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This Week's Citation Classic

Havel R J, Eder H A & Bragdon J H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum.
J. Clin. Invest. 34:1345-53, 1955.
[Lab. Metabolism, Natl. Heart Inst., Natl. Insts. Health, Dept. Health, Education, and Welfare, Bethesda, MD]

A versatile method for isolating lipoprotein fractions from blood serum according to their hydrated density is presented, enabling them to be characterized and quantified by ordinary procedures. Results are given for normal humans and animals and human hyperlipidemics. [The *SCI*® indicates that this paper has been cited in over 2,160 publications since 1961.]

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"When the National Institutes of Health Clinical Center opened in 1953, I came from Cornell Medical School to the National Heart Institute with a group of young physicians recruited by James Shannon. I joined a small group organized by Christian Anfinsen to work on lipids and chose to work in the laboratory of Joseph Bragdon, a pathologist who was interested in the pathogenesis of atherosclerosis. One year later, Howard Eder, also an internist from Cornell, joined the group. He had worked with David Barr and Ella Russ on the distribution of cholesterol and phospholipids in two lipoprotein fractions separated from human blood plasma by 'Cohn fractionation'; they had made a number of interesting observations on alterations of lipoproteins in several diseases, including atherosclerosis.[1] I was by then separating lipoproteins for metabolic studies by preparative ultracentrifugation, based upon the pioneering work of Gofman, Lindgren, and their associates at the University of California at Berkeley.[2] They had used analytical ultracentrifugation extensively to characterize human plasma lipoproteins and their alterations in patients with atherosclerosis and other diseases. What appeared to be needed was a straightforward method of isolating and characterizing a larger number of fractions than provided by Cohn fractionation. A combination of preparative ultracentrifugation and chemical analysis appeared to have promise as a reproducible, quantitative procedure. A preliminary study by Eder and others demonstrated that the ultracentrifugal fraction containing high density lipoproteins corresponded closely to the Cohn fraction that contains alpha lipoproteins.[3] The procedure that we designed, now known as 'sequential preparative ultracentrifugation,' yielded clean lipoprotein fractions that corresponded to those defined by the work of Gofman and Lindgren.[2] To illustrate the usefulness of the procedure, we provided limited normative data and some values for patients with various forms of hyperlipoproteinemia. We also tested the method in six other mammals and demonstrated large variations in lipoprotein concentrations and distributions which are now widely appreciated. We separately reported the detailed composition of those lipoprotein fractions that are now analyzed routinely in clinical and epidemiological research.[4]

"Although we referred in the laboratory to the fraction that we separated at a nonprotein ('background') solvent density of 1.019 g/ml as 'very low density lipoproteins' to distinguish them from those subsequently separated at 1.063 g/ml (low density lipoproteins), such terminology was not permitted by the editors of the *Journal of Clinical Investigation* until 1957.[5] Since then, this terminology has 'stuck,' and 'VLDL,' 'LDL,' and 'HDL' have become the standard jargon of the field.

"Our description of this procedure evidently helped to make the study of lipoproteins readily accessible to both clinical and basic scientists. Several modifications have been published, some of which are also cited frequently, and the phenomenon of 'extinction' is increasingly evident. I was among the first to point out that ultracentrifugation dissociates certain proteins from lipoproteins[6] and I have advocated gentler methods that avoid ultracentrifugal artifacts! At the University of California, San Francisco, we now make extensive use of chromatographic and immunoadsorption methods to characterize lipoprotein particles that are modified or even destroyed by ultracentrifugation.

"None of the authors of this *Citation Classic* felt that it represented a major conceptual advance and we were initially surprised by the wide attention that it received. Evidently, even rather straightforward methodological efforts can sometimes help to open up a fruitful field of research."

1. **Barr D P, Russ E M & Eder H A.** Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. *Amer. J. Med.* 11:480-93, 1951. (Cited 245 times.)
2. **Lindgren F T, Elliott H A & Gofman J W.** The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins, with applications to the study of atherosclerosis. *J. Phys. Colloid Chem.* 55:80-93, 1951.
3. **Eder H A, Russ E M, Pritchett R R A, Wilber M A & Barr D P.** Protein-lipid relationships in human plasma: in biliary cirrhosis, obstructive jaundice, and acute hepatitis. *J. Clin. Invest.* 34:1147-62, 1955. (Cited 90 times.)
4. **Bragdon J, Havel R J & Boyle E.** Human serum lipoproteins. I. Chemical composition of four fractions. *J. Lab. Clin. Med.* 48:36-42, 1956. (Cited 135 times.)
5. **Havel R J.** Early effects of fat ingestion on lipids and lipoproteins of serum in man. *J. Clin. Invest.* 36:848-54, 1957. (Cited 70 times.)
6. **Faimaru M, Havel R J & Imaizumi K.** Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. *Biochem. Med.* 17:347-55, 1977. (Cited 60 times.)