thryotropin (TSH) and in 34 samples from 34 patients hospitalized for a nonthyroidal illness, whose thyroid function was normal on the basis of normal values for TSH and FT₃ (group B). Serum TSH was measured with IRMA RIA GNOST (Behringwerke, Marburg, Germany) (normal reference range, 0.1–4.5 mIU/L) and FT₃ with the FT₃ Amerlex MAB kit (10.3–25.6 pmol/L). This method for measuring FT₃ has been well validated in nonthyroidal illness (2, 3). Normal reference intervals for FT₃, determined by the two kits in 68 euthyroid subjects (normal TSH and FT₃) without serious extrathyroidal illness were 2.2–7.1 pmol/L (mean±2SD) for FT₃ Amerlex and 4.1–8.0 pmol/L for FT₃ Amerlex MAB, a range slightly different from that determined by the manufacturer on a larger sample of subjects (3.4–7.2 pmol/L).

Thirty (86%) of the samples drawn in group A had a low FT₃ when measured by Amerlex and 34 (97%) were low by Amerlex MAB. This difference in sensitivity is small, and not significant considering the small number of control subjects and hypothyroid patients. In this group, the two FT₃ estimates were significantly correlated (r = 0.69; Fig. 1A).

In contrast, 14 of the 34 hospitalized patients (44%) with an extrathyroidal illness and normal TSH and FT₄ had a low FT₃ (0.5–2.5 pmol/L) by the FT₃ Amerlex (specificity vs hypothyroidism in this population: 56%). By the FT₃ Amerlex MAB, FT₃ estimates were normal in 13 of these patients and slightly increased in 1. The two FT₃ estimates were not correlated in this group (Fig. 1B), indicating that the Amerlex FT₃ estimate does not reflect thyroid function in sick patients.

Thus Mullinger and Gates are right when stating that the FT₃ estimate by FT₃ Amerlex MAB will lose its prognostic significance in severely ill patients, but FT₃ Amerlex MAB gives a better estimate of thyroid function. Our results also confirm that FT₃ is less sensitive than FT₄ with regard to the diagnosis of hypothyroidism, because some patients of group A with a low FT₄ had normal FT₃ estimates by both methods, particularly when the manufacturer’s range for FT₃ Amerlex MAB was used.

References

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Colorimetric Quantification of Urinary Iron During Deferoxamine Therapy

To the Editor:

The concentration of iron in urine is of interest in monitoring deferoxamine therapy for acute iron poisoning (1) or iron overload (2). Atomic absorption spectrophotometry (AAS), the Reference Method for iron measurement (3), is usually not available in most routine laboratories. Routine automated methods for measuring serum iron with use of a chromogen do not detect iron in these samples (1), because the complex of deferoxamine and Fe²⁺ is not dissociated. Several types of pretreatment of urine samples to dissociate the deferoxamine–Fe²⁺ complex have been described (1, 4).

The need for a sensitive, (semi)-

automated procedure to monitor deferoxamine therapy in urine, we combined a ferrozine-based method for iron measurement (kit 1127683/691; Boehringer Mannheim, Mannheim, Germany), implemented on an automated analyzer (Hitachi 747; Hitachi, Tokyo, Japan), with a pretreatment. We optimized and compared two types of pretreatment: thioglycolate–trichloroacetate (1) and ascorbate–citrate (4).

To measure the recovery of iron, we added FeCl₃ to normal urine with or without equimolar concentrations of deferoxamine. Urine samples of patients with proteinuria or in deferoxamine therapy were collected. Each sample was thoroughly mixed, and aliquots were stored at 4°C and analyzed within 4 h.

In the thioglycolate–trichloroacetate pretreatment, the reagent (0.29 mol/L thioglycolic acid and 0.61 mol/L trichloroacetate in water) is added to an equal volume of fresh urine. In the ascorbate–citrate pretreatment, 3.5 volumes of reagent (0.48 mol/L ascorbate and 0.57 mol/L citrate in water) is added to 1 volume of fresh urine. These pretreatments are done at room temperature, and the pretreated samples are analyzed after 15–60 min on the automated analyzer. A control urine sample is processed the same way for a reagent blank.

We tested the linearity of the procedures from 0.41 to 1000 μmol/L iron. In the absence of deferoxamine, recovery of added iron (95% at 1000 μmol/L) and linearity (r² > 0.9999) were excellent without pretreatment. In urine containing both deferoxamine and iron (Fig. 1), iron could not be detected without pretreatment of the samples. Pretreatment with thioglycolate–trichloroacetate yielded poor recovery (19%) and linearity (r² = 0.81). Pretreatment with ascorbate–citrate was superior in both recovery (92%) and linearity (r² = 0.9996) and was thus adopted for further experiments and routine analysis. The ascorbate–citrate reagent solution was stable for at least 3 days at 4°C, although values for the reagent blank tended to increase. The reagent can be stored for longer periods at –18°C.

Because the molar absorptivity of the Fe⁺⁺–ferrozine complex (ε = 28 000 L·mol⁻¹·cm⁻¹) exceeds that of the Fe⁺⁺–tripyridyltriazine complex (ε = 22 600) (5), the detection limit for chelated iron was 0.8 μmol/L. This analytical sensitivity, although inferior to that of AAS [0.08 μmol/L (3)], allows our procedure to be used in the deferoxamine–iron excretion test (positive result: chelated iron excr-
tion >36 μmol/day (2) and in monitoring deferoxamine therapy. At a concentration of chelated iron of 50 μmol/L, the within-run CV was 3.4%, and the between-day CV 4.0%. Iron recovery was similar in urine containing various amounts of protein (0.5–7 g/L).

Urine samples of 33 healthy volunteers (ages 38 ± 9 years; 22 women, 11 men) not treated with deferoxamine were collected according to the guidelines of the local ethical committee. In all samples, the free iron concentration was <2.15 μmol/L—in the range of the reference values found in literature [free iron >35 μmol/L (6) to <1.25 μmol/L (7)]. Acidification or alkalization of urine in vitro (pH 4.5 to 9.5) did not alter the test results significantly.

In conclusion, urinary iron concentration in patients receiving deferoxamine therapy can be determined accurately with a routine automated, ferrozine-based method after a simple pretreatment of the samples with a reagent of 0.48 mol/L ascorbate and 0.57 mol/L citrate. This procedure has been valuable for monitoring deferoxamine therapy in our laboratory.

References

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Therapeutic Monitoring of Sertraline

To the Editor:

Sertraline (M₄, 306.2), one of the newer antidepressants, was recently approved in Canada for clinical use. Sertraline, like fluoxetine, is believed to be a specific serotonin-uptake inhibitor. It undergoes extensive first-pass metabolism to form N-desmethylsertraline, which is essentially an inactive metabolite (1, 2). Preliminary clinical trials have shown that sertraline is similar in therapeutic efficacy to traditional antidepressants but has significantly fewer side effects. No correlation has been reported so far between serum sertraline concentrations and therapeutic response. However, with the increased use of the drug, the demand for serum sertraline assays is increasing to monitor compliance or to detect unexpected toxic concentrations after chronic use.

Sertraline has been determined by gas chromatography with mass spectrometry (3) or electron-capture detection (4). Now we have applied a column liquid-chromatographic (LC) procedure, recently described for the determination of antidepressants (5), to assay sertraline. A 0.5-mL aliquot of the serum sample is mixed with 200 μL of internal standard solution (0.5 mg/L CP₅₃,630-1, an analog of sertraline, in 10 g/L potassium bicarbonate solution) and 0.5 mL of acetonitrile. After centrifugation, the supernate is applied to a 1-mL BondElut C₁₈ extraction column (Varian Associates, Harbor City, CA) that has been previously activated by washing successively once with 1 mol/L HCl, twice with methanol, and once with water. The sample is passed slowly through the column (by mild suction), and the column is washed twice with water and once with acetonitrile. The column is then eluted by gravity (no suction) with 250 μL of methanol containing 25 mL/L of 35% perchloric acid, the eluate being collected directly into an autosampler cup. A 20-μL aliquot of this elute is injected onto a 15 cm × 4.6 mm (i.d.) Ultrashphere ODS reversed-phase silica column packed with 5 μm particles (Beckman, San Ramon, CA). The column is eluted at ambient temperature with a mobile phase of 750 mL of water + 400 mL of acetonitrile + 0.5 g of tetramethylenamine perchlorate + 0.5 mL of 7% perchloric acid at a flow rate of 1.8 mL/min. The peaks were monitored at 205 nm with a Model 10A absorbance detector (Shimadzu, Columbia, MD).

The chromatogram of a drug-free sample (Fig. 1A) shows a stable baseline and only a few extraneous peaks, even at 205 nm. A chromatogram of an extract of a serum standard supplemented with 100 μg/L of the drug and the desmethyl metabolite (Fig. 1B) shows good separation of the drug, metabolite, and the internal standard—from each other and from the solvent and extraneous peaks. A chromatogram of a serum sample extract from a patient receiving sertraline (Fig. 1C) does not show any additional peaks. The standard curve is linear for the range tested, 15 μg/L to 2 mg/L. The linear regression of peak-area ratios of drug/internal standard (y) vs drug concentration (x) is excellent: y =