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This Week's Citation Classic

Sperry W M & Webb M. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187**:97-106, 1950. [Depts. Biochemistry, New York State Psychiatric Inst., and Coll. Physicians & Surgeons, Columbia Univ., New York, NY]

A revision of the Schoenheimer-Sperry method for the determination of total and free cholesterol in blood serum is described. [The *SCI*® indicates that this paper has been cited in over 1,650 publications since 1961.]

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"In 1930, when I moved from the University of Rochester to the Babies' Hospital at the Columbia Medical Center, nothing was known about free and esterified cholesterol in children's blood serum because the only available method, gravimetry of the digitonide, required large amounts of blood. I undertook an attempt to adapt this method to a microscale.

"In 1932, Rudolf Schoenheimer of Freiburg, knowing my interest in a micromethod for cholesterol, invited me to collaborate in the development of a method based on his brilliant idea of applying the widely used colorimetric method to the digitonide. He realized that a procedure which works in one laboratory may fail in another.

"There followed a voluminous correspondence in which Rudolf in long letters described his procedures and the good results they gave, and I in equally long letters described my utter failure to obtain such results. The impasse was ended by Rudolf's move from Freiburg to the department of biochemistry at Columbia University. He came to my laboratory to show me where I had gone wrong. He failed as miserably as I had to reproduce his Freiburg results. Although this was a severe setback to our plans, I must confess that it did much to restore my ego, which was at a low ebb. Rudolf was already deeply immersed in plans for his pioneering work on the use of isotopes as metabolic indicators, and he turned the further development of the method over to me.

"I went back to square one and began a study in which I varied solvents, reagents and their concentrations, temperatures and times at various steps, mechanical procedures, etc. That was about 50 years ago, and I have forgotten the details. Finally, a method which gave accurate results on 0.2 ml. of serum was achieved.[1]

"In several studies, hundreds of samples of blood serum were analyzed. No trouble was encountered until our supply of digitonin was interrupted by the war. Aqueous solutions of domestic digitonin were unstable, but solutions in 50 percent ethanol were found to be stable. This and several other changes which facilitated the technique were incorporated in a revision of the method, which was published in the paper cited above.

"I was surprised by the number of citations. Because the method requires considerable time and effort, it has been used in few, if any, clinical laboratories. It has been used as a standard for calibration of rapid colorimetric methods which determine only total cholesterol. It made possible the finding that the percentage of esterified in total cholesterol in healthy humans is relatively constant, varying only from about 70 to 75 percent.[2] In contrast, the level of total cholesterol in the same subjects varied by nearly 200 percent. Studies of the mechanism by which the ratio between the cholesterol fractions is governed and its significance in high- and low-density lipoproteins should be rewarding."

1. **Scboenheimer R & Sperry W M.** A micromethod for the determination of free and combined cholesterol.
J. Biol. Chem. **106**:745-60, 1934.
[The *SCI* indicates that this paper has been cited in over 355 publications since 1961.]
2. **Sperry W M.** The relationship between total and free cholesterol in human blood serum.
J. Biol. Chem. **114**:125-33, 1936.