We describe a rapid, reliable staining procedure for lipoproteins on paper electrophoreograms. Staining time is 2.5 h in an acetone-water (1:1, by vol) solution of Oil Red O dye (0.4 g/liter). Results agree well with those obtained by the conventional overnight staining technique.

**Additional Keyphrases:** screening for hyperlipemia • speed lipoprotein measurement • Oil Red O staining

Paper electrophoresis is widely used in clinical medicine as a screening test for hyperlipidemia. It is a simple, reproducible procedure, but requires 36 h to complete. Aliquots of sera are electrophoresed 16–18 h, paper strips are dried for 30 min, followed by overnight staining (1, 2). Recently it was reported that staining time could be decreased to 2 h by using Oil Red O stain in acetone-water solvent (3). By this technique of staining we found that the alpha/beta lipoprotein (α/β) ratio was not consistent with that obtained by either the conventional staining technique (1, 2) or ultracentrifugal method (4). The alpha-lipoprotein band, in particular, stained less intensely than by the other techniques (1, 2).

The cause of this could be (a) staining time is not optimal for this less stainable fraction (5), or (b) the lipoprotein or lipid of the lipoprotein is eluted by the dye-solvent mixture. Prolonging the staining time of the method (3) to 4, 8, or even 16 h did not result in appreciably more intense staining in the alpha region, so it seemed that factor b above probably accounted for the lack of dye uptake by alpha-lipoprotein. The high organic solvent concentration (68.6 volumes of acetone to 31.4 volumes of water) of the method (3) tends to support this idea.

The staining procedure described here results in optimum staining in 2.5 h of lipoproteins on paper-electrophoretic strips.

**Materials and Methods**

**Samples and Procedure**

**Sera.** Blood samples were obtained from patients who had fasted 12–16 h. Ethylenediaminetetraacetate, Na₂ salt, was added to the prepared sera (1 mg/ml). Serum triglycerides and total cholesterol were determined (6).

**Electrophoresis.** Paper electrophoresis was done by a modification of the Lees and Hatch method (1, 2) in Durum cells filled with sodium barbital buffer (0.05 mol/liter, pH 8.6). Paper strips (Scheicher and Schuell 2043A, No. 320046; Beckman Instruments, Inc., 25511 Southfield Rd., Southfield, Mich. 48075) were presoaked in a solution containing 1 g of bovine albumin (Fraction IV powder, No. 81-033; Miles Lab., Inc., Kankakee, Ill. 60901) per 100 ml of the barbital buffer. They were mounted on the glass rods of the electrophoresis cells, and were allowed to stand for 30 min before 20 μl of sera was applied to each strip. After electrophoresis at 110 V for 18 h, the strips were oven-dried for 30 min at 100°–110°C, and then stained with Oil Red O stain.

Staining. The lipid stain was prepared by adding 0.4 g of Oil Red O dye (No. 320051, Beckman Instruments, Inc.) to 500 ml of reagent-grade acetone and heated to 50°C for 2 h. After adjusting the volume of the dye-acetone mixture to 600 ml by adding acetone, 500 ml of distilled water (50°C) was added, and the mixture was incubated at 37°C for at least 4 h before use. (Elimination of this incubation step results in unsatisfactory brownish background-staining of the electrophoretograms). Acetone-water Oil Red O stain (0.4 g/liter) containing 600, 400, or 300 ml of acetone per final 1000 ml was also prepared by the procedure just described. Staining by the reference method (1, 2) had previously been modified as follows: Ethanol-water (1:1, by volume) solution of Oil Red O dye (0.4 g/liter) rather than the 60:40 (by volume) Oil Red O stain they described. All staining was done at 37°C. After the staining period the strips were removed from the air-tight staining containers, washed in running tap water for 15 min, blotted dry with paper towels, and then allowed to air dry. The dried strips were scanned with the “Analytrol” (Model R; Beckman Instruments, Inc.) with use of a B-5 cam, slit width of 3, and 550-nm filters.

**Experimental Variables**

**Optimum solvent concentration.** Optimum solvent concentration was determined by staining pairs of electrophoretograms of the same serum sample (triglyceride = 85 mg/dl, cholesterol 245 mg/dl) in the 60, 50, 40, and 30% (acetone-water, by volume) Oil Red O stains. Because staining time is inversely related to solvent concentration, the paired strips of electrophoretograms from the four stains were removed from the staining tanks when the intensity of the beta-lipoprotein band matched the intensity of the beta-lipoprotein band of the reference strips stained by the modified overnight-staining method of Lees and Hatch (1, 2). The electrophoretograms were then scanned to obtain the α/β ratios, which were compared to the α/β ratio as determined by analytical ultracentrifugation (4).

**Optimum staining time.** Because our preliminary studies showed that a staining time of less than 2 h results in an α/β ratio unlike those for the reference stained strips, optimum staining time was ascertained by staining for various lengths of time—2.0, 2.5, 3.0, 6.0, 12.0, 24.0 or 96.0 h—electrophoretograms of a given serum that were run at the same time in the same cell. Triglyceride and total cholesterol concentrations of this serum were 155 and 225 mg/ml, respectively.

**Reproducibility.** Using a 2.5-h staining time, we tested

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From the Division of Research, The Cleveland Clinic Foundation, Cleveland, Ohio 44106.

Research Fellow of the American Heart Association, Northeast Ohio Chapter, Inc.

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reproducibility by staining seven strips that were electrophoresed in the same cell at the same time. Serum triglyceride and total cholesterol concentrations of this serum were 150 and 220 mg/dl, respectively. The $\alpha/\beta$ ratios for the seven strips were compared. The eighth strip was stained by the modified conventional overnight-staining method (1, 2) and compared to the $\alpha/\beta$ ratio for the above seven strips.

Repeatability. Repeatability was tested by comparing the $\alpha/\beta$ ratios for the electrophoretograms of a normal serum sample (triglyceride = 155 mg/ml and cholesterol = 225 mg/dl) that were stained by the present and by the modified reference method. Four runs were made in which half of the strips in each cell (four per cell) were stained by the 2.5-h method and the other half (four per cell) were stained by the overnight procedure.

Hyperlipemic sera. Patients' sera containing either abnormally high or low amounts of lipids were electrophoresed and stained by the 2.5-h staining procedure. The strips were compared to those stained by the modified Lees and Hatch method (1, 2). Electrophoretograms of hyperlipoproteinemic sera (Types I to V) were also stained by the 2.5-h staining method.

Results and Discussion

Staining the electrophoretograms in 60, 50, 40, or 30% (by volume) acetone–water solution of Red O stain (0.4 g/liter of solvent) demonstrated the optimum solvent proportions to be 1:1. The $\alpha/\beta$ ratios of the electrophoretograms agreed well with the $\alpha/\beta$ ratio obtained with the analytical ultracentrifuge (Table 1). Other proportions of acetone and water resulted in a lower percentage of alpha lipoprotein.

Within 1 h a visible lipoprotein pattern was produced when the 1:1 acetone-water Red O stain was used. However, optimum staining time was between 2.5 and 24 h, as judged by the consistent $\alpha/\beta$ ratios (range = ±0.21 to 0.23). Two-hour staining produced a slightly lower $\alpha/\beta$ ratio, 0.19. As the staining time was increased from 1 to 96 h, each of the lipoprotein bands took up a proportionate amount of the lipid dye, except for the 96-h strips (Figure 1), which had an $\alpha/\beta$ ratio of 0.12. After 24 h of staining, a slight but noticeable background appeared in the strips. The $\alpha/\beta$ ratios, when the strips were stained by the present method, were comparable to the $\alpha/\beta$ ratio of the strip stained by the modified method of Lees and Hatch (1, 2).

The $\alpha/\beta$ ratios for the seven electrophoretograms stained for 2.5 h were comparable for reproducibility. The mean ratio was 0.25 and the standard deviation ±0.02, which indicates that the present staining procedure is quite reproducible. The eighth strip stained by the modified reference method (1, 2) had an $\alpha/\beta$ ratio of 0.27. The similar $\alpha/\beta$ ratio of the two staining procedures demonstrates that the new rapid-staining method is reliable.

### Table 1. Alpha/Beta Lipoprotein Ratios for the Same Serum Sample, a Stained by Using Different Solvent Concentrations, as Compared with Conventional and Analytical Ultracentrifuge Methods

<table>
<thead>
<tr>
<th>Acetone-water solvent method</th>
<th>Modified ref. staining method</th>
<th>Analytical ultracentrifuge</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 ml acetone/dl</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.20</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*aPatient's serum triglycerides = 85 mg/dl and total cholesterol = 245 mg/dl.

### Table 2. Repeatability Test of the Present and Reference Method a

<table>
<thead>
<tr>
<th>Method of staining</th>
<th>Run no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>$\alpha/\beta$ Ratio</td>
<td>0.19 ± 0.02</td>
<td>0.20 ± 0.03</td>
<td>0.21 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>0.17 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.17 ± 0.02</td>
</tr>
</tbody>
</table>

*aPatient's serum triglycerides = 155 mg/dl and total cholesterol = 225 mg/dl.

Also, the values (0 to 4+ grading) by the visual scoring technique (4) of strips from the present and reference methods of staining were ±, 3+, 3+, 4+, 4+ for the chylomicrons, beta, pre-beta, and alpha fractions, respectively, which indicates that the two staining methods produce similarly stained electrophoretic patterns.

The test of repeatability (Table 2) demonstrated that the 2.5-h staining method is as repeatable as is the modified reference staining method of Lees and Hatch (1, 2). The slight difference in $\alpha/\beta$ ratios between runs and between the two different staining procedures was not found to be significant by Student's $t$-test (7).

Sera of patients having low and high lipid concentrations were electrophoresed and stained by the short method and by the reference method (Figure 2). Proportionate dye uptake by each lipoprotein fraction was comparable in the two procedures. The electrophoretograms of hyperlipoproteinemic sera are shown in Figure 3.
The above results indicate that paper electrophoretic strips, stained 2.5 h in the staining mixture we describe, give reliable, accurate patterns with minimal background, suitable for densitometric scanning (Figure 4) for semi-quantitation of the lipoprotein fractions. The staining intensity of the lipoprotein bands corresponds well with that obtained with the reference method.

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References