A METHOD OF LIPOPROTEIN ELECTROPHORESIS

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The analysis of serum lipoproteins has been shown to be important for numerous clinical purposes[1,2]. Despite its clinical value, however, lipoproteins are still not widely determined for clinical purposes on a routine basis. Since limitations of methods may be partly responsible, further methodological developments could be of much value in this field.

Filter paper electrophoresis is a relatively simple method for lipoprotein determinations, but has the disadvantage that some lipoproteins are highly adsorbed by the filter paper. This results in tailing and errors in quantitation as well as changes in relative mobility of the adsorbed fractions[3].

The electrophoretic method utilizing a semi-fluid or thixotropic buffer solution as medium appeared suitable for lipoproteins, since adsorption effects are largely avoided[4]. The procedure can be conducted in an apparatus which combines electrode compartments and the running surface in a single vessel. This reduces the preparations necessary for the analysis of a number of samples, primarily, to pouring a stabilized solution in the vessel[5].

Staining the lipoproteins in the serum before the separation, as described by McDONALD AND RIBEIRO[6], permits them to be followed during the migration, and to be quantitated without errors due to rinsing excess stain from the background. In order to separate enough serum to obtain accurate optical density values, however, it was found necessary to pour the stabilized buffer, not as a thin film, but as a solution 1/4 inch thick. A larger quantity of serum could be applied with the thicker solution[7]. The electrophoresis of lipoproteins by this procedure was investigated. The experimental methods used and the results obtained are described below.

MATERIALS AND METHODS

Pre-staining of serum lipoproteins

A saturated solution of Sudan Black B in ethylene glycol was prepared according to the method of McDONALD AND RIBEIRO[6]. This solution was stable at room temperature. One volume of the Sudan Black B solution was added to 2 volumes of serum, at least 1 day before the electrophoretic separation. The serum was stored in a refrigerator at 3°.

Electrophoretic method

The electrophoresis was conducted as reported previously, except for the modifications described below. The apparatus can easily be constructed of plexiglas or other inert material. A number of 1/4 in. thick glass strips were positioned on the level, vinyl-covered platform so as to produce a series of parallel migration paths (Fig. 2). A volume of 0.05 *M* veronal buffer, pH 8.6, sufficient to fill the electrophoretic vessel, was prepared, and enough Difco Purified agar was weighed out to make a 0.16% solution in this volume. (Difco Bacto agar cannot be substituted for this procedure, as will be discussed later.) The agar was heated to an active boil in about 1/10 of the buffer and was immediately poured back into and mixed with the remaining 9/10 of the buffer. The buffer was poured into the electrophoretic vessel to a depth of 1/4 in. over the platform, level with the tops of the glass strips. In order for the solution to acquire sufficient rigidity to control convection, it was permitted to stand for 30 min without any current (this wait is not necessary when the buffer is layered as a thin film).

At the end of this period, the temperature of the solution should be between 25–30°. If it is higher, the agar may have been boiled in too large a portion of the buffer. A potential gradient of 4 V/cm was applied. A strip of Whatman No. 17 filter paper, 3/16 in. wide, was dipped in each of the prestained sera and blotted briefly. Each strip was placed with a forceps in the buffer solution with its width in the vertical plane, and its length perpendicular to the direction of the current, as shown in Fig. 2. The strips should not protrude above the surface, and will remain in position when released. The Whatman No. 17 paper retains about 0.5 ml of serum per sq. in., and the volume of serum to be separated can be varied according to the length of the strip.

The samples were permitted to migrate until a distinct separation between α_2- and β-lipoproteins was obtained, which required about 3 hours. The power source was turned off, and each lipoprotein band was lifted between two aluminum sheets and inserted into a 10 ml graduated test tube. This operation is illustrated in Fig. 1. The two sheets are pushed down vertically into the solution, one on each side of the band to be isolated. They are brought together at the bottom, and lifted up to the mouth of the tube. A bottom corner of the two sheets is inserted into the mouth. Separation of the sheets then permits the band to fall into the tube. Any remaining drops may be wiped into the mouth of the tube.

Each of the tubes containing the lipoprotein bands was diluted to 4.5 ml with the ethylene glycol. Drops on the side of the tube can be washed down with the diluent. The tubes were mixed and placed in a hot water bath for about 5 min. After the blue particles dissolved to form a clear solution, the optical density was determined at 590 mμ in a Beckman D.U. spectrophotometer. The per cent of each lipoprotein was calculated from the ratio of its optical density to the total optical density of all three bands.

As an alternative method of quantitation, the lipoproteins could also be measured by direct photometry by a procedure previously reported[8]. Wavelengths of both

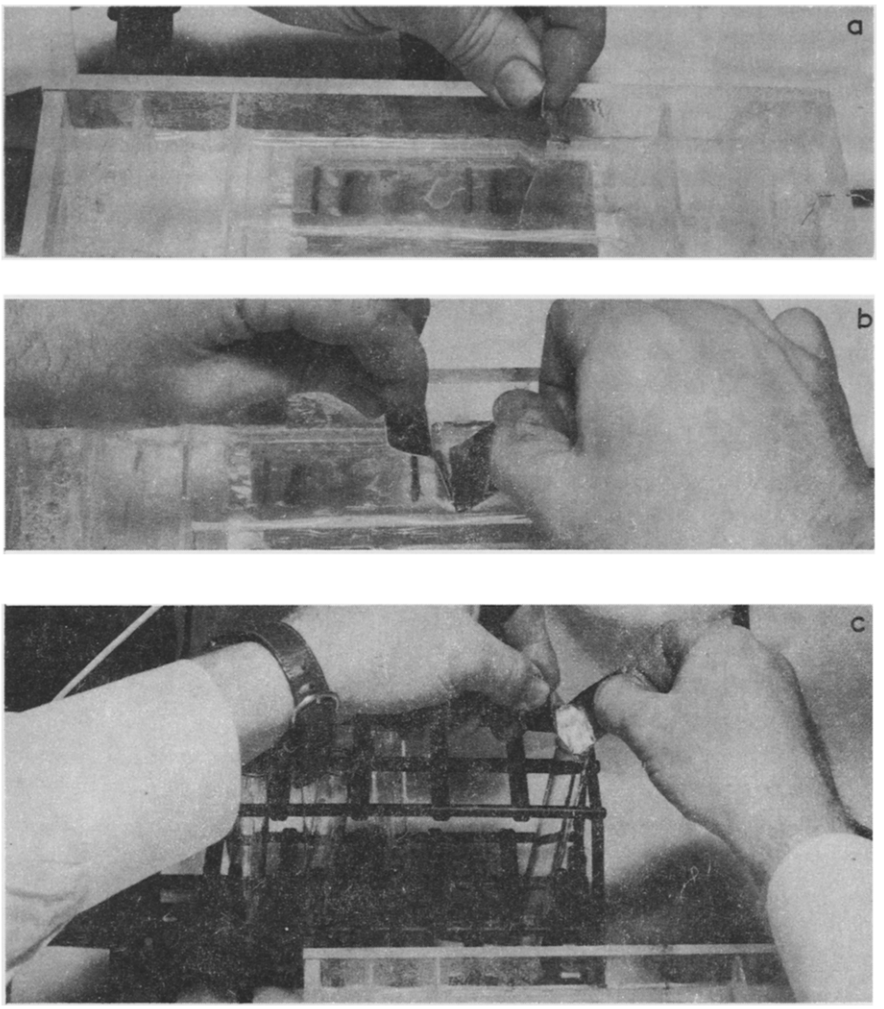

Fig. 1. Transfer of lipoprotein band. An aluminum sheet is inserted down on one side of the band in (a), and another one is inserted on the other side in (b). They are brought together at the bottom and lifted over a graduated test tube in (c). The sheets are parted, releasing the band into the tube.

590 and 200 mμ were used in order to detect both lipid stain and total serum proteins. The buffer used for this purpose contained 0.066 *M* of boric acid and 0.027 *M* of LiCl per l, and was adjusted to a pH of 8.6 with NaOH.

RESULTS

An estimate of the reproducibility of the method of removing each band and measuring its optical density was obtained by analyzing 8 samples of the same serum. The results are shown in Table I.

In order to determine whether this reproducibility is adequate for clinical pur-

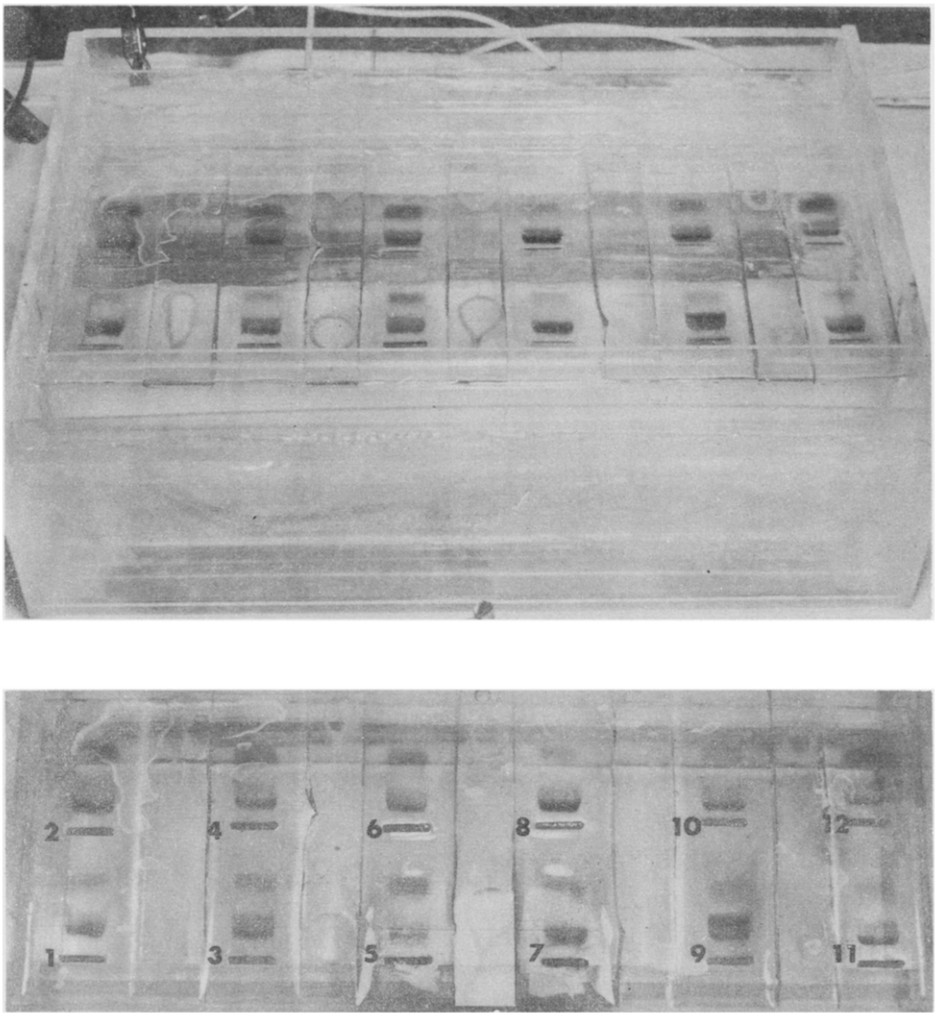

Fig. 2. Lipoprotein analysis of 12 sera. Entire apparatus is shown above, close up of sera is shown below.

TABLE I

REPRODUCIBILITY OF MULTIPLE LIPOPROTEIN DETERMINATIONS ON A SINGLE SERUM

Sample No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
% β-Lipoprotein	53.3	55.8	53.3	54.4	50.2	52.8	53.0	56.9
% α_2-Lipoprotein	22.5	17.1	18.2	19.8	19.4	21.9	22.9	19.1
% α_1-Lipoprotein	24.2	27.1	28.4	25.9	30.4	25.3	24.2	24.0

poses, the serum lipoproteins were analyzed in 12 fasting individuals, 6 of whom were apparently in good health, and 6 of whom were patients expected to have abnormal lipoprotein patterns. The results are presented in Table II and in Fig. 2. The magnitude

TABLE II

NORMAL AND ABNORMAL SERUM LIPOPROTEIN VALUES

Serum No.	% β	% α_2	% α_1	*Age*	*Sex*	*Clinical description*
1	53.3	22.5	24.2	28	M	apparently in good health
2	51.1	17.3	31.6	27	F	apparently in good health
3	50.8	24.8	24.4	27	M	apparently in good health
4	49.2	21.5	29.3	35	F	apparently in good health
5	45.5	19.7	34.8	32	F	apparently in good health
6	42.8	23.6	33.6	23	F	apparently in good health
7	81.0		19.0	25	F	infectious hepatitis
8	70.0	21.6	8.4	37	F	essential hyperlipemia, myocardial infarction
9	52.3	34.8	12.9	38	M	hyperlipemia, angina pectoris
10	59.2	23.0	17.8	51	M	myxedema
11	59.9	27.9	11.7	69	F	intrahepatic obstructive jaundice secondary to chlorpromazine
12	36.0	22.4	41.6	26	F	sarcoidosis

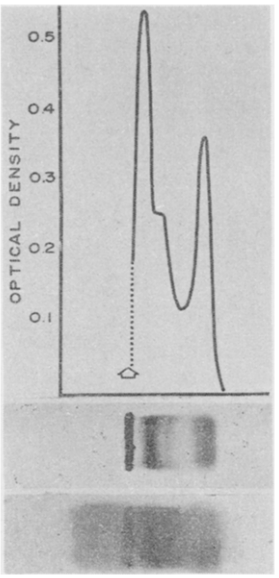

Fig. 3. Photoelectric curve and photographic film at 590 mμ. Bottom film was obtained at 200 mμ.

of the differences between the presumably normal and abnormal values demonstrates that the method is sufficiently reproducible to distinguish them.

Fig. 3 presents the results obtained when the lipoprotein bands were separated and measured directly without removal. A monochrometer light source was positioned above a quartz cell and photographic film or a photocell was used underneath. The volume of serum applied with the Whatman No. 17 paper, in the 1/4 in. thick solution, resulted in suitable optical densities for accurate measurement at 590 mμ. At 200 mμ, however, the optical densities were too great for application of Beer's law.

For purposes of comparing relative mobilities of lipoproteins to other proteins in the same serum, better resolution can be obtained by applying less serum, and reducing the thickness of the buffer solution to a 1.5 mm thick film[8]. This was done and Fig. 4a illustrates the relative mobility of lipoproteins when 0.16 % Difco Bacto agar was substituted for Difco Purified agar. The β-lipoprotein migrated more rapidly, and all the lipoproteins formed a single, diffuse band. This confirms what has been

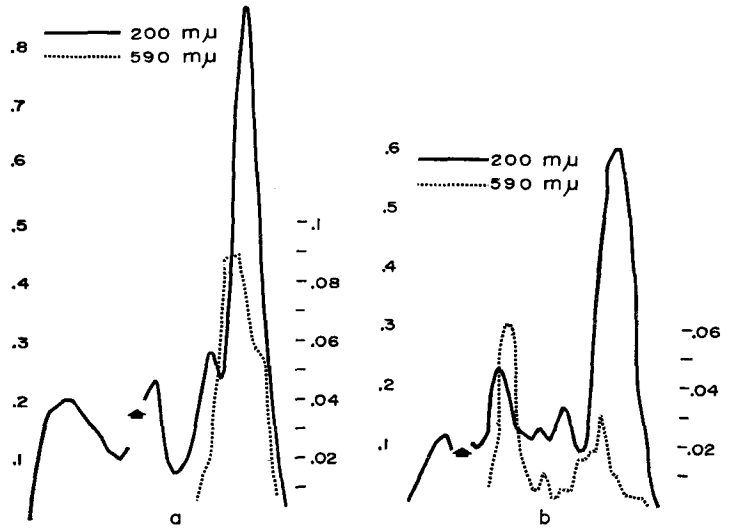

Fig. 4. Optical density distributions obtained with 0.16 % Difco Bacto agar in (a), and with 1.5 % potato starch in (b). Scales at 200 mμ are on the left, and at 590 mμ are on the right.

previously reported by WIEME[9]. When 1.5 % potato starch was substituted for the 0.16 % Purified agar, the results shown in Fig. 4b were obtained. The results with starch appear comparable to those obtained by others workers[10].

DISCUSSION

In the present procedure, the lipoprotein bands appear to migrate without significant tailing, since little density can be detected between the β-lipoprotein and the line of application (Fig. 2).

The resolution of the α_2-lipoprotein may be important in view of the investigations which indicate that this fraction is elevated in patients with arteriosclerotic diseases[11,12].

The only serum shown in Fig. 2 which did not reveal a separation between α_2- and β-lipoproteins was from a patient with infectious hepatitis (No. 7 in Table II and Fig. 2). Since atypical lipoproteins have been found in individuals with this disease[13], the lack of separation may be due to the production of heterogenous lipoproteins of intermediate mobility.

SUMMARY

An electrophoretic method is described for the routine analysis of lipoproteins. Use of a semi-fluid medium for the migration minimizes adsorption of lipoproteins by the medium. α_1-, α_2-, and β-Lipoproteins are separated by this procedure. Little working time per sample is required when a number of sera are analyzed together.

The lipoprotein bands can be quantitated by isolation and spectrophotometric measurement. It is also possible to quantitate the bands by direct photometry. The use of light at wavelengths of both 200 and 590 mμ permits comparison of the relative mobilities of the lipoproteins to other proteins in the serum.

The separation allows recovery of the lipoprotein fractions in quantities which may be sufficient for further uses or investigations.

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