Modified Determination of Total and Inorganic Mercury in Urine by Cold Vapor Atomic Absorption Spectrometry

David Littlejohn, Gordon S. Fell, and John M. Ottaway

In this procedure a single-beam spectrophotometer is used without background correction. By the method of Magos [Analyst (London) 96, 847 (1971)] mercury in undigested urine is complexed to L-cysteine in acid solution. At high pH and in the presence of stannous ions, mercury ions are reduced to elemental mercury. The mercury vapor is partitioned above the reagent solution in a specially designed chemical reduction apparatus similar in principle to that used by Kubasik et al. [Clin. Chem. 18, 1926 (1972)]. The vapor is then flushed by air through an "absorption" cell, where the absorption of the mercury line at 253.7 nm is measured. The value obtained for inorganic mercury subtracted from that for total mercury gives a value for organic mercury. CV's for the inorganic mercury procedure at 40 and 5 μg/liter concentrations were 3.1% and 7.5%, respectively. The detection limit is 0.82 μg/liter. The CV for the total-mercury procedure (20 μg/liter) was 2.6%. Mean analytical recoveries of organic and inorganic mercury were 96.5% and 101%, respectively. We investigated storage conditions for urine and compared results by the present technique with those by activation analysis. Our method is a convenient way to screen individuals who have been exposed to a mercury hazard.

Additional Keyphrases: screening, environmental hazards, trace elements, activation analysis, optimum sample preservation

The concentration of mercury in urine, both as inorganic and organic mercury, is used as a guide to excessive exposure. Although correlation between the extent of increased urinary mercury excretion and clinical symptoms of mercury poisoning is generally considered to be poor, until a more satisfactory index is available, analysis of urine for mercury will be the best way to screen for and assess occupational or accidental exposure (2). This is particularly so for inorganic mercury; organic mercury in urine is not thought to reliably reflect the level of exposure (1). However, in the absence of any other convenient procedure, threshold values for both inorganic and organic mercury have been suggested (3): for inorganic mercury in urine 300 to 600 μg/liter, and for organic mercury 30 μg/liter. This concentration of inorganic mercury in urine is well above that found in non-exposed subjects, which is less than 20 μg/liter (4).

Several methods for determination of mercury in urine have been published that are based on the adsorption of the mercury line at 253.7 nm by monatomic mercury vapor (5-10). Such "cold vapor" procedures require that the mercury be in the elemental state. This is accomplished by reducing mercury with stannous ions. In addition, cadmium salts can react with organomercury compounds to produce inorganic mercury ions, cadmium replacing mercury in the organic compound. The mercury ions are then reduced by stannous ions.

We describe here a modification of the procedures of Magos (5) and Kubasik et al. (9) and show its application to determination of both inorganic and organic mercury in urine. As in the method of Magos (5), analyses were performed without predigestion of urine samples. A single-beam atomic absorption spectrophotometer was used without background correction. Use of a chemical reduction cell, similar to that used by Kubasik et al. (9) and designed specifically for "cold-vapor" analysis, has allowed development of simple procedures that are sufficiently sensitive and accurate for use in screening subjects suspected of exposure to mercury.

Methods and Materials

Principle

Mercury compounds in urine are complexed with L-cysteine in acid solutions. At high pH and in the presence of stannous ions, mercury is released only from inorganic mercury compounds and reduced to elemental mercury vapor. If a stannous chloride/cadmium chloride reagent mixture is used at high pH, mercury from both inorganic and organic mercurials will be released and reduced to elemental mercury vapor. Thus by performing separate analyses on two 5-ml portions of a urine sample, total mercury and inorganic mercury concentrations can be determined, and subtraction of the inorganic mercury from the total mercury concentration gives a value for the organic mercury concentration in the urine. In either reaction, the atomic mercury vapor is measured quantitatively by the cold vapor atomic absorption technique.

Apparatus

The apparatus was assembled as illustrated in Figure 1. The spectrometer was a Pye Unicam SP90 Series 1 Atomic Absorption Spectrophotometer, and a Pye Unicam Mercury Hollow Cathode Lamp was used as light source. The burner head was removed and a 15-cm absorption cell positioned in the light path. A Charles Austen Capex Mark II pump was used to generate the flushing air flow and the flow rate was monitored with the air flowmeter on the spectrophotometer. Absorbance was either measured on the meter of the spectrophotometer or was recorded on a strip-chart recorder.

Operating instrumental conditions were:

- Mercury lamp current: 5–6 mA
- Mercury lamp wavelength: 253.7 nm
- Slit width: 0.2 mm
- Meter response: position 1
- Scale expansion: none
- Air flow rate: 1 to 1.25 liter/min

The different parts of the apparatus were connected with polyvinylchloride tubing, the length of tubing between the

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chemical reduction vessel and the absorption cell being made as short as possible. The system is "open ended"; that is, no circulation of mercury vapor occurs. Magnesium perchlorate, used as a drying agent, was placed in the bulb of a 10-ml glass pipette. Cotton wool was loosely packed in the tubing between the reduction and absorption cells. Both the cotton wool and magnesium perchlorate must be changed at weekly intervals or once every 60 to 70 analyses.

A special reduction apparatus was constructed for the generation of mercury vapor (Figure 1). Reaction flasks of 50-ml capacity were used. A 5-ml dropping funnel was used to add the sodium hydrosulfite solution that initiated the reduction reaction. Stopcock valves A and C were made of polytetrafluoroethylene Pyrex 4 and valve B of polytetrafluoroethylene Pyrex 2. The ground-glass "Quick Fit" joint size was 1/4. The taps on the reduction cell are closed during the conversion of mercury compounds to atomic mercury to allow maximum partition of the mercury vapor before it is flushed through the absorption cell.

Reagents

All reagents were of "Analar" grade. De-ionized water was used in the preparation and dilution of all solutions.

L-Cysteine (or L-cysteine HCl), 10 g/liter of nitric acid, 1 mol/liter.

Stannous chloride solution, 100 g/liter of hydrochloric acid (2 mol/liter).

Stannous chloride (100 g/liter), cadmium chloride (100 g/liter) reduction solution (for organic mercury), in hydrochloric acid, 2 mol/liter.

Sodium hydroxide, 300 and 450 g/liter.

Sulfuric acid, 100 and 500 ml/liter.

Magnesium perchlorate, anhydrous granules.

Stock inorganic mercury solution, 10 mg Hg/liter. Dilute "atomic absorption spectroscopy grade" (British Drug House) mercuric chloride solution (1 mg of Hg per milliliter) to a concentration of 10 μg/ml with de-ionized water. This solution is stable for several weeks. Each day, prepare working standard solutions in the range 10 to 50 μg of mercury per liter by dilution of this stock solution.

Stock organic mercury solution, 100 mg Hg/liter. Dissolve 17.1 mg of methyl mercury iodide or 16.8 mg of phenyl mercuric acetate (equivalent to 10 mg of mercury) in a small volume of methanol. Transfer the solution to a 100-ml calibrated flask containing 20 to 30 ml of de-ionized water, and dilute to the mark with water. Prepare working standard solutions daily by dilution of the stock solution with de-ionized water.

Procedure for Inorganic Mercury Analysis

Into a 50-ml "Quick Fit" reaction flask pipette 2 ml of the cysteine solution and 5-ml of the urine sample, inorganic mercury standard, or blank (de-ionized water), and add 0.5 ml of the stannous chloride solution. Connect the reaction flask to the upper part of the apparatus, which should have valves A and C open and valve B closed. Switch on the magnetic stirrer and air pump and adjust the air flow to 1 to 1.25 liter/min. Pipette 3 ml of the 300 g/liter sodium hydrosulfite solution into the funnel. Turn off the air pump, close valve C, and open valve B to allow the sodium hydrosulfite to enter the reaction vessel. When all the sodium hydrosulfite solution has been added close valve A and then valve B in quick succession.

Allow the reduction reaction to proceed for 2 min, then in rapid succession open valves A and C and switch on the air flow, which should be at 1 to 1.25 liter/min. Measure the absorbance on the instrument meter or record the peak absorbance as the mercury is flushed through the absorption cell.

Switch off the magnetic stirrer but continue to flush the system with air until the absorbance reading returns to zero. Remove the 50-ml flask from the apparatus and wash it out with the more dilute sulfuric acid solution, tap water, and finally de-ionized water. The system is now ready for the next sample. About 12 to 15 analyses for inorganic mercury can be performed per hour.

Procedure for Total Mercury Analysis

Into a 50-ml "Quick Fit" flask pipette 2 ml of the cysteine solution and 5 ml of urine, organic and (or) inorganic mercury standard, or de-ionized water blank, 1 ml of the 500 ml/liter sulfuric acid, and 1 ml of the stannous chloride/cadmium chloride reduction solution. Proceed as described for inorganic mercury analysis, except that 3 ml of 450 g/liter sodium hydrosulfite should be added instead of 3 ml of 300 g/liter sodium hydrosulfite. About 10 to 12 analyses for total mercury can be performed per hour.

Results

Instrumental Settings

Instrumental conditions were chosen to give maximum sensitivity and precision. The slit width of 0.2 mm was chosen because we found that the CV at this slit width was about half that at 0.05 or 0.40 mm. Sensitivity was maximum at a flow rate of 1 to 1.25 liter/min.

The effect of varying reaction time is shown in Figure 2. The reactions appear to be complete and sensitivity maximum.
Table 1. Recoveries of Inorganic and Organic Mercury Added to 15 Urine Samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Hg added as HgCl₂</th>
<th>Hg added as CH₃Hg³ or PhHgAc⁴</th>
<th>Hg found by total procedure ng</th>
<th>Hg found by inorganic procedure ng</th>
<th>Organo-Hg found by difference</th>
<th>Total Hg</th>
<th>HgCl₂ or PhHgAc¹</th>
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<tbody>
<tr>
<td>1</td>
<td>50</td>
<td></td>
<td>48, 51</td>
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<td></td>
<td>96, 102</td>
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<td>2</td>
<td>100</td>
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<td>100, 104</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>150</td>
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<td>145, 144</td>
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<td>97, 96</td>
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<tr>
<td>4</td>
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<td></td>
<td></td>
<td>100, 97.5</td>
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<td>11</td>
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<td></td>
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<td>50*</td>
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<td>100*</td>
<td>194, 194</td>
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<td>87, 92</td>
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<tr>
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<td>50</td>
<td>150*</td>
<td>185, 190</td>
<td>54, 51</td>
<td>131, 139</td>
<td>92.5, 95</td>
<td>108, 102</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Results for Analysis of Urine Samples for Total Mercury by Neutron Activation Analysis (NAA) and Atomic Absorption (AA)¹

<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg Hg per liter</td>
<td></td>
</tr>
<tr>
<td>110.1</td>
<td>110.0</td>
<td></td>
</tr>
<tr>
<td>63.0</td>
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<td></td>
</tr>
<tr>
<td>54.9</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>36.5</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>29.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>18.3</td>
<td>18.0</td>
<td></td>
</tr>
</tbody>
</table>

¹ In all samples, the mercury was present in inorganic form.

Analytical Variables

Precision and limit of detection. The precision of the method for inorganic mercury was assessed from results for 15 analyses of two urine samples at concentrations of 40 and 5 µg/liter. CV's of 3.1% and 7.5% were obtained, respectively. The detection limit, based on 2 SD of the determination at 5 µg/liter, was 0.82 µg/liter.

The precision of the total mercury method was similarly assessed and the CV for 10 analyses of a 20 µg/liter urine sample was 2.6%. This precision for both procedures is adequate for diagnostic purposes.

Recovery. To assess the accuracy of the methods, we performed recovery experiments in duplicate on urine samples, as follows:

(a) Known amounts of inorganic mercury were added to urine samples and determined by the procedure for inorganic mercury analysis (urine samples 1 to 4 in Table 1).

(b) Known amounts of methyl mercury and phenyl mercury were added to urine samples and determined by the procedure for total mercury analysis (urine samples 5 to 12 in Table 1).

(c) Known amounts of both inorganic and methyl mercury were added to urine samples and inorganic and total mercury determined by the respective procedures. The value for the methyl mercury concentration was obtained by subtracting the inorganic mercury value from that for the total mercury concentration (urine samples 13 to 15 in Table 1).

The results (Table 1) were generally satisfactory, but it is clear from samples 13 to 15 that in a mixture, recovery of methyl mercury is slightly incomplete and recovery of inorganic mercury is greater than expected. Based on all the results, the mean recoveries of inorganic mercury and methyl mercury were 101% and 96.5%, respectively.

Comparison with Neutron Activation Analysis

Urine samples were analyzed by the procedure reported in this paper and by neutron activation analysis (Table 2). The activation analysis was performed at the Scottish Universities Research and Reactor Centre, East Kilbride, Scotland, with a 300 kW reactor. The procedure followed was an adaptation of the method of Johansen and Steinnes (11), with use of the equipment illustrated in a paper by Sjostrand (12).
Urine samples were irradiated for 12 h in a flux of $3 \times 10^{12}$ n/cm$^2$ per second and then left for 2 to 3 days, to allow short-lived isotopes to decay. The samples were then acid-digested and mercury was precipitated as the sulfide. Gamma radiation at 77.9 kiloelectron volts from the 65-8 $^{197}$Hg isotope was counted with a 25 ml Ge/Li detector. In a previous collaborative exercise, samples of urine were found to exhibit a change in apparent mercury content on storage after neutron activation analysis had been done. The atomic absorption results were subsequently found (about two months later) to be of a similar order of magnitude, but generally lower. In this experiment analyses by the two methods were performed within 10 h of one another and, as can be seen from Table 2, the results were then found to be in good agreement.

**Storage of Urine Samples**

The prevention of losses of mercury during storage is one of the main practical problems in urinary mercury analysis. Skare (7) suggested that this disappearance was attributable to bacteria in the urine converting mercury in solution into volatile mercury compounds. To overcome this he proposed that 1.0 g of sulfamic acid and 0.5 ml of Triton X-100 detergent be added per 500 ml of urine, as a preservative. Magos (5) suggested that mercury standard stock solution could be preserved by adding 9.0 g of sodium chloride, 743.5 mg of di-sodium ethylenediaminetetraacete, and 63 mg of L-cysteine hydrochloride to 1000 ml of a 0.5 g/liter stock solution of mercury.

To evaluate the best way of storing urine samples before analysis, we stored batches of a urine containing 40 $\mu$g of Hg per liter in the laboratory bench at 22 °C, (b) in a cold room at 4 °C, and (c) in a deep-freeze at −10 °C. The urine samples were: untreated; at pH 1.7; at pH 12.7; with added cysteine, disodium ethylenediaminetetraacetate, and sodium chloride in the concentrations suggested by Magos (5); or contained sulfuric acid and Triton X-100 detergent in the concentrations suggested by Skare (7). The test was run over a period of 24-days. Our results indicate that untreated urine, even when stored in a deep-freeze, losses up to 20% of the mercury during the first few days of storage. Either storing the urine in the presence of sulfamic acid and Triton X-100 detergent or adjusting the urinary pH to 1.7 prevented losses of mercury at all three storage temperatures, as did making the urine strongly alkaline and storing in either the freezer or cold room. Addition of L-cysteine hydrochloride, as suggested by Magos, restricted losses to 10% over a 24-day period if samples were stored in the freezer or cold room.

**Measurement of Mercury in Urine from Laboratory Personnel Possibly Exposed to Mercury**

Using the total mercury procedure, we measured mercury in specimens of urine from nine laboratory workers who were using volumetric gas-analysis techniques where there is a potential hazard of exposure to mercury vapor (2). Eight subjects had urinary mercury concentrations between 3.5 and 12 $\mu$g/liter; only one had a value (26 $\mu$g/liter) exceeding the accepted upper normal limits, 20 $\mu$g/liter. In all the samples, mercury was present only in the inorganic form.

**Discussion**

The chemical reduction cell introduced in this paper was designed to allow the partition of the mercury vapor in a fixed volume above the reagent solutions. This apparatus has two advantages over other systems (5, 8–10): (a) the valve arrangement allows the mercury to be trapped and concentrated during the reduction reaction, thus increasing sensitivity, and (b) the reaction vessels can be varied in size and easily removed for cleaning.

The system is open ended and no circulation of mercury vapor occurs. Others (6) have used closed-loop pumping systems, in which mercury is constantly recycled until a peak of absorbance is reached. Use of an open-ended system shortens analysis time, and with the mercury collection apparatus described no loss of sensitivity or reproducibility is observed as compared to closed-loop systems.

Hwang et al. (8) previously reported that when using open-ended systems for mercury analysis, it is necessary to optimize conditions such as the flow rate of the flushing gas and the reaction mixing time to yield maximum sensitivity and precision. In general, we agree. In open-ended systems it is to be expected that if the air flow rate flushing the mercury vapor through the absorption cell is too fast, insufficient time may be available for a maximal response of the instrument. If it is too slow, the mercury may diffuse.

In the present system, the valve arrangement prevents diffusion of mercury from the reduction apparatus during the chemical reaction. If the reaction is complete, the time at which the analytical measurement is made need not be as precise as in the system used by Hwang et al. (8). The design of their reduction apparatus did not incorporate a valve arrangement, and they found it necessary to make the absorbance readings at exactly the same time interval for each analysis, to achieve maximum sensitivity and avoid diffusional losses of mercury from the reduction cell.

It is important that as little water as possible enters the absorption cell with the mercury vapor, because nonspecific background absorbance can be produced by either light scattering from aerosol droplets or by broad-band absorption by water vapor. It is therefore necessary to blow the air over the top of the solution rather than through it to decrease entrainment of water, and to dry the vapor with magnesium perchlorate.

The method outlined in this paper is at least as precise at the 40 $\mu$g/liter concentration (i.e., $2 \times 10^{-4}$ g of Hg) as other commercially available systems in which double-beam instruments with continuous background correction are used (6). A lower working limit, 1.0 $\mu$g/liter, can be achieved without increasing sample volume.

In general, the analytical recovery of added mercury is satisfactory, indicating acceptable accuracy. However, when a urine sample contains appreciable amounts of organomercury as well as inorganic mercury compounds, the recovery of organomercury is slightly incomplete and recovery of inorganic mercury is greater than expected. These results as indicated on Table 1, for the recovery of methyl mercury and inorganic mercury, suggest that either the methyl mercury added is contaminated with inorganic mercury compounds or that the methyl mercury is partly reduced during the inorganic procedure. When a urine containing 40 $\mu$g of methyl mercury per liter was analyzed by the inorganic mercury procedure, we obtained an absorbance equivalent to 2 $\mu$g/liter. The actual cause of these small discrepancies is unknown. In practice, however, this will not seriously affect the value of the method as a screening procedure for detection of exposure to mercury.

The results of the storage tests suggest that if mercury is not to be analyzed immediately after collection of a urine sample, some method of preserving mercury should be used to prevent losses of mercury during storage. The addition of sulfamic acid and Triton X-100 detergent to urine stored in a cold room proved to be the most convenient method of preserving urinary mercury, and all samples for mercury analysis have been subsequently stored in this manner at the Pathological Biochemistry Laboratory at Glasgow Royal Infirmary.

The method proposed can be used either to determine total mercury (i.e., inorganic plus organic mercury) or inorganic mercury alone with acceptable precision and accuracy. No
Use of Values for Calcium and Protein in Serum, and of a
Derived Index Obtained from a Probability Population Sample

Anthea Kelly, Louis Munan, Claude PetitClerc, Kok Ping Ho, and Bernard Billon

We measured serum protein and calcium concentrations in 2340 individuals between 10 and 96 years of age from 900 families chosen by probability methods to give a representative population. These values were used to calculate an index, based on a regression analysis of serum protein on calcium, which was then treated as a new variable. Age-sex specific reference values and frequency distributions are presented for this index as well as for protein and calcium calculated by both parametric and nonparametric methods.

Additional Keyphrases: reference values • percentile levels • gaussian and nonparametric methods • bivariate analysis • influence of age and sex • evaluation of renal and endocrine function

Calcium and total protein concentrations are among the measurements that are routinely available to the practitioner for the evaluation of certain renal and endocrine functions, and albumin is not.

We report here the results of our examination of the relationship between calcium and protein as found in a representative noninstitutionalized population, unselected as to disease or disease factors, and describe an index of this relationship that may be used before requesting more specific determinations such as albumin and ionized calcium.

Materials and Methods

Calcium and protein concentrations in serum, among other biochemical variables, were measured in 2340 individuals (over 10 years of age) from 900 families chosen by probability sampling methods from a total population of slightly more than half a million, living in almost equal numbers in urban and rural regions. The sampling scheme and other details of the population are those reported by Kelly and Munan (1) and Munan et al. (2).

Blood specimens were collected into evacuated tubes with the subject in the sitting position, after an overnight fast. These were transported to the laboratory and analyzed in the SMA 12/60 system (Technicon Instruments Corp., Tarrytown, N. Y. 10591). The method of analysis for calcium is a modification reported by Gitelman (3) of the Kessler and Wolfman (4) method. The SMA 12/60 method for measuring total protein is a standard AutoAnalyzer "N" procedure, which utilizes a modified biuret reaction.1