Electrothermal Atomic Absorption Spectrophotometry of Cadmium in Semen

Lawrence T. Wetzel and John U. Bell

We describe the electrothermal atomic absorption spectrophotometry of cadmium in rabbit semen collected before and after seven days of subcutaneous administration of 0.5 mg of cadmium per kilogram body weight per day. The analytical technique involves combining an aliquot of an acid-digested semen sample with an equal volume of an (NH₄)₂HPO₄ solution (50 g/L), to allow an increase in charring temperature, which results in a nearly complete destruction of matrix. Cadmium values as determined by this method correlated well with those determined by the method of standard additions. The dosing regimen resulted in a statistically significant (p <0.01) increase in cadmium concentrations in semen (and whole blood) sampled just after the last day of cadmium administration.

Additional Keyphrases: rabbits - trace elements - fertility in the male - occupational hazards - variation, source of.

Measurement of heavy metals in various biological materials is considered to provide an index to environmental, industrial, or occupational exposure to them. Electrothermal atomic absorption spectrophotometry is a sensitive, accurate, and relatively simple way to make such measurements.

Lead concentrations in semen and blood from healthy men with no previous occupational exposure to lead have been measured by atomic absorption spectrophotometry (1). Those measurements were made in an attempt to relate seminal lead concentrations with hypofertility in men.

Cadmium, a ubiquitous environmental contaminant, significantly decreases fertility of male laboratory mice (2). It also exerts deleterious effects on the testes of laboratory mammals receiving it in various doses. Histologically, cadmium in the testes is mostly associated with vascular endothelium and interstitial tissue (3). It adversely affects spermatogenesis in several species (4).

Electrothermal atomic absorption techniques for determining cadmium in semen have apparently not been proposed. Here we describe a relatively simple procedure for measuring cadmium in rabbit semen by atomic absorption spectrophotometry. Additionally, using this technique, we found that subacute administration of the metal results in a significantly increased cadmium content in semen (and blood).

Materials and Methods

Apparatus

Cadmium analyses were done with a Model 305B atomic absorption spectrophotometer equipped with an HGA 2200 graphite furnace, a temperature ramp accessory, and a deuterium arc background corrector (all from Perkin-Elmer Corp., Norwalk, CT 06666). Microliter volumes of sample (maximum, 5 µL) were injected directly into the graphite tubes with Oxford micropipets (Fisher Scientific Co., Pittsburgh, PA 15230). Peak heights were recorded with a Recordall 5000 (Fisher) at a chart speed of 25 cm/min. Tissues were thermally digested in a multiblock heater (Lab-Line Instruments, Inc., Melrose Park, IL 60160).

Reagents

"Ultrax" grade HNO₃ (Baker Chemical Co., Phillipsburg, NJ 08865) was used for all sample digestion. All water used was de-ionized, then glass distilled. Cadmium standards were prepared from certified standards (Fisher). ACS certified (NH₄)₂HPO₄ (Baker) and (NH₄)₂SO₄ (Fisher) were used to prepare the matrix solutions tested. All glassware was washed and immersed in 4 mol/L HNO₃ for 24 h, rinsed thoroughly, inverted, air dried, and stored in covered containers to prevent contamination.

Animals

Sexually mature New Zealand White male rabbits (Hilltop Lab Animals, Inc., Scottdale, PA 15683) were used in this investigation. Body weights ranged from 3.9 to 5.2 kg.

Cadmium Administration

Seven-day osmotic minipumps (Model 1701; Alza, Palo Alto, CA 94304) were used to deliver 0.5 mg of cadmium (as cadmium chloride) per kilogram body weight per day for seven consecutive days to each rabbit. Pumps were implanted subcutaneously in the interscapular region of each rabbit. The dose was based on each animal's body weight at the time the pump was implanted.

Sample Collection

Semen and blood were sampled from each rabbit immediately before and seven days after minipump implantation. Each rabbit therefore served as its own control. Semen samples were obtained by using an artificial vagina (5) and the ejaculate was collected into a graduated centrifuge tube. Blood was collected from the marginal ear vein with disposable polypropylene plastic syringes and stainless-steel hypodermic needles and dispensed immediately into heparinized cadmium-free glass tubes.

Preparation of Semen and Blood for Cadmium Determination

All pre-treatment and post-treatment semen samples were wet-digested in HNO₃ (one part sample to three parts HNO₃) immediately after collection. These proportions gave the most consistent results for semen analysis. Because rabbit semen coagulates quickly, it was necessary to digest the entire volume collected. The specimens were placed in screw-cap vials, the
acid was added, and the vials were tightly capped and heated in a heating block for 30 min at 80 °C.

Immediately preceding analysis, an aliquot of each digested semen sample was combined with distilled water or various concentrations of (NH₄)₂HPO₄ or (NH₄)₂SO₄. The combination was thoroughly mixed on a vortex-type mixer immediately before electrothermal cadmium determination.

Cadmium in each sample was evaluated by two procedures. In the first, peak heights were compared with those used to prepare a linear standard curve obtained under identical matrix conditions. In the second, the method of standard additions (6) was used. Reagent blanks were prepared and analyzed in the same manner as the samples. Each analysis was conducted in duplicate or triplicate.

Blood samples were prepared and analyzed in the same manner as the semen samples.

**Results**

A previously described regimen for electrothermal cadmium detection in digested tissues suggests a 100 °C drying temperature, a 300 °C char (ash) temperature, and a 1900–2100 °C atomization (7). The charring stage is very important in that, ideally, the tissue matrix should be destroyed without loss of metal. It has been suggested (8) that use of (NH₄)₂HPO₄ or (NH₄)₂SO₄ helps decrease matrix interference in analysis by allowing use of a higher char temperature, even temperatures at which an unprotected cadmium sample would volatilize. Char time and temperature must be optimized to promote maximum matrix destruction.

The protection afforded by a matrix solution is illustrated in Figures 1 and 2. An aqueous cadmium standard diluted with HNO₃ to a final concentration of 12.5 µg/L shows a continual decrease in peak height at charring temperatures > 300 °C (Figure 1). However, combining the standard with various concentrations of (NH₄)₂HPO₄ or (NH₄)₂SO₄ to yield a final cadmium concentration of 12.5 µg/L reduces cadmium loss at the higher charring temperatures (Figure 1). Similarly, when a semen sample known to contain cadmium was digested as described and analyzed with or without matrix solutions, the results were identical (Figure 2). From the data in Figures 1 and 2 it was determined that adding a 50 g/L solution of (NH₄)₂HPO₄ to an equal volume of the sample to be analyzed gave the most nearly consistent results over the range of charring temperatures investigated. Thus we selected this combination as the matrix solution of choice in subsequent semen and blood analysis. Table 1 shows the experimentally determined times and temperatures we used.

Despite the increased charring temperatures, sample matrix effects can still cause errors. A potential solution to this problem is the use of a deuterium arc background corrector. Figure 3 shows the effect of background correction on cadmium atomization peaks for a semen sample obtained from a rabbit treated with cadmium. Clearly, there is non-atomic
absorption occurring coincident with the atomization peak for cadmium. Figure 3 also illustrates a second peak that is present in tracings from the uncorrected analysis, which is not seen when background correction is used. Background correction is therefore mandatory if results are to be accurate in these analyses.

The reproducibility of the technique is shown in Figure 4, which shows tracings of 10 replicate cadmium determinations from a semen sample combined with (NH₄)₂HPO₄ and ashed at 500 °C. The coefficient of variation was 5.85%.

In Table 2, cadmium values for semen sampled from five rabbits immediately before and after seven days of cadmium treatment are compared, as are cadmium values determined by using a linear standard calibration curve and those determined by using the method of standard additions. Least-squares and pair analysis of the two sets of data is shown in Table 3. Results obtained by the two methods correlate well. Semen cadmium concentrations were increased significantly after treatment. These increases are significant for either method of detection, standard curve or standard addition. Evidently for semen cadmium, the easier analytical procedure, comparison with a standard curve, gives the same values as the more time-consuming method of standard additions.

### Table 2. Cadmium Analyses for Five Samples of Digested Semen and Digested Blood before and after Cadmium Administration

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<thead>
<tr>
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<th>Standard curve</th>
<th>Standard addition</th>
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<td>Control 7 day</td>
<td>Control 7 day</td>
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<tr>
<td>Semen</td>
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<tr>
<td>7.1</td>
<td>27.2</td>
<td>6.5</td>
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<td>9.0</td>
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<td>8.0</td>
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<td>8.0</td>
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<tr>
<td>Blood</td>
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<td>2.0</td>
<td>195.0</td>
<td>25.6</td>
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* Cd concon, µg/L. Data from a normal distribution as assessed by the Kolmogorov–Smirnov test (11).

* Line fitted by regression analysis.

Table 2 also shows cadmium concentrations in whole blood, as determined by the two methods. These values were subjected to the same statistical analyses as the values for semen, and although there was a statistically significant increase in blood cadmium after the seven-day treatment, there was not a good correlation between the two analytical procedures (Table 3). Therefore, when blood is to be analyzed for cadmium, more complicated methods involving standard addition or extraction with organic solvents (9, 10) evidently must be used.

With either method there was no correlation between the increased cadmium values in semen and blood.

### Discussion

This method is simple, rapid, precise, accurate, and repeatable for the analysis of cadmium in semen. Results obtained by the proposed technique correlated acceptably with those by the method of standard additions. However, the inconsistent results obtained for acid-digested blood suggest that the method of standard additions or further extraction techniques are required for the analysis of cadmium in blood.

The observed increase in semen cadmium concentration after its subcutaneous administration may have toxicological implications for the reproductive process.

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### References


