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Atomic Absorption Determination of Zinc, Copper, Cadmium, and Lead in Tissues Solubilized by Aqueous Tetramethylammonium Hydroxide

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Rat liver and kidney homogenates and homogeneous rat hair samples were prepared for atomic absorption spectrophotometric analysis by digestion with an appropriate concentration of aqueous tetramethylammonium hydroxide (TMAH). The endogenous tissue levels of Zn, Cu, Cd and Pb, the reproducibility of the analyses, and the recovery of added metal standards compare favorably with the results obtained by standard wet ashing procedures using concentrated nitric acid or nitric-perchloric acids. The use of 5% HNO_3 standard curve in calculations for the TMAH-treated samples leads to slightly lower results compared to the method of additions due to viscosity and surface tension effects on the aspiration rate of these samples. Moreover, the TMAH digestion method allows faster and safer processing and handling of samples in comparison to acid digestion procedures.

Methods presently available, which we have used frequently for the determination of trace metals in some biological materials by atomic absorption spectrophotometry require long digestion or extraction procedures (1–3). These are not only tedious, but may require rather stringent safety precautions as well, particularly when using strong acids. Experience with tissue samples in our laboratory has shown that an aqueous solution of tetramethylammonium hydroxide can be used for solubilizing some tissues for the determination of sodium and potassium by flame photometry and for estimation of protein content by the Biuret method. Tetramethylammonium hydroxide and other alkaline digestants also have been found useful in liquid scintillation counting (4) and gas chromatographic identification of tissues (5). As a consequence, an investigation was undertaken to determine the suitability of tetramethylammonium hydroxide for digesting rat tissues in the determination of zinc, copper, cadmium and lead by atomic absorption spectrophotometry.

MATERIALS

A Perkin-Elmer Model 303 atomic absorption spectrophotometer with a Boling air-acetylene burner and recorder readout was used.

Tetramethylammonium hydroxide (TMAH), 10% and 25% aqueous solutions (Eastman Organic Chemicals); concentrated nitric acid (HNO_3), 70–71% reagent grade (du Pont); concentrated perchloric acid ($HClO_4$), 60–62% reagent grade (Allied Chemical); and standard metal solutions (Fisher "Certified") of zinc, copper, cadmium and lead were used. The metal solutions were diluted as required with 5% HNO_3 to make working standards containing 10, 20, 40 or 50 μg/ml of Zn, Cu, Cd and Pb, or with deionized water for use in the method of additions (6).

All glassware used was washed with 10% HNO_3 to remove metallic contaminants, rinsed thoroughly with deionized water, and dried.

METHODS

Digestion of Tissue with TMAH. One gram of liver or kidney was homogenized in 4 ml of deionized water using a Bronson sonifier, and the volume was adjusted to 5.0 ml with deionized water. Larger quantities of tissue were prepared as required, maintaining the ratio of one gram of tissue per 5.0 ml of homogenate. Aliquots (0.5 ml) of the tissue homogenate were pipetted into 10-ml graduated cylinders, along with 0–0.5 ml of an appropriate metal working standard and 1.5 ml of 10% TMAH. A series of solutions, with varying amounts of added metal standards, was thus obtained and used to construct a calibration curve for use in the method of additions (6). The volume was adjusted to 2.5 ml with deionized water, the contents mixed, and allowed to stand at room temperature for 30 min. The samples were then incubated in a thermostatted water bath at 65°C for 25 min agitating with a vortex mixer every 5 min.[1] Upon cooling, each solution was made up to 5.0 ml with deionized water.

Digestion of Rat Hair with TMAH. Hair from several rats was finely cut and combined. After washing the samples according to the procedure of Petering *et al.* (7), they were homogenized in deionized water with a Waring blender. The homogenized hair was then filtered, washed with deionized water, rinsed with reagent-grade acetone, and dried between filter papers in an oven at 70°C for 1 hr. Samples of 100 mg of hair homogenate were transferred into 10-ml glass-stoppered, graduated cylinders together with 0–1 ml of an appropriate metal working standard solution (for the method of additions) and 3.0 ml of 10% TMAH. The

[1] The same digestion can be accomplished without incubation by increasing the TMAH content to 2 ml, shaking and leaving the stoppered or covered samples overnight at room temperature. Such treatment is also suitable for thin tissue slices, if shaken continuously.

total volume was brought to 4.0 ml with deionized water, the contents mixed with a vortex mixer, and the mixture allowed to stand at room temperature for 4 hr or longer. The samples were then incubated in a thermostatted water bath at 65°C for 50 min, agitating every 5 min. After making up the volume to 5.0 ml with 25% aqueous TMAH, the incubation and agitation were continued for an additional 15 min.[2] The contents were cooled and readjusted to 5.0 ml with deionized water. Because of high sample viscosity, the samples were further diluted with an equal volume of deionized water prior to analysis.

HNO_3 Digestion of Tissue and Hair. Samples identical to those treated with TMAH were digested with 10 ml of concentrated HNO_3 (1). The digestion was carried out in 30-ml Pyrex beakers covered with watch glasses on a hot plate until complete dissolution had occurred and the solution had become colorless. The acid solution was evaporated to less than 0.5 ml and hot, 5% HNO_3 was used to redissolve the residue. The solution was cooled and made up to 5.0 ml with 5% HNO_3.

HNO_3–$HClO_4$ Digestion of Tissues. The procedure of the preceding section was followed, except that 8 ml of concentrated HNO_3 plus 2 ml of concentrated $HClO_4$ was the digesting agent (1).

Analysis. After digestion, the sample solutions were transferred to 10-ml polypropylene test tubes. In order to avoid losses of metals by surface adsorption, the samples were analyzed the same day by atomic absorption spectrophotometry. The metal concentrations were determined by the method of additions and also by comparison with a curve based on 5% HNO_3 solutions of mixed metal standards.

RESULTS AND DISCUSSION

Metal Determinations and Reproducibilities. Metal concentrations in the TMAH-digested solutions giving a % absorbance twice the noise were 0.01 μg/ml of Zn, Cu or Cd and 0.02 μg/ml of Pb. These detection limits correspond to 0.5 μg/g of Zn, Cu, or Cd and 1.0 μg/g of Pb in the original liver or kidney samples, and to twice these limits in the case of hair samples.[3]

Table 1 shows the average levels of trace metals expressed as μg ± standard deviation of metal per gram of tissue in liver samples containing endogenous zinc, copper and cadmium and in hair samples containing

[2] Digestion can also be accomplished by adding 5.0 ml of 10% TMAH to the hair in 20-ml inert plastic beakers, making sure that all hair is submerged, and leaving the beakers covered with a watch glass overnight at room temperature.

[3] Lower detection limits of 0.2 μg/g of Zn, Cu, and Cd, and 0.4 μg/g of Pb, in liver and kidney can be obtained by solubilizing 2.5 times more tissue with a proportionately larger amount of TMAH. However, this will result in a higher protein content and therefore increased viscosity compared to less concentrated samples.

TABLE 1
Determination of Zn, Cu, Cd, and Pb in TMAH-Digested Samples

Sample	Standard	Metal in sample (μg/g)[a]			
		Zn	Cu	Cd	Pb
Liver[b]	Method of additions	38.6 ±0.8	6.0 ±0.4	4.2 ±0.2	<1
Liver[b]	5% HNO_3 standards	35.0 ±0.5	5.6 ±0.4	4.0 ±0.2	<1
Hair[c]	Method of additions	228 ±3.4	10.7 ±0.4	<1	47.2 ±1.7
Hair[c]	5% HNO_3 standards	201 ±3.8	10.2 ±0.4	<1	42.8 ±1.8

[a] Data are given as mean ± standard deviation of eight determinations.
[b] From rats with Cd added to their diet.
[c] From rats with Pb added to their diet.

endogenous zinc, copper and lead. The results obtained using 5% HNO_3 standards were approximately 8.8–9.5% lower than those using the method of additions. This was more evident with hair samples and was probably related to their higher viscosity. Further dilution of the samples before aspiration would have resulted in better agreement, but at the expense of the sensitivity of the analyses.

Figure 1 shows typical calibration curves for metal standards dis-

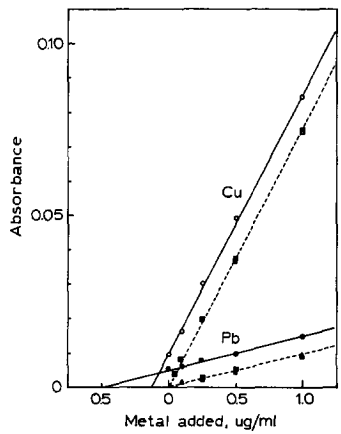

FIG. 1. Calibration curves for low levels of copper (open symbols) and lead (solid symbols). Solid lines: Method of additions to TMAH-digested hair, giving 0.107 μg Cu/ml (10.7 μg Cu/g hair) and 0.472 μg Pb/ml (47.2 μg Pb/g hair). Dashed lines: Metal standards added to 5.5% TMAH (triangles) or 5% HNO_3 (squares), with no tissue present.

TABLE 2
Recovery of Metals Added to TMAH-Digested Samples[a]

Sample	Standard	Metal recovered (μg/g)				% Recovery			
		Zn	Cu	Cd	Pb	Zn	Cu	Cd	Pb
Liver	Method of additions	25.1 ±1.1	25.2 ±0.9	25.5 ±0.5	24.8 ±1.4	100.3	100.7	102.0	99.3
Liver	5% HNO_3 standards	23.1 ±1.0	24.7 ±0.9	25.7 ±0.3	24.8 ±1.5	92.3	98.7	102.7	99.3
Kidney	Method of additions	25.1 ±1.6	25.0 ±1.0	24.9 ±1.0	24.0 ±1.2	100.3	100.0	99.7	95.7
Kidney	5% HNO_3 standards	23.7 ±1.1	23.6 ±1.1	25.4 ±1.0	24.7 ±0.5	94.7	94.3	101.7	98.7
Hair	Method of additions	245 ±6.9	26.5 ±0.5	26.2 ±1.2	25.2 ±2.0	97.9	106.0	104.7	101.0
Hair	5% HNO_3 standards	244 ±3.8	22.9 ±0.6	21.5 ±0.8	20.5 ±1.2	97.5	91.7	86.0	82.0

[a] The amount of added metal was 25 μg/g of tissue, except in the Zn in hair, where 250 μg/g were added. Data are given as mean ± standard deviation of six determinations.

TABLE 3
Recovery of Metals Added to Acid-Digested Samples[a]

Sample	Digestion	Metal recovered (μg/g)				% Recovery			
		Zn	Cu	Cd	Pb	Zn	Cu	Cd	Pb
Liver	HNO_3	23.5 ±0.7	25.0 ±0.8	25.4 ±0.8	24.8 ±1.5	94.0	100.0	101.5	99.3
Liver	HNO_3–$HClO_4$	24.0 ±0.4	23.6 ±0.6	24.4 ±0.6	25.0 ±2.1	96.0	94.5	97.5	100.0
Kidney	HNO_3	24.2 ±1.1	24.2 ±0.7	25.6 ±0.8	21.6 ±1.1	96.5	97.0	102.5	86.5
Kidney	HNO_3–$HClO_4$	23.0 ±2.0	21.2 ±0.4	24.5 ±0.6	25.6 ±0.3	92.0	85.0	98.0	102.5
Hair	HNO_3	248 ±8.2	24.8 ±0.5	22.4 ±0.8	22.8 ±2.1	99.0	99.0	89.7	91.3

[a] The amount of added metal was 25 μg/g of tissue, except in the Zn in hair, where 250 μg/g were added. Data are given as mean ± standard deviation of four determinations, computed from 5% HNO_3 standard curves.

solved in 5% HNO_3 or 5.5% TMAH (with no tissue), and for the method of additions to TMAH-digested hair. The linearity of these plots at low metal concentrations and the similarity of the absorbance of metal standards in dilute HNO_3 and TMAH suggest that there is no nonlinear dependence of absorbance on viscosity in the solutions containing TMAH, and that interferences due to TMAH are minimal. From Fig. 1, it is evident that the hair digest contains 0.107 μg Cu/ml and 0.472 μg Pb/ml, corresponding to 10.7 μg Cu/g hair and 47.2 μg Pb/g hair.

Recoveries. Known amounts of zinc, copper, cadmium and lead (25 μg in all cases except hair Zn, where 250 μg of Zn were added) were added to 1.0-g portions of liver, kidney and hair and the samples processed using TMAH and acid digestion procedures. Data are presented in Tables 2 and 3. Analysis of six replicates by TMAH digestion and using the method of additions showed an overall mean recovery of 100.6 ± 2.7% for the metals. Use of 5% HNO_3 standards for computation yielded recoveries of 97.2 ± 3.8% for all samples, with the exception of the low recoveries of 86.0% for Cd and 82.0% for Pb in hair. When HNO_3 alone was used for digestion, with 5% HNO_3 standards, the recoveries were 98.0 ± 3.4% for all samples, other than Pb in kidney at 86.5% and Cd in hair at 89.7%. For the digestions with HNO_3–$HClO_4$ mixture, the overall mean recovery was 97.2 ± 3.5%, except for Cu in kidney, at 85.0%. The TMAH method thus shows a favorable comparison with the acid digestion methods in the recoveries of Zn, Cu, Cd, and Pb.

TABLE 4
Comparison of Digestion Methods for Metal Analysis

Sample	Digestion	Standard	Metal in sample (μg/g)[a]			
			Zn	Cu	Cd	Pb
Liver	TMAH	Method of additions	28.5	5.5	<0.5	<1
Liver	TMAH	5% HNO_3 standards	26.0	5.0	<0.5	<1
Liver	HNO_3	5% HNO_3 standards	27.5	7.5	<0.5	<1
Liver	HNO_3–$HClO_4$	5% HNO_3 standards	28.5	8.0	<0.5	1.0
Kidney	TMAH	Method of additions	29.5	19.0	<0.5	<1
Kidney	TMAH	5% HNO_3 standards	27.5	17.5	<0.5	<1
Kidney	HNO_3	5% HNO_3 standards	25.0	16.5	<0.5	<1
Kidney	HNO_3–$HClO_4$	5% HNO_3 standards	26.0	17.0	<0.5	<1
Hair	TMAH	Method of additions	235.0	21.0	<1	<2
Hair	TMAH	5% HNO_3 standards	188.0	18.0	<1	<2
Hair	HNO_3	5% HNO_3 standards	178.0	16.0	<1	2.0

[a] Concentrations per ml of aspirated solution (corrected for reagent blank) were multiplied by a factor of 50 to obtain μg of metal per gram of wet liver or kidney; acid-digested hair also required a factor of 50; TMAH-digested hair required a factor of 100. All results are the means of six determinations.

Comparison of the TMAH Method with Acid Digestion. To determine whether the results obtained by TMAH digestion are comparable to those obtained by other methods, samples of liver, kidney and hair from rats on diets without added trace metals were processed and the results shown in Table 4. Agreement is generally good between methods, and the previously mentioned differences between the calculations based on the method of additions and those based on 5% HNO_3 standards are again evident.

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