generally has a polarization less than that of the zero-standard response in PFIA systems. Drug-free urine will therefore tend to depress the overall polarization signal from an immunoassay mixture, such that a small concentration of drug may appear to be present. The extent and variability of this effect can be characterized by testing urines from normal subjects to establish a positive/negative discriminatory cutoff concentration (10–12). With a cutoff of 1 mg/L, the methamphetamine PFIA reliably identified urines that had been reported positive by gas–liquid chromatography.

The PFIA for methamphetamine enables the specific detection of abuse of this drug and provides a companion test to the previously developed PFIA specific for amphetamine (11). An assay that would detect either amphetamine or methamphetamine could be developed by combining the two sets of specific reagents (18).

References
complexing agent (5). These properties of DDTC suggested that the metalloprotein, alcohol dehydrogenase (EC 1.1.1.1) (6), is a likely target for DSF interaction, and we initiated this work.

Here we report the findings of a study of the time-dependent inhibition of alcohol dehydrogenase. The results of this and another investigation (7) indicate that DSF interacts with serum albumin (7), alcohol dehydrogenase, and ADH (7).

Materials and Methods

Apparatus. We measured NADH formation at 340 nm in a model 410 spectrometer (Gilford Instrument Labs., Oberlin, OH) at room temperature (25 ± 1°C). We processed the reaction-rate data by using a standard least-squares fit with a Zenith Model 158 minicomputer.

Reagents. Equine hepatic alcohol dehydrogenase and di-thiothreitol (DTT) were obtained from the Sigma Chemical Co., St. Louis, MO. The enzyme solutions (40 mg/L) were prepared in pH 8.8 (50 mmol/L) pyrophosphate buffer, from an enzyme preparation having a specific activity of 1.7 kU/g.

Procedures. The solutions for the time-dependent study contained 40 mg of ADH per liter. This corresponds to 0.6 μmol/L, assuming a specific activity of 2.6 kU/g for the pure enzyme (5) and a relative molecular mass of 83 000 (5). Stock DSF was added to 5 mL of ADH solution to produce samples containing 0.6, 1.2, 1.8, and 2.4 μmol of DSF per liter; DDT/ADH solutions contained 60, 120, 180, and 240 μmol of DDT/ADH per liter. These solutions were stored at 4°C, then assayed during several days. The assay solution contained 100 μL of enzyme solution (enzyme, enzyme–DDT, enzyme–DDTC), 100 μL of NAD+ (1.5 mmol/L), 100 μL of ethanol (2 mol/L), and 2.7 mL of the pyrophosphate buffer.

Solutions for inhibition kinetics included 100 μL of enzyme solution; 15, 25, 50, 65, or 100 μL of 1.5 mmol/L NAD+ solution; 0, 10, or 20 μL of DSF solution (3.0 mmol/L); 100 μL of ethanol (2 mol/L); and enough pyrophosphate buffer to make a final volume of 3.0 mL. The absorbance at 340 nm was recorded during 3 min. We calculated the resulting velocities, using a molar absorptivity of 6.22 mL·mmol⁻¹·cm⁻¹ for NADH (9).

Results and Discussion

Time-dependent studies. We observed that DSF is a reasonably strong inhibitor of ADH activity and is considerably stronger than DDTC when these two reagents are incubated with similar concentrations of alcohol dehydrogenase (40 mg/L). The results in Table 1 suggest that there is a reaction between DSF and a thiol group, e.g., Cys-46 (6). This particular cysteine residue is coordinated to an active-site zinc ion, which should readily react with the DDTC molecule formed by the Cys–DSF reaction. Correspondingly, the reaction between DDTC and zinc in the absence of DSF is considerably more difficult, because the metal is already firmly coordinated to the protein (ADH).

With the above in mind, we repeated our previous experiment in the presence of DTT, a well-known thiol-group protecting reagent (Cleland's reagent). After 72 h, the presence of 4.8 μmol of DSF per liter, which ordinarily inhibits ADH activity by about 60%, had no effect on ADH (40 mg/L) in a solution that also contained DTT, 24 μmol/L.

Kinetics of inhibition. The results of our time-dependent study led to the determination of a K for the DSF–ADH reaction. The ADH reaction is an ordered one, in which NAD+ binds first to the active site of the enzyme, where zinc is coordinated to a cysteine residue (6). We recorded the resulting linear-mixed inhibition kinetics shown in Figure 1. These results are consistent with the reaction sequence (9) shown in Figure 2.

In this reaction sequence, only the enzyme that does not have an inhibitor of some type bound to it can catalyze the

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Table 1. Effect of Incubation Time on Inhibition of Alcohol Dehydrogenase Activity by DDTC and DSF

<table>
<thead>
<tr>
<th>DDTC, μmol/L</th>
<th>Incubation, h</th>
<th>DSF, μmol/L</th>
<th>Incubation, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>0</td>
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<tr>
<td>240</td>
<td>0</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

*% of inhibition.

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*Fig. 1. 1/V vs 1/[NAD+] plot with disulfiram concentrations of 0 (.), 10 (O), 10 (with 10-min incubation) (L), and 20 μmol/L (A).*

*Fig. 2. Proposed reaction mechanism for alcohol dehydrogenase-disulfiram (inhibitor, I) interaction where a > 1 and b = 0.*
reaction and produce acetaldehyde. This proposed sequence does not answer all of the questions concerning the actual mechanism of inhibition, but does provide a general model. During the acquisition of the data shown in Figure 1, we added DSF to the enzyme solution just before addition of NAD⁺ and ethanol, except in one experiment. In this latter case, we allowed a 10-min incubation period for DSF–ADH, after which we obtained the kinetic data. The time dependence of the DSF–ADH reaction was obvious: the $K_v$ value obtained from the 10-min incubation study was 21 (SD 2) μmol/L, as compared with 34 (SD 4) μmol/L obtained in the routine measurements.

From these results we conclude that the possible effects of DSF on alcohol dehydrogenase should also be considered when a clinical diagnosis is undertaken in Antabuse treatment of alcoholics. Further study is necessary if we are to understand the exact nature of DSF–ADH interactions and their resulting effects on patients treated with DSF.

This study was supported by grants from NINCDS (NS 23645) and NIDDKD (DK 38853).

References

Role of Qualitative Choriogonadotropin Assays in Diagnosis of Ectopic Pregnancy
Anne LeMaistre,1,2 Arthur Bracey,1 Allan Katz,2 and Alan H. B. Wu1,4

We compared the clinical sensitivity and cost-effectiveness of a qualitative assay for choriogonadotropin in human urine ("Icon" hCG) with a quantitative assay of serum from 142 women with pathologically-diagnosed ectopic pregnancy. Results show that although the qualitative assay had a clinical sensitivity for pregnancy of 98.6%, as compared to 100% for the quantitative assay, it was more economical to use, and had a significantly shorter turnaround time. We conclude that qualitative hCG assay of either urine or serum is a good screening method for detecting pregnancy, and can replace the stat quantitative assay in women with suspected ectopic pregnancy. We present a diagnostic algorithm to illustrate the role of qualitative and quantitative hCG assays in conjunction with ultrasonography, culdocentesis, and laparoscopy for diagnosis of ectopic pregnancy.

Additional Keyphrases: emergency procedures · enzyme immunoassay · cost-effectiveness

Ectopic pregnancy, first described in the tenth century (1), remains a diagnostic dilemma. The total number of ectopic pregnancies reported in the United States from 1970 to 1980 rose dramatically from 17 800 to 52 200 (2). Despite this increase, the death rate has declined to ~50/y (3). Although the early detection of ectopic pregnancies with laparoscopy, ultrasound, and choriogonadotropin (hCG) measurements have helped decrease mortality, they have also contributed to an increase in reporting of ectopic pregnancy (4–5). The development of rapid, highly sensitive, and specific qualitative assays for hCG in urine and serum requires a re-evaluation as to their role in the sequence for diagnosis of ectopic pregnancy in the emergency situation. This study was performed because physicians at our institution were requesting both stat qualitative urine and quantitative serum hCG determinations. We wanted to determine if the stat qualitative serum hCG assay was needed in this clinical setting or if it unnecessarily delayed treatment and added to the hospital costs these patients are charged.

Materials and Methods

Patients. We qualitatively screened for hCG 3790 women with history or physical findings suggestive of ectopic pregnancy who presented to the emergency room of Hermann Hospital from November 1984 to April 1986. This study was conducted under the auspices of the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston. Quantitative serum hCG, ultrasound, and culdocentesis were also performed when necessary. During the first six months of the study, the urines that tested negative by the qualitative hCG assay were retested with a stat quantitative serum hCG assay. Thereafter, the quantitative assay was routinely performed the next morning.

CLIN. CHEM. 33/10, 1908–1910 (1987)