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Broadening perspectives of electrophoresis—as seen from twenty-five years of use at the Cleveland Clinic

LENA A. LEWIS, PH.D.

Division of Research

ELECTROPHORESIS—the migration of particles under the influence of an electric field—has been applied to the study of the proteins of serum and other body fluids, and over the years has become increasingly important to the physician. Electrophoretic patterns of serum proteins have diagnostic significance, and can provide additional information in some cases concerning the progress of the disease. This report briefly traces the history of electrophoresis and the research—clinical development of electrophoresis during a quarter of a century of use at the Cleveland Clinic.

The development of moving-boundary electrophoretic methods from the early mobility studies of Picton and Linder[1] in 1892, was gradual until 1937 when Tiselius[2] perfected an apparatus that permitted quantitative estimation of the mobilities of various components in a mixed protein solution such as plasma. In 1941 no electrophoretic apparatus for the study of plasma proteins was available commercially, so an apparatus similar to that of Longsworth and MacInnes[3] was constructed (*Fig. 1*) in the instrument shop at the Cleveland Clinic under the supervision of Roy McCullagh, Ph.D., head of the biochemistry department of the Division of Research. Because many materials were unavailable during World War II, progress was slow, but satisfactory analyses of some plasma samples were obtained early in 1942. Free moving-boundary electrophoresis using phosphate buffer, pH 7.8, as was performed in the early studies, resolved plasma proteins into five fractions: albumin, α_2-globulin, β-globulin, ϕ-globulin and γ-globulin.

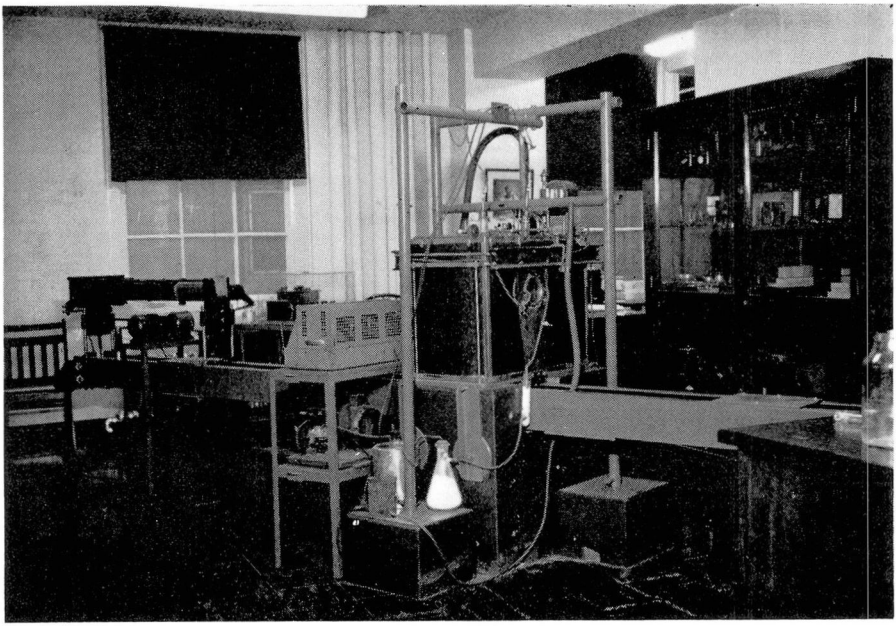


Fig. 1. Free moving-boundary electrophoretic apparatus constructed at Cleveland Clinic, Division of Research, in 1941.

In collaboration with colleagues E. Perry McCullagh, M.D., and Robert W. Schneider, M.D., studies were made of changes in the plasma proteins of patients with diabetes.[4] In patients with severe uncontrolled diabetes the total plasma protein was within normal limits, but the albumin was greatly decreased and the β-globulin was increased (*Fig. 2*). It was suggested that the high β-globulin content was probably due to increased content of blood lipids. After treatment for several months and good control of the diabetes, the plasma protein patterns returned to normal, except in those patients in whom there were complications such as diabetic retinitis; their protein electrophoretic patterns failed to attain normal levels.

Changes in the proteins of plasma, urine, and body fluids of patients with lupus erythematosus were studied to determine whether there were or were not changes that might account for the property in the plasma which caused the development of the lupus erythematosus cell (L.E. cell). Study of various protein fractions isolated after electrophoresis showed that the L.E. cell factor migrated with the γ-globulin.[5]

While the concentrations of the plasma proteins change in many clinical entities (*Table 1*), among the most interesting alterations observed are

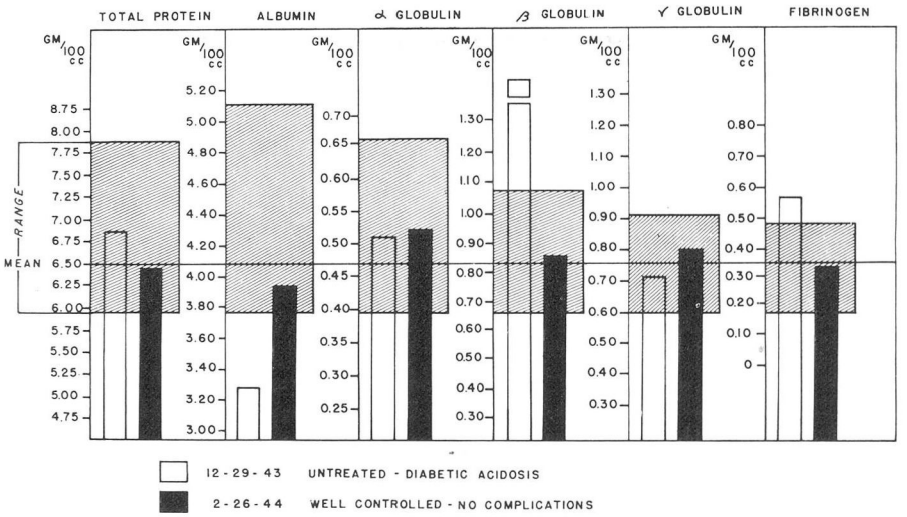


Fig. 2. Plasma protein changes observed in uncontrolled and controlled diabetes. (Courtesy of Lewis, L. A.; Schneider, R. W., and McCullagh, E. P.: Tiselius electrophoresis studies of plasma proteins in diabetes mellitus. J. Clin. Endocrinol. **4:** 535–539, 1944; and of *The Journal of Clinical Endocrinology and Metabolism.*)

those that occur in multiple myelomatosis. The serum electrophoretic protein pattern may show a sharp spike due to increase in a specific protein, with mobility of γ_2-, γ_1-, β-, or α_2-globulin. In some cases the abnormal protein was found to have a rapid sedimentation rate, characteristic of macroglobulins.[6]

The usefulness of the free moving-boundary technic was limited by the small number of studies that could be made in a specific time, and by the relatively large amount of material required for analysis. Introduction of the paper electrophoretic technic and use of other types of supporting media have revolutionized the situation. In studies using supporting media, most of the same basic principles apply as in moving-boundary procedures.[7] Demonstration, by specific stains, of resolved components after electrophoresis, permits estimation of different parts of complex proteins. Thus, after electrophoresis the strip may be stained with bromphenol blue for protein, oil-red-O for lipid, or Schiff reagent for polysaccharide.[8]

The increased information that can be obtained by improved electrophoretic technics is perhaps best demonstrated by a study of the results obtained on a severely hyperlipemic patient whose progress has been followed at the Clinic for more than 15 years.[9] Electrophoretic analysis of the serum by free-moving boundary technic showed an extremely high concentration of β- and α-globulins; however, the resolution of α- from β-globulin

Table 1.—*Summary of changes in various diseases in blood serum protein and lipoprotein patterns determined by paper electrophoresis**

I. Diseases in which changes are specific

A. Protein changes

		Globulin				
	Albumin	α_1	α_2	β	γ_1	γ_2
1. Multiple myeloma	0 to 2−	0*	0	0	0	1+ to 4+†
	0 to 2−		0	0	1+ to 4+	0
	0 to 2−		0	1+ to 4+	0	0
	0 to 2−		1+ to 4+	0	0	0
	(† Typical narrow sharp protein fraction.) Clinical picture apparently independent of type of protein abnormality.					
2. Waldenstrom's disease	0 to 2−	0	0	0	0	1+ to 4+
	0 to 2−	0	0	0	1+ to 4+	0 to 2−
3. Aggammaglobulinemia	Nonspecific changes			0	0	1− to 4−‡
				−1 to −2	0	1− to 4−
	(‡ Further evaluation by immunoprecipitin technics using anti Ig − G, anti Ig − A and anti Ig − M antisera necessary.)					
4. Nephrotic syndrome	2− to 4−	1+	4+	4+	0	2− to 4−

B. Lipoprotein changes genetically determined

	Lipoprotein			
	Chylomicron	β	Pre β	α
1. Hyperlipoproteinemia				
I§	↑	0 or ↓	0	0 Decreased post-heparin lipoprotein lipase activity; familial fat-induced type of hyperlipemia.
II		↑	0	↑, 0 or ↓ Familial "hypercholesteremic" type of hyperlipemia.
III	0	Broad β extending into pre β band		0 or ↓ Familial hypercholesteremia with hyperlipemia. Wide fluctuations in concentrations of cholesterol and triglyceride. Greater than normal cholesterol in d < 1.0006 fraction.
IV	0	0 or ↓	↑	0 or ↓ "Carbohydrate" induced type; frequently associated with abnormal glucose tolerance.
V	↑	0 or ↓	↑	0 Possibly may be combination of types I and IV; combination of fat- and carbohydrate-induced hyperlipemia.

(§ Modified from Fredrickson & Lees.[13])

2. Hypolipoproteinemia				
a. Hypo β-lipoproteinemia				
Total a-β-lipoprotein	None	None	None	Normal
	Results by paper electrophoresis must be confirmed by immunochemical analysis using anti β-lipoprotein and anti α-lipoprotein antisera.			
Hypo-β-lipoprotein	0	↓	↓	Normal
b. Hypo-α-lipoproteinemia				
Tangier disease		↓Δ		Almost complete absence
	Abnormal lipid composition of β-lipoprotein, but reacts antigenically with anti β-lipo antiserum. Requires immunochemical analysis for α- and β-lipoproteins to establish diagnosis.			

* 0 indicates no consistent change from normal; 0 to 4 − = decreases; 0 to 4 + = increases from normal level.

Δ Some decrease in d 1.019 to 1.063 fraction.

Table 1.—*Concluded*

II. Diseases in which changes are nonspecific but significant

A. Protein changes

		Globulin				
	Albumin	α_1	α_2	β	γ_1	γ_2
Liver disease						
Cirrhosis	2−	0	1− to 1+	2+	—	4+
Acute toxic hepatitis	2− to 4−	1+	1+ to 2+	2+	—	2+ to 4+
Infectious hepatitis	1− to 2−	1+	1+	1+	—	2+ to 3+
Myocardial infarction	1− to 2−	1+	1+ to 3+	1+ to 2+	—	0 to 2−
Hypertension						
Malignant	1− to 3−	1+	0 to 2+	1+ to 3+	—	1− to 1+
Essential	0 to 1−	0	0 to 1+	0 to 1+	—	0
	(No changes in mild essential hypertension)					
Infectious disease						
Pneumonia	0 to 1−	+	1+ to 3+	0	—	0
Tuberculosis	0 to 2−	1+ to 2+	0 to 3+	0 to 2−	—	1+ to 3+
	(Change depends on stage of disease)					
Syphilis	0− to 2−	1+	1+	0	—	1+ to 3+
	(Change depends on stage of disease)					
Leprosy	0 to1−	—	2+ to 4+	0 to 1+	—	2+ to 4+
Endocrine disease						
Diabetes (without vascular complications)						
Acidosis	0 to 1−	0	0 to 2+	2+ to 3+	0	0 to 1+
Well controlled	0	0	0	0	0	0
Diabetes (with vascular complications)						
Well controlled	0 to 2	0 to 1+	1+ to 3+	2+ to 3+	0	2− to 2+
Cushing's syndrome	0 to 2−	0 to 1+	2+ to 3+	1+ to 2+	0	2− to 4−
Collagen diseases					(L.E. factor in γ-globulin fraction.)	
Lupus erythematosus	1− to 3−	0 to 1+	1+ to 3+	0	0 to 1+	1− to 4+
Rheumatoid arthritis	0 to 1−	1+ to 3+	1+ to 3+	0	0 to 1+	1+ to 4+
Malnutrition						
Anorexia nervosa or famine	0	0	0	0	0	0
Vitamin deficiency	1− to 2−	0	0 to 1−	0 to 1−	0	0
Aging	1− to 2−	0	0	1+ to 2+	0	0

B. Lipoprotein changes not genetically determined, associated with disease

		Lipoprotein		
	Chylomicron	β	Pre β	α
Renal disease				
Nephrosis	↑	↑	↑	0 or ↓
Renal failure or arenal	0	0	0	↓ to trace amounts
Sprue and malabsorption syndrome	0	↓	0	0 or ↓
Thyroid disease				
Hyperthyroid	0	↓	0	0
Hypothyroid	0 or ↑	↑	0 or ↑	0 or ↑
Liver diseases				
Cirrhosis	0	↑	↑	↓, 0, or ↑ (Changes dependent on stage of disease)
Infectious hepatitis	0	↑ or 0	↓	0 or ↓ Abnormal migration rate
Liver failure	↓	↓	↓	↓
Infectious diseases	0	↓	0	0 or ↓
Diabetes (acidosis)	↑	↑	↑	0

* 0 indicates no consistent change from normal; 0 to 4− = decreases; 0 to 4+ = increases from normal level.

Δ Some decrease in d 1.019 to 1.063 fraction.

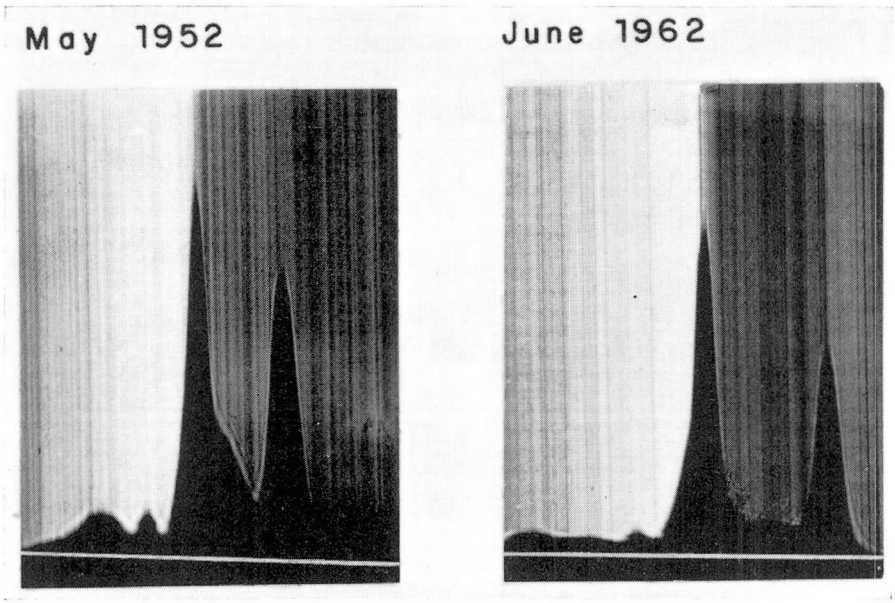


Fig. 3. Plasma protein pattern of patient with severe hyperlipemia, studies of 1952 and 1962. (Courtesy of Lewis, L. A., and Page, I. H.: An unusual serum lipoprotein-globulin complex in a patient with hyperlipemia. Am. J. Med. **38:** 286–297, 1965; and of *The American Journal of Medicine.*)

was poor (*Fig. 3*). Greatly elevated concentrations of low density (−S 20–400) lipoproteins were demonstrated ultracentrifugally; the isolated liproprotein concentrate had an unusual syrupy consistency when removed from the preparative ultracentrifuge tube. When the serum or lipoprotein concentrate was studied by paper electrophoresis and stained for lipid, a broad, intensely stained lipid band with mobility between that of α- and of β-globulin and a faintly stained α-lipoprotein band were demonstrated. These results offered no explanation for the unusual nature of the lipoprotein.

Smithies[10] found that additional resolution of proteins was accomplished when electrophoresis was carried out using starch-gel as a supporting medium. With this medium at least 21 protein fractions could be demonstrated in human serum. Separation is dependent not only on the electric charge but also on the size of the protein molecules. Grabar and Williams[11] about the same time combined immuno-precipitin technics and electrophoresis and thus provided a means for more accurate identification of proteins, in mixtures such as serum, than those previously used. Application of these two specific electrophoretic technics demonstrated that the lipoprotein concentrate of the hyperlipemic patient's serum contained not only proteins

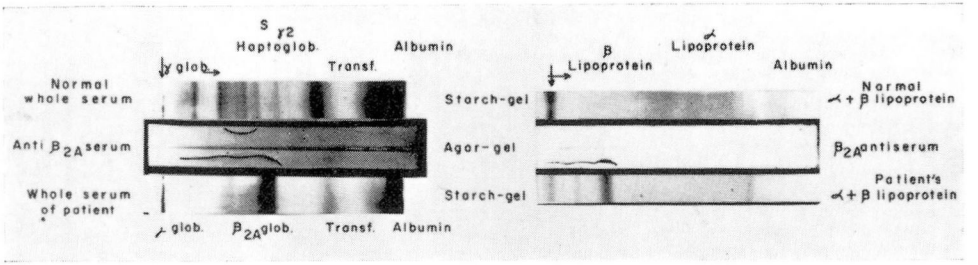


Fig. 4. Starch-gel immunoelectrophoretic pattern of hyperlipemic patient's serum [antisera against γ-A(β_2A) globulin was used; tris-borate double buffer system was used]. (Courtesy of Lewis, L. A., and Page, I. H.: An unusual serum lipoprotein-globulin complex in a patient with hyperlipemia. Am. J. Med. **38:** 286–297, 1965; and of *The American Journal of Medicine.*)

with electrophoretic and immunologic properties of β- and α-lipoproteins, but an additional component that migrated faster than β-lipoprotein on starch-gel. It was found by immunoelectrophoresis and ultracentrifugation to be a γ-A (i.e., β_2-A)-globulin with a sedimentation constant of 12S (*Fig. 4*). Thus, the unusual characteristic of the lipoprotein was explained by a complexing of β-lipoprotein with γ-A globulin. The complexing of γ-A-globulin with the β-lipoproteins in the patient's serum may explain the lack of any evidence of atherosclerosis in the patient, despite the extremely high serum lipid levels that have been present for more than 15 years; the molecular aggregate may be so large that it is unable to traverse the blood vessel wall.

A simplified technic for thin-layer starch-gel electrophoresis,[12] which requires no special scanning equipment in addition to that used for paper electrophoretic strips, makes this method an important aid in identification and quantitation of proteins of serum and other body fluids and of abnormal hemoglobins such as sickle cell hemoglobin, type C, A_2, and other more unusual hemoglobulins. The method has also been used in the study of saline-soluble extracts of tissues. Other types of stains, including those for various enzymes, lipids, and haptoglobins increase the usefulness of the method. The starch-gel electrophoretic technic has been widely used in genetic studies,[10] since the types of haptoglobin and of transferrin are genetically controlled and these fractions are well resolved by this method.

For studies of some materials, small modifications in electrophoretic technics may improve resolution and greatly increase the information derived. Thus, the use of albuminated buffer[13] in the fractionation of serum lipoproteins reduces the "trailing" effects that frequently occur when usual buffer systems are used. The method is especially helpful when high concentrations of low-density lipoproteins are present. Paper electrophoresis has also been useful in screening of family members in a study of hypo β-lipoproteinemia.[14]

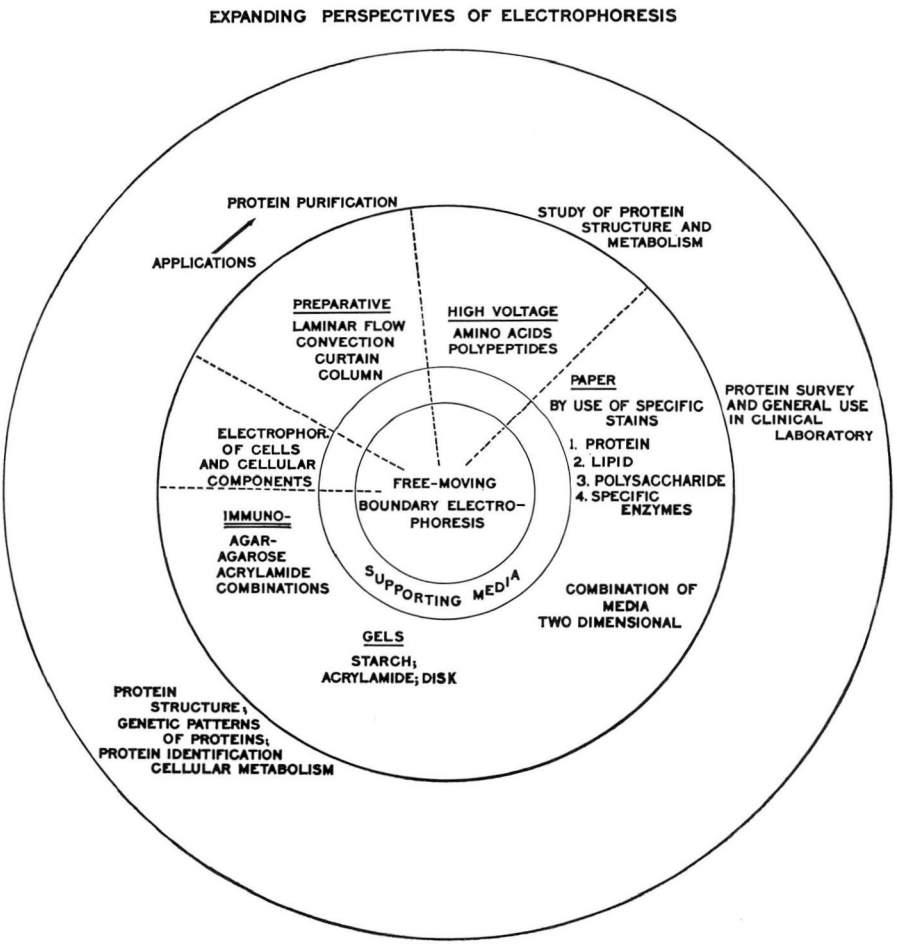


Fig. 5. Expanding perspectives of electrophoresis. From free moving-boundary electrophoresis as center or nucleus, the orbits (i.e., applications and technics) are constantly enlarging.

Improved electrophoretic methods have been developed for preparative purposes,[15] and the use of high-voltage electrophoresis[16] permits study of small molecular materials such as polypeptides and amino acids. A combination of chromatography and gel-electrophoresis has been effectively used in determining structure of immune globulins and hemoglobin. (Fig. 5.)

SUMMARY AND CONCLUSIONS

The broadening orbit of electrophoretic technics is depicted in *Figure 5*. From the basic studies that used free moving-boundary electrophoresis as a

nucleus, the field has expanded to include the use of many types of supporting mediums, high-voltage, immuno-, and preparative electrophoresis, and combinations, to help in identifying and in characterizing of proteins, polypeptides, and amino acids, and in understanding their physiologic roles.

Some of these methods are now applicable only in the research laboratory, but future developments and applications may mean that many more electrophoretic studies of many types will be essential and available for help in diagnosing and understanding clinical problems.

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