Determination of Cholesterol in Blood.
Part 3

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Abstract—The chronological development of procedures for determining the concentration of cholesterol in plasma, serum, and whole blood is presented in the review. It is stated that, since the correlation between the risk of development of cardiovascular diseases and the concentrations of total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in human blood has been established by numerous medical studies, procedures for the measurement of these parameters have been developed most actively. A brief overview of these procedures and the results of their comparative tests in medical examinations of patients are given. Classifications are also proposed for procedures that determining total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in blood. The mechanism of action of the chemical reactions taking place in these procedures, the advantages and disadvantages, and prioritization of the field of their application are considered. Promising directions in the development and improvement of procedures are mentioned, ensuring more accurate measurements of the blood cholesterol concentration, and alternative means of determining cardiovascular disease risk are discussed.

Keywords: cholesterol, plasma, serum, blood, measurement, concentration, procedure, analysis, clinical diagnostic laboratory.

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1. RESEARCH IN THE 1990s

In 1990, Warnick et al. studied the effect of triglycerides on the determination of LDL cholesterol concentration in blood plasma samples by a procedure using the formula proposed by Friedewald et al. The relative error of measurement of the LDL cholesterol concentration did not exceed 10% for more than 90% of samples at a plasma triglyceride concentration of less than 2 g/L, for 72% of samples at a triglyceride concentration of 2–4 g/L, and for 39% of samples at a triglyceride concentration of 4–6 g/L [1].

In 1990, Sandkamp et al. determined the concentrations of total cholesterol and HDL and LDL cholesterol in blood serum during their medical studies. The total cholesterol concentration in serum was measured by a spectrophotometric procedure implemented in an automatic flow-through device. The determination of HDL cholesterol in serum was carried out by enzymatic spectrophotometry with preliminary sedimentation of VLDL and LDL with a solution of phosphotungstic acid and magnesium chloride. The LDL cholesterol concentration in serum was measured by the procedure based on the formula of Friedewald et al. [2].

In 1990, McNamara et al. also studied the effect on triglycerides on the determination of the LDL cholesterol concentration in blood plasma samples by the procedure using the formula developed by Friedewald et al. At a triglyceride concentration in plasma up to 4 g/L, a slight difference was observed between measurements of the LDL cholesterol concentration by this procedure and a procedure based on ultracentrifugation [3].
In 1990, Asayama et al. proposed an enzymatic spectrophotometric procedure for determining the concentration of HDL, HDL$_2$, and HDL$_3$ cholesterol in blood serum during their medical investigations. The serum was initially treated with solutions of heparin and manganese(II). The resulting mixture was centrifuged to remove sedimented VLDL and LDL, and the HDL cholesterol concentration in the blood serum was measured spectrophotometrically in one part of the centrifuged liquid ($c_{HDL}$). Another part of the centrifuged liquid was mixed with potassium bromide. The mixture was ultracentrifuged to separate HDL$_2$ and HDL$_3$, and the HDL$_2$ cholesterol concentration in serum was determined in the ultracentrifuged measurement liquid by spectrophotometry ($c_{HDL2}$). The HDL$_3$ cholesterol concentration in serum ($c_{HDL3}$) was calculated by the equation $c_{HDL3} = c_{HDL} - c_{HDL2}$. The mean-square deviation of the relative error of determination was 3.4% for the HDL$_2$ cholesterol concentration in serum and 5.9% for the concentration of HDL$_3$ cholesterol [4].

In 1990, Tetrault et al. assessed the results of the determination of total and HDL cholesterol in blood plasma samples obtained in 12 clinical diagnostic laboratories. In all laboratories, the total cholesterol concentration in serum was measured by enzymatic procedures. The determination of HDL cholesterol in serum was performed according to the procedures with sedimentation of LDL and VLDL with solutions of dextran sulfate, phosphotungstic acid or heparin, and manganese(II). The mean-square deviation of the relative error of measurement of the total cholesterol concentration in serum ranged from 1.4 to 4.2% (with an average value of 2.3%). The mean-square deviation of the relative error of the determination of the HDL cholesterol concentration in serum was at a level of 2.4–7.2% (with an average value of 4.3%). The relative systematic error of measurements of the total cholesterol concentration did not exceed 5% [5].

In 1990, Brown et al. determined the concentrations of total cholesterol and HDL, HDL$_2$, and HDL$_3$ cholesterol in blood plasma during their medical investigations. The total cholesterol concentration in plasma samples was measured by an enzymatic procedure. HDL cholesterol was determined in blood plasma ($c_{HDL}$) after centrifugation of plasma mixed with solutions of dextran sulfate and manganese chloride (to sediment LDL and VLDL). Measurement of the HDL$_3$ cholesterol concentration in blood plasma ($c_{HDL3}$) was performed after sedimentation of HDL$_2$ cholesterol from the centrifuged liquid during further treatment with solutions of dextran sulfate and manganese chloride. The HDL$_2$ cholesterol concentration in serum ($c_{HDL2}$) was calculated by the equation $c_{HDL2} = c_{HDL} - c_{HDL3}$ [6].

In 1990, Bachorik et al. compared the determinations of the total cholesterol concentrations in whole blood by an enzymatic spectrophotometric procedure (Procedure 1) and an enzymatic reflectance photometry procedure using test strips (Procedure 2). The mean-square deviation of the relative error of measurements of the total cholesterol concentration in blood was 1.3–2.1% by Procedure 1 and 4.0–7.1% by Procedure 2. The correlation coefficients between the determinations of total cholesterol in whole blood by Procedures 1 and 2 corresponded to 0.906 (blood from a vein) and 0.893 (blood from a finger) [7].

In 1990, Ellerbe et al. compared measurements of the total cholesterol concentration in blood serum by the following procedures:

- Spectrophotometric procedure with Liebermann–Burchard reagent (Procedure 1);
- Chromatography—mass spectrometry.

The discrepancy between the measurement results of total cholesterol in serum by these procedures was estimated at 1.6% [8].

In 1990, Greenland et al. compared the determination of the total cholesterol concentration in blood plasma and serum by an enzymatic procedure. The mean-square deviation of the relative error of the determination of the total cholesterol concentration in plasma and serum was less than 3% [9].

In 1990, Poon and Hinberg reported on the effect of sodium azide on the determination of total cholesterol in blood serum by an enzymatic reflectance photometry procedure using test strips (Table 1).