Clinical Chemistry Journal Has Contributed to Progress in Lipid and Lipoprotein Testing for Fifty Years

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The journal Clinical Chemistry has played a major role in facilitating the progress that has occurred in lipid and lipoprotein testing over the past 50 years. In celebration of the 50th anniversary of the Journal, this Special Report highlights five selected areas of highly significant accomplishments during the past 50 years contributed by key researchers, some whose careers have spanned the entire history of modern lipid analysis and research.

An overview of the history of lipid analysis from nonspecific strong acid methods to modern enzymatic assays, fully integrated lipoprotein methods, and the remarkable instrumentation developed by industry is presented by G. Russell Warnick.

Dr. Bill Friedewald, whose name is associated with the widely recognized calculation of LDL-cholesterol (1), will relate the genesis of this important development. His 1972 report in Clinical Chemistry (1) has become the most cited ever in the Journal, with more than 6000 citations.

Dr. Richard Havel developed an ultracentrifugal method in 1955 that continues to be the common citation for preparative isolation of the lipoproteins (2). The Lipid Research Clinic (LRC)4 laboratories, in addition to assaying for cholesterol and triglycerides, separated VLDL from the bottom layer of LDL and HDL for HDL analyses by ultracentrifugation, following this method. Dr. Havel, who is still active in lipid research, provides his views on the history of the lipoproteins and separation methodology.

Drs. Gerald Cooper and Gary Myers present the story of how the CDC/National Heart Lung and Blood Institute (CDC-NHLBI) Lipid Standardization Program was developed over a period of nearly 50 years. The LRC program also focused attention on the need for standardization of lipid and lipoprotein measurements. Dr. Cooper of the CDC, one of the co-authors of the standardization article (3), has been a leader in the standardization field for 52 years. The CDC standardization effort, led since 1985 by Dr. Myers, is extensively involved in national and international programs to standardize lipoprotein and other clinical chemistry methods with AACC, NCCLS, IFCC, and WHO.

The NIH, through the LRC Program, undertook the largest and most expensive clinical trials up to that time, attempting to demonstrate the efficacy of decreasing cholesterol concentrations (1970) in reducing risk to heart attacks in an effort to alleviate the scourge of heart disease. The NIH programs also created an interest in and a need for driving improvements in the lipoprotein cholesterol measurement technology. Dr. Virgil Brown relates this history, having trained during these years at NIH and later directing one of the LRCs. He worked closely with two of the most important pioneers in lipid technology and clinical applications at the NIH, Drs. Donald Fredrickson and Robert Levy, both recently deceased, who were truly the prime contributors to the science, technology, and clinical applications of lipids and lipoproteins. Dr. Brown is also a well-recognized contributor of important technical papers to Clinical Chemistry.

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These articles, presented in honor of the 50th anniversary of the journal Clinical Chemistry, remind us of the nature of scientific life, laboratory conditions, and the remarkable accomplishments of scientists in the cholesterol and lipoprotein field during the past 50 years.

A Partner in Improving Lipid Analysis:
From Acids to Enzymes
—G. Russell Warnick

Considering the ease with which cholesterol is measured today by use of enzymatic reagents on automated chemistry analyzers, one might overlook the fact that modern technologies are relatively new. My first experience with cholesterol analysis was in 1970. Fresh out of graduate school, I was an ROTC Captain, a Laboratory Officer in the US Army assigned to a military medical laboratory near San Francisco. Cholesterol had not yet reached the mainstream; only a few samples were being received. The assay was still performed manually, with serum and strong acid reagent pipetted by mouth into test tubes and the color read one tube at a time in glass cuvettes in a spectrophotometer. The first common automated instruments had recently become available: the Technicon AutoAnalyzer, a continuous flow system, and the Abbott ABA 50, a rudimentary discrete analyzer. Intrigued by the potential, I adapted a strong-acid cholesterol assay to the AutoAnalyzer and was eventually successful, but not without several incidents involving squirting acids and the characteristic holes in lab coats and shoes. Old-timers will recall licking flow tubing sections to make the connections, and tubing coming loose, spraying acid reagents: conditions unimaginable today.

Production of a characteristic color with sulfuric acid by cholesterol in gallstones was first recognized as early as 1872 by Salkowski. Compared with the rapid pace of development today, progress back then was relatively slow. By the 1890s, first Lieberman and then Burchard had refined, again for analysis of gallstones, the combination of sulfuric and acetic acids with acetic anhydride still used today in the cholesterol reference method. The first colorimetric assay for cholesterol in serum, reported by Grigaut in 1910, combined purification by digitonin with an acid reagent. The same year, Windaus introduced a saponification step for esters. A 1977 review article in Clinical Chemistry [reviewed in Ref. (4)]. Innovators including Richmond, Flegg, Allain, and Roschlau led the way with the LRC laboratories playing an important role. In 1975, my laboratory received for evaluation one of the first enzymatic cholesterol reagents from Boehringer-Mannheim, one of the early innovators in Germany. Abbott in the US also pioneered a direct enzymatic assay. The mild reagents were much better suited for the increasingly sophisticated automated chemistry analyzers; in truth, without the innocuous enzyme reagents, the move to full automation would have been much more challenging. Their improved specificity also avoided the need for extraction, streamlining and simplifying the methodology. But the new reagents were not perfect. In the early years, the cholesterol lipase enzymes did not fully hydrolyze all of the esters, which together with interference from compounds such as bilirubin required the use of serum-based calibrators. However, the methods still gave erroneous results on unusual specimens. At least the solvents and the acids became obsolete, except in the LRC laboratories; to avoid biasing ongoing studies we had to keep the archaic methods until the mid-1980s. The Liebermann-Burchard acid reagent persists today in the cholesterol reference method.

Enzymatic cholesterol reagents have continued to im-
prove. Today they are commodities with little to distinguish one vendor’s reagent from another. Triglyceride reagents followed a similar evolution. The LRCs extracted triglycerides over zeolite to remove free glycerol. Zeolite, obtained from deposits of minute marine organisms and apparently available in the essential quality only from a small family business in rural Kentucky, was extremely temperamental, making the assay as much art as science. Fortunately, enzymes came to the rescue with triglyceride lipases and first coupled hydrogenases and later oxidases. The earliest hydorgenase chemistries required an inconvenient ultraviolet reading, but the oxidases facilitated the more convenient peroxidase endpoint. The direct enzymatic determinations are still subject to interference from free glycerol, a challenge complicating interpretation of the measurements to this day.

With increasing awareness of the differing risk implications of the various lipoprotein particles, focus shifted to measurement of the major lipoproteins, accelerated by acceptance of the protective role of HDL. At the NIH, a combination method, designated acceptance of the protective role of HDL. At the NIH, a combination method, designated acceptance of the protective role of HDL. At the NIH, a combination method, designated acceptance of the protective role of HDL. At the NIH, a
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The LRC studies, completed during the mid-1980s, culminated in the NIH-sponsored National Cholesterol Education Program (NCEP), which was successful in bringing cholesterol into the public mainstream. The NCEP convened two successive expert laboratory panels, which developed recommendations for reliably measuring the lipids and lipoproteins. As panel reports were completed, each was published either in full or in executive summary in Clinical Chemistry. Much of the knowledge base underlying the NCEP laboratory recommendations came from ad hoc panels organized by the Lipids and Lipoproteins Division of the AACC, approved as a permanent Division in 1989 after being granted provisional status in 1987. This AACC Division, recently renamed as the Lipoproteins and Vascular Diseases Division, has continued to be a key forum for developing and disseminating guidelines for lipid measurement. For example, members of the Division instituted a book series published by AACC Press, culminating in the fourth edition, the Handbook of Lipoprotein Testing, released in 2000 and still a key reference today (6).

The attention generated by the NCEP also led to a revolution in the measurement technology, with instruments and even noninstrument devices allowing measurement of lipids and lipoproteins outside of the traditional laboratory, in clinics, physician offices, and even the home. Another, more recent and very important innovation has been fully integrated homogeneous assays first reported for HDL in 1994 and then for LDL in 1998 [reviewed in Refs. (5, 7)]. The homogeneous reagents were developed in Japan, through the joint efforts of academic scientists such as Dr. Suiguchi and several diagnostic companies as a response to declining reimbursements by the Japanese Ministry of Health. Reimbursements became so low that the manual pretreatment methods had to be automated. Although controversy remains about the specificity of the methods, their improved efficiency and convenience have mandated adoption by most clinical laboratories throughout the world.

The protein constituents of the lipoproteins are also interwoven in this history, with pioneering work at the NIH, contributions by academic and industry scientists, and many important publications in Clinical Chemistry. One interesting anecdote involves the nomenclature for the apolipoproteins (8). When I entered the field in the early 1970s, the apolipoproteins were named by their C-terminal amino acids, e.g., C-gln II and C-Threo, a convention advocated by the NIH-based scientists. Another group in Oklahoma City led by Pierre Alaupovic advocated a more efficient terminology based on lipoprotein families designated by the letters of the alphabet. For a time in the late 1960s, parallel systems existed, but finally in the early 1970s the NIH group relented and for pragmatic reasons adopted the simpler letter nomenclature. A fortunate compromise; otherwise apolipoproteins today might be designated by the more complicated terminology, making even more difficult their already challenging interpretation.

The science and technologies associated with the lipoproteins and their lipid and protein constituents have experienced dramatic changes in recent years. Building on the foundation laid by the early pioneers, our generation has seen considerable progress, much of it documented in the journal Clinical Chemistry. Important to note, however, is that notwithstanding the tremendous advances in recent years, our present state of knowledge still leaves
much to learn about these important and complicated biological entities. Considerable work remains for future generations.

**Comments on the Development of the Friedewald Equation**

—William Friedewald

In most research stories, there are elements of chance and opportunity combined with effort. The story behind the report that developed a procedure to estimate LDL-cholesterol, now known as the Friedewald equation, is just such a story. I had just finished my Internal Medicine training and a year of biostatistical training when I returned to the NIH in 1969. Shortly thereafter, I was approached by Bob Levy to see if I could solve a statistical problem that had not been solved with an earlier attempt. Bob understood that for wide acceptance and usefulness of LDL-cholesterol concentrations in clinical practice, a non-ultracentrifuge-based methodology was needed. He was also aware of two important observations: (a) there is a 5:1 ratio between the triglyceride and cholesterol concentrations in the VLDL in normo- and all hyperlipoproteinemic patients, except type IIIs; and (b) in the absence of chylomicrons, most of the triglycerides in plasma are contained in the VLDL. Thus, with direct measurement of plasma total cholesterol and HDL-cholesterol concentrations and dividing the measured triglyceride value by 5, perhaps one could derive a reasonable estimate of the remaining cholesterol component, LDL. The problem that stopped the earlier attempt was that the correlation between measured and estimated VLDL-cholesterol is not very good. The new insight was that because the VLDL component of total cholesterol is small with regard to the LDL, using triglycerides/5 as an estimate for VLDL-cholesterol in the equation gave surprisingly good estimates of the LDL-cholesterol, which was the measure of real interest.

When analyzing data from normo- and type II hyperlipoproteinemic patients, we obtained correlation coefficients of 0.98 and 0.99 between the estimated and ultracentrifuge-measured LDL-cholesterol values. However, for type IV patients we had a correlation coefficient of only 0.85. As I reviewed the data, it was apparent that the problem seemed to rest with those patients who had triglycerides \( \geq 4000 \) mg/L. I therefore simply removed these patients’ data, and now the correlation coefficient increased to 0.94. On reflection and after examining the data from the type IIs and type IVs, we opined that if a patient had a triglyceride \( > 4000 \) mg/L, then he or she would most likely be a type IV. Regardless of this caveat of not using the estimation equation for patients with triglycerides \( \geq 4000 \) mg/L, we felt that the estimation equation should work quite broadly across the population. Patients with chylomicronemia could be excluded, and because type III patients represent such a small percentage of the general population, we thought that they would not present a major problem in the usefulness of the equation. Our other concerns were that the data were from patients who were referred to the NIH, and thus represented a heavily selected population, and that the sample sizes were relatively small. Nonetheless, we were obviously quite encouraged by the excellent correlation coefficients and by the belief that there was sound stoichiometry behind the estimation procedure.

Because Bob Levy and Don Fredrickson were heavily involved with their research into the phenotypes of hyperlipoproteinemia, they suggested that I work on this LDL-cholesterol estimation article and that I could be first author. Little did they realize that this small methodology article \( (1) \) would be one of the major surviving efforts of that intense period of lipid research and would end up with my name attached to the equation. Regardless, the major outputs of their work during this period turned out not to be the classification of hyperlipemias, but rather a national focus on lipid research with the establishment of multiple lipid centers of excellence around the country and a strong and lasting commitment to high-quality laboratory methodology.

So what has happened to the three authors of that early article. Don Fredrickson became the Director of the NHLBI and later the Director of NIH, eventually retiring from NIH and holding several outside positions. Unfortunately, he died a couple of years ago. Bob Levy went on to also become the Director of the NHLBI, and from there he moved to academia with his last position at Columbia University before joining the pharmaceutical industry. He also unfortunately died a few years ago. I moved through several administrative positions in the NHLBI until 1986 when I moved to the Office of the Director of NIH and became the Associate Director for Prevention. Then, in 1989, I retired from NIH and became the Medical Director at the Metropolitan Life Insurance Company. In 1999, I also retired from there and have been on the full-time faculty at Columbia University ever since. My main research interest throughout has been randomized clinical
trials, and I am now involved with two large multicenter clinical trials, one studying diabetes and heart disease and the other traumatic brain injury. In addition, I teach a course in randomized clinical trials and run the biostatistical consulting service. Through all of these moves, however, the one effort that gets the most prominent recognition is that one little 5-page article, now published 31 years ago in Clinical Chemistry.

**Separation of Lipoproteins by Ultracentrifugation**
—Richard J. Havel

The existence of a lipid–protein complex in blood plasma was initially demonstrated in 1929 when Macheboef isolated a lipoprotein from horse serum, shown later to be a HDL (9). During World War II, Edwin Cohn’s laboratory at Harvard Medical School developed refined precipitation methods to fractionate plasma proteins, leading to the separation of two lipoprotein fractions migrating with α- and β-globulins (10). The ultracentrifuge was invented in Sweden by the physical chemists Svedberg and Rinde (11). Svedberg’s group then developed an analytical ultracentrifuge for protein analysis, which used an optical system to detect moving boundaries (12). The first application of ultracentrifugation to separation of lipoproteins occurred when Pederson showed that raising the density of human plasma with salt permitted the flotation of a lipid–protein complex with a density of ~1.03 kg/L that migrated with β-globulins (13). This complex was thought to be the mysterious “X protein” seen in the region of the albumin boundary in analytical ultracentrifugation (14). Pederson’s work led him to conclude that this material is a labile complex that readily dissociated with serum dilution. The breakthrough with this method occurred in 1949 when John Gofman and his graduate students, Frank Lindgren and Harold Elliott, at the Donner Laboratory of the Berkeley campus of the University of California showed that the X protein could be resolved in the analytical ultracentrifuge by increasing the density further, to ~1.06 kg/L, and was in fact a stable lipoprotein (15). During the next several years, Gofman’s group did pioneering research on the relationship of lipoproteins with a density <1.06 kg/L to atherosclerosis, which energized this field of research (16–19).

In 1949 I began my medical internship at New York Hospital–Cornell Medical Center, where David Barr (Chair of the Department of Medicine), Howard Eder (a young Assistant Professor), and Ella Russ (a medical technologist) were applying a Cohn fractionation method to measure cholesterol in α- and β-lipoproteins. Howard’s laboratory was located adjacent to the medical wards, and it was there that I first learned that there is such a thing as a lipoprotein. I also remember David Barr’s excitement when he showed me a milky serum sample from a woman who had just delivered an infant whose serum was crystal clear. This was my first introduction to “idiopathic hyperlipemia”. Because of my interest in biochemistry [I had published an article on lactate and pyruvate metabolism in congenital heart disease (20)], Barr recommended me for a position at the National Heart Institute of the NIH, where the new Clinical Center was soon to open. I arrived in Bethesda in early 1953 as a Clinical Associate, where I admitted the first patient to the new hospital. I was told to “look around” to decide which laboratory group I might join. I selected Christian Anfinsen’s new Section on Metabolism in the Laboratory of Cellular Physiology and Metabolism, which had recently initiated research on plasma lipoproteins and the “clearing factor”, soon thereafter identified by Ed Korn as a lipoprotein lipase (21). Space was available in Joseph Bragdon’s laboratory. Bragdon was a pathologist and then the only National Heart Institute investigator actually studying atherosclerosis! He had recently acquired a preparative ultracentrifuge (an analytical ultracentrifuge had also been acquired and was being set up by another investigator, Edwin Boyle). For reasons of space, Bragdon’s ultracentrifuge was next to my desk. I became interested in chylomicrons and the role of clearing factor in their metabolism and began to study triglyceride metabolism in experimental animals, together with Bragdon (22) and, later, Robert Gordon (23) and Donald Fredrickson (24). Little was then known about lipoprotein-lipoprotein metabolism in general, and I also set about to develop a practical method to separate lipoprotein fractions by ultracentrifugation, mainly to study triglyceride metabolism. Bradgon had developed an indirect method to measure triglyceride concentrations, by subtraction of phospholipids and esterified and free cholesterol from “total lipids” (estimated by reduction of dichromate ion). Howard Eder then joined Anfinsen’s group; like myself and others, including Fredrickson and Gordon, he was meeting his military obligation during the Korean War in the Public Health Service. So it happened that Bragdon, Eder, and I joined forces to quantify lipoproteins in blood plasma and measure their major chemical components.

From the work of the Donner Laboratory group, it was known that the “low-density” lipoproteins included a major cholesterol-rich species with a density between 1.019 and 1.063 kg/L and triglyceride-rich components with lower densities (25). Accordingly, we added concentrated salt solutions to float in the preparative ultracentrifuge three lipoprotein fractions, with densities <1.019, 1.019–1.063, and 1.063–1.21 kg/L, from serum of humans and other mammals. We developed a technique of “sequential preparative ultracentrifugation” that was made quantitative by use of a tube-slicing device. This method, which required three overnight “spins”, provided sufficient material from 10 mL of serum for detailed chemical analysis of these fractions (26), which we identified as “VLDL”, “LDL”, and “HDL”. For routine work, we omitted the last centrifugal step and analyzed lipids in the infranate of the 1.063 kg/L spin as the HDL fraction. In our original publication of 1955 (2), the editors of the
The Journal of Clinical Investigation did not permit us to use these names, which first appeared in an article a year later (27). These editors later relented in part, allowing these names to be placed in a separate table in an article published in 1957 (28). Independently, Lindgren, Nichols, and Freeman developed a similar sequential flotation method at the Donner Laboratory and provided compositional data for human serum lipoproteins based on chromatographic separation of lipids and infrared absorption analysis (29).

We later subfractionated the VLDL fraction by floating the more triglyceride-rich components at a density of 1.006 kg/L (which did not require addition of concentrated salt solution), and a fraction that was designated “intermediate-density lipoproteins” (IDL) of density 1.006–1.019 kg/L. Of note, the later application of the standard “beta-quant” procedure used the 1.006 spin to separate VLDL, so that LDL came to include IDL in clinical chemistry applications.

In retrospect, earlier use of the preparative ultracentrifuge could have readily resolved the long-standing anomaly of the X protein. If one simply subjects human serum to ultracentrifugation at its native density, LDLs (which contain most of the serum carotenoids) are concentrated in a yellow band that is readily seen just above the amber (bilirubin-stained) band of albumin. The floating VLDLs are seen as a faintly yellow, opalescent band at the top of the centrifuge tube. With ultracentrifugation at a density of 1.063 kg/L, the combined VLDL/LDL fraction is obviously concentrated at the upper surface. With most animal sera, LDLs are not colored, but can be visualized by Tyndall scattering, as originally noted in rabbit sera by the Donner Laboratory group (31).

Although preparative ultracentrifugation is by no means a perfect method for lipoprotein isolation or quantification, particularly for applications involving apolipoprotein analysis, it continues to provide a useful frame of reference for the many other methods that have been used to characterize and quantify these fascinating macromolecular complexes.

**Cholesterol Standardization: Leading the Way to Laboratory Improvement**

—Gary L. Myers and Gerald R. Cooper

Clinical interest in the role of cholesterol in cardiovascular disease has grown tremendously since Chevreul first isolated cholesterol from gallstones in 1815 and coined the term “cholesterine”. Interest in measuring cholesterol concentrations in humans has also grown similarly. In 1952, Abell et al. (32) published an analytical method for measuring serum total cholesterol that would later become the “gold standard” for one of the most extensive laboratory improvement efforts in the past 50 years. We both have been intimately involved in this effort at different times and at the same time during our careers at the CDC. We would both like to share just a few highlights of our experiences in this amazing story.

**Comments from Dr. Cooper**

The first concern about the reliability of cholesterol measurement occurred in 1957 when the Conference on Longitudinal Cardiovascular Studies received disparate results from laboratories participating in longitudinal studies of heart disease. This lack of interlaboratory comparability, especially not knowing which laboratory result was correct, became a major problem for clinical investigators conducting epidemiologic work. These early investigators soon realized that the value of study results would be seriously limited if laboratories did not agree and comparisons with similar studies would not be possible. I was invited by the Conference to speak on how the CDC could assist in assuring the quality of their cholesterol results. The increasing interest and activity in heart disease epidemiology demanded assurance that cholesterol measurements were reproducible within and comparable between laboratories. Such requirements for laboratory accuracy and agreement were a new challenge for the laboratory community.

As a result of my discussions with the Conference, the National Heart Institute (now the NHLBI) in 1957 approached the Communicable Disease Center (now the CDC) to serve as a neutral laboratory to help “standardize” cholesterol measurements among cardiovascular epidemiologic laboratories. Thus began a partnership in cardiovascular laboratory standardization that continues today. We were approached for assistance because in 1946, over a decade earlier, CDC had already established a Clinical Pathology Section in its Laboratory Branch to develop a laboratory improvement program in clinical chemistry to conduct quality-control research and prepare reference materials. In response to this request, I was asked to establish a cholesterol methodology development laboratory and standardization office at CDC. I remember visiting Liese Abell and Forest Kendall at their laboratory on an island in the Hudson River to learn how to perform their cholesterol method. In 1961, we initiated the first Cooperative Cholesterol Standardization Program (CCSP). Seven cardiovascular laboratories participated in that first effort at cholesterol standardization.

From this meager beginning we launched a concept and an approach to laboratory improvement that has had an astounding impact on cardiovascular laboratory medicine specifically and clinical chemistry in general. The “cholesterol model” is the standard by which other programs are modeled and judged. Beginning with only 7 laboratories, the CCSP (now known as the CDC-NHLBI Lipid Standardization Program) has grown into an internationally respected program that standardizes more than 100 lipid research laboratories and associated clinical investigations annually. After more than 50 years, I continue to work for the program that started from such meager beginnings.
Although the CCSP planted the seed that spawned the concept, it is by far not the only factor to consider in the standardization story. Today, much attention is focused on the need for traceability of laboratory results to standards of higher order. However, the focus on producing traceable results in laboratory medicine is not a new concept. It was, in fact, the underlying premise on which the cholesterol standardization program was built. The foundation of the cholesterol model has always been a well-defined and accepted reference system. With the cooperation of CDC, the National Bureau of Standards [now the National Institute for Standards and Technology (NIST)] and the AACC, the National Reference System for Cholesterol (NRS/CHOL) was established as the first nationally accepted reference system in clinical chemistry. The NRS/CHOL has served as the hierarchy of approved methods and materials for the measurement of cholesterol, developed and credited after peer review within the voluntary consensus process of the NCCLS. It comprises the CDC-modified Abell–Kendall cholesterol method (33), the isotope-dilution mass-spectrometry definitive method for cholesterol developed by the NIST (34), and the NIST Standard Reference Material 911b (pure cholesterol primary standard). These two methods have provided laboratory medicine with a consistent and stable accuracy base on which to anchor cholesterol measurement results. The epidemiologic studies and clinical trials that were and continue to be standardized and traceable to the NRS/CHOL provide cardiovascular medicine with a reliable scientific database for evaluating risk factors associated with coronary heart disease. This database led scientists to the conclusion that increased blood cholesterol is a significant risk factor in the development of heart disease and that lowering total cholesterol prevents or delays heart disease. As interest grew to other lipids and lipoproteins, reference methods for HDL, LDL, and triglycerides were developed, and standardized services were provided through the CDC-NHLBI Lipid Standardization Program (35).

COMMENTS FROM DR. MYERS

The standardization story does not end here. I joined CDC in 1976 and assumed responsibility for the CDC cholesterol standardization activities in the mid-1980s. In 1985, the NCEP was established and launched its public health campaign “Know Your Cholesterol Number”. This national effort to reduce high blood cholesterol and reduce the risk of heart disease focused unprecedented attention on the laboratory measurement of cholesterol and a need to measure cholesterol in clinical laboratories at a higher level of performance. I was now responsible for the CDC-NHLBI Lipid Standardization Program and realized this was again an opportunity to apply the principles of standardization that I had learned from Dr. Cooper to meet a pending need for laboratory improvement. Because of the focus directly placed on the clinical laboratory, the accomplishment of such laboratory improvement and standardization required unified national support by government agencies, manufacturers of diagnostic products, the medical profession, and the laboratory community.

From 1985 to the present, we have made many noteworthy accomplishments that originated from the cholesterol model. Probably the most profound challenge facing us was how we were going to standardize thousands of clinical laboratories and establish traceability to the NRS/CHOL to properly implement the medical decision points recommended by the Adult Treatment Panel. The traditional approach had been to simply assign values to reference materials and use these materials for measurement calibration and performance assessment. It was assumed that these reference materials would recover cholesterol similarly to patient specimens. Unfortunately, with the advent of enzymatic cholesterol assays, results from commutability studies with available reference materials quickly demonstrated that this approach would not work in most cases. The problem lay in matrix effects caused by the processes used in preparing the materials, e.g., lyophilization. I vividly remember giving a presentation at an NCCLS meeting about our efforts to standardize cholesterol measurements, when Dr. Roy Rand (Kodak Diagnostics) asked me if we had experienced any problems with lack of commutability or matrix effects with any of the reference materials being used for standardization. Without thinking much about it, I quickly responded “No”. Little did I know that the problem with matrix effects alluded to by Dr. Rand would shape my program efforts for nearly 20 years to come. As a result of the problems encountered in attempting to standardize routine cholesterol measurements, matrix effects drew center stage attention. As we learned more about matrix effects and cholesterol measurement, it became obvious that noncommutability was not limited solely to cholesterol but was also an issue for many other clinical measures. Matrix effects took on such importance that the College of American Pathologists held a major conference in 1993 on matrix effects and accuracy assessment in clinical chemistry (36), and later the NCCLS developed a matrix effects guideline (37). In 1997, I chaired a NCCLS subcommittee that prepared the first consensus guideline for preparing commutable frozen reference materials for cholesterol measurement (38).

The cholesterol standardization effort generated still another first. Because the only acceptable approach to establishing traceability to the NRS/CHOL involved split-sample comparisons between a routine method and the reference method using patient samples, an approach to facilitate access to the cholesterol reference method was needed. In response, we established the Cholesterol Reference Method Laboratory Network (CRMLN) (39). The CRMLN was truly the first international reference method laboratory network. It is a network of specialized laboratories that perform the Abell–Kendall cholesterol reference method standardized by CDC. CRMLN programs
are directed at the manufacturers of cholesterol diagnostic products designed to facilitate split-sample comparisons for purposes of assessing assay performance and validating calibration traceability. By focusing on the manufacturers, the standardization effort directly impacts the quality of millions of cholesterol tests performed annually in clinical laboratories. The CRMLN also provides reference method services for HDL, LDL, and very soon for triglycerides.

The impact of the cholesterol standardization effort is certainly far reaching. Standardization has not only served to improve the measurement of cholesterol; it has also established a path that, if followed, can lead to excellence in laboratory testing for all measurands. For example, programs to improve the measurement of apolipoproteins (40, 41), lipoprotein(a) (42), and C-reactive protein (43) as emerging risk factors for heart disease; glycated hemoglobin for assessing glycemic control in diabetes (44); and the recent effort to improve serum creatinine measurement for estimating glomerular filtration rate for assessing early kidney disease are examples of recent programs that owe their beginnings and success to the lessons we learned in cholesterol standardization. Cholesterol standardization has set a standard for nearly 50 years that will continue to guide laboratory improvement efforts well into the future.

Clinical Impact of the Lipid Research Clinics Program
—W. Virgil Brown

In the Spring of 1969, as I was attempting to complete a series of experiments on the apolipoprotein composition of VLDL in the laboratory of Drs. Robert Levy and Donald Fredrickson, I was summoned to Don’s (as we all called him) office and asked to consider working with Bob Levy on a new project to learn more about lipoprotein distributions in the community and their impact on disease. This discussion reflected their early thoughts about the formulation of what became the LRC program. I was very appreciative of this offer but refused because I had committed to joining Dan Steinberg at the new University of California San Diego Medical School in La Jolla, where I planned to continue more basic studies. However, this new “community program” was to reappear in the fall of 1970 as a Request for Proposal from the NIH as the LRC. With Dan’s help, I wrote a proposal describing a laboratory incorporating much of the technology developed at the NIH, including our concept of a community-based study to measure the distribution of lipoprotein values using the methodology of Levy, Lees, and Fredrickson (incorporating the Friedewald calculation) as well as the \( \beta \)-quantification method of LDL-cholesterol measurement as the “gold standard”. The University of California San Diego team and 11 sister universities, across the country and Canada, were funded to begin a saga lasting over two decades with continuing ramifications. It took almost 1 year and the help of the CDC laboratory (described above by Drs. Cooper and Myers) to develop a functioning laboratory meeting the high standards set by the laboratory committee. During this year, we recruited an infectious disease epidemiologist, Dr. Elizabeth Barrett Connor, who took primary responsibility to plan the community study to be carried out in Rancho Bernardo, CA. My basic studies of lipoprotein structure took a back seat for many months as we met with community leaders, set up a local clinic, and hired staff that ultimately examined 6155 citizens of Rancho Bernardo. We later repeated the examination in a 15% random sample of the initial cohort plus those with “hyperlipoproteinemia”, using the \( \beta \)-quantification procedure. This same process was ongoing in the other LRC locations. The result was a huge body of data providing an assessment of the new technology for lipoprotein measurement with precision and accuracy without comparison. We now had, for the first time, large data sets for free-living men and women across North America of all ages (including children). This provided a relational database that has been invaluable for examining lipoprotein correlations with other diseases, dietary habits, use of prescription drugs, and seasons of the year (45). It led to outcome data relating many baseline measures to all-cause and cardiovascular mortality as well as a variety of other degenerative diseases. Hundreds of publications and scores of academic careers have been built on those initial LRC scientific programs, but this was not the end of the story.

In the fall of 1972, as the community study was getting underway, the various university groups participating in the LRC program were asked to develop a plan for a definitive trial of the primary prevention of coronary artery disease by cholesterol reduction in hypercholesterolemic men. This was met with disbelief because the work load related to the community studies seemed totally consuming. However, it was clear that “No” was not an appropriate answer. As with the community studies, there were many committees requiring representatives of our faculty and staff to attend repeated meetings for logistical discussions and, ultimately, training for individual procedures. The LRC Coronary Primary Prevention Trial (LRC-CPPT) was begun in 1974 with the goal of recruiting 3600 men between the ages of 35 and 59 years whose baseline LDL-cholesterol was \( >190 \text{ mg/dL} \). The participants could have no history or clinical findings diagnostic of coronary artery disease at baseline and had to be willing to take 12 g of cholestyramine twice daily for more than 5 years. Furthermore, the presence of major risk factors was a cause for exclusion because we thought at the time that these would overwhelm the risk contributed by the LDL-cholesterol increases. The recruitment was modeled after another study done on familial hypercholesterolemia in which patient referral from local physicians had provided the few score patients (46). It quickly became evident that this model did not work in the nationwide study, and new strategies were debated and
adopted in each clinic. In San Diego, we measured cholesterol concentrations in over 60,000 individuals during 1975 to provide our cohort of 299 patients meeting all inclusion and exclusion criteria. Nationally, over 300,000 individuals were screened by equally Herculean efforts to recruit the total study population of 3806 men 35–59 years of age. Many thought that more elderly men would have irreversible arteriosclerosis and would therefore be unsuitable for study. During the trial, we debated adding niacin when the incidence of endpoints was not developing at the rate anticipated, but we decided instead to simply extend the length of the study to 7 years of observation, which led to publication of the results in the JAMA in 1984 (47, 48). It became clear that the difficulty with maintaining compliance to cholestyramine therapy was underestimated. The reduction in LDL-cholesterol was only 11% instead of the anticipated 25% or more. The result was a reduction in myocardial infarction and coronary death of 17%, which was shown to be significant using a “one-tailed Student t-test.” This became the source of controversy because some statisticians felt that we should have used the traditional and more stringent “two-tailed t-test”. However, the double-blind placebo-controlled design and the consistency in the reduction of several other measures of clinical vascular disease won the day, and the LRC-CPPT was widely accepted to be the most convincing evidence to date that reducing LDL-cholesterol was an effective maneuver in the prevention of coronary artery disease. After this publication, it became clear that we had to have additional policies that would further drive efforts to identify and treat those with hypercholesterolemia as an important strategy in preventing the huge tide of coronary disease sweeping through the industrialized world. In the US, development of the NCEP was a direct outgrowth of having this compelling evidence (49).

The NCEP also began with guidance from Don Fredrickson, Robert Levy, and Basil Rifkind. Basil had been the NIH staff director of the LRC Program during its mid and latter years as Bob Levy had become the Director of the NHLBI. The appointment of a Steering Committee consisting of representatives of every national medical organization that had an interest in the treatment or prevention of vascular disease provided a forum for debate and for settling of issues that has been extremely effective in gaining acceptance of the various recommendations and guidelines that subsequently were issued. Several groups of experts were appointed and asked to develop recommendations on (a) improving the precision and accuracy of critical lipid measures, (b) improving public health approaches to reducing the population-wide excess concentrations of blood plasma cholesterol present in the US, (c) improving the assessment of children and providing guidelines for management of hypercholesterolemic children, and (d) improving the clinical assessment and management of lipid abnormalities in all American adults with the aim of reducing vascular disease risk. This latter group, under the initial guidance of Dewitt Goodman and later Scott Grundy, became known as the Expert Panel on Assessment and Treatment of Hypercholesterolemia in Adults, or in the vernacular, the “Adult Treatment Panel”. More recently it has become known simply as the ATP, and with the last iteration of the group, ATPIII. I had the privilege of serving on the first ATP beginning in late 1985 and continuing until the first report was published in 1988 (49). Subsequent revisions in 1993 (50) and 2001 (51) have kept these guidelines up-to-date and maintained their very significant impact on the management of lipid disorders in the US and in many other countries across the world.

References

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