In 1998 the AACC celebrates its 50th anniversary. Thus, we are honored to call attention to the outstanding professional contributions of the journal Clinical Chemistry over the years by pointing out the citation classics that have been published in its pages. By the end of 1995, 31 articles in Clinical Chemistry had been cited more than 300 times (1). Thirty of these articles were still being cited in 1995, and some are still cited to the present day. These 31 papers are distributed in multiple areas of biological sciences, and in particular: lipids, 8 papers (2–9); endocrinology, 2 papers; clinical enzymology, 4 papers; intermediary metabolism, 6 papers; renal function, 2 papers; cancer, 1 paper; and calcium metabolism, 3 papers. Publications on methodology research and laboratory applications of lipid, lipoprotein, and apolipoprotein measurements in clinical investigations and epidemiologic trials have had a major impact on research of lipid metabolism and cardiovascular medicine (2–9). The combined efforts of epidemiologists, clinicians, and laboratory scientists, have produced classic publications on the understanding of lipid metabolic disorders, have stimulated development of novel equipment and methodology, and have helped to establish the development of beneficial preventive and control measures for diseases of the heart and blood vessels.

The cited lipid papers published in Clinical Chemistry included mainly the original papers on measurement of lipoproteins, enzymatic measurements of cholesterol and triglycerides, and immunoassays of apolipoproteins. The method used most widely today for estimation of the concentration of LDL-cholesterol (LDL-C) in both clinical and research laboratories arose from the publication of Friedewald et al. (2) that showed that the serum concentration of LDL-C can be estimated from available established measurements of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglyceride (TG) in serum (Fig. 1). Prior to this, only time-consuming and expensive ultracentrifugal measurements were used to determine LDL-C in serum. The ability to calculate LDL-C thus permitted greater worldwide access to LDL-C estimates and allowed for the greater application of the more sensitive and specific LDL-C estimates in lieu of, or in conjunction with, TC in the assessment of coronary heart disease risk. The Friedewald equation has since been used as the basis of LDL-C estimation in several landmark trials of the clinical benefits of lipid modification, thus demonstrating its important role in the epidemiology of coronary heart disease.

HDL-C has become recognized as an important independent risk factor for coronary heart disease. Two highly cited publications in Clinical Chemistry have contributed methods that have provided valid HDL-C laboratory measurements for clinical and epidemiologic investigations. Lopes-Virella et al. (5) reported the analytical performance characteristics of three different methods for the information of lipid laboratorians (Fig. 2). Warnick et al. (6) published a dextran sulfate method for quantification of HDL-C that has become a method preferred by many research and clinical laboratories.

Around 1960, colorimetric TG measurements were of questionable accuracy and lacked desired precision. Van Handel (8) optimized the colorimetric zeolite extraction, alcoholic KOH saponification, periodate, and chromotropic acid reagent method. He purified corn, cotton, and olive oil by shaking with ground zeolite after dissolving the oil in chloroform for use as a primary standard. A modification of this colorimetric method is the TG reference method for the Centers for Disease Control and Prevention.

A major breakthrough in cholesterol analysis resulted from development of enzymatic reagents for analytical measurements of TC. Richmond (7) isolated a cholesterol oxidase enzyme from a species of Nocardia, using a surfactant for solubilizing and ion-exchange chromatography for purifying the cholesterol-oxidizing enzyme. He determined the stability, substrate specificity, and optimal conditions under which the enzymatic oxidation reaction could occur and later combined alkaline ethanolic hydrolysis, enzymatic oxidation, and colorimetric estimation for automation of the total cholesterol analysis. Allain et al. (3) developed an automated method for total serum cholesterol using three enzymes (Fig. 3): cholesteryl ester hydrolase was used for hydrolysis of cholesteryl esters, cholesterol oxidase for oxidation of cholesterol to form hydrogen peroxide, and peroxidase for oxidative coupling to develop color with 4-aminoantipyrine and phenol. Allain et al. (3) also developed assays for standardization of the enzymes for optimization experiments. These enzymatic cholesterol measurements are applicable to measurements of low cholesterol concentrations of HDL-C in serum.

Bucolo and David (4) improved the TG ultraviolet test by using a lipase together with a protease to replace chemical hydrolysis for formation of glycerol from the TG (Fig. 4). The determination of glycerol by the enzymatic method with three coupling enzymes, glycerol kinase, pyruvate kinase, and lactic dehydrogenase, measured the decrease in the absorbance of NADH at 340 nm. This procedure became the most popular ultraviolet technique for measurement of the glycerol freed from TG because it could be used with most manual and automated laboratory instruments.

The development of immunochemical procedures permitted laboratory measurements of apolipoprotein (apo).
Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge

William T. Friedewald, Robert I. Levy, and Donald S. Fredrickson

A method for estimating the cholesterol content of the serum low-density lipoprotein fraction (S<sub>2</sub>-0-20) is presented. The method involves measurements of fasting plasma total cholesterol, triglyceride, and high-density lipoprotein cholesterol concentrations, none of which requires the use of the preparative ultracentrifuge. Comparison of this suggested procedure with the more direct procedure, in which the ultracentrifuge is used, yielded correlation coefficients of .94 to .99, depending on the patient population compared.

In the cited article by Curry et al. (9), electroimmunoassay was applied to the measurement of apo A without requiring the use of a radioisotope in the assay. apo A and its components apolipoprotein A-I and A-II are quantified by electrophoresis of the antigen into agarose containing its corresponding antibody, a process that yields precipitates that resemble ascending rockets. Washed antigen-antibody precipitates are stained with lipid stains. This electroimmunoassay along with developed immunonephelometric assays became widely used across the United States to describe complexes, associations, and reference values for different apos.

Outstanding scientific articles continue to be published in and cited from Clinical Chemistry. The Institute for Scientific Information reported that in 1996, Clinical Chemistry was cited 14 144 times, mostly in general medical and specialty journals, and had an impact factor of 3.422, which was double that of the nearest journal in the same category. Lipid articles cited widely by investigators in the cardiovascular lipid discipline recognize the following

Cholesterol Determination in High-Density Lipoproteins Separated by Three Different Methods

Maria Fernanda Lopes-Virella, Pamela Stone, Shelton Ellis, and John A. Colwell

We describe a simplified method for measuring high-density lipoprotein cholesterol in serum after very-low- and low-density lipoproteins have been precipitated from the specimen with sodium phosphotungstate and Mg<sup>2+</sup>. Values so obtained correlate well with values obtained with the heparin–Mn<sup>2+</sup> precipitation technique (r = 0.95, CV <5% in 66% of the subjects studied and between 5 and 10% in the remaining ones) or by ultracentrifugal separation (r = 0.82, CV <5% in 80% of the subjects studied and between 5 and 10% in the remaining ones). Our precipitation technique is more appropriate for routine clinical laboratory use.
Enzymatic Determination of Total Serum Cholesterol

Charles C. Allain,1 Lucy S. Poon,1 Cicely S. G. Chan,1 W. Richmond,2 and Paul C. Fu3

An enzymatic method is described for determination of total serum cholesterol by use of a single aqueous reagent. The method requires no prior treatment of sample and the calibration curve is linear to 600 mg/dl. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (EC 3.1.1.13). The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500 nm. The method is reproducible, and the results correlate well with those obtained by automated Liebermann–Burchard procedures (AA-2 and SMA 12/60) and the method of Abell et al. The present method affords better specificity than those previously reported and has excellent precision.

Quantitative Determination of Serum Triglycerides by the Use of Enzymes

Giovanni Bucolo and Harold David

We describe a novel method for determining serum triglycerides, in which an enzymatic hydrolysis replaces the more commonly used saponification procedure. Under the conditions of the assay, the enzymatic hydrolysis can be completed in less than 10 min by the combined action of a microbial lipase and a protease. We have been able to demonstrate complete hydrolysis of triglycerides by thin-layer chromatography of the reaction products, by recovery of glycerol from sera of known triglycerides content, and by comparison of triglyceride assays on a number of sera assayed by our method vs. the AutoAnalyzer procedure. The hydrolysis is directly coupled to the enzymatic determination of glycerol, and is followed through absorbance changes at 340 nm. The assay is simple, rapid, and requires only 50 μl or less of sample. Because the enzymes used do not release glycerol from other compounds in serum, the hydrolysis can be considered specific for triglycerides.
or statins) (21); and (ii) identifying and documenting new risk factors beyond those associated with lipid concentra-

tions (22).

In the future, Clinical Chemistry will be an excellent journal in which to explore important new coronary risk factors, such as homocysteine and lipoprotein remnants. The precise mechanisms whereby lipid-lowering drugs exert their antiatherogenic effects in the arteries, particularly how statins help prevent heart attacks and stroke, will also need to be addressed. Quantitative measurements of oxidized lipids and lipoproteins may help elucidate the relationship between these particles and endothelial dysfunction, inflammation, and atherosclerosis. Laboratory measurements of lipid risk factors will be aided by mass spectrometric methods to provide improved reference materials. Homogeneous methods will keep improving automated methods for lipid measurements for clinical and epidemiologic investigations. Inflammation and infectious disease sources of coronary disease risk will be detected and documented. Research on gene therapy also shows promise of producing exciting ways to reverse atherosclerosis. For example, in the laboratory of Weill Medical College of Cornell University, gene therapy has been used to induce new blood vessel formation in a pig model of total arterial occlusion, thus restoring impaired blood flow completely.

Without doubt, Clinical Chemistry will continue to be a highly desirable forum for publications by laboratories, clinicians, and epidemiologists of the scientific results of methodology research and collaborative laboratory, clinical, and epidemiologic studies. Although the achievements of the journal have been excellent, we predict that its future contributions and continued commitment to the field of lipid and atherosclerosis research will be even more impressive.

References


Antonio M. Gotto
Gerald R. Cooper

1 Department of Medicine
Weill Medical College of Cornell University
New York, NY 10021

2 Division of Environmental Health Laboratory Sciences
National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, GA 30341-3724

*Address correspondence to this author at: c/o Jesse Jou, Weill Medical College of Cornell University, 445 East 69th Street, Olin Hall, Room 205, New York, NY 10021. Fax 212-746-8200; e-mail jjou@mail.med.cornell.edu.