

Disc electrophoresis of rat plasma lipoproteins

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ABSTRACT The disc electrophoresis of lipoproteins of unfractionated rat plasma is described. The plasma was pre-stained with Sudan black B and electrophoretically separated at polyacrylamide gel concentrations of 7.5, 5, and 3.75%. At least four lipoprotein components were observed, and an additional 2-3 components in the main gel and 2-5 components in the spacer gel possibly were present. Densitometry of the resolved gel patterns indicated good reproducibility. Thin-layer chromatography of lipids extracted from the Sudan black B-binding components confirmed the lipoprotein nature of these components of rat plasma.

A comparison of the disc electrophoretic patterns of human serum and rat plasma suggested that the low-density lipoprotein components of rat plasma are smaller in size than those of human serum.

KEY WORDS electrophoresis · disc · acrylamide gel · plasma · lipoproteins · rat · Sudan black B · densitometry · proteins · human serum · ultracentrifugation

WE RECENTLY REPORTED a high degree of resolution of human serum lipoprotein fractions by a disc electrophoretic procedure (1). The theoretical basis and the practical aspects of disc electrophoresis have been described by Ornstein and Davis (2, 3). In connection with experiments in progress in this laboratory, it was necessary to determine the lipoprotein pattern in small volumes of rat blood. Ultracentrifugal methods are time consuming, require expensive equipment, and call for sizable quantities of plasma. The commonly used zone electrophoretic methods which use paper, starch gel, agar, or starch granules as the supporting media suffer from several disadvantages. These are low resolution, relative invariability of pore size of the media compared to polyacrylamide gel, the requirement for rather large quantities of sample in high concentration, and diffi-

culty in obtaining satisfactory densitometric traces (1-7). While standard polyacrylamide gel electrophoresis is superior in many respects to starch gel electrophoresis, it also suffers from the drawback that the separated protein bands tend to be diffuse and, in general, do not approach the clarity of the disc electrophoretic patterns. We describe here the disc electrophoresis of unfractionated rat plasma lipoproteins using polyacrylamide gels at three different main gel concentrations. The results indicate the presence of between four and twelve electrophoretically resolvable lipoprotein components in rat plasma.

EXPERIMENTAL PROCEDURE

Collection of Rat Blood and Separation of Plasma

Blood was obtained from the abdominal aorta of rats anesthetized with Nembutal (sodium pentobarbital). The rats had been maintained for over a year on a normal synthetic diet containing casein 20.0%, cerelose 66.1%, corn oil 10.0%, Wesson salt 3.5%, choline chloride 0.3%, and all the necessary vitamins. The blood was drawn into syringes and centrifuged immediately. Unless otherwise specified, heparin (25 mg/100 ml of 0.9% NaCl) was used as anticoagulant and the separated plasma was used immediately for electrophoretic experiments.

Staining of Plasma Lipoproteins by Sudan Black B

This was accomplished according to the method of Ressler, Springgate, and Kaufman (8). Generally, 0.6 ml of the plasma was taken up in a glass vial and mixed gently with 0.3 ml of Sudan black B-ethylene glycol solution, prepared according to the procedure of McDonald and Ribeiro (9). The vial was filled with N₂ and was placed at room temperature away from light for 1 hr and then transferred to a refrigerator maintained at 2°. The electrophoretic experiments were usually conducted about 24 hr later.

Abbreviation: TLC, thin-layer chromatography.

Disc Electrophoretic Procedure and Equipment

The equipment used in these experiments was the Model 12 Disc Electrophoresis Unit and the Model E Microdensitometer purchased from Canalco (Bethesda, Md.).

Disc electrophoresis was conducted at pH 9.5, essentially as described by Ornstein and Davis (10), except that the sample gel was not used and potassium ferricyanide was omitted in the formulation of the main gel solutions. Solution B of Ornstein and Davis was mixed with 20% (w/v) sucrose solution and water in order to obtain the following solutions (each with a total volume of 80 ml), which were used instead of upper gel solution for the sample layer. Solution B-sucrose (2 X): Solution B, 20 ml; 20% sucrose solution, 60 ml. Solution B-sucrose (1 X): Solution B, 10 ml; 20% sucrose solution, 40 ml; water, 30 ml. Light Solution B-sucrose (1 X): Solution B, 10 ml; 20% sucrose solution, 20 ml; water, 50 ml.

Acrylamide, N,N'-methylenebisacrylamide, N,N,N',N'-tetramethylene diamine, riboflavin, and glycine were purchased from Eastman Kodak Co. (Rochester, N.Y.); 2-amino-2-(hydroxymethyl)-1,3-propanediol from Sigma Chemical Co. (St. Louis, Mo.); Oil red O (lipid stain) and Buffalo black NBR (protein stain) from Allied Chemical Corp. (New York, N.Y.); and Sudan black B (lipid stain) and ethylene glycol from Fisher Scientific Co. (Chicago, Ill.). The various solutions required for disc electrophoresis such as gel solutions, buffer solutions, staining solutions, and solution B-sucrose solutions were all prepared under carefully controlled conditions and were stored at 2°. The concentration of the buffer used in the upper and lower bath was five times the concentration recommended¹ (10). The tracking dye (bromophenol blue) was not used, because, when the gels were placed in acetic acid solution, the tracking dye diffused out of the gels and became bound to the serum proteins and thus interfered with the visual observation of the lipoproteins.

The composition of the main gel solution (7.5% acrylamide) and of the upper gel (spacer) solution was the same as described by Ornstein and Davis (10). Main gel concentrations of 5 and 3.75% were obtained by suitable adjustment of the volumes of solution C and water in the formula provided by these investigators. A longer spacer (typically about 1.8 cm) and a correspondingly shorter main gel were used in order to obtain a better stacking of the plasma proteins, which

¹ In disc electrophoresis conducted at constant current, the voltage increases as the boundary sweeps across the spacer and into the main gel. The starting voltage at 2.5 ma per tube is usually 60 volts and increases to 120 volts when the boundary enters the main gel. When the dilute buffer rather than the concentrated buffer was used the starting voltage was observed to be 160 volts at 2.5 ma per tube and increased to 240 volts during the course of the run.

were used at a much higher concentration than that originally proposed (10).

The prestained plasma was mixed with an equal volume of solution B-sucrose (2 X). Unless otherwise specified, 30 μ l of the mixture was applied on top of the spacer and was carefully layered with light solution B-sucrose (1 X). The tubes were carefully introduced into the upper bath containing the buffer, and the plastic stoppers were removed with extreme caution in order to prevent mixing of the solutions. Electrophoresis was performed using a current of 2.5 ma per tube; under these conditions the heat generated was negligible.

When a suitable resolution of the lipoprotein components had been achieved, usually after 35–40 min, the tubes were removed and placed in screw-capped test tubes filled with cold (2°) 7.5% acetic acid solution. The tubes containing the gels were photographed immediately and placed in the refrigerator at 2°. They were removed one at a time and measured on the densitometer as rapidly as possible. The peak areas were determined by counting the number of integrator pips under each peak in a manner similar to that illustrated by Ferris, Easterling, and Budd (11). Only the four major lipoprotein peak areas were determined and the albumin component (peak to the far left in all traces) was not included in the measurements. The major peaks have been numbered successively starting with the albumin component as peak 1.

In some experiments, disc electrophoresis was conducted with 10- μ l samples of unstained rat plasma mixed with solution B-sucrose (1 X) and the gels were stained for lipid with an Oil red O solution (12).

Slicing of the Gels and Fraction Identification

In some experiments, each gel was removed from the tube and placed on a Petri dish over a light box. The various bands were sliced with the aid of a clean razor blade, which was repeatedly rinsed with methanol between slicings to avoid contamination. The sliced gel sections (about 1–3 mm thick) were immediately transferred to separate round bottom flasks containing 10 ml of absolute methanol. All the 12 gels from run E 143 (Fig. 2A) were sectioned in order to determine the lipid composition of the various components. The gels were sliced starting with the fastest-moving component. Fraction identification was as follows: fraction 1, the zone ahead of the albumin band; fraction 2, the albumin band; fraction 3, the lipoprotein band just behind zone 2; fraction 4, the hazy sudanophilic zone behind zone 3; fraction 5, the lipoprotein band behind zone 4; fraction 6, the lipoprotein band behind zone 5; fraction 7, the slow lipoprotein component near the top of the main gel; fraction 8, the region of Sudan black dye precipitation near the top of the spacer gel.

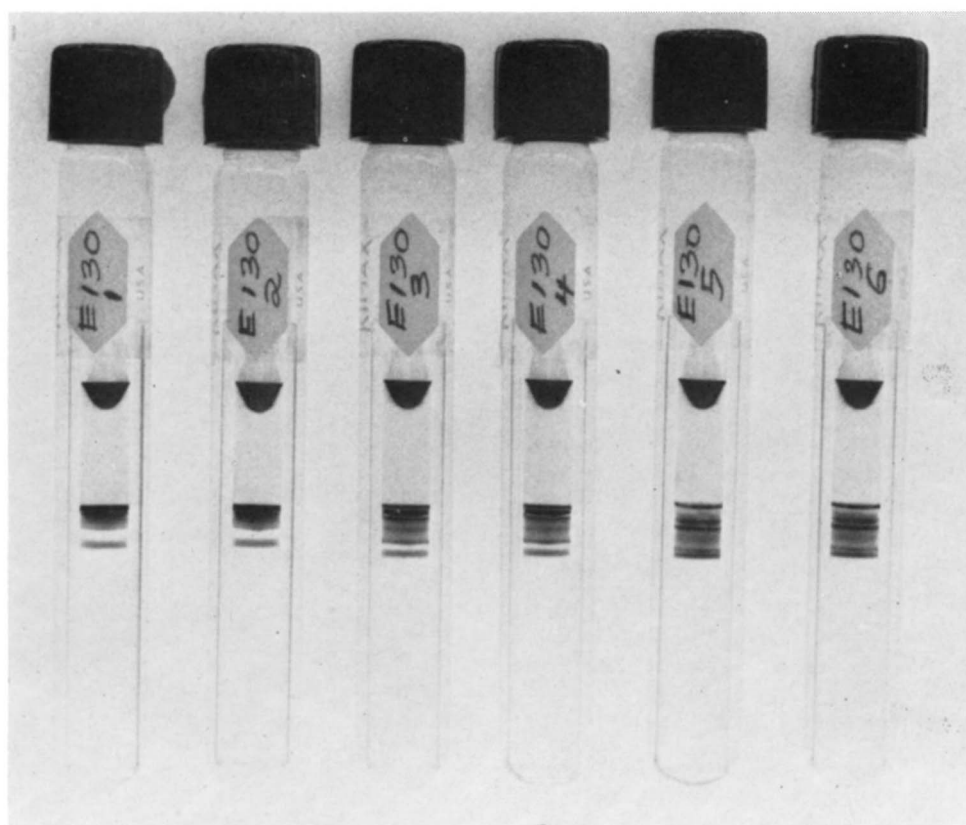


FIG. 1. Disc electrophoretic patterns of prestained rat plasma lipoproteins at different gel concentrations. E 130-1, 2 with 7.5%, E 130-3, 4 with 5% and E 130-5, 6 with 3.75% main gel concentration, 10 μ l of plasma, 2.5 ma per tube for 39 min. Migration from top to bottom.

Lipid Extraction of the Sections and TLC

Each of the gel sections was refluxed with 20 ml of chloroform for 1 hr. The cooled extract was filtered and washed with distilled water in order to remove the non-lipid constituents. The chloroform layer was dried and evaporated under N_2 to a very small volume, which was applied in its entirety to a thin-layer plate. Precautions were taken in order to keep contamination to a minimum.

For the TLC, Silica Gel D-5 (Arthur H. Thomas Co., Philadelphia, Pa.) which had been previously extracted with chloroform-methanol 2:1 was used. This extraction eliminated the solvent front impurities often encountered with commercial absorbents.

The solvent system used was Skelly F-diethyl ether-acetic acid 90:10:1. The standards used for identifying the lipid components were (a) cholesterol; (b) linseed oil; (c) cholesteryl laurate; (d) stearic acid; (e) corn oil; (f) a mixture of a, b, c, and d; and (g) rat plasma lipids obtained in the same manner as described above for the gel sections. The spray used was 50% sulfuric acid, which showed up triglycerides and fatty acids better than the dichromate-sulfuric acid spray recommended by Mangold (13).

RESULTS

The results indicated that rat plasma contains 4-7 lipoprotein components in the main gel and 2-5 lipoprotein constituents in the spacer gel.

Effect of the Pore Size of the Gel on the Resolution of Lipoproteins

The pore size of the main gel could be altered greatly by changes in the concentrations of the polyacrylamide. A significant portion of the lipoproteins penetrated very little into the 7.5 and 5% gel, but they migrated appreciably in the 3.75% gel (Fig. 1). The number and concentration of the various components seemed to depend upon the gel concentration. The leading component was albumin, whose mobility was not greatly increased by increasing the pore size of the gel. It appeared as a brown band and did not bind Sudan black B. The number of Sudan black B-bound protein components observed in each of the three main gels was: 7.5% gel 3-4; 5% gel 6-8; 3.75% gel 4-6; and it depended on whether the hazy blue areas between the bands could be considered as separate components.

Influence of the Quantity of Plasma on the Electrophoretic Patterns

The quantity of plasma used in these experiments was at least three times that recommended by Ornstein and Davis (10). From a theoretical standpoint, based on Ornstein's equations (2), there appears to be no reason why a greater quantity of plasma cannot be used in disc electrophoresis provided that a long spacer is employed and that a high background is of no consequence or can be tolerated; but it was necessary to determine the influence of the quantity of plasma on the electrophoretic patterns. The quantity of plasma used ranged from 3 to 83 μl , corresponding to 10–250 μl of the plasma-dye-solution B-sucrose mixture. The best separation resulted with 10 μl of plasma when a gel concentration of 3.75% was used. However, with larger amounts of plasma, additional components were observed: one just inside the main gel, 1–3 near the top of the spacer gel, and 1–2 near the bottom of it. The top spacer bands became more evident when the gels were removed from the tubes and placed in acetic acid solution. Other blue zones appeared to be present in the main gel when an amount of plasma greater than 50 μl was used; however, their identification was difficult because of the intense background in these gels. The pattern observed with 3 μl of plasma was essentially the same as that observed with 10 μl of plasma except that the leading lipoprotein component was very faint in the former case.

The above experiments clearly emphasized that the lipoprotein components were well resolved when large amounts of plasma were used. However, staining the gels with protein stain (Buffalo black) revealed that the other protein constituents did not separate into distinct lines when more than 10 μl of plasma was used.

Reproducibility of Patterns with Fresh and Frozen-Thawed Sample

We investigated the reproducibility of the method using 10 μl of plasma and a main gel concentration of 3.75%. Twelve tubes were run simultaneously with 30- μl portions from the same prestained plasma-solution B-sucrose mixture. Only one of the tubes from this run is shown in Fig. 2A but the other eleven tubes were very similar to this and indicated good reproducibility of the patterns. Experiments have also been conducted with several other prestained samples of rat plasma or serum, and the electrophoretic patterns were similar to those indicated in Fig. 2. In order to investigate the effect on the electrophoretic patterns of freezing the plasma, a portion of the sample of plasma used in run E 143 (Fig. 2A) was frozen and maintained at -18° for about 5 months. The electrophoretic pattern of this sample, shown in Fig. 2B, indicates that prolonged freezing did

not significantly affect the resolution or the dye-binding capacity of the lipoproteins of rat plasma.

Identification of the Dye-Binding Components of Plasma

In a main gel concentration of 3.75%, one slow component near the top of the main gel and 3–5 faster components were observed with rat plasma. In previously reported experiments using 5% gel and human serum, we have not observed more than two or possibly three fast lipoprotein components (1). In 3.75%

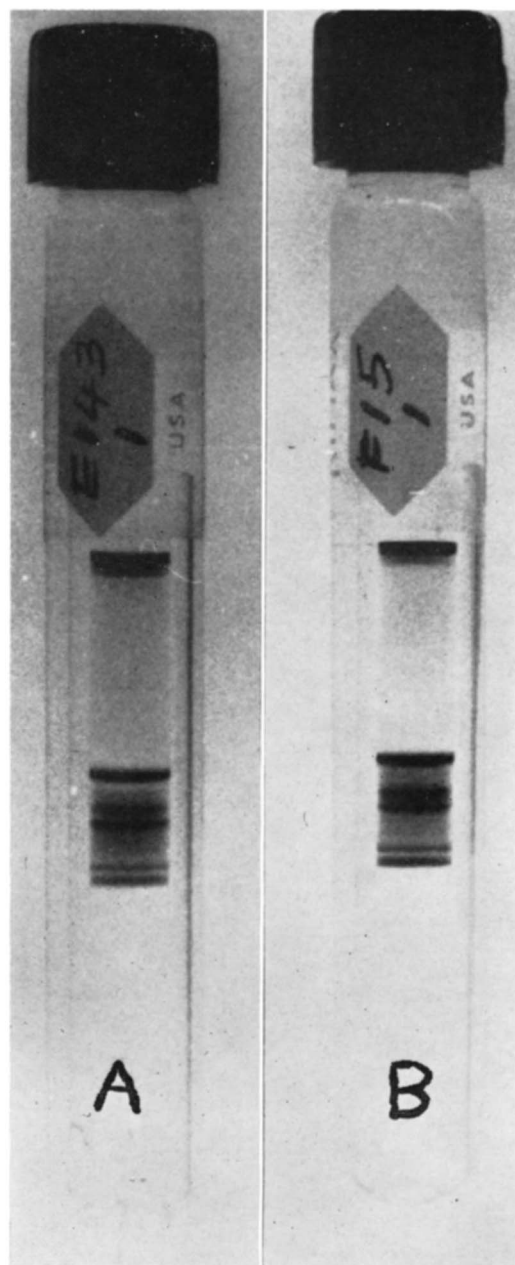


Fig. 2. Reproducibility of patterns after freezing and thawing. 10 μl of rat plasma using 3.75% main gel concentration at 2.5 ma per tube for 40 min. A, fresh sample of plasma; B, frozen and thawed portion of the same plasma.

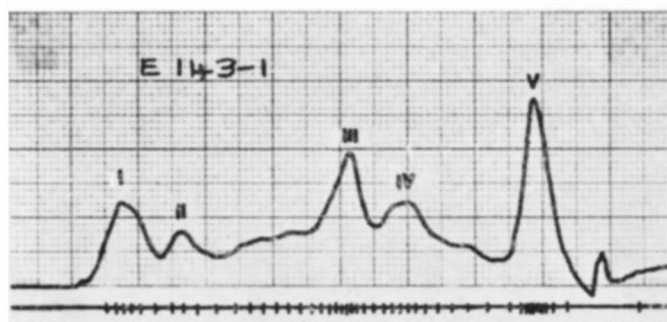


FIG. 3. Typical densitometer trace of a run on 3.75% gel. The pattern is shown in Fig. 2A. Chart speed 1; gain 5; integrator 20 mm² for each pip. The peak to the far left (peak I) is albumin and the other peaks represent the lipoprotein components of rat plasma.

gel, human serum fast components were unresolved and were very close to the albumin component, so that there was always a large clear zone between the slow component and the fast components.

The Oil red O stain and TLC were used to establish the presence of lipids in the Sudan black-binding components. When an Oil red O stain was employed on gels which had been used for the separation of unstained rat plasma or human serum proteins, the patterns were very similar to those observed with the corresponding blood fractions prestained with Sudan black B.

A tentative identification of the lipids of the lipoprotein fractions of rat plasma separated by disc electrophoresis is given in Table 1. The solvent system used did not satisfactorily resolve the triglycerides of linseed or corn oil from stearic acid. TLC of the lipid extract of rat plasma used in this investigation indicated the presence of phospholipids, cholesterol, triglycerides, and cholesterol esters. TLC of the extracts of electrophoretic fractions indicated the presence of cholesterol esters in fractions 3–8 and of free cholesterol in 3–6 and possibly in 7 and 8. Triglycerides appeared to be abundant

in fraction 8 but were also present to a lesser extent in the fractions 4, 5, 6, and 7. The identification of phospholipids was difficult because most of the dye remained at the origin, together with the phospholipids, as a nonmigrating component. Other unidentified components were observed in small amounts in all the fractions. The TLC of fractions 1 and 2 also indicated the possible presence of cholesterol esters, free cholesterol, and phospholipids; although compounds with similar R_F were detected in trace amounts in the extracting solvent system used, the intensities of the charred zones obtained by applying the concentrated impurities from 30 ml of the solvent mixture were lower than those observed in the TLC of fractions 1 and 2.

Densitometry and Quantification

The dye in the fast lipoprotein components tended to fade rapidly when the gels were removed from the tubes and placed in acetic acid solution. A further difficulty was that the 3.75% gels were rather soft and much more difficult to handle than the standard 7.5% gels. In an attempt to solve these problems, we conducted densitometry on the tubes containing the gels, rather than on the gels removed from the tubes. When the tubes were used, the plastic insert inside the specimen holder had to be removed. Preliminary experiments had indicated² that, while there were small variations, the traces obtained by both the procedures were essentially the same.²

The relative percentages of the major peaks representing the lipoprotein components are shown in Table 2. A typical densitometric trace is shown in Fig. 3. The slowest component (Peak V) contained approximately

TABLE 1 TENTATIVE IDENTIFICATION (BY TLC) OF LIPID COMPONENTS OF DISC ELECTROPHORETIC FRACTIONS OF RAT PLASMA

Fraction No.	Phospholipids*	Cholesterol	Triglycerides	Cholesterol Esters
1	+	+	+	+
2	+	+	+	+
3	+++	++	+	+++
4	++++	++	++	++++
5	++	++	++	+++
6	++	++	++	+++
7	+	+	+++	+++
8	+++	+	++++	++
Total rat serum lipids	+	+++	++++	++++

Intensities of the spots as assessed visually are indicated by plus signs.

* Includes the nonmigrating component of Sudan black B, except in the case of total rat serum lipids (bottom line).

² The differences in the relative percentages of the various peaks obtained by both the methods were generally within 10%. However, a 20% increase in the apparent absorbance of the bands was noticed when the tubes containing the gels rather than the gels alone were used. We would explain this as due to a tighter "fitting" of the specimen in the sample compartment, thereby minimizing the amount of unabsorbed light passing along the sides of the gel.

TABLE 2 REPRODUCIBILITY AND RELATIVE SIZE OF LIPOPROTEIN PEAK AREAS

Run No.	Number of Determinations	Peak II		Peak III		Peak IV		Peak V		Total Area ($\times 5$)
		Area ($\times 5$)	% Total Area	Area ($\times 5$)	% Total Area	Area ($\times 5$)	% Total Area	Area ($\times 5$)	% Total Area	
1 (Fig. 2A)	12	3.9 ± 0.5	12.3 ± 1.1	9.8 ± 0.5	30.9 ± 0.9	7.2 ± 0.5	22.5 ± 1.0	10.9 ± 0.5	34.3 ± 1.3	31.9 ± 1.5
2 (Frozen and thawed, Fig. 2B)	6	3.8 ± 0.5	12.1 ± 1.4	8.1 ± 0.8	25.9 ± 1.7	7.9 ± 0.5	25.2 ± 1.7	11.5 ± 0.7	36.8 ± 1.5	31.5 ± 1.6

Electrophoretic conditions in legend to Fig. 2. Values given are means \pm SD. Peak I, which is due to albumin, has been excluded.

one-third of the total quantity of the dye bound by the rat plasma lipoproteins. Satisfactory reproducibility (within 4.4%) was obtained for the three major lipoprotein components (III, IV, and V), but the variation in amount of the fast lipoprotein component was about 9%. This could be due to its unsatisfactory separation from the albumin component. In other experiments conducted with four different prestained samples obtained from a single sample of rat serum, the variation in the relative amounts of dye carried by the lipoprotein components was 4–12%. Table 2 also shows similar data for a frozen-thawed sample of plasma (Fig. 2B). Since the differences observed in the two runs were small, it could be concluded that freezing of rat plasma under the present conditions did not produce significant alterations in the lipoprotein structure, at least as determined by solubilization and electrophoretic char-

acteristics. The reproducibility of the individual peak areas was such that it would presumably be possible to determine the concentration of lipoprotein components in mixtures, provided that rigidly controlled conditions were used and that suitable standards of known dye-binding capacity were run simultaneously. Curves obtained at the three different gel concentrations are exemplified in Fig. 4. Some of the traces indicated the presence of components that could not be clearly distinguished visually in the gels themselves. For example, in the trace E 130-3, the major (penultimate, reading from left to right) lipoprotein band was apparently resolved into three components whereas this resolution was not apparent in the gel (Fig. 1). The trace E 130-5 was similar to that shown in Fig. 3 and those analyzed in Table 2, but there was poor resolution between fastest-moving peaks (first two on the left).

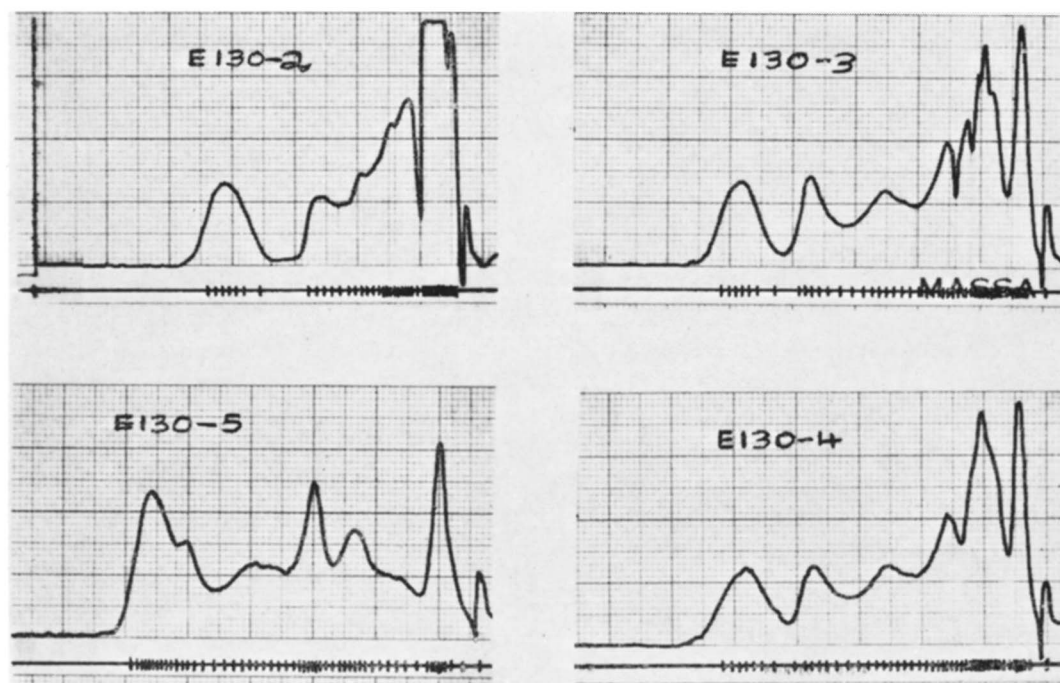


FIG. 4. Densitometer traces of runs at different gel concentrations (see Fig. 1). E 130-2, 7.5% polyacrylamide; E 130-3 and 130-4, 5%; E 130-5, 3.75%. Chart speed 1; gain 5; integrator 20 mm² for each pip.

DISCUSSION

Comparison with Other Methods of Lipoprotein Fractionation

The previously reported results of zone electrophoresis of rat serum on starch gel and paper have indicated the presence of two or three lipoprotein components (14, 15). Under the present conditions, at least four distinct Sudan black B-binding protein components could be clearly seen in the 3.75% gel. The possible presence of another two or three components in the main gel and 2–5 components in the spacer gel may also be inferred from the experiments with heavy loading of the gel. Hillyard, Entenman, Feinberg, and Chaikoff (16) showed that rat serum lipoproteins could be fractionated by ultracentrifugal methods into at least three fractions, but the presence of 4–12 lipoprotein components in rat serum has not been previously indicated. Preparative ultracentrifugal methods of separation are based essentially upon differences in density of the lipoprotein components. It is thus conceivable to have in the same density fraction several components of approximately the same density but of different size (17). We have previously reported the resolution of human serum HDL₃ ($1.125 < d < 1.21$) into three components by disc electrophoresis, whereas it was unresolved either by analytical ultracentrifugation (1) or by starch gel electrophoresis (5).

In disc electrophoresis, the separations could be attributed to the compression of the protein components in the spacer gel into extremely thin discs or starting zones of thickness of the order of 10 μ before entry into the main gel and to differences in charge as well as the size of the ionic species (2). Furthermore, the sensitivity of detection of components in the disc electrophoretic method is many times greater than that of other methods. Therefore, it was not surprising that several lipoprotein components were detected by this technique. Sudan black B does not bind the nonlipoprotein components of human serum, which are more numerous than those of rat serum (4); ovalbumin and bovine serum albumin were also shown not to bind Sudan black B under the present experimental conditions. Hence, each of the blue bands and blue areas observed in disc electrophoresis of rat plasma probably represents a lipoprotein. Partial confirmation was obtained by TLC, which indicated the presence of one or more of the major lipid classes in each of the six lipoprotein fractions investigated.

Relationship of Disc Fractions to Ultracentrifuge Density Classes

Preliminary experiments to relate the disc electrophoretic bands of rat plasma with the usual ultracentri-

fuge density classes indicate that the slow components (peaks III, IV, and V) form part of the low-density lipoprotein (LDL) fraction ($d < 1.063$) and that the fast components (peak II and the small peaks between peak II and peak III) form part of the high-density lipoprotein (HDL) fraction ($1.063 < d < 1.21$). Full particulars regarding these experiments and results will be presented in a subsequent paper. On the basis of the glyceride content of the fractions as judged by TLC, we suggest that the slowest component (peak V) represents the very low-density lipoprotein (VLDL) component. It should be emphasized that this component (peak V) has a mobility slightly lower, and the other components (peaks III and IV) have mobilities considerably higher, than the human serum LDL. Thus these results suggest that the rat plasma LDL contains components that are considerably smaller in size than the human serum LDL. Other unpublished experiments have indicated that the human VLDL ($1.006 < d < 1.019$) migrates slower than the human LDL. An accurate determination of the mobilities of rat and human VLDL in 3.75% gel is under investigation.

Quantitative Determination

Although reproducible densitometric traces have been obtained, there are several problems to be solved before one can obtain absolute or even accurate relative amounts of the various lipoprotein components. Some of the uncertainties are caused by: *a*) the background stain and lack of knowledge of what it really represents; *b*) the overlapping components and those with skewed boundaries; *c*) the possibility of interactions between proteins and lipoproteins and between lipoproteins and the dye solution, with the production of artifacts; *d*) the different dye-binding capacities of each of the various protein components, and their variation with alterations in structure or chemical composition of the lipoproteins or with changes in the conditions used. With respect to the last problem, satisfactory correlation has not been obtained between lipoprotein concentration and stain intensity, because the dye uptake is apparently dependent upon the lipid composition of the lipoproteins (18, 19).

Modification of the Original Method

In the original method proposed by Ornstein and Davis (10) or in their recently revised method (3), it was not possible to increase the quantity of serum applied beyond 4 μ l because this increase inhibited the polymerization of the sample gel. Furthermore when handling labile constituents such as lipoproteins, it may be best to avoid polymerization of the acrylamide solution in the presence of the labile constituent. It is for this reason that we eliminated the sample gel layer and resorted to the use of

sample in a dense medium such as 10% sucrose (4). The reproducibility obtained in the present experiments also proved that the technique for inserting the tube into the upper bath without significant loss of the sample was satisfactory.

Although the patterns obtained by staining with Buffalo black after photography and densitometry indicated considerable overlapping, 3.75% gel is not necessarily unsuitable for obtaining total protein patterns. The normal rat plasma protein pattern obtained according to our modified method (4) with 2–3 μ l of sample and a gel concentration of 3.75% was sharp; five major components and six minor components were observed (unpublished results). When sample quantities greater than 10 μ l of plasma were used, the number of Sudan black B-binding protein components was observed to increase. This could be explained as owing either to the presence of these components in such minor amounts that they could be detected only at large sample quantities, or to the production of artifacts. The artifacts may be the result of incomplete compression of the various proteins in the starting zone or of the degradation of some of the more labile lipoprotein constituents. However, the lipoprotein pattern observed with 10 μ l of plasma was essentially the same as that observed with the standard quantity of 3 μ l of plasma, so that at least this quantity (10 μ l) could be successfully used under the present experimental conditions. Since the pattern of the gel stained with Oil red O after electrophoresis was very similar to that of rat plasma prestained with Sudan black B, it may be concluded that neither Sudan black B nor ethylene glycol produces artifacts at the concentrations recommended.

Identity of the Top Spacer Bands

It was interesting to note the presence of 1–3 very closely spaced bands near the top of the spacer gel, which may be due to large-sized lipoproteins, possibly the very low density lipoproteins or chylomicrons. It is also possible that one or more of these bands may be due to large molecular weight proteins such as the 19S gamma globulins. Since these components were observed even with 10 μ l of plasma, the possibility that they were artifacts could

be overruled. TLC of this fraction (fraction 8) indicated the presence of triglycerides in large amounts. Experiments are under way to determine the disc electrophoretic patterns of isolated, very low density lipoproteins and chylomicrons.

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REFERENCES

1. Narayan, K. A., S. Narayan, and F. A. Kummerow. *Nature* **205**: 246, 1965.
2. Ornstein, L. *Ann. N.Y. Acad. Sci.* **121**: 321, 1964.
3. Davis, B. J. *Ann. N.Y. Acad. Sci.* **121**: 404, 1964.
4. Narayan, K. A., S. Narayan, and F. A. Kummerow. *J. Chromatog.* **16**: 187, 1964.
5. Scanu, A., and W. L. Hughes. *J. Clin. Invest.* **41**: 1681, 1962.
6. Smithies, O. *Advan. Protein Chem.* **14**: 65, 1959.
7. Raymond, S., and M. Nakamichi. *Anal. Biochem.* **7**: 225, 1964.
8. Ressler, N., R. Springgate, and J. Kaufman. *J. Chromatog.* **6**: 409, 1961.
9. McDonald, H. J., and L. P. Ribeiro. *Clin. Chim. Acta* **4**: 458, 1959.
10. Ornstein, L., and B. J. Davis. In *Disc Electrophoresis*, preprinted by Distillation Products Industries, Rochester, New York, 1962.
11. Ferris, T. G., R. E. Easterling, and R. E. Budd. *Anal. Chem.* **36**: 955, 1964.
12. Beaton, G. H., A. E. Selby, and A. M. Wright. *J. Biol. Chem.* **236**: 2001, 1961.
13. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38**: 708, 1961.
14. Neuhaus, O. W., R. Havez, and G. Biserte. *Arch. Biochem. Biophys.* **106**: 159, 1964.
15. Erwin, E. S. *Proc. Soc. Exptl. Biol. Med.* **103**: 396, 1960.
16. Hillyard, L. A., C. Entenman, H. Feinberg, and I. L. Chaikoff. *J. Biol. Chem.* **214**: 79, 1955.
17. Gurd, F. R. N. In *Lipide Chemistry*, edited by D. J. Hanahan. Wiley and Sons, Inc., New York, 1960, p. 268.
18. Cornwell, D. G., and F. A. Kruger. *Proc. Soc. Exptl. Biol. Med.* **107**: 296, 1961.
19. Schjeide, O. A., A. U. Rivin, and J. Yoshino. *Am. J. Clin. Pathol.* **39**: 329, 1963.