Gradient Gel Electrophoresis of Human Plasma Lipoproteins

G. J. Bautovich, M. J. Dash, W. J. Hensley, and J. R. Turtle

A rapid and simple method for separating and identifying plasma lipoproteins is described. The technique utilizes the "molecular sieving" properties of continuous concave gradient electrophoresis on a slab of polyacrylamide (4 to 26%) gel. Gradient gel electrophoresis is particularly suitable for separating lipoproteins according to their molecular weight. The technique can be used to demonstrate the major lipoprotein classes in normal plasma and to identify abnormalities of lipoprotein metabolism. Components of a sample are better resolved by gradient gel electrophoresis than by electrophoresis on paper or cellulose acetate. The results obtained compare favorably with those achieved by preparative ultracentrifugation. The technique is easily and rapidly performed in a routine clinical laboratory.

**Additional Keyphrases:** diagnostic aid, differentiation of hyperlipoproteinemias, polyacrylamide gel, ultracentrifugation compared lipoprotein heterogeneity

The major lipoprotein classes are subdivided according to their physical and chemical characteristics. They are usually separated into the VLDL,1 LDL, HDL, and chylomicrons. The molecular weight of each lipoprotein class varies according to its density and composition (1–3). Since abnormalities of lipoprotein metabolism may be demonstrably associated with an increased incidence of vascular disease, it has become necessary for routine clinical laboratories to have simple and accurate methods available for lipoprotein analysis.

Fredrickson's classification (1–4) of the disorders of lipoprotein metabolism is based on separation of the various lipoprotein classes in plasma by use of paper electrophoresis (5). This technique is not satisfactory for demonstration of the pre-beta or VLDL band in normal plasma. Beta (LDL) and pre-beta (VLDL) lipoproteins have been separated by plasma electrophoresis on cellulose acetate (6), agarose gel (7), or polyacrylamide gel (8, 9). Although there have been some improvements in resolution, these techniques produce a uniform mechanical hindrance to the passage of lipoproteins through the supporting medium, which limits their use in routine diagnosis.

In the technique to be described (10), we used a vertical polyacrylamide gel slab with a continuous concave concentration gradient from 4 to 26%, resulting in a progressively smaller pore size along the slab. The extent of migration of molecules in the electrophoretic field is determined by the "pore limit" (11). High-molecular-weight lipoproteins move slower as the "pore size" diminishes, and thus remain close to the point of application, whereas low-molecular-weight lipoproteins travel further through the gel. The resulting separation of plasma lipoproteins has been compared with the results obtained by preparative ultracentrifugation.

**Materials and Methods**

**Apparatus**

The instrument used for lipoprotein electrophoresis is the "Gradipore" electrophoresis apparatus, based on that described by Margolis and Kenrick (10, 11) and distributed by Townson & Mercer Pty. Ltd., Lane Cove, N.S.W., 2066, Australia. The electrophoresis tank has been modified to accept four gels and is supplied in kit form, together with polyacrylamide gradient gel (4–26%) slabs. Spacers are provided in order that 14 separate applications may be made on each gel.

**Reagents**

The buffer used for electrophoresis was Sigma "7–9" (Sigma Chemical Co.), 0.06 mol/liter, pH 9.0. This buffer may be used for at least three electrophoresis runs.

Lipoproteins were stained with Oil Red O (G. T. Gurr, C.I. 26125) (12), 0.4 g dissolved in 630 ml of 95% ethanol. The mixture is heated with vigorous

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1 Nonstandard abbreviations used: VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; EDTA, ethylenediaminetetraacetate.

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stirring, then 370 ml of warm distilled water is added. A reflux condenser is fitted and the solution is boiled gently for 1 min. It is stored at 55°C and can be used for several weeks.

Procedure

Blood was taken by venipuncture from patients who had been fasting for 9 h. Specimens were collected in glass tubes containing EDTA at a final concentration of 1 mg/ml. After centrifugation, plasma was stored at 4°C and processed within three days. Serum cholesterol and triglycerides were estimated by AutoAnalyzer techniques (13), after extraction with isopropanol and shaking with zeolite (14).

A polyacrylamide gel slab was placed in the cell and the current was run for 30 min before the samples were applied. After 30 min, 10 μl of plasma was applied to each slot with a Hamilton syringe. Electrophoresis was performed at a constant voltage of 150 V for 5–6 h, at 4°C. The gels, removed from the cell and separated from the glass casing, were stained overnight in Oil Red O at 55°C.

Lipoprotein classes were separated from plasma after precipitation with heparin–manganese chloride (15) to precipitate LDL, leaving HDL in the supernatant fluid. The LDL precipitate was dissolved in sodium citrate solution (100 g/liter) (16) and dialyzed extensively against 0.15 molar NaCl/0.001 molar EDTA, pH 7.0. Various density fractions (<1.006, 1.006–1.019, 1.019–1.050, >1.050) were separated from the LDL fraction by repeated ultracentrifugation for 24–48 h at 4°C, using a No. 50 rotor at 120,000 × g in a Beckman Model L2 preparative ultracentrifuge (12, 17).

Results

Normal Patterns

The positions of the normal major lipoprotein classes are shown in Figure 1. The VLDL class formed one or more bands in the 4 to 5% polyacrylamide concentration region, close to the point of application. Chylomicrons did not enter the gel, but remained at the point of application. With normal plasma, there may be some staining at the point of application owing to the presence of large VLDL particles.

The LDL class extended from the 5 to 7% region. The presence of the following features should be noted in the LDL region:

- The principal LDL band was densely staining, varying slightly in position in different plasma samples. This suggested some variation in molecular weight of the principal LDL band in different individuals.
- Several secondary bands stained lightly and were adjacent to the main band. Low-molecular-weight LDL migrated further down the gel, comprising a more diffuse region towards the anode.

It is significant that the LDL class was spread over a wide area of the gel, permitting identification of several components with different molecular weights.

Electrophoresis of Fractions Separated by Preparative Ultracentrifugation

Fractions of various densities were obtained by preparative ultracentrifugation of a heparin–manganese chloride precipitate from plasma. The pattern obtained on electrophoresis on each of these fractions is shown in Figure 2.

The LDL fractions (densities 1.006 to 1.020, 1.020 to 1.050, and >1.050) migrated separately towards
the dense section of the gel. This resolution permitted examination of LDL subclasses in whole plasma according to their molecular size.

The heparin-manganese chloride supernate (HDL) migrated over a broad region close to the anode, and comprised the alpha-lipoproteins. This diffuse broad band could just be detected by electrophoresis of normal plasma.

Abnormal Lipoprotein Patterns

Hyperlipoproteinemias of types IIa, IIb, IV, and V (Fredrickson classification) were compared with normal plasma (Figure 3, Table 1).

Both types IIa and IIb demonstrated a marked increase in the intensity of the principal LDL band. Type IIb was differentiated from IIa by the presence of increased staining close to the point of application, consistent with an increase in VLDL.

In Fredrickson type IV hyperlipoproteinemia, the following features were observed:

- The presence of increased intensity of staining in the 4 to 5% polyacrylamide region, consistent with an increase in VLDL.
- The principal LDL band was of lower molecular weight than normal, and migrated further down the gel.
- Additional lightly staining low-molecular-weight LDL bands appeared in front of the main band.

The pattern obtained from type V plasma was similar to that from type IV, but it was associated with increased staining at the point of application owing to the presence of chylomicrons, which had failed to enter the gel.

Discussion

The molecular weight of each lipoprotein class is variable and has a direct relationship to density (2, 3). For this reason, separation of the lipoproteins according to molecular size can be achieved satisfactorily by using pore-limit electrophoresis (10, 11). The results obtained, particularly in the separation of LDL subclasses, are comparable with those achieved by the more expensive and complicated ultracentrifugation technique. Until now, the only method for the definitive typing of lipoprotein disorders has been ultracentrifugation, which requires several days to complete and the availability of expensive instrumentation. Gradient gel electrophoresis requires only 5 to 6 h, and provides detailed information, comparable to that obtained over several days by ultracentrifugation. The technique is reproducible and can be used for the densitometric assessment of lipoproteins when a known density sample is run on each gel for comparative purposes.

Subclasses of the LDL lipoproteins in plasma have been better separated than was possible by other electrophoretic techniques. VLDL remain close to the point of application and HDL distribute broadly in lower part of the gel. The HDL class are the smallest of the lipoproteins and their diffuse spread on gradient gel electrophoresis may be explained by the ellipsoidal shape of these molecules (2).

Although it is theoretically possible that VLDL and LDL could be more widely separated by decreasing the initial polyacrylamide concentration to 3% instead of 4%, the gel structure is not satisfactorily stable at the low concentration, limiting the range of application to 4 to 26%.

Plasma from patients with disorders of lipoprotein metabolism, particularly those associated with hypercholesterolemia and (or) hypertriglyceridemia, produces recognizable and reproducible patterns. With plasma of Fredrickson type IV, an intense VLDL band appeared close to the point of origin but the pattern also showed distinct differences in the LDL region from that of normal serum. There is greater molecular heterogeneity of the LDL in type IV plasma than occurs in normal plasma, type IIa or IIb. Plasma obtained from type II hyperlipoproteinemia shows an intense LDL band with a varying number of subsidiary bands, indicating less heterogeneity in this region than is seen in type IV. There are minor variations in molecular weight of the principal LDL

Table 1. Classification and Cholesterol and Triglyceride Values for the Plasma Samples Illustrated in Figure 3

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Fredrickson type</th>
<th>Cholesterol</th>
<th>Triglyceride mg per 100 ml</th>
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<tr>
<td>1</td>
<td>Normal</td>
<td>140</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>230</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>IIa</td>
<td>317</td>
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<td>4</td>
<td>IIa</td>
<td>290</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>IIb</td>
<td>290</td>
<td>560</td>
</tr>
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<td>6</td>
<td>IV</td>
<td>240</td>
<td>600</td>
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<td>8</td>
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<td>850</td>
</tr>
<tr>
<td>9</td>
<td>V</td>
<td>300</td>
<td>1,050</td>
</tr>
</tbody>
</table>

Fig. 3. Gradient gel electrophoresis of abnormal plasma lipoproteins classified according to Fredrickson 1 and 2, normal; 3 and 4, type IIa; 5, type IIb; 6, 7, and 8, type IV; 9, type V. Corresponding cholesterol and triglyceride values are shown in Table 1.
fraction in patients with type II hyperlipoproteinemia.

In type IV hyperlipoproteinemia, the principal LDL band is of lower molecular weight than normal. It is associated with multiple, less-intense LDL bands extending throughout the LDL range. Fisher et al. (18, 19) have described the presence of a family of LDL's of different densities and molecular weights throughout the LDL range in type IV hyperlipoproteinemia. They claim that this pattern contrasts with normal and type II hyperlipoproteinemia, where the heterogeneity is less marked. The staining of the LDL fractions obtained on gradient gel electrophoresis supports their findings.

The lipoprotein classes are easily, rapidly, and cheaply separated by gradient gel electrophoresis. It is suitable for regular use in a routine clinical laboratory and provides information comparable with that obtained from more expensive and prolonged studies by preparative ultracentrifugation.

References