mens (Figure 1). However, clinically important differences between results by the two techniques appear only in very rare situations, such as hyperlipidemia, paraproteinemia, and myelomatosus, among others. In practice, this would seldom present any clinical problem.

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References


Fluorometry of Selenium in Serum or Urine
L. Lalonde, Y. Jean, K. D. Roberts, A. Chapdelaine, and G. Bleau

This fluorometric procedure for determining selenium in human serum or urine is sensitive (requiring only 0.4 mL of sample), accurate, simple, and can be performed on several samples concurrently. Using this technique, we found a mean selenium concentration in the serum of normal Canadian men of 142.9 (SD 16.1) μg/L. The mean urinary excretion rate was 124.5 (SD 76.0) μg/day.

Additional Keyphrases: trace elements • reference interval • methods for the small laboratory

The toxic effects of selenium are well described, particularly in livestock, where acute and chronic poisoning have been reported (1). Selenium is also recognized as an essential micronutrient, and this element can protect rats against experimental liver necrosis (2). Since this early observation, several pathological conditions in animals have been shown to result from selenium deficiency (3). It has also been suggested that a selenium deficiency could be associated with human diseases such as kwashiorkor (4, 5), sudden death syndrome in infants (6), cancer (7), and multiple sclerosis (8). This prompted us to develop a rapid, reliable procedure for measuring trace amounts of this element in human body fluids. Fluorometry offers many advantages because it can be adapted to the assay of nanogram amounts with a minimum of labor and equipment. Here we present a fluorometric technique that allows measurement of selenium in many samples with the required sensitivity and accuracy.

Materials and Methods

Apparatus

We digested samples in disposable 18 × 150 mm borosilicate glass tubes, heated in a sand bath. This bath was custom-made from a hot plate (Lindberg Co., Watertown WI 53094; Model 53014; 2800 W, 240 V) around which a metal border, 7.5 cm in height, was soldered directly to the plate; into this container was placed a 2.5-cm layer of fine sand. Fluorescence was measured with an Aminco Bowman spectrophotofluorometer equipped with a xenon lamp.

Reagents

All aqueous solutions were prepared in doubly distilled, doubly demineralized water. High-quality nitric acid (Ultrex, J.T. Baker) was used for preparing the digestion mixture. Diaminonaphthalene dihydrochloride (DAN) was purchased from Aldrich Chemical Co. and solutions were prepared immediately before use. DAN (99% pure) did not require further purification because blank values were consistently lower than 5 arbitrary units of relative fluorescence intensity, i.e., equal to cyclohexane itself.

Standard Solutions

Standard A was prepared by dissolving in HCl (0.1 mol/L) known amounts of selenium oxide (Puratronic; Johnson Matthey Chemicals Limited); standard B was purchased as selenium acid solution (Ventron Alfa Division); standard C was purchased as a selenium oxide reference solution. All other reagents were of “Fisher Certified” grade.

Procedure

Digestion of samples. To 0.4 mL of standard solutions or urine or serum, add 1.0 mL of an equivolume mixture of HNO3.
and HClO₄. This volume of acid is more than sufficient to allow complete digestion. Slowly heat for 30 min at 150 °C, then increase the temperature to 190 °C and maintain it for 2 h. Increase the temperature to 210 °C and maintain it for 1 h. Then cool the tubes. Add 0.2 mL of 6 mol/L HCl and heat at 150 °C for 5 min. Repeat this step if fumes of NO₂ are detected (reddish vapors). Cool the tubes.

Reduction of selenate to selenite. Add 2 mL of a solution containing, per liter, 20 mmol of ethylenediaminetetraacetate, 10 mg of bromcresol purple, and 7 mol of NH₄OH. Heat at 140 °C until a distinct yellow color is observed (pH 1 to 2). Cool the tubes. Add 5 mL of HCl (0.1 mol/L) and adjust the volume to 10 mL with water. Let stand overnight at room temperature. This interval allows complete reduction and is convenient.

Formation of the piazselenol complex. Under subdued light, add 0.5 mL of DAN (4 g/L solution in 0.1 mol/L HCl). Incubate for 30 min in a water bath at 40 °C. Extract the complex with 5 mL of cyclohexane, and measure the fluorescence of the extract with excitation wavelength set at 360 nm and emission wavelength at 520 nm.

Results

The standard curve of fluorescence vs selenium concentration (Figure 1) is linear (coefficient of correlation: 0.999) up to a concentration of 400 μg/L. We tested three different selenium standard solutions (Table 1). All three gave this linear relation, with high correlation coefficients, but the slopes (S) were different (S₁ = 0.28; S₂ = 0.29; S₃ = 0.12). Standard C, purchased as a prepared solution, was rejected because it differed significantly from standard A, which we prepared in our laboratory from pure solid selenium oxide. Standard B was satisfactory because its selenium concentration was accurate. The mean concentration of selenium in the serum of 15 normal men was 142.9 (SD 16.1) μg/L. Selenium was also measured in the 24-h urine samples obtained from 10 men on three consecutive days. The mean daily urinary excretion was 124.5 (SD 76.0) μg/day.

![Graph of fluorescence intensity vs selenium concentration](image)

**Fig. 1.** Representative standard curve

| Table 1. Relative Fluorescence Intensity of Three Different Standard Preparations |
|-----------------------------------|------------------|------------------|------------------|
| Se, μg/L  | Std. A           | Std. B           | Std. C           |
|          | Relative fluorescence intensity | Relative fluorescence intensity | Relative fluorescence intensity |
| 0        | 5.16 ± 0.76      | 5.16 ± 0.76      | 5.16 ± 0.76      |
| 100      | 32.33 ± 0.57     | 34.00 ± 1.00     | 18.00            |
| 200      | 59.33 ± 0.57     | 64.00            | 30.83 ± 1.04     |
| 300      | 87.33 ± 1.52     | 92.33 ± 2.08     | 43.33 ± 0.57     |
| 400      | 119.33 ± 1.15    | 123.00           | 56.33 ± 1.15     |
| S¹       | 0.28             | 0.29             | 0.12             |

* Arbitrary units. ¹ Standard deviation. ² Slope of the curve.

Analytical-recovery studies demonstrated that a mean of 99.6% of the selenium added (50 or 100 μg/L) to a control pooled serum was accounted for after digestion. The mean within-assay variation (CV) of this technique was 2.93% (SD 1.45%); the between-assay variation was 2.67% (SD 2.16%).

Discussion

Several techniques for measuring selenium have been described. However, these techniques could not be applied in a clinical laboratory where many samples had to be assayed on a routine basis. The fluorometric technique described here is an adaptation of the method of Watkinson (10). It is simple, sensitive, reproducible, precise, and requires relatively small sample volumes. Thus, the selenium content of urine can effectively be measured in 0.4 mL of sample with this technique, as compared with 10 mL required with neuron activation analysis (9). Moreover, many samples can be digested concurrently, a characteristic shared only by the semi-automated fluorometric method (10). The digestion unit is readily made, and the technique requires little instrumentation. The simplicity of this procedure renders this technique accessible to many laboratories where sophisticated apparatus is not available. Blank values are very low, representing about 5 arbitrary units of relative fluorescence intensity. The accuracy of this method, as reflected in the high recoveries, compares well with that of any other technique.

All steps in the procedure should be considered critical. The digestion must be carefully controlled to avoid changes of temperature by more than approximately 10 °C. Also, as pointed out by Watkinson (10), all residual nitric acid must be removed if reduction of selenate to selenite is to be complete. The pH adjustment with bromcresol purple as indicator allows rapid adjustment of acidity in the range of pH 1 to 2.

Levesque and Vendette (11) have commented upon the importance of a proper system to reduce selenate to selenite and to provide suitable conditions for the formation of the selenium complex. The present system, which allows an overnight reduction step in hydrochloric acid, fulfills this prerequisite.

It is noteworthy that selenium reference standards can vary considerably from one supplier to another. Also, it is advisable to have periodic quality-control assays of these standard solutions and compare the results with those for freshly prepared solutions of pure solid selenium oxide.

The mean value for selenium in serum, 142.9 μg/L, that we obtained for Canadian men with this method corresponds well with reported values. For example, Dickson and Tomlinson (12) reported a mean plasma value of 144 μg/L in 254 normal individuals in a Canadian population.

The selenium in 24-h human urine specimens was reported to be 79.3 (SD 38.7) μg/L, with a range of 21.5 to 203.0 μg/L (13), but the daily excretion in micrograms was not reported. For comparison purposes, one needs such data. In our study this value is 95.5 (SD 47.2) μg/L, range 29.1–198 μg/L. This
Radioimmunoassay and Chemical Ionization/Mass Spectrometry Compared for Plasma Cortisol Determination

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We describe a method for determination of cortisol in plasma and urine, based on chemical ionization/mass spectrometry with deuterium-labeled cortisol as the internal standard. The within-run precision (CV) was 2.5–5.7%, the between-run precision 4.6%. Results by this method were compared with those by a radioimmunological method (RIANEN Cortisol, New England Nuclear) for 395 plasma samples. The latter method gave significantly higher (approx. 25%) cortisol values.

Because of its speed and the sensitivity with which a large number of samples can be analyzed, radioimmunoassay (RIA) is commonly used in routine laboratory work for measuring cortisol in plasma. As compared with other methods, such as fluorometry, RIA also has a relatively high selectivity; however, there is some cross reaction with other steroids—e.g., cortisol metabolites and synthetic corticosteroids, which may become important in the diseased state and during drug treatment (1).3 Gas chromatography/mass spectrometry (GCMS) with selected-ion monitoring is recognized as one of the most selective analytical techniques available today and has been suggested as a "definitive" method in clinical chemistry (2). Björkhem et al. (3) described a GCMS method with electron-impact ionization for the determination of plasma cortisol. Cortisol labeled with 14C was originally used as internal standard, but has recently been replaced with a deuterium-labeled analog (4). We present here a modified method based on chemical-ionization (CI) GCMS, which we used to measure cortisol in plasma and urine during an investigation of the systemic effects of glucocorticoid ointments. We used RIA to rapidly check if plasma cortisol suppression was severe, in which case the steroid treatment was discontinued. For the final evaluation of cortisol concentrations, the same plasma samples were analyzed at a later date by the more laborious GCMS method. The data compared are those for 395 plasma samples from 16 healthy men.

Materials and Methods

Subjects and blood collection. Sixteen healthy men gave

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3 Nonstandard abbreviations: RIA, radioimmunoassay; GC, gas chromatography; MS, mass spectrometry; CI, chemical ionization.