the defining feature may be missed without special precautions to compensate for the instability of thiol compounds in blood.

One method for avoiding this mistake is to deproteinize the plasma or serum specimen before transport. In this study, rapid deproteinization preserved the disulfides as free analytes for at least 7 days in storage at −20 °C. However, plasma tHcy measurement is an even more effective method for assuring accurate diagnosis of the homocystinurias. After a week of storage without deproteinization, we found that virtually all tHcy could be recovered by a method of preparation that includes a reducing agent such as dithiothreitol (9). This procedure accommodates specimens transported by mail or courier at room temperature, as well as those in which the homocysteine disulfide might not be detectable for biologic reasons, without the danger of a missed diagnosis.

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Early Historical Milestones in HDL-Cholesterol Assay

To the Editor:

As discussed in a recent editorial in this journal (1), the establishment of the plasma HDL-cholesterol assay as a substantial negative risk factor in the development of coronary heart disease (CHD) has lead to many recent proposals for modifying or replacing the simple but elegant polyanion precipitation technique. Lest the contributions of the pioneers in this field get lost in the literature, a little historical perspective is warranted.

The technique originated in 1948 when workers noted that the addition of phenol to plasma caused a turbidity that was proportional to the concentration of total lipid (2). It was also noted that this effect was decreased in plasma from heparin-treated patients. Paradoxically, when heparin (a natural polyanion) was added to plasma in vitro in conjunction with phenol, an increase in turbidity was obtained. (This discrepancy was attributed to the fatty acids produced in vivo by lipoprotein lipase). Because a positive clinical correlation had already been made between LDL concentrations and CHD (3), in 1957 Scanu et al. (4) suggested that the technique could be a rapid, inexpensive tool to clinically monitor LDL.

Although Oncley et al. (5) showed that high molecular weight dextran sulfate (a synthetic heparinoid) could be used for the bulk isolation of β-lipoprotein from plasma in the absence of phenol, the first description of the precipitation technique still used today (in modified form) can be attributed to the French workers, Burstein and Samaille (6). The key to their innovation was the introduction of a complexing divalent cation (calcium, initially), which formed a cross-link between the polyanion and the lipoprotein macromolecule. They used an electrophoretic technique to verify separation of the α- and β-lipoproteins by this method. I was unable to find any reference to this seminal publication in the recent HDL literature. Sadly, to my knowledge, no clinical laboratory in those early days had the foresight to add the test to their lipid panel, although HDL was already considered to have a protective role against CHD (3). In 1964, a slightly modified version of the precipitation technique emerged (7), which we used to study lipoprotein metabolism in isolated liver perfusions (8).

The next milestone in the HDL-cholesterol story occurred in 1970 when Burstein et al. (9) reviewed the methodology and introduced sodium phosphotungstate/Mg2+, which are still the preferred reagents. The introduction in 1972 of the “Friedwald” formula to estimate plasma LDL concentrations gave laboratories a further justification to introduce the test because HDL-cholesterol concentrations were needed in the calculation. However, the technique still made little headway because of the popularity of the “Fredrickson” phenotyping, which was based on electrophoresis. After Hazzard et al. (10) found inconsistencies between the electrophoretic...
phenotypes and the familial hyperlipidemic disorders, laboratories gradually began to add LDL and HDL-cholesterol to their test menu by using the relatively simple “Burststein” method, which did not require expensive equipment or a pathologist’s interpretation.

During the next two decades, the findings of the famous long-term Framingham and Helsinki epidemiological studies confirmed the association between low HDL-cholesterol concentrations and CHD. This information coincided with the publication of numerous modifications to the polyanionic procedure, which resulted in improved reliability and specificity. Because the new technique required precise cholesterol measurements, the concomitant development of the new, enzyme-based cholesterol assay for automated analyzers made possible the establishment of reliable reference ranges for HDL-cholesterol assay. In 1982, the prolific Dr Burstein, in collaboration with P. Legmann (11), published an excellent monograph reviewing almost three decades of studies into the mechanisms of lipoprotein precipitation. The use of polyelectrolytes, polyphosphates, surface-active agents, and neutral polymers in the differential precipitation of plasma lipoproteins was thoroughly discussed.

Recently, homogeneous assays that do not require centrifugation have been proposed as cost-saving substitutes. However, until the long-term reliability of these proprietary reagents has been fully documented, I would caution against abandoning the simple polyanionic procedure that has withstood four decades of scrutiny and refinement. Because the HDL-cholesterol assay has the goal of long-term risk assessment, the test is most suited to a regional laboratory where large batches are prepared using robotic technology. The reliability of the assay in the presence of hypertriglyceridemia is mostly an academic issue, in my view, because any increased long-term risk of CHD posed by reduced HDL-cholesterol concentrations would be overshadowed by the ominous implications of a fasting triglyceride concentration in excess of 4000 g/L. The physician would likely concentrate his/her efforts into diagnosing and treating the cause of the lipid abnormality. The HDL assay can be performed at a later date, after the triglyceride concentration has been brought within range of the assay.

References

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