CRC Handbook of Electrophoresis

Volume II
Lipoproteins in Disease

Editors

Lena A. Lewis

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CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging in Publication Data

Lewis, Lena Armstrong, 1910-
CRC handbook of electrophoresis.

Bibliography: p.
Includes index.
1. Contents: v.1. Lipoproteins. — v.2. Lipoproteins in disease.
Electrophoresis. I. Opplt, Jan J., joint author.
II. Title.
QD117.E45L48 543'.087 78-10651
ISBN 0-8493-0572-1.

Direct all inquiries to CRC Press, 2000 N.W. 24th Street, Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-0572-1

Library of Congress Card Number 78-10651
Printed in the United States

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_{1-1}, 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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Lipoprotein Changes Induced by Physiological and Disease Processes

Genetically Directed Hyperlipoproteinemias

FAMILIAL HYPER-α-LIPOPROTEINEMIA

C. J. Glueck and P. M. Steiner

More than 25 years ago in the early investigations of the interaction of lipoproteins with ischemic heart disease (IHD), several groups reported that α-lipoprotein cholesterol (C-HDL) correlated negatively and β-lipoprotein cholesterol (C-LDL) positively with development of IHD.[1-4] The "anti-risk" nature of HDL and its hypothesized role in mobilizing cholesterol from the artery wall has been recently reviewed by Miller and Miller.[5] In population studies, C-HDL is "inversely related to CHD prevalence . . . this relationship is essentially independent of total and LDL cholesterol,"[6] CHD being coronary heart disease. In population groups having low IHD event rates, C-HDL levels are considerably higher than in males of comparable age who have higher IHD mortality.[7] While controlling for serum cholesterol, an inverse relationship between IHD prevalence and HDL cholesterol was reported in Hawaiian Japanese men.[8] In a very dissimilar population group from Evans County, Georgia,[9] Tyroler et al. observed that black males had lower frequencies of coronary heart disease, "controlling for the standard risk factors in univariate and in multivariate logistic risk function analyses." Tyroler et al. observed significantly higher LDL cholesterol and total triglycerides in whites and higher HDL cholesterol in blacks, in comparisons matched for age, sex, and total serum cholesterol.[9] Tyroler et al. concluded:[9] "the black-white lipoprotein fraction differences in Evans County are consistent with a negative coronary risk factor role of elevated HDL cholesterol ..."

C-HDL is inversely correlated with plasma low- and very low-density lipoprotein cholesterols (C-LDL and C-VLDL, respectively)[3,10,11] In subjects having familial hypercholesterolemia,[12-15] C-LDL elevations are accompanied by low levels of C-HDL. Accelerated IHD in the familial hypercholesterolemias and hypertriglyceridemias is associated with both elevated C-LDL and C-VLDL and subnormal C-HDL.[12,16] This provides a much higher than normal ratio of atherogenic (C-LDL, C-VLDL) to "anti-atherogenic" lipoproteins (C-HDL).[17-20]

The authors recently described a new type of familial dyslipoproteinemia, familial hyper-α-lipoproteinemia, in 18 kindreds.[18-20] In extensive prevalence studies of lipids and lipoproteins in family units in the Cincinnati area (5000 kindreds), qualitative increments in α-lipoproteins which appeared to aggregate in certain families were first observed by paper lipoprotein electrophoresis. Putative normal limits for C-HDL were then established for 168 free-living control subjects, with the 90th percentile 70 mg/dl and the mean ± 1 SD C-HDL being 55 ± 12 mg/dl. As an upper normal limit for C-HDL, 70 mg/dl was arbitrarily chosen.[18] Affected probands and relatives had distinctive elevations of C-HDL, slight elevations of total cholesterol, no elevation of C-LDL and C-VLDL, and normal to low triglyceride levels. Simple segregation analysis involving 84 offspring of 22 hyper-α × normal α matings revealed a ratio of hyper-α to normal of 37:47, a ratio not significantly different from 1:1 ($X^2_1 = 1.2$), that ratio which is consistent with autosomal dominant transmission.[18] Males and females from kindreds with familial hyper-α-lipoproteinemia had life expectancies which were 5 and 7 years longer, respectively, than others in U.S. (life table) populations ($P < 0.002$).[18-20] Combined morbidity and mortality from myocardial infarction was threefold greater in normal control kindreds than in kindreds with familial hyper-α-lipoproteinemia.[18-20] The (mean ± SE) ratio of C-LDL to C-HDL in kindreds with familial hyper-α-lipoproteinemia was 1.21 ± 0.06, twofold lower than the ratio, 2.4 ± 0.12, in a control population, $P < 0.01$.[20] This relatively low ratio of C-LDL to C-HDL, which is qualitatively

similar to the "protective" cholesterol/phospholipid ratio reported 25 years ago,[1,2] probably relates to prolonged longevity and reduced morbidity and mortality from myocardial infarction in familial hyper-α-lipoproteinemia.[18-20]

Recognition of hyper-α-lipoproteinemia, until C-HDL determinations are done routinely as part of lipid and lipoprotein sampling, will inevitably be accomplished by electrophoresis, coupled with appropriate subsequent quantitative C-HDL determinations. Qualitative and quantitative aspects of C-HDL determination are summarized below.

QUALITATIVE AND QUANTITATIVE ASPECTS OF C-HDL DETERMINATION IN NORMAL AND HYPERALPHALIPOPROTEINEMIC SUBJECTS

Hyper-α-lipoproteinemia is often detected initially through routine paper[21] or agarose-gel[22] lipoprotein electrophoresis by increased staining of the band with α_1-globulin mobility. Quantitation of C-HDL requires separation of HDL and subsequent measurement of C-HDL. HDL isolation may be accomplished either by precipitation of particles, VLDL and LDL,[23,24] or by sedimentation of HDL in the preparative ultracentrifuge.[25]

Plasma VLDL and LDL are precipitated by sodium heparin and manganese chloride according to Burstein and Samaille.[23] Plasma samples are obtained from fasting subjects in vacuum tubes containing crystalline disodium EDTA (1mg/ml).[26] Plasma in 2-ml aliquots is placed in 15-ml conical, glass-stoppered centrifuge tubes. Samples are maintained in an ice bath or at 4°C throughout the procedure. With a microliter syringe, 0.08 ml of sodium heparin (5000 U.S.P. units per milliter and 0.10 ml of 1.0*M* $MnCl_2$ are added by vortex mixing after each addition. Tubes are allowed to stand for 30 min and then centrifuged for 30 min at 1600 G. The high-density lipoprotein (HDL)-containing supernatant is removed with a Pasteur pipette, and analyzed for cholesterol.[26] Frequently, when samples have increased concentrations of VLDL and triglyceride, sedimentation of the precipitate will not be complete. If this occurs, HDL must be separated by preparative ultracentrifugation.[26]

It is advantageous to monitor supernatants by performing agarose-gel lipoprotein electrophoresis to ensure complete precipitation of LDL. Immunodiffusion against antiserum to β-lipoprotein[25] is an even more sensitive method for checking supernatants for LDL. Because of differences in the preparation of heparin from different sources and the considerable molecular weight variation of commercial heparin,[27] it is necessary to check each individual lot for its ability to completely precipitate VLDL and LDL by agarose-gel electrophoresis.[26] Some HDL has been reported to precipitate with heparin and manganese chloride:[28] however, this has not been found to be a cause of underestimation of HDL in this procedure.[29]

HDL is separated in the preparative ultracentrifuge by adjusting plasma density to 1.063, ultracentrifugation, and recovery of the infranatant. Plasma density is adjusted by addition of potassium bromide according to the following formula:

$$X = \frac{V_i (d_f - d_i)}{1 - \bar{V} d_f}$$

where X is grams of solid KBr added, V_i is initial volume of the solution, d_f is the final density desired, d_i is the initial density, and $\bar{V}$ is the partial specific volume of KBr.[25,30] Densities of the solutions should be confirmed by pycnometry. A convenient procedure is to add 5 ml of plasma to a cellulose nitrate ultracentrifuge tube into which 0.417 g KBr has previously been weighed. The KBr is dissolved, and the tube, filled