E, and 4.7% for DA (n = 10); intra-assay CVs were 4%, 3.8%, and 5%, respectively (n = 10). Analytical recovery for each analyte was 64% (SD 4%; n = 10).

"Normal values" so obtained were:

\[
\begin{align*}
NE &= 231.5 \pm 33.8 \text{ pg/mL (1.37 \pm 0.21 pmol/mL)} \\
E &= 56.7 \pm 25.5 \text{ pg/mL (0.31 \pm 0.14 pmol/mL)}
\end{align*}
\]

same range as Foti et al. (3), and

\[
DA = 39.9 \pm 13.3 \text{ pg/mL (0.21 \pm 0.07 pmol/mL)}
\]

The specific advantage of this method is the simultaneous measurement of NE, E, and DA in plasma, also valid for low concentrations.

References

Modified Assay for Blood Coagulation Factor XIII with Use of Antibody for Enzyme Immobilization, Terry C. Whyard and Richard J. Ablin (Immunology Unit, Dept. of Urology, State Univ. of New York at Stony Brook, Stony Brook, NY 11794-8093)

We developed a modified enzyme assay for blood coagulation Factor(F) XIII (EC 2.3.2.13) to complement the established conventional filter paper assay (1). We used the concept of enzyme immobilization (retaining the enzyme in a stationary phase, while permitting biological activity and allowing conformational changes) to facilitate detection of the FXIII in a mixture of serum proteins. *Staphylococcus aureus* (Staph-A) armed with specific antibody was used to remove the FXIII selectively from the sample mixture. Important advantages of this modified assay appear to be: (a) increased sensitivity as compared with the conventional filter paper assay and (b) alleviation of the necessity for the inactivation of fibrinogen, as required by the conventional filter paper assay (2) or other assays for the determination of FXIII.

Staph-A (Calbiochem, La Jolla, CA; lot no. 645032) was armed with antibody reactive to Factor XIII A-subunit or S-subunit (Calbiochem, lots 702581 and 703581). After three wash cycles in buffer (Tris HCl, pH 8.0, 150 mmol of NaCl and 5 mmol of EDTA per liter), the armed Staph-A was reacted with the test material. After incubation at room
temperature for 30 min and three additional wash cycles, the enzyme, now in a stationary solid phase, was added to a radioactive amine substrate mixture and incubated (I, 2). The FXIII activity was then evaluated by measuring the amount of radioactive amine incorporated (I, 2).

Representative data, comparing the conventional filter paper assay with the modified Staph-A filter paper assay for FXIII, are presented in the following tabulation.

<table>
<thead>
<tr>
<th>Sample tested</th>
<th>Conventional filter-paper assay (a)</th>
<th>Modified filter-paper assay (b)</th>
<th>Sensitivity increase (v = a/b, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Human VIII</td>
<td>0.45 ± 0.05</td>
<td>6.82 ± 0.42</td>
<td>1415%</td>
</tr>
<tr>
<td>Normal human plasma</td>
<td>6.78 ± 0.24</td>
<td>30.20 ± 1.38</td>
<td>345%</td>
</tr>
</tbody>
</table>

*Mean of two replicate counts per sample.
*Using Staphylococcus aureus armed with rabbit anti-human FXIII S-subunit.
*New York Blood Center, New York, NY, lot L15-84, adjusted to 1 unit/ml.

Interassay variation of the modified assay appears to be a function of antibody specificity. For example, evaluation with an antibody to the S-subunit of FXIII was observed to be approximately two times more sensitive than antibody to the A-subunit of FXIII (data not shown).

The protein that has been captured by antibody may be further conveniently assayed by electrophoresis on polyacrylamide gel.

References

Absorbance was measured at 520 nm instead of 500 nm. This change does not alter results, as shown by the good correlation between theoretical (x) and measured (y) DMF concentrations: y = x – 0.406 (r = 0.98; n = 19).

To evaluate within-run precision, we used two batches of Precinorm U control serum, with the following results for fructosamine concentrations: 2.8 ± 0.02 (n = 8, CV = 0.9%) and 3.4 ± 0.03 (n = 8, CV = 1.1%) mmol of DMF equivalents per liter.

Between-run precision was evaluated during three months by assaying Precinorm U. The results: 2.6 ± 0.05 mmol/L (n = 30, CV = 1.8%).

Accuracy of the method was examined by measuring the serum fructosamine reagents, with the following results: y = 1.006x – 0.051 mmol/L (r = 0.99; n = 12).

A comparison between results by the present method (y) and those obtained with the Cobas Bio centrifugal analyzer with use of the Glycoprobe GSP kit protocol (x) resulted in the following regression equation: y = 0.98x – 0.08 mmol/L (r = 0.97; n = 22).

The normal reference interval for this method is 1.8 to 2.2 mmol DMF equivalents per liter. Standard reagents given in the Glycoprobe GSP kit are stable for only two weeks after reconstitution. This modified procedure is well suited for laboratories that do few such analyses, because only one of the three standard reagents is used at a time.

References

GlycoProve Fructosamine Kit Used with the Rotochem CFA 2000 Centrifugal Analyzer, P. M. Dancé, J. Rousseaux, and M. Doutrevaux (Lab. de Biochimie, Hôpital B, Centre Hospitalier Regional, Lille Cedex, France)

A new approach for monitoring diabetes mellitus is determination of fructosamine concentration in serum (I, 2). We have adapted a fructosamine method (GlycoProve GSP kit; Eurobio, Paris, France 75005) for use on Rotochem CFA 2000 Centrifugal Analyzer (Kontron Analytique, Velizy, France 78140). This method is derived from the one initially described by Johnson et al. (3). Serum and standard are incubated with tetrazolium in the presence of a high molarity bicarbonate buffer, excluding the interference caused by the pH.

We modified the manufacturer's protocol as follows. The reaction was calibrated with one of the three serum fructosamine reagents given in the kit. For quality control we used DMF (1-deoxy-1-morpholinofructose) as the primary standard and Precinorm U batches (Boehringer Mannheim, Mannheim, F.R.G.). This control serum, with no assigned values for DMF, is a very stable control serum.

Use of Overlap Frequencies between Normal and Pathological Ranges to Compare Affinity Chromatography with Ion-Exchange Chromatography for Determining Glycated Hemoglobin, F. Ziegler, C. Boileau, C. Paulin, J. A. Alexandre, and P. Giraudet (Lab. Central de Biochimie, Hôpital Ambroise Paré, 92104 Boulogne Cédez, France)

Glycated hemoglobin (GHB) is being monitored with increasing frequency for long-term management of diabetes mellitus (I, 2). GHB is usually measured by either ion-exchange chromatography (IEC) or affinity chromatography (AC) (2–4). Because AC seemed to have fewer drawbacks than IEC, we compared the two methods, analytically and clinically, including a particular study of the overlap frequencies between normal and pathological ranges, given that the two methods do not measure the same molecular species (2).

The population studied included 100 diabetic patients and 30 control subjects. For both methods we used minicolumns: for AC the Glyc-Hb Kit (Merck, Darmstadt, F.R.G.); for IEC, the HbA1c Kit (Bio-Rad, Richmond, CA). Results for GHB by the AC method (y) correlated well with HbA1c results by the