A Rapid Method for Cholesterol Determination with a Single Reagent

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A method for the determination of serum cholesterol, using a single reagent, is described. This method is simple, rapid, and economical, and prevents some of the errors inherent in the manipulative process of earlier procedures.

In 1953 Pearson et al. (1) described a method for the determination of serum cholesterol in which extraction and evaporation were not necessary and in which only a single test tube was employed. Subsequently Rappaport et al. (2) modified this method and used sulfosalicylic acid instead of p-toluensulfonic acid. Boetzeler et al. (3) later published a modification of Pearson's method, in which a mixture of all the reagents was added at once.

In our laboratory, we introduced a method which also requires only a single reagent. The density of the green color (Lieberman-Burchard reaction) is read in the Klett-Summerson photocolorimeter, using a 600-mg filter. In the normal range (140–280 mg./100 ml.), the values obtained with the new method are nearly identical with those obtained by the original method of Pearson and its modification by Rappaport.* If the serum is icteric, 5 mg./100 ml. cholesterol are subtracted for 1 mg./100 ml. bilirubin (3, 4). This correction is necessary as bilirubin forms a green color with the reagent. With different concentrations of standards the Beer-Lambert law is observed up to levels of 750 mg./100 ml. cholesterol.

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*At elevated cholesterol levels the new modification gives results 5–10% higher.
Reagents

Sulfosalicylic acid Dissolve 50 gm. in glacial acetic acid, and make solution up to a volume of 1 L. (Solution 1).

Acetic anhydride, reagent grade (Solution 2).

Sulfuric acid, conc., reagent grade (Solution 3).

Cholesterol standard Add 250 mg. cholesterol to 100 ml. absolute ethanol. It is advisable to purify the cholesterol utilizing three recrystallizations from absolute ethanol. Store in refrigerator.

The single reagent Prepare before use by mixing 35 ml. of the sulfosalicylic acid solution (Solution 1) with 65 ml. acetic anhydride (Solution 2). Add 10 ml. H$_2$SO$_4$ (Solution 3), constantly rotating. Cool the reagent to room temperature before use.

Procedure

Place 0.1 ml. serum, 0.1 ml. standard, and 0.1 ml. water (blank) into 3 test tubes. To each tube, add 5 ml. of the reagent combination mix, and read color after 10 min. in a photoelectric colorimeter at 600 m$\mu$. (Adjust the zero point to the blank.)

Discussion

During the past few years, determination of serum cholesterol has changed from a comparatively involved and time-consuming procedure to a simple and rapid technic which requires the use of only one reagent. The method presented in this paper has the advantages of simplicity and economy of time, glassware, and personnel, and avoids some of the drawbacks of earlier methods, such as errors from inaccurate pipetting, from insufficient mixing, and from inexact temperature control and timing. Because of the high dilution of the serum (1:50) and the proportionality of the absorbance up to relatively high values of cholesterol, an additional dilution for photocolorimetric reading is rarely necessary.

The use of the red filter, as well as the higher dilution of the serum, permits one to omit the correction (necessary in earlier methods) for noncholesterol chromogens and other interfering substances. Thus no correction need be made for slight hemolysis. However, correction must be made for the presence of bilirubin in the serum, as was
stated above. Consequently, a further simplification is obtained by the omission of all serum blanks. In comparison with former methods, the slight differences in the high values of cholesterol obtained by this new method present no problems in its use for clinical purposes.

References