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SERUM LIPOPROTEIN ESTIMATION BY POLYACRYLAMIDE GEL DISC ELECTROPHORESIS

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ABSTRACT

A simple, rapid method is described for separating the serum lipoproteins into clear, discrete and reproducible bands by polyacrylamide disc gel electrophoresis. The sample is prestained with Sudan Black B in a sample gel and resolved by electrophoresis in a discontinuous pH system consisting of a sample gel, concentrating gel and separating gel. 30 young healthy males and 20 young healthy females were examined with the method. The examination of 22 young people of them showed trace amount of chylomicrons. No significant sex difference could be demonstrated in the plasma concentrations of chylomicrons, β- and pre-β- and α-lipoproteins as a whole.

INTRODUCTION

It has been known for a long time that liver diseases are frequently accompanied by marked changes in plasma lipid concentrations. The example best known to clinicians is the hypercholesterolemia in cholestasis or fatty liver. Plasma lipoprotein estimations are still more important in the investigation of disorders of lipid metabolism. However, there are few methods of lipoprotein analysis by ultracentrifugation and electrophoresis. Paper electrophoresis[1] is in general use and has, due to the works of Fredrickson *et al.*[2], received great clinical attention, but there are certain disadvantages in this method. One of them has been the poor separation between β- and pre-β-lipoproteins, which makes difficult the exact interpretation and the quantitative evaluation of the results. Many investigators have studied the electrophoresis supporting medium for lipoprotein analysis. Polyacrylamide gel is a useful medium, because of good separation and the need of only a small amount of sample[3]. Disc electrophoresis sharply delineates lipoprotein bands and is more rapid than other electrophoretic method. This paper deals with the estimation of lipoprotein by a prestaining method of polyaclamide gel disc electrophoresis as a quantitative method for clinical purpose, and the results

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obtained by this method on 50 young healthy persons were studied.

MATERIALS AND METHODS

Reagents

Solution A: Dissolve 36.6 g of Tris and 0.23 ml N, N, N, N-tetra methylethylendiamin in water. Add 48 ml of 1 N-HCl and dilute to 100 ml with water. The pH should be 8.9.

Solution B: Dissolve 5.98 g of Tris and 0.46 ml of TEMED in water. Add 48 ml of 1 N-HCl and dilute to 100 ml with H_2O. The pH should be 6.7.

Solution C: Dissolve 15.0 g of acrylamide and 0.4 g of bis-acrylamide in H_2O and dilute to 100 ml with water.

Solution D: Dissolve 20.0 g of acrylamide and 5.0 g of bis-acrylamide in water and dilute to 200 ml with water.

Solution E: Dissolve 4.0 mg of riboflavin in 100 ml of water.

Solution F: Dissolve 200 g of sucrose in water and dilute to 500 ml with water.

Solution G: Dissolve 0.14 of ammonium persulfate in water in a 100 ml volumetric flask and dilute to volume with water.

Dye Solution

Absolute ethanol saturated eith Sudan Black B.

Separation Gel Solution

Combine solutions A, C and G in the ratio of 1 : 1 : 2, by volume prepare approximately 1.2 ml of separating gel solution per separating gel required. The separating gel solution should be prepared just before use.

Concentrating Gel Solution

Mix solutions B, D, E and F in the ratio 1 : 2: 1 : 4, respectively, by volume.

Sample Gel Solution

Just before use add 8 parts of concentrating gel solution to 1 part of dye solution and mix by gentle inversion.

Reservoir Buffer

Dissolve 6.0 g of Tris and 28.8 g of glycine in about 850 ml of H_2O. If necessary, adjust the pH to 8.3 with Tris or glycine. Dilute to 1 liter with water.

PROCEDURE

Preparation of Gels

1. Use glass tubes (10 cm long, 6 mm inside diameter, with smoothed ends) which have been soaked overnight in chromic acid cleaner, rinsed well with

distilled water, and oven dried.

2. Close one end of each tube with parafilm and place the tubes in vertical position in a rack. Gel tubes are held in the vertical corrugations with rubber bands. Alternatively, terry clamps screwed into a wood block may be used to hold the tubes.

3. Using a propipette, fill about 1.0 ml of freshly prepared separating gel solution to each tube.

4. Carefully layer a small volume of distilled water on top of the gel solution in each tube.

5. After 30 minutes at room temperature an interface will be seen indicating that the gel has solidified. Invert the tubes and blot on absorbent paper.

6. Add 0.1 ml of concentrating gel solution to each tube. Layer a small amount of distilled water on top of the solution.

7. Allow each tube to remain undisturbed for 30 min. under intense ilght.

8. After photopolymerization, shake the water layers from the gels.

9. Mix 20 μl of serum and 2 μl of dye solution for 30 min. and then add this solution and 0.2 ml of sample gel.

10. Carefully layer a small amount of reservoir buffer.

11. Photopolymerize under intense light.

Electrophoresis and Scanning

Place the tubes in the electrophoresis apparatus, add reservior buffer to the upper and lower tray. Connect the power supply to the electrodes, with the anode in the lower tray, and begin electrophoresis. This is performed in the cold room. We use a current of 5 mA per gel 30 min. The pH of the reservoir buffer changes appreciably during prolonged electrophoresis. The use of large trays or of several changes of the buffer can eliminate the problem. With in 15 min. after electrophoresis, electrophoresed gel tube is scanned in the Johko densitometer equipped with a 578 mμ. Longer than 15 min., sharp delineated bands are diffused in the course of time.

Lipid, cholesterol, triglyceride concentration

Plasma total lipid concentration was determined gravimetrically[4]. Plasma total cholesterol concentration was determined according to Rude[5]. Plasma triglyceride was estimated with an enzymatic glycerol determination[6]. Phospholipids were determined as lipid phosphorus.

Blood Samples

Specimens of blood were drawn from 30 young healthy males and 20 females after an overnight fast, allowed to clot and the serum separated by low speed centrigugation. Electrophoresis was carried out within 2 days.

RESULTS AND DISCUSSION

Fig. 1 shows an electropherogram of lipoproteins pattern on "polyacrylamide gel disc electrophoresis". The lipoprotein zones were well separated and sharply delineated. Usually four fractions could be observed. The fastest moving fraction for routine use was called α-lipoprotein. After this, β- and pre-β-lipoprotein fractions are always seen, well separated from the β-lipoprotein band. The positions of the β- and pre-β-lipoprotein bands on polyacrylamide gel are reversed, compared to their positions after paper electrophoretic mobility and molecular size, while separation on paper depends only on electrophoretic mobility. The electrophoretic mobility is attributed to the net charge of the protein and to the electroosmosis of the supporting medium, but there is little electroosmosis on polyacrylamide gel.

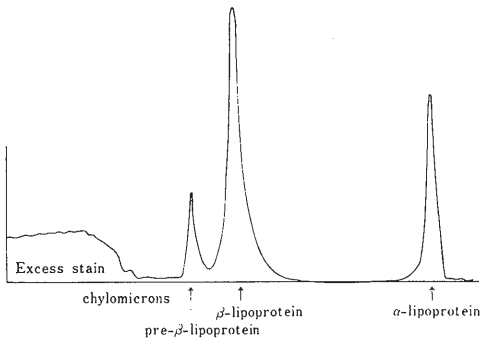

FIG. 1

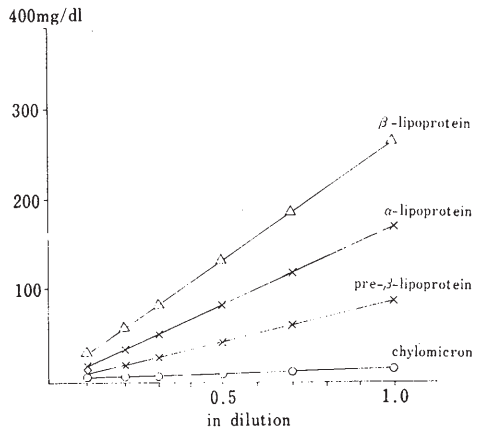

FIG. 2

The slowest moving fraction is due to chylomicrons, and trace amounts of chylomicrons were often observed in the normal serum.

Hyperlipoprotenemia showed the prominent chylomicron fraction in types II, III, IV, V according to the classification of Fredrickson *et al.*[2] For quantitative lipoprotein estimation by the staining method, it is a problem whether the linearity between lipoprotein amount and dye uptake can be demonstrable for all fractions. Electrophoretic separation methods for the lipoproteins have yielded quantitative information that is sufficient to lead to the establishment of clinically useful classification. Variable degrees of absorption to filter paper, lipoprotein interaction with gel, and staining irregularities have made precise and reproducible densitometric estimates difficult. Fortunately, however, present clinical use does not requires complete quantification. These problems are similar to those of the generally accepted electrophoretic estimation of plasms

protein fractions.

Fig. 2 shows the linearity between lipoprotein and dye uptake, in the dilution of a plasma preparation with saline. From this lipoprotein estimation could be expressed by the ratio of dye uptaking of the individual frations to the total dye uptake. The above method was applied to estimate the plasma lipoprotein of normal young people. The results of lipoprotein and lipid estimations are shown in Table 1.

TABLE 1

	Males (mg/dl)	Females (mg/dl)
α-lipoprotein	170±30	158±34
β-lipoprotein	250±47	274±89
pre-β-lipoprotein	88±31	76±38
chylomicrons	9± 8	12± 9
cholesterol	164±22	170±28
triglyceride	90±24	81±24
phospholipid	149±28	158±30
total lipid	522±74	524±78

means±SD

The examination of 22 young people after an overnight fasting showed trace amount of chylomicrons. Pre-β-lipoprotein was observed in all, the content being 17% of total lipoprofeins in males and 15% in females. Our estimations of β- and pre-β-lipoprotein were found to be smaller than the results of Postma *et al.*[7] and Dyerberg *et al.*[8]. One reason is the difference in sensitivity of the two methods, the other the difference between the plasma total lipid concentration in their material and in our's.

No significant sex difference could be demonstrated in the plasma concentrations of chylomicrons, β-, pre-β- and α-lipoproteins as a whole. The procedure can be carried out rapidly and will yield reproducible results even in the hands of an individual. The method is readily adaptable for use in routine diagnostic laboratory or in the field to elacidate many problems of lipid metabolism.

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