CRC Handbook of Electrophoresis

Volume I
Lipoproteins: Basic Principles and Concepts

Editors

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PREFACE

The concept of a handbook of electrophoresis, comparable to the *Handbook of Chromatography,* appeared to be a sound and plausible idea, when first suggested. Some modification of this idea, however, resulted after consideration of the vastness of the electrophoretic literature which has accumulated during the 40 years since the monumental work of Arne Tiselius. Thus, it was decided to give in-depth coverage to electrophoresis as applied to the study of lipoproteins in the human being. This aspect was selected because of the many different electrophoretic techniques which have been employed in elucidating physiological variations and biochemical and physical properties of lipoproteins and of apolipoprotein molecules. Knowledge in the lipoprotein field has developed as in electrophoresis, rapidly, and has in some aspects been significantly affected by the application of developments in electrophoretic techniques.

The *Handbook of Electrophoresis,* Volumes I and II, provides basic information concerning lipoprotein molecules, their electrophoretic properties, basic principles of electrophoresis, and the techniques employed in studying lipoproteins. Alterations in lipoprotein electrophoretic patterns in many diseases states are presented, and their significance, discussed. A detailed bibliography of the literature dealing with lipoproteins is included in the appendix, which provides invaluable information for all who want to pursue in-depth investigation of particular aspects of the lipoprotein literature.

EDITORS

Dr. Lena A. Lewis is Emeritus Consultant at The Cleveland Clinic Foundation in the Division of Laboratory Medicine and in the Division of Research. She is a Clinical Professor of Chemistry at Cleveland State University. Dr. Lewis has an AB from Lindenwood College (1931), an MA from Ohio State University (1938), and Ph.D. from Ohio State University. She served for 10 years on the editorial board of Clinical Chemistry, is author of Electrophoresis in Physiology (1950), 2nd edition, 1960, Charles C Thomas, Springfield, Illinois. Dr. Lewis received an honorary LLD degree in 1952 from Lindenwood College, was elected a fellow in the New York Academy of Science in 1977 and received the Boehringer-Mannheim Award from the American Association of Clinical Chemists for outstanding contributions to Clinical Chemistry in the field of lipids and lipoproteins.

She is a contributor to *Endocrinology, Clinical Endocrinology and Metabolism,* the *American Journal of Medicine, Clinical Chemistry,* and the *American Journal of Physiology, Science.*

Dr. Lewis was the President (1970-1971) of the Northeast Ohio chapter of the American Association for Clinical Chemists. She also is a member of the American Physiological Society, the Endocrine Society, The AAAS; Fellow Atherosclerosis Council of the American Heart Association, the New York Academy of Science (Fellow), and the Ieota Club (International Women's Service Club.

She is listed the *American Men of Science, Who's Who in America* (Midwest section), the *Who's Who in American Education,* the *American Women in Science, Personalities of the West & Midwest,* and the *Who's Who in the World of Women.*

Dr. Jan J. Opplt received his education at the Charles University in Prague, Czechoslovakia. He was awarded the following degrees: M.S. (1942), M.D. (1949), Ph.D. (1952), Scientific Degree in Medical Science (1966), and Scientific-Educational Degree-Docent (1969).

He specialized in Clinical Pathology (1956) and in Clinical Chemical Pathology (1968).

He served as Assistant Professor in the Department of Medical and Clinical Chemistry School of Medicine, Charles University (1948—1950). Thereafter, he was appointed as Chairman of the Department of Clinical Chemistry and later as Associate Professor (Docent) at the School of Medicine and Hygiene, Charles University (1951—1969).

From 1970 to the present he has been serving as Clinical Professor in the Department of Chemistry, Cleveland State University. Since 1971, he has been acting as Director of the Division of Clinical Chemistry, Cleveland Metropolitan General Hospital and as Associate Professor of Pathology, Case Western Reserve University.

He acted as a Member of the Czechoslovak Medical Society J.E. Purkyne's (1948—1969) and on its Board of Clinical Biochemistry (1962—1969). He was also a member of the European Diabetes Society (1962—1969). He is presently active in the following professional societies: Cleveland Academy of Science (member since 1969); American Heart Association (Fellow since 1970, Scientific Committee, Ohio Chapter, 1976—1978; American Association of Clinical Chemists (member since 1970, Chairman of the Cleveland Section, 1975) and National Academy of Clinical Biochemistry (Fellow since 1979).

His awards and honors include: Prize of J.E. Purkyne, ACPS (CPSP, 1952, Prague, CSR; Fellowship Award, WHO, 1959, (Professors Tiselius and Svedberg) Uppsala, Sweden; Fellowship Award, CCF, 1970, (Dr. L.A. Lewis), USA; Gold Award, American Society of Clinical Pathologists (ASCP and CAP), 1971, USA; Honorable Men-

tion Award, Ohio State Medical Association, 1973, USA; Certificate of Recognition, CSU, 1977, USA and Service Award, American Heart Association, 1978, USA.

His main professional interests are in the fields of Internal Medicine, Clinical Chemical Pathology, Biochemistry, and Physical Chemistry. He developed courses for medical students, residents and graduate students in differential diagnostic procedures and clinico-pathologic correlations as well as clinical chemical pathology, used in therapeutic and preventive medicine.

He has devoted 27 years, investigating the physiological and pathological metabolism of plasma proteins and lipoproteins. He developed specific techniques for separation and physical analyses, which opened his study of plasma lipoproteins and apolipoproteins on a molecular basis. His publications include 87 scientific papers, 22 abstracts and contributions in seven books.

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NOMENCLATURE OF LIPOPROTEINS

The nomenclature of lipoproteins has developed over the years as knowledge of their physical, chemical, and immunological properties has been elucidated. The plasma lipoproteins have been known for many years to have different electrophoretic properties. The major plasma lipoproteins of normal, healthy human beings have the electrophoretic mobility of α- and of β-globulins, respectively. In addition to the major components, additional fractions of characteristic mobility and lower concentration may be demonstrated in some plasma.

The various nomenclatures of lipoproteins are based chiefly upon the method of isolation or characterization of the fraction, e.g., (1) chemical procedures, involving precipitation, electrophoretic, and chromatographic procedures; (2) ultracentrifugation using density gradients, and (3) immunologic techniques. The following table includes *most* of the names which are currently (1977) being used to identify lipoproteins and which have been used by various authors of this volume. Elucidation of the complexity of the lipoprotein structure has resulted in a much more involved nomenclature than in the early days, and extensive discussions of preferred terminology have taken place.

Table 1
VARIOUS NOMENCLATURES OF LIPOPROTEINS

Method of study on which nomenclature based	Name of fraction
	α-Lipoproteins
Electrophoresis	α-lipoproteins, α-Lp, lipoproteins with electrophoretic mobility of α_1-globulins; they may be resolved as single or multiple bands depending on type of support media
Ultracentrifugation	HDL, high-density lipoprotein (d 1.063—1.21 g/ml); HDL_2, subclass of HDL (d 1.063—1.125 g/ml); HDL_3, subclass of HDL (d 1.125—1.21 g/ml); flotation rate at d 1.21 of −S 0—10.
Apolipoprotein composition — chemical, immunologic	Apo A, apolipoprotein A consisting of two nonidentical polypeptides, A-I and A-II: A-I contains glutamic acid as C-terminal and aspartic acid as N-terminal amino acid A-II contains glutamic acid as C-terminal and pyrrolidine carboxylic acid as N-terminal amino acid apo D, apolipoprotein D present in HDL_3; apo E_{1-3}, apolipoprotein E_{1-3}, present in HDL; apo C-II, apolipoprotein C-II present in HDL
	Pre-β-Lipoproteins
Electrophoresis	Pre-β-lipoproteins, pre-β-Lp, lipoproteins with electrophoretic mobility of α_2-globulins; they have this mobility when agarose, paper, or starch powder is used as support medium; they migrate slower than β-lipoprotein when gels with sieving effect, such as acrylamide or starch gel, are used; pre-β-Lp may be resolved as single or multiple bands.
Ultracentrifugation	VLDL, very low-density lipoproteins (d < 1.006 g/ml); isolated from serum after previous removal of chylomicron; flotation rate at d 1.21 of −S 70—400

Table 1 (continued)
VARIOUS NOMENCLATURES OF LIPOPROTEINS

Method of study on which nomenclature based	Name of fraction
Apolipoprotein composition — chemical, immunologic	Apolipoprotein of VLDL contains apo C-II, B, and E_{1-3} polypeptides; apo C is an apolipoprotein consisting of 3 nonidentical polypeptides: C-I is characterized by *N*-threonine and *C*-serine, C-II by *N*-threonine and *C*-glutamic acid, C-III by *N*-serine and *C*-alanine
	β-Lipoproteins
Electrophoresis	β-lipoproteins, β-Lp, lipoproteins with electrophoretic mobility of β-globulins; β-Lp may be resolved as single or double bands, depending on buffer and support medium used
Ultracentrifugation	LDL, low-density lipoproteins (d 1.006—1.063 g/ml); LDL_1, subclass of LDL (d 1.006—1.019 g/ml); LDL_2, subclass of LDL (d 1.019—1.063 g/ml); flotation rate at d 1.21 of −S 25—40; at d 1.063, S_f0—20 may be divided into −S 40—70, i.e., S_f 12—20, intermediate density fraction and −S 25—40, S_f0—12 LDL
Apolipoprotein composition — chemical, immunologic	Apo B, apolipoprotein B is major apoprotein of β-lipoprotein; β-Lp also contains C-II and E_{1-3}
	Other fractions
Electrophoresis on agar	Lp-X, lipoprotein X, a lipoprotein of β- or slow β-globulin mobility and low density, characteristically found in obstructive jaundice patients' sera; best identified by electrophoresis on agar, where it has unusual property of migrating to γ-globulin position
Immunologic and genetic studies	Lp(a) is a polymorphic form of β-Lp which is of importance in genetic studies, and in those patients who have received multiple transfusions

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VOLUME II

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History

HISTORY OF ELECTROPHORESIS

M. Reiner

It may be timely in this book of recent methods and applications in the field of electrophoresis of proteins to pause and contemplate the origins of a method which has made possible the mass production of serum protein analyses and important associated substances, such as lipids, immune bodies, and radioactive substances.

Reuss, a Russian physicist, performed the first electrophoretic experiment on record in 1809.[1] His apparatus consisted of two pieces of glass tubing driven into a block of wet clay and filled with water. A layer of sand was put on the bottom of the tubes which were then connected with the poles of a voltaic pile made of 74 silver-zinc couples. When the circuit was closed, the water in the tube connected with the positive pole became milky due to the electrophoretic migration of colloidal clay particles through the sand layer. At the negative pole, the water remained clear but increased in volume, which showed the phenomenon of electro-osmosis. From this early experiment, theories and techniques have been developed which have placed the unique tool of electrophoresis at the disposal of biochemists, allowing them to investigate complex mixtures of proteins.

Porett,[2] in 1816, communicated with T. Thomson, the editor of *Annals of Philosophy*, about a series of experiments dealing with water transport by galvanic currents and defined the role played by the surface in contact with the liquid. These experiments were of particular biological interest since he was the first to use an animal membrane in electrical transport experiments. Historically, they are of special interest, because he described a charged, protein-coated membrane, foreshadowing by almost a century the electric mobility experiments of protein particles by Hardy[3] and the protein-coated surfaces of Loeb.[4]

Hardy,[3] in 1899, reported that negatively charged particles of denatured egg albumin could be made positive by addition of acid; the concentration of acid required to produce zero mobility was called the isoelectric point. He also showed that these particles possessed amphoteric surfaces.

Some of the early quantitative studies were undertaken by Quincke[5] in 1861, who showed that the rate of migration of particles in a field is a linear function of the potential gradient. Helmholz[6] and Smoluchowski[7] worked on theories and solutions of the complicated equations, valid for all large particles with smooth surfaces and uniform distribution of charge.

The first workers to experiment with a V-shaped cell, the forerunner of the U-tube, were probably Picton and Linder (1892—97).[8,8a] They studied the movement of boundaries of As_2S_3, shellac, ferric hydrate, hemoglobin, and other colloidal sols in relation to the direction of the current and to the acidic or basic character of the colloid. By changing the direction of the current, they demonstrated the reversibility of the phenomenon and their amphoteric nature.

Ellis[9] and Powis[10] studied electrophoretic migration, the relationship between mobility and surface charge or surface potential, by relatively simple means. This property is of fundamental importance to the stability of many colloidal systems, especially those of a hydrophobic type.

Michaelis,[11] in 1909, named the migration of colloid ions in the electrical field "electrophoresis". He determined the isoelectric points of the enzymes, invertase and catalase, by their migration at various pH in a U-tube cell with stopcocks, even though the pure substances were not available at that time.

Abramson[13] alone and with Michaelis[12] performed numerous experiments to show the influence of size, shape, and orientation on the electric mobility of microscopic particles coated with protein film. They found that even though there was wide variation in ther characteristics, shape, size, etc., there was no difference in electrophoretic mobility. Studies have been made of white and red blood cells[13] and bacteria.[14] Electrophoretic scanning patterns of whole tobacco leaf cytoplasm have been made at various intervals after infection with tobacco mosaic virus.[15] Electrophoresis has proved a very sensitive technique for detection of differences brought about by biological variation or chemical treatment in the surfaces of viruses, bacteria, sperms, or cells. Differences in surface-charge density of mutants or chemically treated particles can be resolved by either microscopic or moving boundary methods.

Using a modified Michaelis cell, Bennhold[16,17] made a large series of experiments on blood sera and serum proteins. He showed that bilirubin was quantitatively linked to the albumin component; whereas, the cholesterol migrates with the globulin fraction.

The greatest impetus to electrophoresis took place in the 1920s and 1930s. Svedberg, working with Jette[18] and Scott,[19] took photographs of egg albumin solutions during electrophoresis, utilizing the green fluorescence emitted by the protein when irradiated with long wave ultraviolet light. The long exposure time required (10 min), the impossibility to immerse the apparatus in a water thermostat, and the risk of contamination by other fluorescent substances made the method seem impractical. Consequently, Svedberg and Tiselius[20] sought to improve the method of optical observation of the electrophoretic migration of egg albumin by direct measurement of the distances on the photographic plate. This procedure gave only approximate values due to the lack of definition of boundaries and the small distances involved.

This apparatus was modified by Tiselius,[21] and the basic ideas underlying the adaptation of the moving boundary method for the study of proteins was due largely to him. He was the first to show that serum is composed of albumin and α-, β-, and γ-globulins, for which, he received the Nobel prize in 1948. The moving boundary method employs a glass U-tube which contains a colloidal solution, such as protein, in the lower part. This protein solution is overlayed with buffer of the same electrolyte content, which is obtained by making up a solution with as nearly the same electrolyte content as the buffered protein and then dialyzing the protein solution against the buffer until equilibrium is reached. Sufficient electrolyte must be present to render the Donnan effect unimportant. The arms of the U-tube are connected to nonpolarizable electrodes, and the motion of the protein-buffer boundaries is observed under the influence of a known potential gradient.

Tiselius[21] divided the U-tube into several sections, which are fitted to each other by ground-glass joints that can slide in and out of place by a mechanical device. The movement of the protein fractions is watched, and when a certain fraction is located in a particular section of the U-tube, the section is moved out and the contents isolated. The most important improvement Tiselius instituted was the method of observing protein boundaries utilizing the so-called "schlieren" or shadow method. When light passes through a boundary separating two liquids of different indices of refraction, the light is refracted or bent in the direction of the liquid of greater refractive index. The buffer and the protein solutions have different indices of refraction, the difference being proportional to the protein concentration; the greater the protein concentration, the greater will be the bending of light rays that pass through the protein/buffer boundary. If an image of the electrophoresis cell is focused on a ground-glass plate and a knife edge is placed in the beam of light emerging from the electrophoretic cell in such a way as to intercept the deflected beam, the boundary of the protein/buffer will appear opaque and the position of the boundary can be accurately located on the