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Sensitive, Selective, and Rapid Procedure for Quantifying Cocaine in Urine

To the Editor:

Cocaine metabolism produces two major metabolites, benzylocgonine (BE) and ecgonine methyl ester (EME) (1-4). BE is the metabolite ordinarily used to identify cocaine misuse in urine (5). However, determination of EME and cocaine in urine can provide valuable and important forensic information as to the source of BE and the time of cocaine ingestion (4-7). Laboratories should therefore be able to provide information on EME and cocaine when requested, in addition to BE. We have already described a sensitive and selective procedure for EME analysis in urine (8). We now present a rapid and sensitive procedure for the identification and quantification of cocaine in urine.

To 2.5 mL of urine (standard, control, or unknown) add 0.2 mL of phosphate internal standard (10 mg/L). Add 2.5 mL of 0.15 mol/L borate buffer (pH 9.2). Vortex-mix and add 5 mL of chloroform/isobutanol (98:2, by vol). Shake for 3 min on a shaker at low speed. Centrifuge tubes for 10 min. Aspirate the top (aqueous) layer, then transfer the lower layer to a small disposable tube and evaporate to dryness. Reconstitute the residue with 50 μL of methanol. Inject 1 μL into a gas chromatograph with a nitrogen-selective detector (NPD).

We purchased cocaine hydrochloride from Sigma Chemical Co., St. Louis, MO 63178 and used a Hewlett-Packard (Palo Alto, CA) 5890-μm megabore column, 10 m × 0.53 mm (i.d.), coated with 35% phenylmethylsilicone, for separating the compounds. Helium was the carrier gas at a flow rate of 20 mL/min. Injector temperatures and detector temperatures were 270 and 300 °C, respectively. The column temperature was programmed as follows: initial temperature 240 °C for 2.5 min, programmed at 70 °C/min to 280 °C for 3.5 min. The retention time for cocaine was 0.46 relative to prazepam, which was eluted at 4.36 min. Linearity studies with samples containing cocaine from 0 to 10 mg/L gave a slope of 0.89 and an intercept of -0.02 (r = 0.999). Analytical recovery of added cocaine at 0.76 and 1.5 mg/L was 100 ±11.2% (n = 7) and 101 (±8.5%) (n = 5), respectively. Within-run precision at 0.75 and 1.5 mg/L (n = 3) was 6% and 12%, respectively. Between-run precision at those concentrations (n = 7) was 11% and 8.5%, respectively. The detection limit of the method was <0.1 mg/L (i.e., the lowest-concentration standard detected was 0.1 mg/L).

Interference studies indicated that the following drugs will not interfere with this procedure: EME, BE, mor- phine, methadone, amitryptiline, nortriptyline, desipramine, amphetamine, methamphetamine, salicylate, aminobiotic, saeobartil, pentobarbi- tal, phenobarbital, ethchlorvynol, doxepin, imipramine, acetaominophen, caffeine, chloridiazepoxide, chlorpro- mazine, clonazepam, codeine, propoxy- phene, diazepam, fluazepam, glutethimide, meperidine, mebrobamate, methaqualone, phencyclidine, primidone, procainamide, quinidine, trimi- pramine, amoxepin, maprotiline, tocainide, theophylline, and mexitel- line. The retention time of pyrilamine, desipramine, and pentazocine is close to that of cocaine under the present chromatographic conditions and may interfere with the analysis for cocaine.

The effect of urine pH on cocaine stability was also investigated. At a urine pH of 8.0, hydrolysis of cocaine occurs, with one-third of the cocaine being lost in 15 days.

The advantages of this method are the lack of requirement for derivatization and the simplicity of sample preparation for gas-chromatographic analysis. Samples that tested positive for BE or EME that were analyzed with this procedure showed that cocaine would be detected only in some urine samples. This is to be expected because of differences in the elimination half-lives of cocaine, EME, and BE, with BE and EME having longer elimination half-lives than cocaine (1-4).

References

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Increased Estradiol Concentration of Unknown Origin

To the Editor:

We report an interesting case of above-normal serum estradiol concentration of unknown origin. A 30-year- old white woman was seen for infertility problems. Hormonal evaluation revealed the following results. Post-ovulatory progesterone concentrations and profile (a plot of hormone concentration versus time of cycle) were within the normal range for our laboratory (Table 1). However, the post-ovulatory estradiol concentrations were ex-
tremely high, although the curve appeared normal in profile (Table 1).

The patient denied the use of exogenous conjugated estrogens or contraceptives. Not only was the estradiol concentration above normal in the samples, but also dilution of the samples gave lower than expected (nonlinear) results. The specimens did not show abnormal nonspecific binding and were negative for heterophile antibodies. Because of the unexplained nature of these increased estradiol concentrations and because of our concern that a possible interference might be occurring, the samples were extracted with ether and assayed by radioimmunoassay (RIA) with an estradiol double-antibody kit (Diagnostic Products Corp., Los Angeles, CA 90045). The same kit was used for both the direct (total) and extracted estradiol analyses. The results for the extracted samples from the patient are given in Table 1. For comparison purposes, samples from normal patients that contained above-normal estradiol concentrations were also extracted with the same procedure. The mean (± SD) extracted estradiol proportion, [extracted estradiol concentration (direct total concentration)] × 100, was then calculated. The mean extracted estradiol proportion in normal patients whose estradiol was increased was 60% (SD 13%, n = 12). The mean estradiol concentration of our patient’s extracted samples was 13% (SD 5%, n = 9).

The estradiol increase in this patient appears due to the presence of an interfering substance that cross-reacts with the estradiol RIA. The substance acts like a conjugated estrogen that is not extracted by ether. However, because the patient denied the use of any exogenous estrogens and because results of all other endocrine tests were normal, we conclude that the observed interference must be some other interfering substance, not exogenous conjugated estrogens.

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False-Positive Titers of Thyroid Autoantibodies in Patients Undergoing Hemodialysis?

To the Editor:

Concentrations of thyroid-related hormones in serum of patients with chronic renal failure are known to be abnormal (1, 2). In our study on thyroid-function testing of patients undergoing hemodialysis, we determined, in addition to concentrations of free and total triiodothyronine, free and total thyroxin, "reverse" triiodothyronine, and thyroxin-binding globulin, the titers of thyroglobulin and microsomal autoantibodies (TGA and TMA, respectively). The study was provoked by the appearance of an uncommon agglutination pattern in the control wells of some samples from patients with chronic renal failure during the standard procedures of detecting TGA and TMA with hemagglutination methods (Thymune-T and Thymune-M assays from Wellcome, London, U.K.). For these samples we were not certain whether positive titers for TGA and (or) TMA represented false-positive or true-positive values. Therefore, we assayed the absorbed serum samples and samples after addition of excess nonspecific immunoglobulin. Furthermore, we wanted to determine the difference in TGA and TMA titers of serum samples before and after hemodialysis.

For 17 patients with chronic renal failure, blood samples were collected before and after hemodialysis; we measured TGA and TMA in all 34 serum samples. In eight of the patients, sera before and after dialysis contained no autoantibodies. In seven patients, autoantibodies were present in sera only before dialysis (in four patients TGA and TMA were positive, and in three patients only TGA), but not after dialysis. In two patients, serum samples contained high titers of both autoantibodies before and after dialysis.

All positive results with an uncommon agglutination in the control wells should be carefully interpreted. According to the Wellcome package insert for determination of TGA and TMA, "the control well (column 2) must always be negative. Heterophile anti-turkey reactions are uncommon at dilutions of 1:10 or greater, but if the control well shows agglutination the serum sample should be absorbed according to the protocol and the repeated test must be negative." When we repeated the test with absorbed sera taken before dialysis, the same uncommon agglutination pattern remained in the control wells of six samples in the Thymune-T test and in one sample in the Thymune-M test. In one patient, serum before and after dialysis showed high positive titers of TGA and TMA after atypical agglutinations in the control wells. These uncommon agglutination patterns did not disappear even after including the excess nonspecific immunoglobulin (normal turkey or rabbit serum) in the assay, as is recommended as the standard approach for reducing heterophile antibody or heteroantibody (3).

The problem of heteroantibody is present in various immunoassays. Bozato and Stuart (4) demonstrated the presence of nonanalyte, antibody-binding substances in almost 40% of serum samples. Testing sera by hemagglutination, Ng et al. (5) indicated that 19% of the sera interfered, but 20% of the results were false negative. In contrast, we found that the hemagglutination methods we used showed false-positive results in at least three samples determined to be negative by another passive particle-agglutination method (Serodia-AMC; Fujirebio).

These results suggest that serum of patients with chronic renal failure might contain some "unknown substance" that interfered with TGA and TMA reactions and induced uncommon agglutination in the control wells, even for absorbed sera. This interfering "unknown substance" was present in normal serum of some patients before dialysis (in six patients, TGA or TMA or both were positive) but disappeared or decreased in serum after dialysis (in one patient, TGA and TMA were positive). With absorbed sera, atypical agglutination remained in six samples before dialysis and in one sample after dialysis. In the TMA