Nonspecificity of Urinary Lead Measurements by Atomic Absorption Spectroscopy

A Spectrophotometric Method for Correction

Robert J. Segal*

Lead measurements by direct AAS at 217 nm on a pooled urine sample gave values of 57 μg/l by the method of standard additions. In contrast, dithizone procedures gave values of 15–20 μg/l. Studies demonstrated that various urine salts and organic compounds contributed to the absorbance at 217 nm. These native urine materials also give a similar absorbance value at 220 nm, a line not related to lead emitted by the lead hollow cathode tube. Thus, correction of the nonlead absorbance was possible by subtracting the A220 from A217. Utilizing this correction, the standard addition procedure gave values of 12 μg/l. The correction technic was also applied to the lead isolation-concentration technic of Kopito and Shwachman (3) which employs bismuth coprecipitation. The simplicity of operations, increased sensitivity and reproducibility obtained by coupling the AAS correction technic with bismuth coprecipitation recommends it as a method for serious consideration.

**Urine Lead Measurements** by atomic absorption spectrophotometry (AAS) should be rapid, sensitive and specific; yet, this had not proven to be so at the low concentrations found in normal individuals. While upper limits of normal have variously been reported at 50 to 80 μg/l, direct aspiration of urine is generally accepted as inaccurate below 100 μg/l by AAS. Thus, with the exception of the rare toxic levels, direct AAS for urine lead is of little value in the clinical laboratory and concentration technics such as extraction, chelation, and coprecipitation are employed (1, 2).

The initial aim of this study was to decrease the detection limit for lead on direct aspiration by utilizing a new spectrophotometer equipped...
with an electronic signal integrator and a highly sensitive ultraviolet detector. The goal was to detect lead in urine at concentrations of 25 μg/l with a precision of ±5 μg/l (one standard deviation)—a goal easily attained with standard dithizone extraction–colorimetric procedures (1).

Methods

Instrumentation

The Instrumentation Laboratories, Inc., AA Spectrophotometer Model 153 with signal integrator and special UV sensitive photomultiplier tube with quartz envelope* was used under the following conditions: (1) Monochromator set at 217.0 nm with spectral slit width of 0.4 nm (setting #5 = 160 μ) and reference filter 405.7 nm; (2) Channel "B" hollow cathode tube unplugged and both "B" channel filters set to block transmitted light; (3) Lamp current was made optimal for drift and noise at 6.5 ma; (4) Aspiration rate was set at 5-6 ml/min; (5) Stoichiometric air-acetylene flame at dial settings of 14 and 5 respectively; (6) Boling type burner adjusted to give minimal absorbance with water; (7) measurements were recorded† using 10-second integration periods after a minimum warmup of lamp and flame of 30 min.

Reagents

Deionized Water. Specific resistance is greater than 3,000,000 Ω.

Glassware was washed and stored in 5% (v/v) nitric acid and rinsed thoroughly with deionized water prior to use.

Working solutions of lead, in concentrations below 1000 μg/l, were prepared daily from a 100 mg/l reference solution (0.1598 g of lead nitrate in 1000 ml of water).

10% (w/v) Bismuth Nitrate. Slowly add 50 ml of 10 N nitric acid to 10 g of bismuth pellets§ and dilute to 100 ml with deionized water.

Concentrated nitric acid, hydrochloric acid, and ammonium hydroxide. The lead content is checked with a water blank. If no measurable signal is produced at 217.0 nm, the reagents may be used.

Procedures

Direct aspiration All direct readings on urine and lead working solutions, at both 217.0 and 220.0 nm, were repeated three to five times and averaged.

*HTV Electronic Tube No. R106, Hamamatsu TV Co, Ltd.
†Westinghouse High Intensity Hollow Cathode Tube.
‡Texas Instrument, Model #FMW06B.
§Metallic Bismuth, certified pure (99.9999 percent)—Spex Industries, Metuchen, NJ.
Bismuth coprecipitation  The bismuth coprecipitation procedure of Kopito and Shwachman (3) was used with minor modifications. The entire procedure can be performed in a 30-ml screw-cap test tube, graduated at 25 and 5 ml. Aliquots of urine and working solutions are measured to the 25-ml line. 1.0 ml of the 10% bismuth nitrate and 0.75 ml of concentrated ammonium hydroxide are added and mixed by inversion. This is centrifuged for 15 min at 2500 rpm and the supernate is discarded. The precipitate is dissolved by adding 2.0 ml of conc HCl and mixing on a Vortex mixer. Deionized water is added to the 5-ml line and AAS readings are taken at 217.0 nm and 220.0 nm, alternating samples with water blanks.

Experimental Results

Sensitivity, Variability, Noise and Detection Limit

The sensitivity was studied at the 217.0-nm and the 283.3-nm lead lines using both the R106 quartz and the 1P28 Vycor photomultiplier tubes. Using the R106 quartz detector, the 217.0-nm Pb line was more than twice as sensitive as the 283.3-nm as shown in Fig 1.

The variability of the signal at the 25, 50, 200, and 1000 μg/l levels was determined at 217.0 nm and 283.3 nm. The results are given in
Table 1. R.S.D.* of Noise to Signal

<table>
<thead>
<tr>
<th>Concentration of lead in water</th>
<th>1P68 217.0 nm</th>
<th>1P68 882.3 nm</th>
<th>RIC68 217.0 nm</th>
<th>RIC68 882.3 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µg/l</td>
<td>20.4</td>
<td>125.0</td>
<td>17.0</td>
<td>32.5</td>
</tr>
<tr>
<td>50 µg/l</td>
<td>13.0</td>
<td>32.5</td>
<td>17.0</td>
<td>32.5</td>
</tr>
<tr>
<td>200 µg/l</td>
<td>5.2</td>
<td>11.2</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>1000 µg/l</td>
<td>2.5</td>
<td>2.4</td>
<td>2.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The R106 quartz photomultiplier tube in combination with the 217.0-nm lead line gives the least variability.

* Relative Standard Deviation = 100 × Standard Deviation of Signal / Average Signal

Table 1. The instrumental-flame noise as tested with the water blanks was 5 µg/l. Using this value, one might expect a lower limit of detection at 10–15 µg/l; however, the best observed during direct aspiration studies was found to be 15–20 µg/l. It is obvious that these results were dependent upon making all operating conditions optimal and would differ significantly with other instruments, components and gas combinations—i.e., single beam units utilizing cooler flames of propane-air or hydrogen-air.

Direct Aspiration of Urine and Standards

The response to 0, 50, 100, and 150 µg/l aqueous working solutions is shown in Fig 2, dotted line. Similar additions were made to a urine pool, giving curve "A". Ideally, these two lines should be straight and parallel. The lead content of the urine pool as determined by this method of standard additions is found by projecting this line to the abscissa. This equals 57 µg/l—a high value compared with those of 15 and 20 µg/l obtained by the two independent outside laboratories.* This suggests that something besides lead was absorbing at 217.0 nm.

Nonspecific Absorbance

To study the question of specificity, solutions of sodium, potassium, magnesium, calcium, urea, and creatinine were prepared in concentrations approximating those found in urine. These were measured at 217.0 nm and the results are shown in Fig 3A. As seen by inspection of these graphs, all of the materials tested absorb to some extent; furthermore, increasing concentrations usually, although not invariably, produced increasing absorbance.

One approach to resolving this problem of nonspecific absorbance would be to measure the concentration of the interfering substances.

and calculate their absorbance at 217.0 nm from these graphs. These calculations were made for the urine pool and the results are shown on the right in Table 2. Here the nonspecific absorbance is estimated to total 60 μg/l for the 6 urine constituents measured.

A more practical approach is to measure the nonspecific absorbance by AAS. This type of absorbance is not restricted to a specific wavelength and should affect all emission lines in the same general area of the spectrum the same way (4). Therefore, absorbance by sodium, potassium, etc. should be approximately the same at the 217.0-nm Pb line as at 220.0 nm, a nearby nonlead line. The similarity of absorbance at these two lines is demonstrated by the data in Table 2. To eliminate the effect of the nonspecific absorbance, the 220.0 nm reading is subtracted from the 217.0 nm reading. Fig 3B demonstrates that the corrected value (217.0 - 220.0) more closely approximates zero interference than the 217.0-nm value and in some instances, false elevations

![Graph](image)
Fig 3. A. Excessive error signal in equivalent concentration of lead due to ions on the non-specific absorbance seen at 217.0 nm. B. Partial correction for this non-specific absorbance by subtraction of absorbance at 220.0 (a nearly non-Pb-line) from that at 217.0 nm. The concentrations of interfering substances equal to unity are as follows: Na = 200 mEq/l, K = 40 mEq/l, Ca = 40 mEq/l, urea = 1500 mg/100 ml, and creatinine = 100 mg/100 ml.

Table 2. Absorbance Due to Interfering Substances

<table>
<thead>
<tr>
<th></th>
<th>Upper limit of normal in urine</th>
<th>Urine pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of interfering substances</td>
<td>Lead equivalent</td>
</tr>
<tr>
<td>Sodium</td>
<td>200 mEq/l</td>
<td>50 µg/l</td>
</tr>
<tr>
<td>Potassium</td>
<td>100 mEq/l</td>
<td>10 µg/l</td>
</tr>
<tr>
<td>Calcium</td>
<td>75 mEq/l</td>
<td>110 µg/l</td>
</tr>
<tr>
<td>Magnesium</td>
<td>25 mEq/l</td>
<td>-5 µg/l</td>
</tr>
<tr>
<td>Urea</td>
<td>1500 mg/100 ml</td>
<td>60 µg/l</td>
</tr>
<tr>
<td>Creatinine</td>
<td>100 mg/100 ml</td>
<td>50 µg/l</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>275 µg/l</td>
<td>310 µg/l</td>
</tr>
</tbody>
</table>

* Concentration of interfering substances were determined by flame photometry (Na, K), AAS (Ca++, Mg++), and AutoAnalyzer (urea and creatinine).

The nonspecific absorbance measured at 220.0 nm closely approximates that at 217.0 nm. The lead equivalent values in columns 2, 3 and 5 are obtained from the graphs in Fig. 3.
Table 3. Urinary Lead Measurements by AAS: A Method for Correction

<table>
<thead>
<tr>
<th></th>
<th>Direct readings</th>
<th>Bismuth method</th>
<th>Reference laboratories' values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value at 217.0 nm</td>
<td>Corrected at 217.0 nm</td>
<td>Value at 217.0 nm</td>
</tr>
<tr>
<td>Urine pool 2</td>
<td>57 µg/l</td>
<td>12 µg/l</td>
<td>29 µg/l</td>
</tr>
<tr>
<td>Lyophilized urine</td>
<td>425 µg/l</td>
<td>192 µg/l</td>
<td>198 µg/l</td>
</tr>
<tr>
<td>Control spec.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The absorbance due to interfering substances is measured at a nearby nonlead line (220.0 nm). This value is subtracted from the absorbance at the lead line (217.0 nm) yielding the corrected value.

are lowered by over 50 µg/l. This correction procedure, when applied to the urine pool with standard additions describes line “B” in Fig 2. The lead concentration is reduced to 12 µg/l.

Bismuth Coprecipitation and Corrections

To further increase the signal, the bismuth coprecipitation procedure of Kopito and Shwachman (3) was employed. Two advantages are gained by this procedure. First, the lead is concentrated by a factor of 5. Second, the concentration of monovalent ions and organic compounds is markedly decreased because they are not precipitated.

This procedure worked surprisingly well from the first trial runs. Aqueous solutions of 15 µg/l were readily detectable, and concentration differences of 8 µg/l were easily measured. Four urine pool measurements at 217.0 nm now gave values of 19, 27, 27, and 30 µg/l—still somewhat higher than reference laboratory results. However, by applying the 220.0-nm subtraction technic, values of 10, 10, 14, and 16 µg/l were obtained.

Similar results were noted using a lyophilized urine specimen with a predetermined value of 180 µg/l.* Direct measurements gave a concentration of 425 µg/l and when corrected, this was reduced to 192 µg/l. The bismuth procedure gave an uncorrected value of 198 µg/l and a corrected value of 178 µg/l. These results are summarized in Table 3.

It is worth noting that Kopito and Shwachman (3) studied this problem of interfering ions in solutions containing 1000 µg/l of lead. They concluded that this problem was “virtually eliminated after precipitation with bismuth”; however, their data show that in the presence of calcium which is completely coprecipitated, recovery was 104%. This

*Dade Laboratories, Miami, Fla (Method given in Reference 5).
represents a false high signal which is equivalent to 40 µg/l of lead—a significant spurious elevation at the normal urine lead level.

Discussion

It is apparent from the foregoing experimental information that lead measurement on direct aspiration of urine gives false high values when compared to the standard colorimetric dithizone method. These high values were shown to be due to nonspecific absorption by urinary constituents such as sodium, calcium, and urea, and might spuriously add more than 100 µg/l to a urine lead determination.

Utilizing a nearby nonlead line at 220 nm as have others (6), measurements were made of the nonspecific absorption. These values closely approximated those at the 217.0-nm lead line, and provided an easy means for correction, i.e., absorbance at the lead line minus absorbance at nearby non-Pb line = absorbance due to lead. As demonstrated in Table 3, this technic is applicable to measurements by direct aspiration of the urine sample and to determinations involving coprecipitation with bismuth.

These experiments have demonstrated that the specificity of direct urine lead measurements by AAS must be questioned. However, by coupling the 220-nm correction technic with the bismuth coprecipitation procedure, the measurement of lead by AAS at levels found in the urine of normal individuals is rapid, sensitive, specific, and precise. This coupled method can be recommended for use in the hospital clinical laboratory for either routine or emergency analytical analyses.

References