates, 8 males and 7 females[25]. Blood were obtained from their fasted mother within 24 hours after delivery. Plasma Apo B concentration of the mothers (n=15) was 263 ±37mg/100ml (Table 2), which was approximately two times of plasma Apo B concentration of control healthy subjects.

Table 2 Concentrations of Apo B, cholesterol, triglyceride and phospholipid in plasma, VLDL, LDL and HDL of pregnant women right after the delivery of the baby. All values are expressed in mg/100ml.

	Plasma	VLDL	LDL	HDL
Apo B	263±37	25±4	214±34	21±8
Cholesterol	256±63	39±24	144±46	73±21
Triglyceride	277±124	130±100	79±33	68±18
Phospholipid	298±71	44±18	112±34	138±43

Table 1 Concentrations of Apo B, cholesterol, triglyceride and phospholipid in plasma, VLDL, LDL and HDL of control healthy subjects. All values are expressed in mg/100ml.

	Plasma	VLDL	LDL	HDL
Apo B	128±40	11±4	103±7	13±5
Cholesterol	179±35	18±17	118±33	44±10
Triglyceride	129±53	59±28	43±32	27±8
Phospholipid	188±38	20±11	84±31	83±14

Table 3 Concentrations of Apo B, cholesterol, triglyceride and phospolipid in plasma, VLDL, LDL and HDL of cord blood. All values are expressed in mg/100ml.

	Plasma	VLDL	LDL	HDL
Apo B	48±16	4±1	42±6	2±0.5
Cholesterol	73±16	3±2	28±7	43±9
Triglyceride	51±10	10±6	16±4	25±5
Phospholipid	110±21	3±3	24±6	83±16

However, there was no difference in Apo B distribution in lipoprotein density classes between pregnant women and control. In cord blood (n=15), plasma Apo B concentration (48±16mg/100ml) was less than one half of control (Table 3). Percent Apo B distribution in VLDL, LDL and HDL were 8.4, 87.7 and 4.0%, respectively. Percent Apo B concentration in LDL and HDL were higher and lower, respectively, than those in LDL and HDL from control healthy subjects.

Discussion

When electroimmunoassay of Apo B in plasma and lipoprotein density fractions is performed, the following items should be tested carefully as in the present study :

1) Characterization of standard
2) Characterization of antiserum
3) Choice of antigen and antibody concentration
4) Time for electrophoresis
5) Precision of the EIA of Apo B

Then, EIA should be applied for quantification of Apo B in plasma and lipoprotein density fractions from healthy control subjects and the patients with various diseases.

Characterization of narrow range of LDL, LDL_2 ($1.030<d<1.050$g/ml) by PAGE and concanavalin A-Sepharose column chromatography exhibited the heterogeneity of apolipoprotein composition, indicating the existence of minor apolipoproteins, such as Apo C, E and A other than major Apo B. Therefore, it is not reasonable in the narrow sense to use LDL_2 as a standard of lipoprotein B. Purification of lipoprotein B by concanavalin A-Sepharose column chromatography was not successful in the present study, though bound fraction of concanavalin A-Sepharose 4B column was mainly composed of lipoprotein B. We used the factor 0.86 (0.94×0.92) for the correction of the lipoprotein B concentration in LDL_2. It is suggested that 4 to 8 percent higher Apo B values for unknown samples is observed when LDL_2 subfraction was used as a standard, owing primarily to the presence of Apo C and Apo E in this density fraction[19]. Previous quantitative data indicated that Apo B account for 94% of the protein content of this density fraotion and there is a minimum amount of other apolipoproteins, as compared to other density classes[19]. Therefore, we used factor 0.94 to correct. Moreover, there was a common disagreement with respect to the protein determination of a standard LP-B. It is suggested that the protein concentration of LP-B standards is overestimated by the method of Lowry, it is necessary to correct for the chromogenecity differences between LP-B and either bovine or human albumin used as standard. We used the factor 0.92 as suggested by Lee D. M.[19]

Characterization of anti-LDL_2 rabbit serum by double immunodiffusion and immunoelectrophoresis disclosed that this rabbit anti-serum induced against LDL_2 was the same as anti LP-B or monospecific antiserum to lipoprotein B. This was confirmed further by immunoprecipin line by electroimmunoassay which showed sharp and fine precipitation peak. This might be explained by stronger antigenecity of Apo B than other minor apolipoproteins such as Apo C, Apo E and Apo A.

In addition to titer of antiserum, there are at least two factors that influence the concentration of antiserum used in the supporting gel[5] :

1) A slight antibody excess had to be

maintained in order to avoid formation of soluble antigen-antibody complexes (the tallest standard should maintain a definite apex after prolonged electrophoresi),

2) The selected concentration of antibody had to allow a reasonable dilution of the sample antigen. In turn, decreasing the concentration of antibodies increases the sensitivity of the assay.

To save antisera and/or antigen, the smallest amount of antigen and the lowest antiserum concentration giving a well precipitation peak 2—5cm high was explored in the present study. Utilizing the antigen, 20 to 160μg/m*l*, appropriate dilution of antiserum was 550-fold. Appropriate dilution of antiserum should be tested, when every new anti-LDL_2 serum is employed.

Curry, M. D. investigated field strength and duration of electrophoresis[5]. He pointed out the followings :

1) The rate of electrophoretic migration of a protein determined the time of assay.
2) The aggregation of antigen-antibody complexes was increased at lower field strengths.
3) High field strengths increased heat production and promoted dehydration of the gel.
4) The temperature of circulating water influenced immunoprecipitation through its effect on the gel (condensation, fluidity of the gel matrix, etc.).

To develop rapid assays without denature of the gels, we utilized the relatively high field strength of 10 V/cm and water circulating platform at 10—15℃. In gels with the largest amount of antigen, the precipitation front continued to migrate for 4—6 hours. It is necessary to explore the appropriate time to obtain the sharp and fine precipitation line, at the concentrations of antigen and antibody used. The data presented indicated that 5 hours for electrophoresis was enough.

Utilizing the optimal condition for EIA of Apo B stated above, we measured Apo B concentrations in plasma and lipoprotein density fractions of healthy control subjects, cord blood and pregnant women. So far, the absolute concentrations of Apo B in lipoprotein fractions have not been reported in Japan and only changes relative to the Apo B levels in a whole serum or the Apo B values expressed in arbitrary units were reported[26][27]. Apo B concentrations in plasma, VLDL, LDL and HDL of normal subjects in USA were 93±26, 80±2.0, 76±20 and 9±3mg/100m*l*, respectively[6]. In the present study, Apo B concentrations in plasma and LDL of the Japanese were slightly higher than those in USA. However, percent distribution of Apo B in lipoprotein density classes from the Japanese and the American were comparable. In normal subjects of USA, percent distribution of Apo B in $d<1.019$, 1.019—1.063 and $d>1.063$ were 8.6, 81.7 and 9.7, respectively[6]. Plasma Apo B concentration of the mothers was 263±37mg/100m*l*, which was approximately two times of plasma Apo B concentration of control healthy subjects. However, there was no difference in Apo B distribution in lipoprotein density classes between pregnant women and control. These observations in percent distribution of Apo B in lipoprotein density classes from control and pregnant women justified our assay system for Apo B concentration.

A unique distribution of lipids in lipoprotein density classes of cord blood has been reported[27] In Japanese cord blood,

total cholesterol of 73.3±15.9mg/100m*l* was distributed in the three lipoprotein density classes as follows : 2.8±2.1mg/100 m*l* (3.7±2.4%) in VLDL, 27.9±7.2mg/100 m*l* (37.9±4.4%) in LDL and 42.8±8.9mg /100m*l* (58.5±4.1%) in HDL. By contrast, in control adults under 40 years old, total cholesterol of 190±29.2mg/100m*l* was distributed in three lipoprotein density classes as follows : 15.3±5.4mg/100m*l* (7.9 ±2.3%) in VLDL, 121.8±27.9mg/100m*l* (63.1±6.0%) in LDL and 54.3±8.7mg/ 100m*l* (29.0±5.8%) in HDL. Fetal HDL are a little over one-half, LDL about one fifth and VLDL about one twentieth of their respective concentrations in maternal plasma at term. In cord serum the concentrations of all lipoprotein classes are lower than those obtained in adults. The greatest difference exists in the levels of VLDL, the fraction of which was found to be only a minor one in fetal blood. In cord blood, plasma Apo B concentration was less than one half of control which is comparable to the report by McConathy W. J. et al.[28] McConathy W. J. et al. reported the reduced levels of apolipoproteins in cord serum. Levels of Apo C-I, C-II and Apo E approached adult levels (83 to 86% of the adult levels), whereas Apo B and D were most reduced when compared to the adult concentrations, 29 and 37%, respectively. The three other apolipoproteins such as Apo A-I, A-II, and C-III were present at approximately one-half the levels found in adults. The present study showed that plasma Apo B level of cord blood was 47.6±15.7mg/100m*l*, approximately one-half that of adults and confirmed the reduced level of Apo B. Apo B distribution in lipoprotein density classes of cord blood has not been reported. Apo B concentrations and distributions in plasma, VLDL, LDL and HDL reported in the present paper confirmed that our method of EIA of Apo B is reasonable.

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