An incubation time >5 min did not increase the GDH activity measured. Due to the typically small amount of GDH activity in a normal serum sample, the somewhat large sample volume (60 μL) was chosen to provide an adequate change in absorbance over the 100 s monitored and thus improve precision. Sera from 37 patients, with GDH values ranging from 0.8 to 56 U/L, were tested by both optimized assays. The assay of Jung et al. yielded GDH values that averaged 49% greater than those obtained by the Ellis and Goldberg assay. Both assays are very similar in formulation but that of Jung et al. contains L-leucine as an enzyme activator in addition to ADP.

I evaluated that assay further and obtained within-run CVs of 2.8% and 8.2% for GDH activities of 10.6 and 23.8 U/L (n = 20). Day-to-day CVs were 13.3%, 8.5%, and 7.6% for GDH of 9.1, 20.5, and 37.9 U/L (n = 20). The assay results varied linearly with activity concentrations to at least 350 U/L and were unaffected by moderate hemolysis, bilirubinemia, and lipemia. GDH activity was stable in serum pools for three days at 4 °C and for a month at -20 °C.

GDH in homogenates of rat brain tissue could be assayed in much smaller sample volumes, owing to the concentrated GDH activity in this tissue. The optimized GDH assay of Jung et al. adapted to the Cobas Bioprovides an acceptable method of measuring GDH activity in both serum and tissue homogenates in an automated fashion.

References

Evaluation of Single-Slide Creatinine Method on the Kodak Ektachem 700 Shows Positive Interference from Lignocaine Metabolites, Kate Couttie, John Earle, and John Coakley (Dept. of Clin. Biochemistry, Royal Children’s Hospital, Parkville, Victoria, 3052, Australia)

The original two-slide creatinine assay in the Ektachem 700 analyzer (Eastman Kodak Co., Rochester, NY 14650), based on the enzymatic hydrolysis of creatinine to produce ammonia, suffers positive interference from 5-fluorocytosine. In the new single-slide method, creatinine is hydrolyzed by creatinine amidohydrolase (EC 3.5.2.10) to creatine, which is further hydrolyzed to sarcosine and urea by creatinase (EC 3.5.3.3). The sarcosine is oxidized by sarcosine oxidase (EC 1.5.3.1) to glycine, formaldehyde, and hydrogen peroxide, which, with peroxidase and a leuco dye, yields a colored product. Although Kodak reports that there are no known interferents, we had a verbal report (Institute of Medical and Veterinary Science, Adelaide, South Australia) of possible interference due to lignocaine (lidocaine) metabolites.

As analyzed by both methods, 91 patients’ samples showed no clinically significant difference. Linear-regression correlation statistics yielded y = 1.03x + 0.00 (r = 0.997). Imprecision studies for the single-slide method yielded: intrabatch: x̄ 0.09 mmol/L, CV 1.2%; x̄ 0.37 mmol/L, CV 0.8% (n = 7); interbatch: x̄ 0.09 mmol/L, CV 2.2%; x̄ 0.60 mmol/L, CV 2.1% (n = 10). Lignocaine added to drug-free serum at five concentrations up to 100 μg/mL showed no interference. Five samples from three patients being treated with lignocaine were analyzed by Ektachem single-slide and Beckman Astra 4 methods (the Ektachem two-slide method being no longer available in our laboratory). All samples showed positive bias, 15%–37%, on the Ektachem compared with results on the Astra (Astra creatinine range 0.10–0.19 mmol/L). Two samples showing the greatest interference were re-analyzed by both methods after enzyme hydrolysis to remove creatinine (1); this confirmed the positive bias of the single-slide method. We conclude that some metabolite(s) of lignocaine, but not the drug itself, produces positive interference in the single-slide creatinine method.

Measurement of follitropin (follicle-stimulating hormone, FSH) and lutropin (luteinizing hormone, LH) in urine provides a convenient, noninvasive alternative to measurements in plasma or serum in evaluation of infertile patients (1). Recently, several immunoassay kits have been developed for simultaneously measuring both hormones in serum (2). In one such kit ("Simultrac," Becton Dickinson) a combined antibody preparation and differentially labeled antigens [57Co-labeled LH and 125I-labeled FSH] are used. Bound and free hormones are separated by double-antibody precipitation. I studied this kit for use with urine samples. I did analytical-recovery experiments and also compared results for dilutions of standard and urine. Mean recovery of FSH and LH standards added to urine was 97% (SD 16%, n = 7) and 27% (SD 9%, n = 8), respectively. Standard curves of percentage tracer binding (B/Bo, logit scale) vs. the log sample volume for dilutions of standard and unknown had similar regression coefficients (FSH: standard −3.19; unknown −2.75; LH: standard −2.79; unknown −2.45), and for neither hormone was the difference in slope between standard and unknown significant.

First morning-urine specimens were collected from one woman who was taking a combined oral contraceptive and from one woman with normal menstrual cycles. FSH and LH concentrations were between <1 and 4.8 int. units/L and between 3.7 and 11.7 int. units/L, respectively, in the contraceptive user, and ranged from 2.7 to 15.0 int. units/L and from 9.1 to 38.8 int. units/L in the normally menstruating woman. There was evidence of an ovulatory gonado-
pin surge in the latter woman but, owing to problems in sample collection, I could not demonstrate this unequivocally. Intra-assay CVs (derived from 10 measurements) for FSH were 14.4% at a sample concentration of 3.1 int. units/L, and 6.1% at a sample concentration of 9.8 int. units/L. For LH the corresponding figures were 11.5% (at 8.6 int. units/L) and 5.5% (at 33.7 int. units/L).

The results of this preliminary study suggest that this assay kit can reliably be used to measure concentrations of LH in urine. Measurements of FSH, however, are likely to be less reliable (as evinced by the wide variation in recovery of FSH standard from urine), given the low concentration of this hormone in urine.

The kits used in this study were kindly supplied by Dr. T. Appleton, Becton Dickinson U.K., Ltd., Oxford, U.K.

References

Adenosine Deaminase Activity and Acquired Immunodeficiency Syndrome (AIDS), Salvatore Delia, Claudio Maria Mastroianni, Anna Paola Massetti, Giuseppe Turbessi, Augusto Cirelli, Salvatore Catania, and Vincenzo Vullo (Dept. of Infectious Diseases, "La Sapienza" University, Policlinico Umberto I, 00161 Rome, Italy)

Adenosine deaminase (ADA; EC 3.5.4.4), a purine enzyme that specifically catalyzes the deamination of adenosine and other adenine nucleoside analogs to inosine, is found in most human tissues, but its physiological role appears particularly relevant in lymphoid cells.

In the absence of ADA, the increased concentrations of adenosine inhibit the in vitro growth of lymphoid cell lines (1). In addition, ADA activity in lymphocytes and erythrocytes, as well as in serum, is absent in about 20%-30% of children affected by a severe inherited T-cell immunodeficiency (2, 3), whose clinical and laboratory findings are similar to those in patients with AIDS. Caused by a retrovirus, the human immunodeficiency virus (HIV), AIDS is characterized by severe impairment of T-cell functions and cellular immune response.

In view of the possible relationship between ADA activity and the immune response, we measured this enzymatic activity in nonhemozyzed sera from patients with HIV infection. The patients were classified according to a recent classification of the Centers for Disease Control (4): 24 patients from Group II (asymptomatic infection), 13 from Group III (persistent generalized lymphadenopathy), and 20 from Group IV (other HIV diseases, including opportunistic infections and malignancies). We also tested sera from 12 HIV-negative male homosexuals and from 15 healthy heterosexuals not at risk for HIV infection. We used the colorimetric method of Giusti (5).

From our results (Table 1), increased ADA activity seems to be correlated with the detection of anti-HIV antibodies, which express the presence of the virus; not only AIDS patients, but also asymptomatic anti-HIV-positive subjects, had high ADA activity. On the other hand, increased ADA activity in serum does not seem to be due to specific factors, such as opportunistic infections, malignancies, etc. Sera from patients with asymptomatic HIV infection and sera from patients of Group IV, who had no opportunistic infections or malignancies at the moment of sample collection, both had high ADA activity.

The enzyme activity seems therefore to be mainly correlated with the retrovirus infection. HIV encodes for a trans-activating factor, which activates the expression of genes linked to the HIV long-terminal repeat (6, 7). Perhaps this factor can also alter the expression of cellular genes (8), including the promoter region of the ADA gene, thus increasing the activity of ADA.

### Table 1. Serum ADA Activity In Different Groups Studied

<table>
<thead>
<tr>
<th>No.</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>24</td>
<td>26.86 ± 16.66</td>
</tr>
<tr>
<td>Group III</td>
<td>13</td>
<td>27.60 ± 13.67</td>
</tr>
<tr>
<td>Group IV</td>
<td>20</td>
<td>30.23 ± 17.14</td>
</tr>
<tr>
<td>Homosexuals</td>
<td>12</td>
<td>9.30 ± 4.29</td>
</tr>
<tr>
<td>Normal controls</td>
<td>15</td>
<td>10.51 ± 4.13</td>
</tr>
</tbody>
</table>

References

Quantification of Glutathione and Glutathione Dioxide in Human Plasma, James D. Adams, Jan N. Johannessen,1 and John P. Bacon2 (College of Pharmacy, Washington State Univ., Pullman, WA 99164; 1 Lab. of Clin. Sci., Natl. Inst. of Mental Health, Bethesda, MD 20892)

Until now, the determination of concentrations of glutathione and glutathione disulfide in human plasma has been