AN INVESTIGATION OF SERUM LIPOPROTEIN DISC ELECTROPHORESIS*

II. A SIMPLE METHOD OF HUMAN SERUM LIPOPROTEIN DISC ELECTROPHORESIS AND IDENTIFICATION OF THE RESOLVED LIPOPROTEINS

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IN THE previous study,¹ the authors investigated general behavior of human serum lipoproteins on acrylamide gel electrophoresis. To obtain an acrylamide gel system (acrylamide gel layers consisting of separating, spacer and sample gel layers) which was suitable for Disc type electrophoresis of human serum lipoproteins, various acrylamide gel systems were evaluated. The study was performed by starting with *Ornstein-Davis'* method² and *McDonald-Ribeiro's* prestaining procedure³.

Acrylamide concentration of separating gel which seemed to be adequate for lipoprotein analysis were concentrations between 4.5% and 3.4%. Concentrations higher than 4.5% gave insufficient migration of S-beta lipoprotein¹ and concentrations lower than 3.4% tended to produce poorly resolved lipoprotein bands. Migration of lipoproteins, especially of alpha-L lipoprotein¹, seemed to be affected by buffer strength of separating gel. As strength was decreased the migration of alpha-L lipoprotein increased.

Sudan Black B in ethylene glycol was employed as a dye for lipoprotein stain. Saturated dye solution which had been employed by previous authors^{3,4} was modified to 0.1% solution, and it

was also proved possible to stain serum lipoproteins directly in a gel tube immediately before photopolymerization of sample gel solution in which dye solution was included.

Sample gel had to be modified considerably from the starting method² because well formed sample gel seemed to be required for lipoprotein electrophoresis to cover all component lipoprotein classes. Lipoprotein particles larger than pre-beta lipoprotein seemed to remain in the sample gel layer. It was shown that control of both the concentration of acrylamide in the sample gel and the ratio of the volume of dye solvent to the total volume of the large pore gel components (acrylamide, Bis-acrylamide, buffer solution, and riboflavin) were critical for obtaining satisfactory results. The optimum acrylamide concentration seemed to be near 3.75% and the ideal ratio, mentioned above, was between 0.6 and 0.7.

On the basis of these observations, the authors concluded that it would be possible to integrate a method of acrylamide Disc electrophoresis of human serum lipoprotein for the purpose of clinical research or routine analysis. The purpose of this study was to refine our previously reported procedures in order to formulate a simple and reliable method for lipoprotein Disc electrophoresis. Identification of the lipoproteins resolved by this method was made by means of electrophoretic and immunological techniques. Clinical application of the method was also tried.

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TABLE I REAGENTS

I. Separating gel reagents		2. Acrylamide solution	
1. Separating gel buffer		Acrylamide 10.0 g	
1N HCl	8.5 ml	BIS	2.5 g
Tris	6.4 g	water to	100 ml
TEMED	0.08 ml	3. Catalyzer*	
water to	100 ml	Riboflavin	4.0 mg
(pH 8.6 to 9.0)		water to	100 ml
2. Acrylamide solution		III. Lipid dye solution	
Acrylamide	15.0 g	1. Stock dye solution	
BIS	0.4 g	Sudan Black B.	0.1 g
water to	100 ml	Ethylene glycol to	100 ml
3. Catalyzer*		2. Working dye solution	
Ammonium persulfate	0.15 g	III-1	6.4 ml
water to	100 ml	Ethylene glycol to	100 ml
II. Spacer and sample gel reagents		IV. Electrophoresis buffer	
1. Spacer and sample gel buffer		Tris	4.0 g
1N HCl	48.0 ml	Glycine	19.2 g
Tris	5.98 g	water to	1000 ml
TEMED	2.3 ml		
water to	100 ml		
(pH 6.4 to 6.8)			

* Actual catalyzer systems are ammonium persulfate-TEMED for separating gel and riboflavin-TEMED for sample and spacer gels.

The source of these chemicals were; Tris: (Tris-(hydroxymethyl)-aminomethane), Sigma Chem. Co., St. Louis U.S.A.; TEMED: N,N,N',N'-tetramethyl-ethylene diamine, Katayama Chem. Co., Osaka Japan; Acrylamide: Wako Pure Chem. Co., Tokyo Japan; BIS: N,N'-methylene bisacrylamide, Wako Pure Chem. Co., idem; Ammonium persulfate: Kayatama Chem. Co., idem; Riboflavin; Wako Pure Chem. Co., idem; Sudan Black B: Merck A. G., Darmstadt Germany; Glycine: Katayama Chem. Co., idem.

MATERIALS AND METHODS

Serum or plasma was obtained at random from subjects with atherosclerotic cardiovascular disorder on whom a routine lipid chemistry including lipoprotein study was requested to the authors' laboratory. All specimens were collected after overnight fast except one (on whom a study was made by postprandial serum, see text). Although serum and double oxalated, or EDTA plasma were used, the term "serum" will be used to cover all three in this report. Samples were kept in a refrigerator at approximately 4 degrees C and electrophoresis was completed within 2 to 4 days.

Reagents employed in this study are shown in Table I. The reagents, I-1, -2, -3, and IV were modified from *Ornstein-Davis'* preprint method². Others were essentially the same as that of these authors, except for those in III. Stock dye

solution was prepared at least 24 hours prior to use in order to achieve complete solubility and was stored in the dark at room temperature. Working dye solution was prepared every one to two weeks by diluting the stock solution with ethylene glycol as given in the table.

Glass gel tubes, 7.0 mm in external diameter and 80.0 mm in length were employed in this study. The tubes were rinsed with a Tween 20 or 40 (Katayama Chemicals, Osaka, Japan) solution which was diluted with distilled water (about 1 : 200), after cleaning with dichromate-sulfuric acid, running tap water, and distilled water.

Paper electrophoresis of serum lipoprotein was performed by *Lees-Hatch's* method⁵ with authors' modification⁶. To obtain lipoprotein fractions separated by paper electrophoresis, the following procedures were followed. A filter paper (*Whatman* 3 MM) was cut to a size 5 cm x 56 cm. On this paper a line was drawn

along its long axis dividing it into two parts; one 2 cm wide and the other 3 cm wide. Over the area where the migration of lipoproteins would occur, lines were drawn perpendicular to the long axis at intervals of 5 mm (beta and pre-beta zone) or 10 mm (alpha zone) as illustrated in Fig.1-b. The serum sample was applied on the paper strip, 20 micro-L/cm, and electrophoresis was performed in a cold room kept at about 5 degrees C. Immediately after electrophoresis was completed, the paper was divided into two strips. The 2 cm strip was stained with Oil Red O^{5,6}. The other 3 cm strip was cut further into pieces (5 mm x 30 mm or 10 mm x 30 mm) along the lines drawn on it. Each piece of filter paper was transferred into small test tube; and 0.2 ml of distilled water was added to it. The lipoprotein fractions (other proteins might be included) thus obtained were submitted to Disc electrophoresis and immunodiffusion.

Twenty five micro-L of these lipoprotein fractions was used for electrophoretic identification. A small portion of these extracted fractions was also used for immunological study.

For immunological identification, lipoprotein bands were cut off from acrylamide gel columns which were extruded from the gel tubes soon after electrophoresis was completed. This acrylamide gel piece in which Sudan Black B stained lipoprotein was included, was then transferred into the holes made on an agarose gel plate (0.1% agarose in barbiturate buffer) as seen in Fig.4-b. Anti human alpha and beta lipoprotein rabbit sera (Hoechst Japan Co., Tokyo, Japan) were introduced into troughs on the plate.

The methods employed for lipid chemical determinations will be given elsewhere as they are referred to in this paper.

Preparation of acrylamide gel layers was made with the authors' apparatus⁷. Maximum capacity of the power source used in this study was 50 mA and 250 V. The Disc electrophoresis cell was a commonly used type, available commercially (Kayagaki Scientific Co., Tokyo, Japan), to which a minor modification was made only for the convenience of connecting gel tubes more easily (rubber stopper type grommets were substituted with soft rubber vacuum tubes).

Separating gel solution and separating gel layer

PREPARATION OF ACRYLAMIDE GEL LAYERS

One part each of separating gel buffer (I-1) and acrylamide solution (I-2) were mixed together, to which two parts of ammonium persulfate

solution (I-3) were added resulting in a concentration of 3.75% acrylamide. Approximately 0.8 ml of this solution was transferred into gel tubes. The height of the delivered solution in the tubes was about 43 mm. After layering with water, polymerization took place at room temperature (20 to 23 degrees C). Gelation was completed usually within 30 min. To proceed to the next step below, excess water and unpolymerized acrylamide monomer were eliminated by inverting the tubes and draining onto filter paper. Spacer gel solution and spacer gel layer

The solution was prepared by mixing gel buffer (II-1), acrylamide solution (II-2), riboflavin solution (II-3), and distilled water in a ratio of 1 : 2 : 0.2 : 4.8. Acrylamide concentration of this solution was 2.5%. About 0.1 ml of this solution was transferred into gel tubes in which separating gel was already prepared, layered with water and exposed to fluorescent light. Photopolymerization was completed within 20 min usually.

Sample gel solution and sample gel layer

The solution containing 3.33% acrylamide was composed of working dye solution (III-2), gel buffer (II-1), acrylamide solution (II-2), riboflavin solution (II-3), and distilled water in a ratio of 2.35 : 1.0 : 2.0 : 0.2 : 0.45. The reagents were added and mixed usually in this order. Preliminary mixing of the components other than working dye solution did not alter the results. The ratio of working dye solution to the total volume of the other four components were 0.64. Sample gel solution was utilized in less 5 hours from the time of preparation.

After complete elimination of water and unpolymerized acrylamide monomer over the spacer gel layer, 10 micro-L of serum was placed on spacer gel layer. In cases where lipoprotein fractions obtained from the sectioned filter paper pieces were analysed, 25 micro-L of the extracts were used. Approximately 0.2 ml of the sample gel solution was then delivered into gel tubes. The sample plus gel solution were mixed well but gently, then layered with electrophoresis buffer (Table I, IV) before exposing to fluorescent light. Time required for preparing the sample gel layer was 35 min or less per tube including photopolymerization.

Electrophoresis

The upper end of gel tube was inserted to rubber vacuum tube of upper buffer reservoir. Electrophoresis buffer was poured into the reservoirs carefully to avoid introducing air bubbles

Fig.1. Paper and Disc electrophoretograms of a patient.

- a: Paper electrophoretogram of routine study.
 b: Paper electrophoretogram used for identification study (see text).
 c: Lipoprotein Disc electrophoretogram (by the authors' method).
 d: Total protein Disc electrophoretogram (same procedure as Fig.1-c except for sample size, 4.0 micro-L, and staining, Amidoblack 10B stain).
 aL: alpha-L position or alpha-L lipoprotein band.
 Sb: S-beta position or S-beta lipoprotein band.
 pB: pre-beta lipoprotein band.

TABLE II LIPID CHEMISTRY AND SOME CLINICAL NOTES OF THE PATIENT PRESENTED IN FIG.1.

Patient's identification: SEKky age 61, sex female
 Clinical diagnosis: Athero & arteriosclerosis complicated with mildly impaired renal function.
 Lipid chemistry

TURB	25×10^{-3} (not turbid)	BLCh	220 mg/dl
TL	659 mg/dl	PL	239 mg/dl
EFA	250 mg/dl	TG	137 mg/dl
TCh	282 mg/dl	NEFA	460 micro-Eq/L

TURB: Turbidity of serum, optical density at 660 mμ in 10 mm light path.
 TL: Total lipid.¹⁸ EFA: Ester type fatty acids.¹⁹ TCh: Total cholesterol.²⁰
 BLCh: Beta lipoprotein cholesterol.²¹ TG: Triglycerides.²² PL: Phospholipids.²³
 NEFA: Non-esterified fatty acids.²⁴

Fig.2. Disc electrophoretograms of lipoprotein fractions.
WS: Original serum sample (4.0 micro-L was applied).
0 to 10: Gel column number 0 to 10. These numbers corresponded to the numbers attached to the sectioned paper pieces shown in Fig.1. Twenty five micro-L each of the paper extract was applied.
Lipoprotein fractions were extracted from the sectioned filter paper pieces and submitted to Disc electrophoresis (see text).

Fig.3. Amidoblack 10B stain (staining for total protein) of the gel columns presented in Fig.2.
 Amidoblack 10B staining followed lipoprotein electrophoresis.
 Sb: S-beta position. aL: alpha-L position.

Fig.4. a: Paper and Disc electrophoretograms of the patient employed for immunological identification (the same patient as presented in Fig.1).

b: Immunodiffusion between serum lipoproteins and anti human lipoprotein antisera.

A, B, and C: Gel pieces from alpha, beta, and pre-beta zones.

a, and b: Anti human alpha and beta lipoprotein antisera.

Into the 11 holes in center, protein extracts from the paper pieces were introduced. There only number 1 and 9 were indicated in this figure, but they were to be numbered, from left to right, as 0, 1, 2, . . . 9, and 10.

c: Additional examples of lipoprotein Disc electrophoresis.

Top: Of a postprandial serum sample; Sudan Black B stained sample gel layer was observed in this case.

Bottom: Of a hypercholesterolemic serum sample; double beta lipoprotein band was observed, but no pre-beta lipoprotein band.

into the gel tubes. The current was set at 4 mA per tube. The negative pole was connected to the upper reservoir and positive to the lower reservoir. Electrophoresis time averaged about 35 min.

Electrophoretograms were evaluated on an immunoviewer (Johko Ind. Co., Tokyo, Japan) with expander lens and recorded either by sketching on paper or by taking 35 mm films.

RESULTS

In Fig.1, serum lipoprotein and total protein Disc electrophoretograms, and lipoprotein paper electrophoretograms of a patient with moderate hyperlipidemia were presented. Lipid chemistry of this patient was shown in Table II. Resolution of serum lipoproteins by Disc electrophoresis was apparently better than that obtained by paper electrophoresis. Recognition of the separated lipoprotein bands on the Disc electrophoretogram was undoubtedly easier than with the paper electrophoretogram and additional lipoprotein bands, not detectable by paper electrophoresis, were resolved as seen in the cases presented here (Figs.1, 5 & 6).

In Fig.2, Disc electrophoretograms of lipoprotein fractions obtained from the sectioned filter paper pieces were presented. The same subject as presented in the previous figure was employed here again for convenience of comparison. Lipoprotein obtained from the sectioned paper piece numbered 0 (5 mm to cathodal from the origin, see Fig.1-b) produced one lipoprotein band at the cathodal end of separating gel layer. Lipoprotein fractions from two pieces of the paper numbered 1 and 2 produced two lipoprotein bands. One was located at the cathodal end of the separating gel layer, and another was located at S-beta position (S-beta lipoprotein¹). Lipoproteins from the sectioned paper pieces numbered 3 and 4 produced a band at the cathodal end of separating gel layer. Lipoproteins from the papers numbered 7, 8, and 9 produced a band at alpha-L position (alpha-L lipoprotein¹). No lipoprotein band was produced by the extracts obtained from the paper pieces numbered 5, 6, and 10.

In Fig.3 Amidoblack 10B stain of the acrylamide gel columns was shown. The gel columns are identical to that presented in Fig.2. Albumin band was marked in all gel columns because albuminated barbiturate buffer⁶ was used for lipoprotein paper electrophoresis.

Other than albumin band, two protein bands

were recognizable in the gel column number 0 (the number corresponds to that of the sectioned paper pieces); one at the cathodal end and the other behind albumin. Four protein bands other than albumin were detectable in gel columns number 1 and 2. One of them was located at the S-beta position and another at the cathodal end of separating gel layer. Six or 7 protein bands were noted in gel column number 4. The slowest and the most dense band among them was observed at the cathodal end of separating gel layer. No protein band other than albumin was detected in gel columns numbered 7, 8, 9, and 10. Lipoprotein seemed to be fused with albumin in these gel columns!

In Fig.4 immunoprecipitations between anti human lipoprotein antisera and electrophoretically separated lipoproteins were shown. The lipoprotein included in the alpha-L zone of the acrylamide gel column and the fraction from the alpha lipoprotein zone of the paper electrophoretogram reacted with alpha lipoprotein antisera. The lipoprotein included in the S-beta zone of acrylamide gel columns and fractions obtained from beta lipoprotein zone of paper electrophoretic strips reacted with beta lipoprotein antisera. Lipoprotein included in the portion of the cathodal end of the separating gel layer showed questionably positive precipitation, although fractions obtained from the pre-beta zone on paper produced a precipitation line with beta lipoprotein antisera. Neither lipoprotein included in the cathodal end of the gel column nor pre-beta lipoprotein fractions from paper showed immunoreaction with anti alpha lipoprotein antisera in this study.

Immunoprecipitation lines on the agarose gel plate showed affinity to lipid dyes when the plate was stained with saturated Oil Red O or Fat Red 7B^{5,6,8}

Disc electrophoresis of postprandial serum (a few hours after eating) was tried. As seen in Fig.4-c, the sample gel layer which usually was not stained by the authors' method was stained diffusely by Sudan Black B suggesting that physiologically increased chylomicrons might be located in this layer, though discrete lipoprotein band formation was not observed there.

In Figs.5 and 6 serum lipoprotein Disc electrophoretograms and paper electrophoretograms of 7 clinical cases were presented. The subjects were picked at random. As mentioned previously, these subjects had some clinical symptoms and findings of atherosclerotic cardiovascular dis-

Fig.5. Paper and Disc electrophoretograms of clinical cases (1).
 Arrowed positions indicate sample gel—spacer gel interfaces. Pre-beta lipoprotein was not detected in the case I and II, and only a trace of it was detected in IV.
 Lipid chemistry and clinical notes pertinent to these subjects were shown in Table III.

Fig.6. Paper and Disc electrophoretograms of clinical cases (2).

Arrowed positions indicate sample gel-spacer gel interfaces. These cases showed marked pre-beta lipoprotein band. Beta lipoprotein band was not detected in VII.

Lipid chemistry and clinical notes pertinent to these subjects were also shown in Table III.

orders. Some information pertinent to these subjects was shown in Table III.

Lipoprotein Disc electrophoretograms of these subjects were consistent with the results of paper electrophoresis and lipid chemistry. In some of the cases, however, discordance between two types of electrophoresis occurred, as seen in

cases 4 and 5. Paper electrophoretograms of these two looked similar, but Disc electrophoretograms were different from each other. It was revealed by Disc electrophoresis that the dominant lipoprotein fraction was beta lipoprotein in case 4, while in case 5 it was pre-beta lipoprotein. Case 7 showed an apparent elevation of pre-beta

TABLE III LIPID CHEMISTRY AND CLINICAL NOTES OF THE CASES PRESENTED IN FIGS.5 AND 6.

Cases	TCh	TG	PL	age	sex	Clinical notes
I*	180	75	174	18	f	erythematoid skin lesion
II	294	84	245	39	m	hypercholesterolemia with palpebral xanthoma
III	194	79	172	55	m	hypercholesterolemia on a drug therapy (anabolic steroid)
IV	246	152	242	41	m	cerebral vascular lesion with left hemiplegia
V	272	175	228	59	f	angina pectoris with moderate hypertension
VI	368	302	290	62	m	angina pectoris with obesity
VII**	582	1515	660	40	m	persistent myocardial ischemia with impaired hepatic function

TCh: Total cholesterol, TG: Triglycerides, PL: Phospholipids.

All in mg/dl, the methods of analyses were same as that in Table II.

* : This case was included for reference to the others.

** : This case has past history of hepatitis. Slight elevation of transaminase activity and moderately delayed dye excretion (BSP and Indocyanine-green) was reported at the time of this lipid and lipoprotein study.

lipoprotein on paper electrophoresis, but the level of beta lipoprotein could not be evaluated adequately because of an overlapping dense pre-beta band. Lipoprotein Disc electrophoresis of this sample revealed marked elevation of pre-beta lipoprotein and an additional band at the anodal end of sample gel layer. Beta lipoprotein band was hardly recognizable.

DISCUSSION

An integrated method for separating human serum lipoproteins by Disc electrophoresis was devised. That the method was simple, reliable and would meet the needs of routine analysis was proved. Electrophoretograms showed clear-cut resolution of serum lipoproteins including beta and pre-beta lipoproteins.

Partial identification of lipoprotein bands on acrylamide gel columns was included in the previous study, and lipoprotein bands were identified tentatively as alpha-L lipoprotein and S-beta lipoprotein because they were located at these positions on serum total protein Disc electrophoretograms^{9,10}. The third lipoprotein band, located at the cathodal end of the separating gel layer, which was observed frequently but not in all cases seemed to be a part of S-beta protein.

In this study, isolated lipoprotein fractions were obtained by paper electrophoresis which has been accepted as one of the standard meth-

ods for separating serum lipoprotein fractions¹¹. These lipoprotein fractions were employed to identify lipoprotein bands on acrylamide gel column.

In terms of lipoprotein paper electrophoresis, the lipoprotein band which appeared in gel columns 7, 8, and 9 was identified as alpha lipoprotein, because lipoprotein fractions obtained from the sectioned papers numbered 7, 8, and 9 contained alpha lipoprotein alone. The lipoprotein bands which appeared in gel columns number 3 and 4 should be pre-beta lipoprotein by the same criterion. Lipoprotein fractions from the sectioned papers numbered 1 and 2 produced two lipoprotein bands on Disc electrophoresis; one of which was pre-beta lipoprotein and other was located at S-beta position. Lipoprotein from paper numbered 0 showed a light lipoprotein band at the pre-beta position. Thus, pre-beta lipoprotein as judged by Disc electrophoresis was included in the lipoprotein fractions obtained from pre-beta zone, beta zone, and the zone near the origin of the paper electrophoresis. This observation, made by Disc electrophoresis, seems to be consistent with the fact that pre-beta lipoprotein is known to spread over the area from the farthest point of its migration back to the origin when serum is subjected to paper electrophoresis^{5,12}.

By acrylamide gel electrophoresis, lipoprotein bands migrated in the order of alpha, beta, and

pre-beta lipoprotein from anodal to cathodal. Pre-beta lipoprotein was not located at the position anodal to beta lipoprotein (pre-beta position) but took the position cathodal to beta lipoprotein (post-beta position). This sequence of serum lipoproteins may be explained from the standpoint of molecular and particle size arrangements of serum lipoproteins¹³

Alpha and beta lipoproteins diffused well from an acrylamide gel column into agarose gel and reacted with their specific antisera. Pre-beta lipoprotein obtained from the sectioned paper showed immunoprecipitation with anti beta lipoprotein antisera sufficient enough to be visualized on an agarose gel plate. However, pre-beta lipoprotein from an acrylamide gel column produced only a trace of immunoprecipitation with anti beta lipoprotein antisera. This phenomenon might be ascribed to limited diffusion of pre-beta lipoprotein, perhaps due to the difficulty for pre-beta lipoprotein to pass freely through the relatively small pores of the separating gel. It was considered, therefore, that partial delipidation or some other procedures might be required to demonstrate immunoprecipitation between them more clearly. Immunoprecipitation was not observed between pre-beta lipoprotein and alpha lipoprotein antisera in this study.

By comparative evaluation of paper and Disc electrophoretograms of clinical cases, identification of lipoprotein bands as described above was supported indirectly. When a sample showed dominant pre-beta or beta lipoprotein by paper electrophoresis it was easily recognized on Disc electrophoretograms producing dense lipoprotein bands at their expected positions on a gel column. Recognition of lipoprotein bands and evaluation of lipoprotein patterns were undoubtedly easier by Disc electrophoresis because of the sharp resolution. As a result of improved resolution of serum lipoproteins, additional lipoprotein bands were detected in some cases, mostly between beta and pre-beta lipoproteins as seen in some of the cases presented (Figs.1, 2, 4-c etc). Identification of these bands has not yet been made. In our experience, these additional lipoprotein bands seemed to be detected more frequently among the subjects with hyperlipidemia. Occurrence of such a lipoprotein band has been described recently in the literature¹⁴ in which the lipoprotein appeared on acrylamide gel electrophoresis between beta and pre-beta bands, and was assumed simply to be a part of pre-beta lipoprotein. However, this band could just as well

be a part of beta lipoprotein as seen in the case presented in this report (Fig.4-c, a double beta band without pre-beta).

Little effort was made in identifying the chylomicron fraction in this study because all sample sera had been obtained after an overnight fast and almost all of our cases showed no abnormal amount of chylomicrons when examined by paper electrophoresis. From the theory of acrylamide gel electrophoresis and from the observations in this study, it was presumed that chylomicrons would be located at a point (or area) more cathodal to the pre-beta band. As seen Fig.4-c, by Disc electrophoresis of post-prandial serum, diffuse color was observed in the sample gel layer which was usually not colored by the authors' method. This suggests that chylomicrons do not migrate out of the sample gel under the conditions employed in this method. However, further study will be required to determine the electrophoretic character of the chylomicron fraction particularly since the homogeneity of this fraction has been questioned¹⁷

SUMMARY

An integrated and simplified method for acrylamide gel Disc electrophoresis was devised in this study based on results obtained by the previous study where general behavior of human serum lipoproteins in various acrylamide gel systems was investigated. Acrylamide gel concentrations employed in this method were 3.75% for separating gel, 2.5% for spacer gel and 3.3% for sample gel. For lipoprotein staining, serum samples were mixed with a sample gel solution in which Sudan Black B in ethylene glycol and buffered acrylamide solution with a combined catalyzer (TEMED-riboflavin) were included.

Identification of lipoprotein bands on acrylamide gel columns was made by means of a combination of paper electrophoretic, acrylamide gel Disc electrophoretic and immunological techniques. It was established that human serum lipoproteins were sharply resolved and were arranged in the order of alpha, beta, and pre-beta lipoproteins from the anodal to cathodal end of the column.

This method could be useful in clinical laboratories as well as in research laboratories for identification of human serum lipoprotein abnormalities.

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