results, we repeated this recovery experiment, using 18 serum samples from postmenopausal women who were not taking exogenous estrogens (native estradiol concentrations undetectable to 43.7 pmol/L; native SHBG 14 to 113 nmol/L). A similar relationship between SHBG and estradiol recovery was found:

Percentage recovery of estradiol = 75.6 - (0.408 · SHBG)

Standard error of the slope = 0.1504, P = 0.015

The standard error of the slope was larger in the second experiment because of two outlying data points for which percentage recovery was high. We could find no explanation for these outliers. Omitting the outliers reduced the standard error of the slope (to 0.0889) and slightly increased the gradient (to 0.532), but there is no substantial difference between the results from the two experiments.

We conclude that SHBG variations within the physiological range have an important effect on this assay.

Reference


In serum of persons receiving dexamethasone (0.5 mg four times a day) to suppress endogenous cortisol, we measured unexpectedly high concentrations of cortisol during a 5-h infusion of hydrocortisone hemisuccinate (Solu-Cortef, Upjohn) at 90 μg/min. As measured with an RIA kit from New England Nuclear (NEN, Boston, MA), cortisol steady-state was apparently reached after only 1 h, the plateau concentration being 3650 nmol/L. The expected values were 3.5 h and 700 nmol/L, respectively, according to the well-known pharmacokinetic parameters. We therefore considered cross-reactivity with hydrocortisone hemisuccinate highly probable.

Indeed, results of the study (Figure 1) indicate that the cross-reactivity of hydrocortisone hemisuccinate exceeded 720%. Thus the affinity of the antisera in the RIA kit appears to be greater for the hemisuccinate form than for cortisol itself. Because the hemisuccinate dilution curve parallels that of cortisol, assay of dilutions of plasma samples would not allow one to detect such a cross-reacting contaminant. This marked cross-reactivity has been repetitively observed over the last two years (lot no. of presently supplied antisera is PH-677). On the basis of these results, we evaluated the interaction of two other hydrocortisone derivatives routinely administered, fludrocortisone acetate (Florinef, Squibb) and hydrocortisone acetate. These compounds also showed high cross-reactivity, 340% and 810%, respectively.

Determination of cortisol concentrations is rarely indicated during acute corticoid therapy, but the above-mentioned hydrocortisone derivatives are present in other pharmaceutical preparations, particularly in some topically applied drugs. Miyachi et al. (1) reported that some topically applied synthetic glucocorticoids reached the blood circulation without hydrolysis. It can thus be suspected that topical application of hydrocortisone derivatives can also produce substantial concentrations of non-hydrated molecules, which could induce an important bias in cortisol determination. We therefore conclude that interpretation of results based on cortisol concentrations measured with the NEN RIA in patients treated with hydrocortisone derivatives could be erroneous.

We thank Drs. S. Glasson and J. Biollaz, Div. Clin. Pharmacol., University Hospital, for their collaboration. This study was supported in part by the Swiss National Science Foundation (grant no. 3.997-0.84).

Reference

Urine Glucose Quantification with the Seralyzer, José M. González Buitrago, Juana Rodriguez, Antonio Gómez del Campo, Fernando Cava, and José A. Navajo (Servicio de Análisis Clinicos, Hospital Virgen de la Vega, Paseo de San Vincente, 58, 37007 Salamanca, Spain)

The Seralyzer (Ames Co., Elkhart, IN) is a test-strip analyzer for use in small laboratories and physicians' offices. The first method introduced by Ames for serum or plasma glucose determination in the Seralyzer was based on the glucose oxidase technique, a method not suited for urine glucose quantification, owing to the high concentration of substances that interfere with the peroxidase reaction. Recently, Ames has replaced this with a new method, based on the hexokinase technique, which is less subject to interference than glucose oxidase methods.

We measured urine glucose with the Seralyzer Glucose (HK) Reagent Strip Test and compared the results obtained with the automated hexokinase assay hitherto used in our laboratory (Boehringer-Mannheim’s “Gluco-quant,” used in an Abbott VP Analyzer).
Glucose (HK) Reagent Strip Test and calibrators (multi-component calibrator kit) for the Seralyzer were purchased from Ames. Precision was assessed for a single operator using one instrument:

<table>
<thead>
<tr>
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<th>n</th>
<th>$\bar{x}$ (g/L)</th>
<th>SD</th>
<th>CV, %</th>
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<tr>
<td><strong>Within-run precision</strong></td>
<td></td>
<td></td>
<td></td>
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<td>Level 1</td>
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<td>Level 2</td>
<td>20</td>
<td>66.5</td>
<td>4.5</td>
<td>6.8</td>
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</table>

We examined correlation between the Seralyzer method and the automated method by assaying 60 urine specimens by both methods. The regression equation for the results was: Seralyzer = 1.33 + 0.99 automated; $r = 0.96$, $S_{yx} = 5.9$ g/L, $n = 60$.

We conclude that the Seralyzer method is acceptably precise (CV <7.0% between-run), that results correlate well with the automated hexokinase method, and that the test is suited for its intended use.

Detection of Papillomavirus DNA, Theodore E. Mifflin, David E. Bruns, and John Savory (Dept. Pathol., Univ. of Virginia, Charlottesville, VA 22908)

Human papillomaviruses (HPV) are associated with various human diseases, including cervical neoplasms, genital warts, and penile intra-epithelial neoplasia (1–4). However, testing for HPV has been restricted to research laboratories because multiple HPV nucleic acid probes are needed to detect the various HPV types. HPV testing also has required the capability to purify and label the probes and to use them in an appropriate detection system.

Recently, a rapid detection system has become commercially available (5). In this method, HPV DNA from the clinical specimen is bound to a nylon membrane by use of a specially designed suction manifold. The bound HPV DNA is detected by hybridization with a mixture of $^{32}$P-labeled riboprobes for seven common types of HPV (6, 11, 16, 18, 31, 33, and 35). Up to 25 samples and controls can be analyzed on a single membrane. The procedure requires approximately 5 h, including approximately 2.5 h for incubations. The autoradiogram can be made visible by exposure for one to five days, depending on the age of the isotope. An analogous procedure (6) allows typing of HPV-positive samples. Specimens can be categorized as type 6 or 11, 16 or 18, or 31, 33, or 35.

We tested the sensitivity of the new screening method and compared it with results of a conventional Southern blot (7). We studied the sensitivity quantitatively, using HPV DNA from clones provided by Dr. H. zur Hausen. When samples of purified HPV DNA were serially diluted, the detection limit was ≤2 pg for each of three HPV types tested (types 11, 16, 18). The new method was used to analyze 32 coded cervical samples, kindly provided by Dr. C. Crum, that had been analyzed by a conventional Southern-blot method (7). It (Table 1) detected 10 of 12 specimens that were positive by the comparison method. (The other two positive samples presumably contained HPV type(s) that the new method is not designed to detect.) Four samples were only weakly positive by the new method. These were not expected to be positive by the less-sensitive comparison technique, and none was positive. In addition, four samples were positive by the new method but negative by the comparison method. Further studies are required to determine whether the positive results on these samples reflect the better sensitivity of the riboprobe method as compared with the conventional DNA test. The remaining 12 samples were negative by both methods.

A preliminary study with the new procedure for typing of HPV's demonstrated sensitivities of 2 pg or less for types 11, 16, and 18. Each of 10 samples that had been typed by the comparison method was correctly classified by the new technique.

These new methods represent attractive alternatives to conventional techniques and hold promise that the new generation of assays will allow wider clinical use of HPV testing.

References
6. Human papillomavirus typing kit ("Viratype"). Ibid.

False-Positive Barbiturate Test in Urine Owing to Phenytoin and 5-(p-Hydroxyphenyl)-5-phenylhydantoin, Kathleen Schiera Siff and Alexander E. Finkler (Toxicol. Lab., Veterans Administration Med. Center, 113 Holland Ave., Albany, NY 12208)

Immunoassay is the favored technique in initial screening for drugs of abuse in urine. We find that phenytoin and its major metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) (1), can give a false-positive reaction in Abbott's fluorescence polarization immunoassay for barbiturates. This can cause problems for laboratories involved in testing for drugs of abuse.

A routine urine screen gave a positive test for barbiturates in the Abbott TDx analyzer, 1.84 mg/L. Attempted