the use of this reagent or the commercial Beckman reagent, showed no significant bias. In addition, we observed no discrepancies in our quality control pool determinations or ADC numbers. We conclude that this creatinine reagent performs well and that the added diethylenetriamine provides a simple and effective means of preventing precipitate formation for at least one month.

References

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Demonstration of Heterogeneity in a High-Density Lipoprotein Subfraction

To the Editor:

High-density lipoproteins (HDL) in human serum are defined as lipoprotein particles isolated by preparative ultracentrifugation from plasma in the hydrated density range 1.063–1.21 g/mL. A previous study (1) reported the separation of HDL by density gradient ultracentrifugation into three subfractions: HDL2a, HDL3b, and HDL3c. Lipoproteins separated by ultracentrifugation in the density range 1.063–1.21 g/mL and lipoproteins having alpha-lipoprotein electrophoretic mobility are considered to be similar. However, a direct comparison has not been reported.

In this study, we subjected each of the three HDL subfractions, separated by ultracentrifugation, to electrophoretic analysis by a sensitive agarose gel electrophoretic technique (2, 3). The electrophoretic patterns of subfractions HDL2a and HDL3c demonstrated a single lipoprotein band with alpha electrophoretic mobility. Two lipoprotein bands were clearly visible in the electrophoretic pattern of the HDL3b subfraction; one with alpha and the other with pre-beta electrophoretic mobility. The demonstration of two electrophoretic bands in the HDL3b subfraction indicates an inherent heterogeneity within HDL particles of density 1.063–1.10 g/mL.

Methods. Blood was collected in EDTA (1 g/L solution), and plasma was separated by centrifugation at 4 °C. Plasma cholesterol, triglycerides, and lipoprotein cholesterol were determined by standard Lipid Research Clinics methodology (4). HDL subfractions: HDL2b (density, 1.063–1.10 g/mL), HDL2c (d, 1.10–1.125 g/mL), and HDL3c (d, 1.125–1.21 g/mL) were isolated by sequential ultracentrifugation as previously described (1). HDL were also isolated in the analytical ultracentrifuge and HDL2b, HDL2c, and HDL3c as total lipoprotein concentration, were quantitated as previously described (5).

The agarose gel electrophoretic technique sharply resolves the lipoprotein fractions and demonstrates additional subfractions (2, 3).

Results. Plasma samples from four fasting normal subjects were analyzed. Table 1 shows plasma lipid and lipoprotein cholesterol values for each. In addition, total lipoprotein values are given for HDL2b, HDL2c, and HDL3c. These determinations include all protein, and lipid constituents within these density fractions. In these subjects, HDL3b averages 13.4 (SD 7.9)% of total HDL.

Figure 1 shows typical lipoprotein electrophoretic patterns of a plasma sample, the 1.063 g/mL supernate, and the HDL2b, HDL2c, and HDL3c subfractions. The electrophoretic patterns of the original plasma shows one beta, one pre-beta, and two alpha lipoprotein fractions; the 1.063 supernate shows one beta fraction, and a trace of the pre-beta band. The patterns of the HDL2b and HDL3c subfractions demonstrate a single lipoprotein band with alpha electrophoretic mobility, but two lipoprotein bands are clearly evident in the electrophoretic pattern of the HDL3b subfraction, one with alpha- and the other with pre-beta electrophoretic mobility. This latter lipoprotein species generally represents less than half of HDL3b mass.

Discussion. Interest in HDL derives from the finding that increased concentrations of HDL cholesterol in serum have been associated with a decreased risk of atherosclerotic cardiovascular disease (ACD). The separation of HDL by ultracentrifugation into three density fractions HDL2b, HDL2c, and HDL3c was followed by population studies that suggest that fluctuations in HDL concentrations are associated with the HDL2 component (6). The demonstration by our electrophoretic technique of two lipoprotein fractions within HDL2b, one with alpha mobility and the other with pre-beta mobility, is of particular importance in view of the opposite roles assigned to these two lipoproteins in the development of ACD.

Plasma HDL (d, 1.063–1.21 g/mL), as isolated by ultracentrifugation, have generally been considered to have alpha mobility on electrophoresis. However,

| Table 1. Serum Lipid Analyses for the Four Normal Subjects |
|------------------|------------|----------------|----------------|----------------|
|                | Plasma     | Lipoprotein cholesterol | Total lipoprotein |
|                | Chol.      | Trig. | VLDL | LDL | HDL | HDL2b | HDL2c | HDL3c |
| 1380           | 800        | 120   | 890  | 370 | 180 | 720   | 1380  |
| 2520           | 1240       | 240   | 2070 | 450 | 220 | 1070  | 1380  |
| 2390           | 1340       | 150   | 1840 | 400 | 590 | 810   | 970   |
| 1630           | 710        | 90    | 110  | 430 | 310 | 1140  | 1120  |

Fig. 1. Lipoprotein electrophoretic patterns of plasma (A), the 1.063 g/mL supernate (B), and of the component HDL subfractions HDL2b (C), HDL2c (D), and HDL3c (E)
Table 1. Apparent Xylose Concentrations Produced by Added Glucose

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, xylose-free</td>
<td>Serum, + 3.34 mmol xylose per liter</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>Serum, + 3.34 mmol xylose and 5 mmol glucose per liter</td>
</tr>
<tr>
<td>0.55</td>
<td>3.48</td>
</tr>
<tr>
<td>0.34</td>
<td>3.17</td>
</tr>
<tr>
<td>Serum, + 3.34 mmol xylose and 10 mmol glucose per liter</td>
<td>Serum, + 3.34 mmol xylose and 20 mmol glucose per liter</td>
</tr>
<tr>
<td>3.69</td>
<td>3.75</td>
</tr>
<tr>
<td>3.35</td>
<td>3.48</td>
</tr>
<tr>
<td>Urine, collected during a</td>
<td>Urine, + 50 mmol xylose per liter</td>
</tr>
<tr>
<td>xylose test</td>
<td>Urine, + 100 mmol xylose per liter</td>
</tr>
<tr>
<td>3.90</td>
<td>108</td>
</tr>
<tr>
<td>3.85</td>
<td>107</td>
</tr>
</tbody>
</table>

Assessment of a New Method for Determination of Xylose in Serum and Urine

To the Editor:

Recently, Eberts et al. (1) described a method for determining D-xylose in blood and urine with use of phloroglucinol. Because this method is faster than the method of Roe and Rice (2) and because they claim that there is little cross reactivity of glucose, we tested this method in our laboratory. According to Eberts et al., glucose, added in a concentration of 5.6 mmol/L (1 g/L), caused an apparent xylose concentration of 0.08 mmol/L. We investigated the influence of this and of higher concentrations of glucose on the determination of xylose.

To determine the concentration of xylose in urine, we had to modify the method of Eberts et al., because of the high absorbance of the final solution. Instead of 5 μL of undiluted urine, we used 50 μL of a 50- or 100-fold diluted urine with a xylose concentration typical of that found during a xylose absorption test. In addition, we compared the results obtained with the methods of Eberts et al. and Roe and Rice in serum, with and without added xylose and glucose.

The results (Table 1) show that with the method of Eberts et al. the apparent xylose concentration in xylose-free serum containing about 5 mmol of glucose per liter is six to seven times that found by these authors, and increases with increasing glucose concentrations. A similar increase in apparent xylose concentrations is found with the method of Roe and Rice. Glucose in urine does not interfere with the xylose determination, probably because of the high dilution.

Eberts et al. suggested determining the apparent xylose concentration of serum before xylose is administered to the patient, to allow for the influence of non-xylose constituents, but even then one should consider with caution the results of xylose determinations in serum of (unstable) diabetic patients. Nevertheless, we find the method of Eberts et al. to be a rapid, simple way of determining xylose concentrations in serum and urine.

References


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Improved Sample Treatment before Liquid-Chromatographic Determination of Anticonvulsants in Serum

To the Editor:

Several “high-performance” liquid-chromatographic methods have been published for simultaneously determining concentrations of phenobarbital, phenytoin, primidone, ethosuximide, and carbamazepine in serum on a reversed-phase column (e.g., 1). I have experienced difficulties with most of these published procedures in the sample-pretreatment step, which generally calls for extraction of the drug from the sample with acetonitrile or some other organic solvent. I find such sample pretreatment unacceptable because of the number of substances extracted that produce irrelevant and interfering peaks.

By using a small reversed-phase Sep-Pak* column (Waters Associates, Inc., Milford, MA 01757) to separate the anticonvulsant drugs from the serum matrix, a “clean” sample is obtained, ready for analysis. The procedure is as follows:

Attach a Sep-Pak to a 20-mL glass syringe and wash it with 5 mL of acetonitrile, followed by 20 mL of 10 mmol/L HCl. Then add 0.5 mL of serum (also any internal standard) to 10 mL of 10 mmol/L HCl mix, and pass it through the Sep-Pak, followed by a 20-mL wash of 10 mmol/L HCl. Pass 2 mL of acetonitrile through the column to elute the anticonvulsant drugs and collect the eluent in a 3-mL sample vial. Evaporate this eluent to about 0.5 mL at room temperature, under nitrogen gas. The sample is then ready for analysis.

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198 CLINICAL CHEMISTRY, Vol. 27, No. 1, 1981