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Improved Determination of Cadmium in Blood and Plasma by Flameless Atomic Absorption Spectroscopy

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Recently reported analytical methods for determining cadmium in biological fluids indicate accurate and reproducible results but are quite limited by a low working range. Scientific interest in cadmium toxicity at low exposure levels and research into the multiple interactions of cadmium in the blood, liver and kidneys has increased the need for measuring minute amounts of cadmium in biological tissue. Many flameless atomic absorption methods for assaying nanogram quantities of cadmium have relied upon extraction procedures prior to analysis (Allain and Mauras 1979; Tully and Lehmann 1982). These techniques can be susceptible to errors by sample contamination or loss while carrying out the many steps of sample preparation (Perry et al., 1975). In the method described here, nanogram quantities of cadmium in blood and plasma are measured by using graphite furnace atomic absorption spectroscopy facilitated by a wet ashing pretreatment of samples. The method appears to be quite accurate and reproducible with an average coefficient of variation of 6.7%. The linear working range of the method was found to extend from 0.4 µg Cd/l to 16.0 µg Cd/l.

MATERIALS AND METHODS

Analyses were performed using a Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Norwalk, CT) with automatic background correction in conjuction with a Model HGA 500 graphite furnace and a Model AS-1 autosampling system. Samples of venous whole blood and plasma were collected from groups of New Zealand white rabbits receiving 0.65 mg and 0.90 mg Cd/kg body weight, daily, as $CdCl_2$, to evaluate the applicability of the method to these biological fluids. The rabbits were dosed with aqueous $CdCl_2$ via subcutaneous injections 7 days/week, for 10 weeks and samples were collected and analyzed every third day.

Distilled, de-ionized water with a minimum resistance of 10 megohms was used throughout the experiment for washing glassware and preparation of solutions. Redistilled cadmium free (< 1 µg Cd/l) nitric acid (J.T. Baker Chemical Co.) and 30% hydrogen

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peroxide (Fisher Scientific Co.) were used in preparation of allstandards and samples. Borosilicate digestion tubes were rinsed three times with dilute nitric acid before use.

Immediately after collection, 0.5 ml rabbit blood was transferred to a digestion tube and 1.0 ml conc. HNO_3 was added. The samples were then digested in an oven for approximately 4 hr. @ 110°C. When the sample volume had been reduced by about 2/3, 0.5 ml 30% H_2O_2 was added and heating of samples was continued until evaporated to dryness. Sample residues were then dissolved in 5.0 ml dilute nitric acid (10 ml/l) prior to analysis. This sample wet-ashing method was similar to that used by Perry et al. (1975).

Standards were prepared by combining 0.5 ml blood or plasma from control animals not receiving cadmium with 20, 60, 120 and 160 µg Cd/l in 1% nitric acid prepared from a 1,000 µg/l stock cadmium solution (Fisher Scientific Co.). Standards were prepared for each set of samples taken from experimental animals and were digested in the same manner as the experimental samples, thereby eliminating correction for cadmium loss in the digestion process. Plasma samples were treated in a manner identical to those of blood after separation from red cells by centrifugation. All reagents and techniques used in sample and standard preparations were duplicated in blanks consisting of fresh blood or plasma from control animals.

Twenty microliters (µl) of sample was transferred to a standard graphite tube by the autosampler. All samples were analyzed in duplicate. The optimal temperatures and times for drying, charring and atomizing, determined experimentally, were found, respectively, to be 110°C for 30 sec, 300°C for 60 sec and 2,000°C for 9 sec. A fourth step requiring 2,400°C for 5 sec as added to completely remove any residue remaining in the graphite chamber from the previous sample. The furnace chamber was purged with argon throughout all four steps of the assay, but during the atomization step the gas flow was increased from 50 ml/min to 200 ml/min. The absorption peak was measured at 228.8 nm with a low slit setting of 0.7 nm. The peak height mode on the spectrophotometer was used for all quantitative measurements.

RESULTS AND DISCUSSION

A representative calibration curve for cadmium standards in blood is shown in Figure 1. Such curves were found to be linear from 0.4 µg to 16.0 µg Cd/l and highly reproducible. Coefficients of variation ($C.V. = \frac{S.E.(s)}{\bar{X}} \times 100$) for the measurement of cadmium at the atomization step, determined from 10 replicate injections each of blood samples containing 1.0 µg Cd/l and 6.0 µg Cd/l were 5.5 and 7.9%, respectively. For

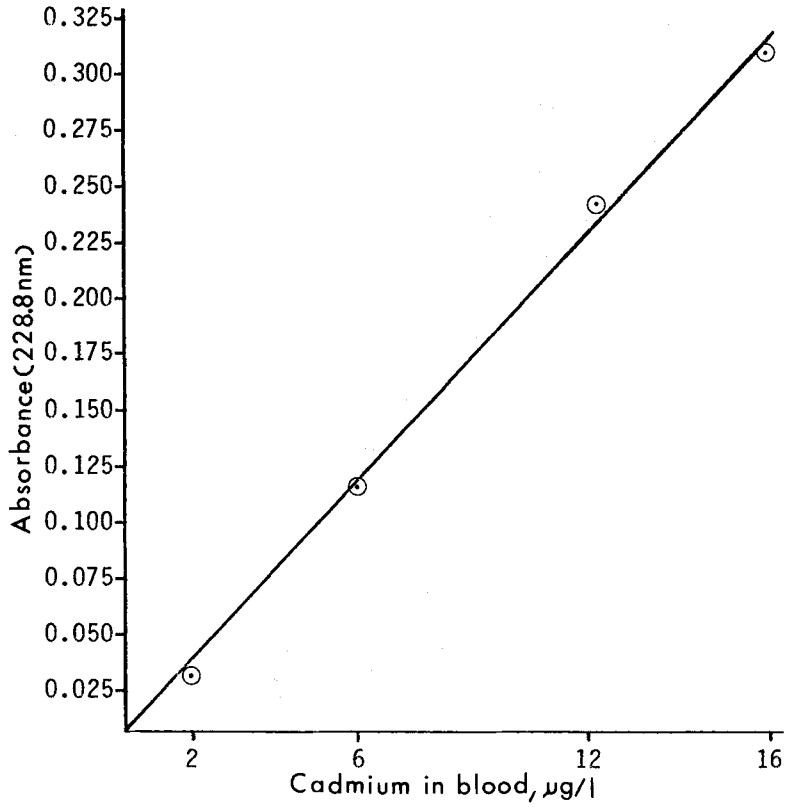

Figure 1. Representative calibration curve for cadmium in blood by atomic absorption spectroscopy (mean ± standard error).

cadmium in whole blood, 3 pg of Cd were needed to produce an absorbance of 0.0044 A. Expressed as concentration, this is equivalent to 0.15 µg Cd/l based upon a 20 µl injection volume. The detection limit (2X blank) was considered to be 8 pg, which corresponds to a concentration of 0.4 µg Cd/l.

Blood and plasma cadmium values obtained from the animals in the study were comparable to previously published values for these biological indices (Friberg, 1955; Piscator, 1974). Our method displayed a limit of detection equal to, or improved over, other published methods (Delves and Woodward, 1981; Huici, 1981), while providing a linear working range that exceeded several existing methods for determining cadmium in blood (Perry et al., 1975; Ikebe et al., 1979). The method described here produced reliable results and worked equally well for detecting cadmium in either blood or plasma. Since the method requires a minimum of complex instrumentation, it could easily be adapted for use in monitoring low levels of cadmium in animals or occupationally exposed humans.

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