AN IMPROVED METHOD FOR THE DIRECT DETERMINATION OF CADMIUM IN BIOLOGICAL MATERIALS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY[1]

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16. Abstract (Limit: 200 words) A method was presented for the determination of metals in mammalian tissues using an atomic absorption method. The procedure calls for digestion of the sample with a quaternary ammonium-hydroxide. With this method one can measure metals which are normally present in low concentrations such as cadmium (7440439) and nickel (7440020) with significant sensitivity and a minimal amount of sample handling. Two month old rats were exposed through inhalation to cadmium-oxide (1306190) aerosols daily for 8 hour periods. The precision of the method was determined in repeated analyses of control and exposed lung tissue. All lungs were divided into approximately five equal portions and each portion analyzed for cadmium, nickel, and zinc (7440666). To determine cadmium and nickel concentrations, 200 to 300 milligrams of wet tissue are needed for precision. These weights can be reduced to 1/20 for the analysis of zinc and copper (7440508). The absorbed amounts were small, suggesting that if the cadmium-oxide aerosol was absorbed, it was also effectively cleared from the system.

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ABSTRACT

An atomic absorption method for the direct determination of metals in mammalian tissues has been developed. The procedure, which involves digestion of the sample with a quaternary ammonium hydroxide, allows one to measure metals normally present in low concentrations, such as cadmium and nickel, with a great degree of sensitivity and a minimal amount of sample handling.

INTRODUCTION

At present atomic absorption is the most commonly used method for the determination of cadmium.[1] Sensitivities are high if a non-biological matrix is present,[2] or the biological matrix is sufficiently dilute to prevent chemical-physical interferences, or a special apparatus is used.[1,3] Since cadmium is present in much lower concentrations than zinc or copper in animal tissues, the common practice has been to increase the sample size or concentrate the sample.

A procedure has been developed in this laboratory for the specific analysis of cadmium and nickel in a variety of tissues. The method utilizes Soluene^{TM} 100 (Packard Scientific) or tetramethyl ammonium hydroxide (Eastman Chemicals) for the dissolution of the tissue, solubilizers originally developed for use in scintillation counting.[4] More recently their usage has been extended to gas chromatographic examination of mammalian tissues[5] and as a medium for sample preparation prior to zinc analyses by atomic absorption spectroscopy.[6] The recognized advantage of minimizing sample handling is supplemented by the increased sensitivity with which certain metals are detected. This method can be extended to analyze those trace metals which do not form insoluble hydroxides under basic conditions.

MATERIALS AND METHODS

White, two month old male rats (Greenacres Controlled Flora) were used for all experiments.

Reagents. All chemicals were reagent grade unless otherwise specified. All water used was triply distilled in a glass still. All glassware

was boiled one hour in aqua regia, rinsed five times with distilled water, immersed in 1% (w/v) ethylene diamine tetraacetic acid (EDTA) overnight and rinsed again five to ten times. Buffer solutions were prepared daily. The buffer used was sucrose (0.17 M), mannitol (0.15 M), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) (0.005 M), pH 7.4.[7]

Standard Solutions of Metals. A stock solution of $CrCl_3 \cdot 6H_2O$ (5.128 gm); $NiCl_2$ (4.05 gm); zinc metal (0.1000 gm) and $CdCl_2$ (0.163 gm) was prepared in 100 ml of (10% w/v) HNO_3. Copper (0.5 gm) was dissolved separately in 10% (w/v) HNO_3 (1 liter). Both solutions were stored at 4°C in acid washed polyethylene bottles. Both solutions were diluted with water at the time of use and aliquots taken for analysis. The final concentrations of metal ions ranged from 5×10^{-8} to 5×10^{-6} gm/ml. Stock solutions are prepared monthly.

Preparation of Lung Tissue. The rat was anesthetized with sodium nembutal, the rib cage opened, and the lungs perfused with buffer through the right ventricle of the heart until the lung visibly cleared of blood. They are then washed four successive times with 10 ml aliquots of buffer according to standard procedures,[8] separated from the trachea and bronchi, and pressed through a tissue press (Harvard Apparatus Company), pore diameter 1.5 mm. The tissue remaining in the press was analyzed separately. The remainder of the lung was divided into approximately equal portions on a wet weight basis, lyophilized overnight in a VirTis UNITRAP apparatus, weighed again, and analyzed for metal content.

Procedure. Dried lung samples weighing two to three hundred milligrams wet weight, (50-100 mg dry weight) were digested with an aliquot of Soluene, in a ratio of 2 ml Soluene for every 100 mg dried tissue, at 60°C for 24 hours in a Dubnoff shaking incubator. After digestion, the sample was diluted 2 1/2 times its volume with toluene and analyzed directly. Reagent blanks and standards were run concurrently and both internal and external standards were used. All metal additions were made prior to the digestion procedure. In the case of cadmium and nickel, the standard curve was not significantly altered by the presence of tissue (the matrix effect is minimal for 200 mgm quantities of tissue) leading ultimately to the use of external standards. The tissue metal concentrations reported were by the method of interpolation. All samples were diluted with toluene for zinc and copper analysis in a ratio varying from 1:2 to 1:20, depending on the weight of the tissue used. The dilution represents a source of error in the final evaluation of concentrations for these two metals. Every metal concentration was run in quadruplet or quintuplet, with and without pressed whole lung. Where internal standards are recorded, they consist of at least three whole lungs, pressed and divided into approximately equal portions, to which the metals were added. The weight of lung present in the standard is listed in the relevant table.

Analyses were performed on a Perkin-Elmer 303 Atomic Absorption Spectrophotometer, equipped with a Boling burner and a Sargent SRG Strip Chart Recorder, using standard methods.[2]

Inhalation Experiments. Cadmium oxide aerosols were generated according to a method established by Bingham et al.[11] and passed into the inhalation chambers. White, male, two-month-old rats were exposed daily for 8-hour periods. The total period is listed for each experiment.

RESULTS AND DISCUSSION

Table One shows data for the working curve of internal standards covering the range 0.1 to 0.5 and 1.0 to 5.0 x 10^{-6} gm/ml for cadmium and nickel, respectively. The means, standard deviations and coefficients of variations are listed for each metal. The coefficient of variation is somewhat larger for nickel for which this method is less sensitive. Although the slope of the standard curves varies slightly from day to day, the coefficients of variation for triplicate analyses performed on one day are generally less than 3%. If one uses external standards, the coefficient of variation is closer to 2%, which is very close to the coefficient of variation for the apparatus in measuring the same sample ten times (1.8%). For cadmium analyses, the average slope using standard acid wet ashing procedures[9] is about 0.357, measuring % absorption against µg metal, whereas, using the present method the average slope is around 1. For nickel, the slope changes from 0.222 in acid, to 0.666 in this organic base.

Since copper and zinc concentrations are determined after dilution, and therefore subject to a certain amount of dilution error, a National Bureau of Standards sample (#1577) of bovine liver was

subjected to the same procedure and analyzed. The copper recovery was 113% ± 5.0%, and zinc 123% ± 6% for three separate analyses.

The precision of the method in repeated analyses of control and exposed lung tissue is presented in Table 2. All lungs were divided into approximately five equal portions and each portion analyzed for Cd, Ni,and Zn. The standard deviations for each lung varied from a low of 0.02 ppm to 1.56 ppm, depending somewhat on the exposure to the inhaled metal. Within the cadmium and nickel concentration ranges measured, 200 to 300 mgs of wet tissue, in an exposed animal, are necessary for precision. If the animal is unexposed, 300 to 400 milligrams are required. These weights can be reduced by as much as 1/20 for the analysis of zinc and copper.[6]

Using the data given in Table 2, the total amount of cadmium recovered from the lung was calculated and compared with the total amount of cadmium present in the inhalation chamber during that period, as well as with the estimated amount of cadmium inhaled by the rats. It can be seen that the percent retained (5%) of that "inhaled", is larger by a factor of 50 than the amount retained from the total exposure. These figures are considerably lower than those reported in the literature for a variety of animals[10] and may reflect the conditions under which these experiments were run. All animals are exposed to a relatively small particle size, (89% are less than 0.53 μ diameter)[11] which would be relatively soluble. The animals were sacrificed 16 hours after removal from the chamber, and the total exposures were not large. However, experiments performed in these laboratories after 5 to 6 week exposure periods, to be reported in the

following paper, do not show any increase in retention. Under the conditions of these experiments then, it would appear that if the CdO aerosol is being absorbed, it is being efficiently cleared from the system.

In summary, data has been presented to illustrate a fast and relatively precise method for the analysis of cadmium and nickel in animals tissues. The technique has been used to measure cadmium concentrations in the rat lung after exposure to CdO aerosols.

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Table 1. Standard Curve for Cadmium and Nickel in the Presence of Lung Tissue[a]

Concentration µg/ml	Avg. Wet Wt. Tissue ± S.E. gms	Mean ± S.D. Absorbance Cd	Ni	Coefficient of Variation % Cd	Ni
1. 0	0.25469 ± 0.00039	0.0292 ± 0	0.0223 ± 0	0	0
2. 0.1 Cd 1.0 Ni	0.25421 ± 0.00119	0.0696 ± .002	0.0622 ± 0.003	2.8	4.8
3. 0.2 Cd 2.0 Ni	0.25600 ± 0.00210	0.1211 ± 0.004	0.0969 ± 0.003	3.3	3.1
4. 0.3 Cd 3.0 Ni	0.24964 ± 0.00894	0.1675 ± 0	0.1378 ± 0.017	0	12.3
5. 0.4 Cd 4.0 Ni	0.25707 ± 0.00043	0.2077 ± 0.01	0.1612 ± 0	4.8	0
6. 0.5 Cd 5.0 Ni	0.25521 ± 0.00316	0.2441 ± 0	0.1838 ± 0.005	0	6.0

a. Average of three determinations

Table 2. Cadmium, Zinc and Nickel Concentrations (Gm/Gm Wet Weight) in Whole Lung as a Function of Exposure to CdO Aerosol[a]

Exposure Time in Hours	Gms Metal/Gms Sample ± S.D.[b]		
	Cd	Zn	Ni
Control	$2.98 \times 10^{-7} \pm 0.4$[c]	$1.47 \times 10^{-5} \pm 0.23$	< limit
54	$3.05 \times 10^{-6} \pm 0.06$	$1.76 \times 10^{-5} \pm 0.04$	$8.01 \times 10^{-6} \pm 0.02$
58	$3.14 \times 10^{-6} \pm 0.44$	$1.62 \times 10^{-5} \pm 0.14$	$1.13 \times 10^{-5} \pm 0.10$
66	$5.79 \times 10^{-6} \pm 0.14$	$1.53 \times 10^{-5} \pm 0.14$	$9.42 \times 10^{-6} \pm 2.10$
72	$6.02 \times 10^{-6} \pm 1.56$	$1.84 \times 10^{-5} \pm 0.11$	$1.14 \times 10^{-5} \pm 0.11$

a. The cadmium concentration in the aerosol varied from 45 to 92 $\mu g/m^3$.

b. The values given are the mean and standard deviations of five analyses.

c. Average of three determinations.

Table 3. Cadmium Retention by the Lung as a Function of Exposure Times to CdO Aerosol[a]

Exposure Period in Hours	Total Cadmium Exposure in Grams[b]	Total Metal Recovered in Grams Cd	Zn	% Retention Cd	Calculated[c] Inhaled Cd in Lungs in Grams	Calculated % Retention of Inhaled Cadmium
54	3.523×10^{-3}	3.68×10^{-6}	2.32×10^{-5}	0.10	1.82×10^{-4}	5.1
58	3.849×10^{-3}	4.29×10^{-6}	2.37×10^{-5}	0.11	2.14×10^{-4}	5.5
66	3.947×10^{-3}	7.68×10^{-6}	2.48×10^{-5}	0.19	2.50×10^{-4}	6.3
72	4.979×10^{-3}	9.67×10^{-6}	3.16×10^{-5}	0.19	3.44×10^{-4}	6.9
Control	0	4.27×10^{-7}	2.30×10^{-5}	-	0	

a. The concentration of cadmium in the aerosols varied from 47 to 90 $\times 10^{-6}$ gms/m^3. The particle mass medium diameter R 0.15 μ, with 97% being less than 0.94 μ, (Ref. 11).

b. The total amount of cadmium to which the animals are exposed is calculated from the concentration of cadmium per cubic meter times the number of cubic meters in the exposure period.

c. The figures are based on an inhalation rate of 80/min. and a lung volume of 2 cc.

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