The three proteins also appear in plasma from the 14-week-old SHR. We therefore used these animals in subsequent studies because they are easier to bleed. After a 2-min equilibration time, the third protein appeared in all of the 16 animals tested but in none of the 15 whose samples were equilibrated for 60 min. In addition, with the 60-min equilibration, the two proteins described by Cloix et al. were evident in only nine of the 15 animals we tested.

The SHR of the Okamoto-Aoki strain were obtained from Taconic Farms, Germantown, NY. The mean systolic pressure was 207 mmHg for the 14-week-old SHR as measured by cuff sphygmomanometry of the tail.

Evidently, the various technological steps involved in 2D- PAGE should be considered carefully so as to retain proteins. The fact that we used less protein per gel than did Cloix et al. (per gel, 5 μL of plasma containing 0.4 mg of protein as determined by a modification of the biuret method (8) vs their use of 15 μL of plasma containing 1.05 mg of protein) eliminates the possibility that the third protein appeared simply because we were using more protein.

Rather, the time allowed for equilibration appears to be the critical factor. Its importance has previously been pointed out. Prolonging the equilibration of the IEF gel to 30 min may result in as much as 40% of the protein in the IEF gel being lost (9, 10).

Thus, the detection of a third protein in the plasma of the SHR not only is important from the pathophysiological and biochemical (e.g., protein purification) point of view; it also emphasizes the importance of attention to technical details in 2D-PAGE.

We thank the Women’s College Hospital Research Fund for supporting this research, and Mr John Hendricks for the photography.

References

Stratus Fluorometric Enzyme Immunoassay System Evaluated for Determination of Total Thyroxin, Gail E. Stahlschmidt, Carl H. Smith, Ralph E. Miller, and Michael Landt (The Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, Children’s Hospital, St. Louis, MO 63110)

RIA is relatively simple, but it is slow and labor-intensive, owing to the need for duplicate analysis, frequent standardization, and long incubations. American Dade (Miami, FL 33152) has developed a solid-phase sequential saturation assay (“Stratus”) for measurement of thyroxin (T4). The manufacturer claims the calibration curve is stable for up to 14 days and that high precision obviates duplicate analysis.

To assess this assay, we assayed for T4 from specimens from patients (newborns to 14 years old) who were undergoing evaluation of thyroid status, using the Stratus fluorometric analyzer according to the manufacturer’s specifications. For comparison we also radioimmunoassayed T4, using reagents provided in the “Total T4,” kit (Clinical Assays, Cambridge, MA) in duplicate. In a comparison of 120 specimens, a coefficient of correlation of 0.924, with a slope and intercept of 0.93 and 3.2 μg/L, respectively, resulted.

Intra-run precision measurements made with use of quality-control materials with mean T4 concentrations of 39 and 145 μg/L (n = 20) produced CVs of 8.1% and 8.0%, respectively, for the Stratus. In day-to-day precision measurements, CVs were <8% at increased or normal T4 concentrations, but precision decreased at low T4 concentrations (CV = 12.2% at T4 = 42 μg/L). In comparison, duplicate analysis by RIA yielded CVs ≤7% for T4 concentrations of 21 to 151 μg/L.

Analytical recovery studies (vs weighed authentic T4) (n = 4) produced a correlation coefficient of 0.997, with slope and intercept 0.94 and 0.3 μg/L, respectively, and close agreement for concentrations up to 200 μg/L, but recovery was <90% at 240 μg/L.

Measured values for T4 were unaffected by added hemolysate (hemoglobin up to 13 g/L) or calf intestinal alkaline phosphatase up to 5000 U/L. With T4 at 89 μg/L, bilirubin
concentrations of 90 and 180 mg/L produced positive interferences of 8% and 16%, respectively. At 45 μg of T₄ per liter, a nonlinear 13–20% increase was produced by addic triiodothyronine over the range of 2.5–10 μg/L.

A preliminary cost comparison estimated a current cost of $3.47 for each Stratus reportable result vs $5.07 for RIA:

<table>
<thead>
<tr>
<th>Stratus cost (per reportable patient result)</th>
<th>RIA cost</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Consumables</strong></td>
<td><strong>$1.83</strong></td>
</tr>
<tr>
<td>Equipment</td>
<td>1.08</td>
</tr>
<tr>
<td>Personnel</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Total costs</strong></td>
<td><strong>$3.47</strong></td>
</tr>
<tr>
<td><strong>$5.07</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Based on 478 specimens in 25 runs/month, six controls/run, 5% repeats; biweekly calibration on Stratus, every-run calibration and duplicate analysis by RIA.

The Stratus procedure appears to be sufficiently accurate for use in the routine clinical laboratory. Standardization is infrequently needed, unstable radioactive reagents are not required, and our cost comparison indicated that the method is economically competitive. However, precision using single analysis was somewhat less than RIA duplicate analysis, and linearity was lost at T₄ concentrations >200 μg/L.

Evaluation of IMACK-MB, an Immunologoic Extension Assay for the MB Isoenzyme of Creatine Kinase, Kenneth Emancipator, Gina Bradford, and M. Desmond Burke (University Hospital L3-532, SUNY at Stony Brook, Stony Brook, NY 11794-7300)

A new commercial kit has been introduced for measuring the cardiac isoenzyme (CK-MB) of creatine kinase (CK; EC 2.7.3.2). A single monocular antibody, which is specific for CK-MB and does not inhibit its activity, is immobilized on a plastic bead and used to extract CK-MB from the sample (1).

We assayed 101 patients' samples and commercial control sera for CK-MB, using the IMACK-MB kit (International Immunoassay Laboratories, Santa Clara, CA 95054); for CK isoenzymes, using the Paragon electrophoresis system (Beckman Instruments, Brea, CA 92621); and for total CK (at 37 °C, with N-acetylcysteine as activator; normal reference interval 35–232 U/L). CK-MB activity, determined by multiplying total CK activity by the proportion of MB as determined by electrophoresis (x), was compared, by linear regression, with the activity [measured in "equivalent units per liter"; EU/L (2)] as determined with the IMACK-MB (y). Electrophoresis results were considered positive if the proportion of MB was ≥4%. IMACK-MB results were considered positive if they exceeded the y-intercept of the regression line and were >0.04m times the total CK, where m is the slope of the regression line. We checked the clinical diagnoses when the two procedures gave discordant qualitative results. The clinicians were unaware of the IMACK-MB results.

The regression-line equation was $y = 0.205x + 4.4 \ (r = 0.97)$. The mean for a control assayed 11 times by IMACK-MB was 7.2 (SD 0.7) EU/L. In our laboratory, electrophoresis has an interassay CV of 16%. There were 49 concordant positive and 42 concordant negative results. There were four false negatives and one false positive by IMACK-MB, as compared with three false negatives and two false positives by electrophoresis. For each false negative by IMACK-MB, there was a true-positive result for a previous or subsequent patient's sample. The IMACK-MB performed well enough in this evaluation to replace electrophoresis for measurement of CK-MB.

References