stabilize, and that “We have been unable to duplicate [these] observations when using various brands of folic acid reagent kits and testing retention samples of the subject control lots.”

Bovine Serum Albumin as a Cause of Misdiagnosis in Tay-Sachs Disease

To the Editor:

In the assay for hexosaminidase A (Hex A) in leukocytes, cultured fibroblasts, and cultured amniotic fluid cells for the detection of Tay-Sachs disease and carrier identification, bovine serum albumin (BSA) is used (1, 2). We find that some BSA preparations are contaminated with hexosaminidase activity. We wish to warn others of this and to show how an incorrect diagnosis could be reached by using contaminated BSA.

The 15 leukocyte samples used in the study had less than 50% Hex A when assayed with BSA (Pentex, Miles Laboratories) having no measurable Hex activity. Assay methods were as described by Kaback (2). The average total activity (Table 1) varied considerably between different BSA preparations; the average percent Hex A varied much less, although significantly.

When assayed for Hex activity, both Sigma products contained significant amounts; whereas, there was no measurable activity in the Pentex sample. Most of the BSA Hex activity was heat inactivated at pH 4.4 and 52 °C, indicating the presence of “A-like” activity. In addition, electrophoresis of the enzyme on cellulose acetate (Cellogel) resulted in a diffuse band of activity having “A-like” mobility.

Because cultured amniotic fluid cells and leukocytes utilize BSA in the assay procedures for Hex A, results could be disastrous if BSA were used that has hexosaminidase activity. A small amount of this “A-like” contaminant could result in the diagnosis of a “normal” couple when actually they were a carrier couple or in the diagnosis of a “carrier” fetus when actually there was no Hex A activity.

References

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Ed. note: A reviewer points out that this message is not new, but will bear repeating because it is mentioned more or less incidentally in the literature.

Quantification of High-Density Lipoprotein Cholesterol in Serum by a Heparin-Mnºº Precipitation Method

To the Editor:

We read with interest the article [Clin. Chem. 22, 1828 (1976)] by Bachorik et al., in which they compared the plasma high-density lipoprotein (HDL) cholesterol concentrations determined by heparin–Mnºº precipitation and by preparative ultracentrifugation. They state that our recent findings (1) on the interaction between HDL and heparin in the presence of Mnºº prompted them to re-examine the heparin–Mnºº procedure, which under their test conditions did not precipitate HDL from whole plasma.

We have been concerned with the problem of specificity of commonly used cations, especially Caºº, Mgºº and Mnºº, in lipoprotein–heparin (and other glycosaminoglycans) interactions. Unlike Caºº or Mgºº, the Mnºº ion produces complexes of varying insolubility between HDL and heparin. Among the subclasses of HDL, 40% of HDL₂ and 10% of HDL₃ can be precipitated in the presence of Mnºº. Therefore, the degree of insoluble complex formation is likely to be determined by the relative proportion of the HDL subclasses rather than a fixed proportion (25%) of HDL being precipitated in each case. It is likely that the magnitude of HDL–heparin interactions in whole plasma may be different from that in isolated fractions. Considering the measurement errors involved in ultracentrifugal isolation and cholesterol determination, the difference between the heparin–Mnºº and the ultracentrifugal procedure may be reasonably small whenever the HDL concentration of the plasma is not too high and HDL₃ is the predominant HDL component or subclass.

Given the wide density ranges of lipoprotein classes and their heterogeneity under different physiological and nutritional states, it is difficult to assume that virtually “all” HDL sediments at relative density 1.07. In human plasma, HDL₃ (hydrated density 1.05) occurs in varying quantities (2). Individual HDL-cholesterol values, by age and sex, and pair-wise statistical analyses of the differences between heparin–Mnºº and ultracentrifugal methods would have been helpful in this regard. It is pertinent to mention the recent observations of Krauss et al. (3) on HDL concentration in plasma of women taking oral contraceptives. While HDL-cholesterol measured after heparin–Mnºº precipitation of whole plasma did not differ significantly from controls, the values for total HDL and HDL₃ measured by analytical ultracentrifugation were higher in users. In unfractonated plasma, the heparin–Mnºº procedure is known to result in coprecipitation of other proteins (albumin, γ-globulins, and fibrinogen), including α-lipoprotein in abetalipoproteinemic patients (4). The authors have elegantly shown that the LDL and VLDL were satisfactorily precipitated by the heparin–Mnºº procedure, and Mnºº concentrations over a wide range did not cause a further decrease in cholesterol content of the HDL-containing supernant fractions. Unfortunately, this does not prove whether LDL was not also completely precipitated. Analyses of the heparin–Mnºº precipitate and supernate from the ultracentrifugal fraction for the presence of HDL (especially for the presence of apo-AI) could have provided further evidence of the desired fractionation.

Although, the heparin–Mnºº procedure can generally be used for estimation of HDL-cholesterol in unfractonated plasma, certain qualitative and (or) quantitative differences among HDL subclasses in an individual’s plasma may alter the specificity of the procedure. In a continuing effort to refine the lipoprotein method used in our laboratory (5–7), we have been comparing the lipoprotein cholesterol values in different groups of individuals (children and adults) using the heparin–Mnºº, ultracentrifug (d 1.063), heparin–Caºº, and other procedures. The mean HDL-cholesterol indicated reasonable agreement among these methods. However, a comparison of individual values showed that there were significant discrepancies.

Table 1. Hexosaminidase Activity in Leukocytes Assayed with Various BSA Preparations

<table>
<thead>
<tr>
<th>BSA</th>
<th>% Hex A activity</th>
<th>Total Hex A activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miles “Pentex Fraction”</td>
<td>44</td>
<td>979</td>
</tr>
<tr>
<td>V</td>
<td>62</td>
<td>1667</td>
</tr>
<tr>
<td>Sigma “Fraction V”</td>
<td>62</td>
<td>1667</td>
</tr>
<tr>
<td>Sigma “Crystallized &amp; Lyophilized”</td>
<td>47</td>
<td>1711</td>
</tr>
</tbody>
</table>

* Activity = nmol 4-methylumbelliferyl released per hour per milligram of protein. Values are average of 15 samples.