Table 2. Results of Analysis of Variance

<table>
<thead>
<tr>
<th>Variation</th>
<th>Mode A</th>
<th>Mode B</th>
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</thead>
<tbody>
<tr>
<td>Between kit</td>
<td>23.0%</td>
<td>6.2%</td>
</tr>
<tr>
<td>Within kit</td>
<td>18.2%</td>
<td>7.6%</td>
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</table>

*The between-kit and within-kit variation was calculated from analysis of variance as described (5) and expressed in terms of coefficient of variation.*

The table summarizes the results of analysis of variance. The "between-kit" variation by the kit standards (Mode A) was 23.3% in terms of coefficient of variation (CV) and the "within-kit" variation was 18.2%. The mean square for the treatment term from which the component of "between-kit" variation was derived significantly (p < 0.05) exceeded the error term from which the "within-kit" variation was derived. The "between-kit" variation by the assay in which the common standards were used was 6.2% and the "within-kit" variation was 7.6% (Mode B), when expressed in terms of CV.

The mean square for the treatment term was not significantly different from that for error term. Thus, use of the common standards significantly decreases the "between-kit" variation.

The common standards improved the precision of the assay in some of the kits. The endogenous CV obtained from the precision profile of kits b and f was smaller when determined by use of the common standards than by kit standards, whereas that of kit e was smaller when determined by kit standards than by the common standards.

The present results make it clear that the common standards were effective in reducing the "between-kit" variation greatly and the "within-kit" variation to some extent. The same effect can be expected in other radioimmunoassays in addition to that for insulin. For example, variations in the assay results for gastrin according to kit were often experienced. Our unpublished observations disclosed that the major cause of the variations was the difference in the concentration of standards. The same may be true of kits for assay of thyrotropin, thyroxin, and cortisol, for which the "between-kit" variation exceeds the "within-kit" variation (1). In fact, it was shown by Wood et al. (5) that the variation of thyroxin and triiodothyronine assays became smaller when the common standards were used.

These results emphasize the importance of establishing international standards for the radioimmunoassay kits that are commercially available in many different countries.

References

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Improved Measurement of Uric Acid in Human Urine by Reversed-Phase Liquid Chromatography

To the Editor:

Recently, we described a reversed-phase liquid-chromatographic procedure for quantifying uric acid in serum (1). When we used the system to measure uric acid in urine, we found that other compounds were eluted close to uric acid. Other authors have also reported that reversed-phase liquid chromatographic systems designed for serum are not directly applicable to urine (2). To avoid interfering substances, a sample-preparation step involving pumping of urine through a "SEP-PAK C18" cartridge (Waters Associates Inc., Milford, MA 01757) has been recommended (2). However, if a liquid-chromatographic method is to be used in routine work in clinical chemistry, sample-preparation procedures must be simple: indeed, injections of diluted urines directly into the liquid chromatograph are desirable.

Reversed-phase liquid chromatography with octadecylsilane (C18) as the functional component of the column has been used in previous work for the separation of uric acid in serum and urine, with spectrophotometric detection (see 1 for references). We now describe an isocratic reversed-phase system with octyl groups covalently bound to the silica particles, i.e., with octylsilane (C8) as the functional component of the column. This modification, an increased surface polarity of the column packing material, resulted in pure chromatograms for uric acid in urine samples, and diluted urines could be injected directly into the chromatographic system.

The materials and liquid-chromatographic instruments were the same as described (1), except that we used the variable-wavelength detector SpectroMonitor III (Laboratory Data Control, Riviera Beach, FL 33404) and integrator Model 3390A (Hewlett-Packard, Avondale, PA 19311). Xanthine oxidase (EC 1.2.3.2) was obtained from Sigma Chemical Co., St. Louis, MO 63178.

Figure 1A shows the chromatogram, obtained at room temperature, of a diluted urine sample with a column (25 x 0.46 cm) packed with LiChrosorb 10 RP, manufactured by E. Merck, Darmstadt, F.R.G. This packing material has an average particle diameter of 10 µm, and octyl groups are covalently bound to the silica matrix. A 7.5 x 0.2 cm precolumn, used to protect the analytical column, was packed with octadecylsilanized LC-8 from Supelco S.A., 1299 Crans, Switzerland. This particle has a diameter of about 40 µm. The mobile phase was a 40 mmol/L solution of sodium acetate, pH 5.0. After incubation of the sample with uricase (EC 1.7.3.3), the uric acid peak was no longer observed (Figure 1B). Xanthine, the precursor of uric acid in the biosynthesis of uric acid, has a retention time of 4.5 min in this chromatographic system (Figure 1C). The identity of this peak was verified by incubation with xanthine oxidase. After incubation with the enzyme, a peak with the retention time 2.8 min, the same as uric acid, was observed (Figure 1D). The following compounds were also tested for possible interference in the system: hypoxanthine, 1,7-dimethylxanthine, 3-methylxanthine, creatinine, ascorbic acid, and allantoin. None of these compounds influenced either the chromatographic quantitation of uric acid or the analytical recovery.

The analytical recovery of a uric acid standard (stock solution, 1000 µmol/L) added to a diluted urine was 100.4% (concentration of uric acid in diluted urine before addition: 124 µmol/L; expected concentration after addition: 562 µmol/L; concentration measured: 564 µmol/L). The within-run coefficient of variation of the present method is 0.5% (n = 12, mean conc 332 µmol/L), which
When the xanthine zmoL/L, for the same range in plasma, the CV was 1.0% daily of the normal system; the CV of the same range in plasma, the CV was 1.0% daily of the normal system. The CV of the normal range was determined at least in quadruplicate as digoxin-equivalent concentration, by comparison with the standard curve for digoxin provided with the kits, which were constructed and used according to the manufacturers' instructions. Radioactivity was counted with an Auto Logic gamma counter (Abbott). Calibration curves for digoxin and concentrations of immunoreactive DHD were determined by use of a custom-made program in an IBM 5110 computer.

As Figure 1 shows, the DHD bound anti-digoxin antiserum minimally in all tested kits. Figure 1 shows the cross-reactivity between the true DHD concentration and the apparent digoxin concentration, as read from the digoxin calibration curve.

In this study we found minor differences among the kits of different manufacturers and among different batches of the same brand, but none of the tested RIA kits showed a significant cross-reactivity to DHD. These results agree with most of the previous observations (e.g., 5), but are at variance with other studies, where cross-reactivity as great as 30% had been reported (3, 6–8). Different immunological and purification procedures are probably the cause of this disagreement. The greatest cross-reactivity observed (about 11% in

Because the formation of DHD and its consequent renal excretion (3, and unpublished ms.) may vary from patient to patient, bioavailability data obtained by means of urinary recoveries may also yield variable information, according to the cross-reactivity of DHD with the RIA kit used: kits with high cross-reactivity would overestimate the bioavailability of digoxin.

In view of the clinical relevance of the problem, we evaluated the cross-reactivity to DHD of commercially available RIA kits for digoxin. We purchased digoxin-I-125 RIA kits through the Italian subsidiaries of the following manufacturers: Abbott Diagnostica, N. Chicago, IL 60064; Becton-Dickinson Immunodiagnostica, Orangeburg, NY 10962; Diagnostic Products Corp., Los Angeles, CA 90064; Mallinckrodt, Dietzenbach, F.R.G.; New England Nuclear, Boston, MA 02118; and Sorin, Saluggia, Italy. When available, both normal and solid-phase kits were tested. At least two kits from the same manufacturer, but with different antibody batch numbers, were tested between January and April 1982. DHD (99% pure at chromatographic analysis; Boehringer, Mannheim, F.R.G.) was dissolved in water/ethanol (70/30 by vol) and appropriately diluted before each assay to obtain standards of 2, 5, 10, 20, 50, and 100 ng/mL. From each dilution, 1 mL was dispensed into neutral glass tubes, lyophilized, reconstituted to a volume of 1 mL with blank plasma, and assayed. Each DHD concentration was determined at least in quadruplicate as digoxin-equivalent concentration, by comparison with the standard curve for digoxin provided with the kits, which were constructed and used according to the manufacturer's instructions. Radioactivity was counted with an Auto Logic gamma counter (Abbott). Calibration curves for digoxin and concentrations of immunoreactive DHD were determined by use of a custom-made program in an IBM 5110 computer.

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