sera with abnormal globulin concentrations. To test this possibility, we analyzed selected samples with greatly elevated globulin concentrations by the kinetic method, with use of bovine albumin standards, and by the comparative method. No significant differences were detected in this group of 12 samples.

The unique capabilities of the centrifugal fast analyzer for this type of analysis should be emphasized. Valid fixed-time kinetic analysis requires that all standards and samples be measured at exactly the same time after the reaction commences (4, 5). The centrifugal analyzer fulfills this requirement for all standards and samples on the same run as a result of its batch, parallel mode of operation. In addition, the desirable feature of blanking the entire sample-reagent mixture can only be fulfilled if that blank can be measured on each solution at an early time during the course of the reaction. Valid data can be taken on Centrifichem as early as 2 s after the reactions commence, thus uniquely eliminating the need for a separate disc for blanks [as was required on the GeMSEAC instrument described by Hatcher and Anderson (1)]

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References


Dual-Precipitation Method for Lipoprotein Quantitation: Improvement by Membrane Ultrafiltration of Lipemic Samples

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Plasma lipoproteins can be measured by the clinical laboratory after the major lipoprotein classes have been separated by two precipitation steps, one of which is the aggregation of very-low-density lipoproteins (VLDL) by the detergent, sodium dodecyl sulfate. Lipoprotein concentrations are expressed in terms of the cholesterol concentration within each class. However, incomplete removal of aggregated VLDL has led to serious analytical errors in a few lipemic subjects. We tested the efficacy of a membrane ultrafiltration step in removal of aggregated VLDL in three patients with plasma triglyceride concentrations of 540 to 12,560 mg/dl. Preliminary membrane ultrafiltration to remove the aggregated VLDL improved the precision of the method and is recommended for the analysis of grossly lipemic samples.

Practical techniques are needed for quantitative lipoprotein determination. We have reported a method for measuring lipoproteins, based on the separation of lipoproteins into classes by two chemical precipitation steps (1). The method, a simplification of a combined ultracentrifugation-precipitation technique (2), measures VLDL, LDL, and HDL cholesterol concentrations (CLDL, and CHDL, respectively). The dual-precipitation method circumvents ultracentrifugation and so brings lipoprotein quantitation within the capabilities of the usual clinical laboratory.

A critical step in this scheme is the aggregation and removal of VLDL in the presence of the detergent, SDS (Figure 1) (3). However, incomplete aggregation or poor separation of VLDL in lipemic samples, noted by Burstein and Scholnick (3) and in our own laboratory (1), leads to erroneously high CLDL and low CVLDL values in comparison with results of the ultracentrifugation-precipitation technique. To extend the usefulness of this method, we examined membrane ultrafiltration as a means for separating the SDS-VLDL aggregates, and find that it improves the precision with which lipoproteins can be measured in lipemic samples by dual-precipitation.

Materials and Methods

Three patients with gross lipemia and plasma triglyceride concentrations of 540 to 12,560 mg/dl were selected for this study. Two of them had type V hyperlipoproteinemia, the other type IV. Patient AB, whose plasma samples could not be analyzed accurately with the original dual-precipitation method (1), was included. All subjects had fasted for 12-15 h before plasma was collected. Venous blood was collected in EDTA, 1 mg/ml, at weekly intervals for 2 Nonstandard abbreviations used: VLDL, LDL, HDL: very-low-density lipoproteins, low-density lipoproteins, and high-density lipoproteins; CVLDL, CLDL, CHDL, cholesterol concentration in the foregoing lipoproteins; CT, total lipoprotein cholesterol; SDS, sodium dodecyl sulfate; TG, total plasma triglycerides.

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during therapy of hyperlipidemia.

The method of analysis is presented in Figure 1; details have been described previously (1). In this study, VLDL was separated by the three methods noted. Each sample was analyzed by ultracentrifugation, with subsequent tube slicing to recover the lipoproteins of density >1.006 g/ml (LDL + HDL). SDS aggregation of VLDL was followed either by routine centrifugation at 10 000 × g for 10 min and aspiration of the lower layer with a Pasteur pipette (original dual-precipitation method), or by membrane ultrafiltration. The cholesterol concentrations in the fractions indicated in Figure 1 were then determined. C_{LDL} and C_{VLDL} were calculated according to the relationships: C_{VLDL} = C_T - (C_{LDL} + C_{HDL}) and; C_{LDL} = (C_{LDL} + C_{HDL}) - C_{VLDL}.

In the membrane-ultrafiltration step, SDS-aggregated plasma was first subjected to routine centrifugation at 2000 rpm for 15 min. The lower layer was gravity-filtered through Whatman No. 1 paper to minimize subsequent clogging of the membrane ultrafilters. Cellulose nitrate filters of 0.5-μm nominal pore size were obtained from Sartorius, Göttingen, West Germany, or from Gelman Instrument Co., Ann Arbor, Mich. 48106. The membrane ultrafilters were held in syringe adaptors, also obtained from Gelman Instrument Co. Two mililitre filters were forced through the filter with hand-held 10-ml disposable Luer-Lock syringes and cholesterol was analyzed in the ultrafiltrate. C_{LDL} was determined in the clear supernatant fluid after VLDL and LDL were precipitated by adding heparin and manganous chloride (1).

Results

Figure 2 compares values for C_{LDL} and C_{VLDL} obtained by the dual-precipitation method, with and without membrane ultrafiltration, with those obtained by ultracentrifugation for VLDL separation. Without ultrafiltration, results of the precipitation and ultracentrifugal methods correlated poorly for both C_{VLDL} and C_{LDL}; as expected, the precipitation method underestimated C_{VLDL} and overestimated C_{LDL}. With preliminary membrane ultrafiltration, there was good correlation between results by the dual-precipitation method and by the ultracentrifugal method. Analysis of the individual data by paired t-test showed that the dual-precipitation method, even with membrane ultrafiltration, underestimated C_{VLDL} and overestimated C_{LDL}. Mean difference from the reference method was 31 mg/dl (P <0.01), acceptable for most clinical purposes.

The relationship between total plasma triglyceride (TG) concentration and VLDL cholesterol by the modified precipitation method was of interest. Calculations were made for the two lipemic patients, who were tested on numerous occasions during several months. In the patient with Type IV hyperlipoproteinemia and TG of 540 to 2025 mg/dl, the correlation between TG and C_{VLDL} was 0.93 (n = 10). The TG to cholesterol ratio, TG/C_{VLDL}, was 4.7 ± 1.0 (SD). In the second patient with Type V hyperlipoproteinemia and TG of 1656 to 11 000 mg/dl, the correlation coefficient was 0.77 (n = 11). In the latter instance the TG/C_{VLDL} ratio was higher and more variable (14.7 ± 6.6 SD). Thus, when the TG/C_{VLDL} ratio remained relatively constant, C_{VLDL} followed plasma TG concentration closely. The correlation was not as good when there were large differences in the relative content of TG and cholesterol in VLDL.

The dual-precipitation method can be used by the clinical laboratory with little additional equipment and standard wet chemical technique. Its limitations, however, must be recognized: (a) It requires accurate cholesterol analyses; (b) low values for C_{VLDL} and C_{LDL} may be imprecise because they are calculated by difference rather than by direct measurement; and (c) incomplete aggregation of VLDL by SDS or inadequate separation of the aggregates in markedly lipemic samples may lead to falsely low C_{VLDL} and falsely high C_{LDL} values. The last of these limitations can be surmounted, at least partly, by use of membrane ultrafiltration to remove aggregated VLDL. The calculated C_{LDL} values are still somewhat high by this method, and this should be taken into account in interpreting borderline values. C_{VLDL} values or TG concentrations may be used to follow lipemic subjects during therapy. In our experience C_{VLDL} is not an adequate substitute for accurate plasma TG determinations in screening for hyperlipoproteinemia.

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