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Interferences in the Determination of Metallic Elements in Human Hair

An Evaluation of Zinc, Copper, Lead, and Cadmium, Using Atomic Absorption Spectrophotometry

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Hair, a metabolic end product, has been used to indicate health status. Our purposes in this study were: (1) to evaluate the hair digest matrix as a source of interferences in the determination of metals using atomic absorption spectrophotometry, and (2) to develop a technique whereby meaningful interlaboratory comparison of analytical data can be made. We have demonstrated that for hair analysis, interferences due to broad-band nonspecific molecular absorption, chemical ionization, flame emmission, flame scatter, light scatter, and matrix matching can be avoided using ordinary methods and commercially available equipment. We have also presented a technique for the preparation of a standard reference hair sample. This standard reference hair was then used to demonstrate that concentrations of copper, zinc, lead, and cadmium in a nitric acid hair digest matrix can be measured with precision and accuracy.

As part of a national study (to be published) to evaluate the influence of the nonessential metals found in man's environment, over 1,500 samples of hair were collected for essential and nonessential metal analysis. In attempting to set up a routine method for analysis of a large number of samples, we decided to use a method that would be accurate, sensitive, rapid, reliable, inexpensive, and conveniently used by other laboratories.

Hair has long been recognized as a metal-containing metabolic end product[1] and is an easily collected specimen. Several papers have described levels of essential[1-3] and nonessential[4,5] metals in human hair. Some reports demonstrated a relationship between hair metal content and nutritional intake as well as environmental exposure.[2,5,6] Harrison et al[3] have suggested collecting the hair from the nape of the neck. Strain and Ponies[7] recently presented a detailed study of the seasonal variation encountered in the use of this collection method.

To analyze hair samples for the metals of interest, it is necessary to remove any metal contaminants from the hair surface. Harrison et al[3] reviewed the methods of hair washing and have suggested the use of a nonionic detergent. However, use of ionic detergents gave similar results. Methods using incipient boiling in ethylenediaminetetraacetic acid solutions were considered and discarded in the belief that hair metal in addition to surface metal would be removed. Washing with organic solvents alone[1] was also considered but was discarded in the belief that inorganic surface contaminants would not be removed. The method used by Petering et al,[8] washes with ionic detergent followed by successive rinses with water, acetone, and ether, was considered best for our purposes, because it is suited to the removal of free oils, dyes, and lacquers commonly used for cosmetic purposes (95% of the hair samples in our national study were obtained from women).

Harrison et al[3] have also reviewed the literature concerned with different methods of determining the concentration of metals in hair and concluded that atomic absorption spectrophotometry is the method of choice. After they washed and dried the hair, it was wet-ashed. This contrasts with the dry-ashing procedure of Schroeder and Nason.[1] Others have routinely employed wet-ashing as the method of choice. We chose wet-ashing to avoid losses due to the possible formation of volatile metal components in dry-ashing. A concentrated nitric acid digesting medium was chosen over a mixture of perchloric-nitric acids, to avoid potential losses of chlorides of cadmium and mercury from the digest matrix. Losses during digestion may be detected by adding a

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known amount of the analyte to several portions of a standard sample prior to digestion. The results of analysis of these samples will show complete recovery of the added analyte if there are no losses. Although recovery of cadmium was satisfactory, recovery of mercury was not. However, a good method for the determination of mercury in hair has subsequently been developed and is published in the following paper (see page 40).

Harrison et al[3] used recovery studies to determine the effect of the digest matrix on absorbance of the analyte, as well as to uncover losses during digestion. In the present work, we have evaluated possible interferences due to broadband nonspecific molecular absorption, chemical ionization, flame emmission, flame scattering, light scattering, and matrix matching interferences.[9] The evaluation was made by applying our method to a standard reference hair sample. The standard reference hair sample was obtained from one individual, finely cut, blended, and washed. A portion of it was also used in the work of Nord et al (see page 40). The standard reference hair was also used to study the recovery of analytes added prior to digestion.

Using a standard reference hair sample, we have shown that the determination of metals in human hair by atomic absorption spectrophotometry is free of interference from the digest matrix when nitric acid is used as the sole digesting agent. In addition, we have established that ionization, chemical, light scattering, and molecular absorption interferences are essentially absent in the determination of copper, zinc, lead, and cadmium when commercially available equipment is used.

Since the concentration of essential and nonessential metals will vary, depending upon nutritional and environmental circumstances, we feel that a firm basis for comparison of data obtained in different laboratories is essential. Analytical methods for the determination of metals in samples of biological origin should be evaluated with respect to the recovery of metal added to a standard sample. This procedure can then serve to improve the validity of comparison of data obtained in different laboratories with regard to biological significance.

Methods

Washing Procedure.—Hair samples were washed according to the method described here and by Petering et al.[8] The sample was covered successively with diethyl ether and acetone. The sample was stirred with each solvent for ten minutes at room temperature and decanted. (Stirring and blending time is dependent upon sample size.) The hair was then stirred with a 50% solution of sodium lauryl sulfate at 35 C for 20 minutes. The detergent solution was decanted and the hair rinsed with deionized water until the detergent was removed. Finally, the sample was rinsed with acetone and then ether, air dried between filter papers, and stored in a plastic bag.

All glassware used in this study was allowed to stand in a 10% nitric acid solution overnight and rinsed thoroughly with deionized water. Cleaned glassware was dried in an oven and stored in a cabinet between layers of paper plastic to prevent dust contamination.

Digestion.—Hair samples weighing 400 mg were placed in 100-ml beakers. Ten milliliters of concentrated nitric acid was then added with an automatic pipette and the beakers covered with watch glasses. The samples were allowed to stand in contact with the acid overnight. On the following day, the mixture was heated on a hot plate to obtain a solution and carefully evaporated to the point of near-dryness. The solution was carefully maintained below the boiling point to permit the loss of vapors but to prevent metal losses due to aerosol formation. With the use of large hot plates, one technician can carry 40 or 50 beakers at a time through the digestion procedure. An additional 5 ml of concentrated nitric acid was added and the procedure was repeated. The residue was transferred to 10-ml graduated cylinders with 10% nitric acid and diluted to 5 ml. Each 5-ml sample was then poured into a numbered disposable plastic test tube and capped for storage.

Measurement of Percent Absorption.—The percent absorption of the prepared samples was measured directly for Pb and Cd. A 1:5 dilution prepared with 10% nitric acid was required to obtain a concentration of Cu and Zn so that the percent absorption was in the range of the instrument. Reagent blanks were analyzed with each wet-ashing to determine if any contamination occurred during the ashing procedure. In all cases the amount of metal in the blank was negligible.

Analyses were performed by standard analytical methods[9,10] with an atomic absorption spectrophotometer equipped with a Boling burner and a strip chart recorder. Continuous-source compensation was provided by a deuterium lamp.[10] The instrument was adjusted according to the manufacturer's directions for each analyte. The percent absorption of each sample was recorded automatically. Before and after each batch of samples was studied the percent absorption of the analyte in standard solutions of several dilutions was recorded. The analytical standard solutions were prepared by dilution of commercially available stock solutions containing 1,000μg of analyte per milliliter, in 10% nitric acid.

Preparation of Standard Reference Hair.—The standard reference hair sample was prepared in the following manner: A large sample of hair donated by one woman in the laboratory was cut into approximately 3-mm lengths with stainless steel scissors. The cut hair was placed in a large beaker and stirred vigorously in deionized water overnight to insure homogeneity. The sample was washed as described above and stored in a paraffin film covered, acid-washed, glass container.

Recovery Study.—Forty samples of the standard reference hair, each weighing 400 mg, were placed in 100-ml beakers. They were divided into four groups. To group one no addition was made. In group two, 50μg, 10μg, 5μg, and 0.2μg of Zn, Cu, Pb, and Cd, respectively, were added to each sample. The additions to the final two groups were, respectively, for Zn, Cu, Pb, and Cd: group three, 70μg, 20μg, 10μg, and 0.5μg; group four, 100μg, 30μg, 20μg, and 1.0μg. The additions were made by pipetting quantities of the analytical standard solutions on the hair samples prior to digestion. Each of the 40 samples prepared for the recovery study went through the digestion and analysis procedure described above. The absorbance of standard solutions of the analytes of known concentration was measured at the same time.

A plot of the absorbance (ordinate) vs the number of micrograms of analyte added (abscissa) was made for the 40 points of each analyte. The intercept of the line through the points with the abscissa is taken as the concentration of the analyte in the sample to which no addition has been made (method of addition[9]). In addition, the concentration of the analytes in the sample solutions was determined by interpolation of the sample absorbance with the absorbance of standard solutions

Table 1.—Recovery of Zinc, Copper, Lead, and Cadmium Added to Standard Hair Samples*

Metal	μg Added	μg Recovered†	% Recovery
Zinc	50.0	48.5 ± 1.5	97.0 ± 3.0
	70.0	74.9 ± 4.0	107.0 ± 5.8
	100.0	100.0 ± 3.6	100.0 ± 3.6
Copper	10.0	9.3 ± 0.5	93.3 ± 5.0
	20.0	21.0 ± 1.0	105.2 ± 5.0
	30.0	29.2 ± 1.0	97.3 ± 3.5
Lead	5.0	4.80 ± 0.16	96.0 ± 3.2
	10.0	9.95 ± 0.15	99.5 ± 1.5
	20.0	19.60 ± 0.28	98.0 ± 4.0
Cadmium	0.20	0.195 ± 0.027	95.5 ± 1.4
	0.50	0.485 ± 0.014	97.0 ± 2.8
	1.00	0.910 ± 0.026	91.0 ± 2.6

*Each entry represents the mean of ten determinations.
†±SE.

Table 2.—Comparison of Hair Metal Concentration (μg/gm)* Using Methods of Additions and Interpolation

Metal	Method of Additions†	Interpolation Method‡
Zinc	215.13 ± 29.06	189.75 ± 44.31
Copper	70.50 ± 9.31	71.25 ± 1.51
Lead	39.00 ± 0.02	43.26 ± 4.32
Cadmium	2.86 ± 0.35	2.60 ± 0.02

*The limits are those obtained at the 95% level of confidence.
†Forty samples used in this determination.
‡Ten samples used in this determination.

Table 3.—Continuous-Source Compensation Comparison of the Analytical Data (μg/gm)*

Element	Without Deuterium Lamp	With Deuterium Lamp
Zinc	189.4 ± 1.0	188.8 ± 0.8
Copper	70.7 ± 0.9	76.3 ± 1.3
Lead	44.7 ± 0.3	45.0 ± 0.3
Cadmium	1.92 ± 0.02	1.93 ± 0.03

*The limits are those obtained at the 95% level of confidence.

of known analyte concentration (interpolation method, see page 40). A least-square plotting program was used to fit straight lines to the absorbance vs concentration data from the analytical standard solutions, and the data from the method of additions. The program computed the concentration values obtained by the methods of additions and interpolation, as well as determined the slopes of the plots.

Interference Study.—The absorbance of additional samples of the standard reference hair was measured at the wavelengths appropriate for the various analytes. The sample absorbance was then measured at nonabsorbing wavelengths: 211.0 nm, 333.0 nm, 288.2 nm, 216.0 nm for Zn, Cu, Pb, and Cd, respectively. These wavelengths were close to the absorbing wavelengths for the respective metals: 213.8 nm, 324.8 nm, 283.3 nm, and 228.8 nm. Absorbance measurements were also made with continuous-source compensation[11] (deuterium lamp).

Results

Results of recovery studies, using the method of additions, are presented in Table 1. Since the recovery is nearly quantitative in each case, these data show that a linear relationship is obtained for concentration vs absorbance. The small standard error observed in each case demonstrates that the variation due to technique is slight. The conclusion is that metal added to the hair matrix can be recovered with precision and accuracy when the samples are wet-ashed with concentrated nitric acid.

The concentrations of Cu, Zn, Pb, and Cd in the standard reference hair obtained with methods of additions and interpolation are presented in Table 2. These data were obtained to compare the results of the additions method with those of the faster, and sometimes more accurate, interpolation method. By inspection, it appears that both methods give the same precise results. Since it was of interest to know whether or not these data were significantly different, the variation at the 95% level of confidence was calculated from fitted, least squares plots of the experimental data. In light of the confidence limits of the concentration values, there appears to be no significant difference between concentration values obtained by the methods of additions and interpolation for these specific metals of interest in hair.

Having established that the data obtained by the two methods were equally precise, we were interested in evaluating the accuracy of our concentration values. To do this, we decided to evaluate possible interferences associated with this form of spectroscopy.[9] Flame emission and flame scatter interferences were considered to be of little consequence in the use of the double beam instrument. The physical properties of the sample digest matrix are often different enough from those of the analytical standard solutions to cause an interference (matrix matching interference[9]). If matrix matching is a factor, the results from the method of additions will differ from those of the interpolation method. As the results were identical, no matrix matching interference was found. However, errors due to light scatter, ionization (enhancement and depression), and chemical and molecular absorption interferences are not eliminated with this method of analysis. The possibility that these interferences exist leaves some doubt as to the accuracy of the analytical results.

Interferences such as light scatter due to particulate material in the light path and molecular absorption by ultraviolet absorbing molecules in the light path have been ruled out. This was done by measuring the absorbance of the standard reference sample at nonabsorbing wavelengths

and the use of continuous-source compensation. In this manner, background absorptions due to light scatter and nonspecific absorption can be approximated. This technique is useful under circumstances where the continuous-source compensation technique is not available. We have also used continuous-source compensation to demonstrate the lack of light scatter and molecular absorption interferences. The data presented in Table 3 demonstrate that background absorption, due to light scatter and molecular absorption interferences, was not observed when nitric acid wet-ashed hair samples were analyzed in 10% nitric acid solutions.

To evaluate the presence or absence of chemical and ionization interferences we decided to compare the slopes of the least squares plots obtained with the data from the two methods of analysis. Chemical interferences are usually caused by anions, which interfere by forming salts that undergo incomplete dissociation, in the flame, to free atoms. In this event the observed absorption due to the analyte is less than expected. Enhancement ionization interferences are caused by elements which have higher ionization potentials than the analyte. This enhancement of absorption is the result of neutralization of the ionized analyte and results in a greater absorption than expected. A depressant ionization interference would be observed if one analyzed for the metal having the higher ionization potential. The depression of absorption is the result of ionization of the analyte, resulting in a lower absorption than expected. The presence of either or both of these interferences would give nonparallel least squares plots for the methods of additions and interpolation with regard to the particular metal of interest. Since there appears to be no light scattering or molecular absorption interferences, only chemical and ionization interferences could cause the slopes of the two plots for each metal to be nonparallel. Statistical analysis of the slopes using the Student *t* test, demonstrated that at the 95% level of confidence, the slopes obtained by the two methods of analysis were the same for Cu, Zn, Pb, and Cd. The results of these analyses demonstrate a lack of chemical and ionization interferences associated with the nitric acid solution of digested hair. This is consistent with the fact that no difference in concentration was observed when these samples were analyzed by the methods of additions and interpolation. Also the metals do not interfere with each other at the levels obtained when metals were added to the reference hair sample.

In summary, our results show that after removal of metal contaminants with an appropriate washing method, a 10% nitric acid solution of the concentrated nitric acid digest provides an interference-free matrix for the analysis of Cu, Zn, Pb, and Cd. In addition, hair analysis can be done with precision and accuracy by means of the interpolation method.

Comment

With the recognition that the concentration of nonessential metals is increasing in our environment, there is a need to accurately monitor these increases to safeguard the health status of the population. Hair is a convenient specimen for the evaluation of essential metal metabolism related to the nutritional and environmental circumstances of individuals in the population. As more and more epidemiologic studies are done by different laboratories, there is an increasing need to be able to relate one study to another.

When the results of two epidemiologic studies on the same metal produce results of different range, one wonders if the difference is of biological significance, or whether there was an analytical bias. The results of work by several laboratories may be adequately compared, if they all report analysis of portions of the same sample. This is particularly important with respect to samples of biological origin, which contain many components. The National Bureau of Standards offers a few samples of biological origin as standard reference material, listing the concentration of many component metals. Ideally it is the analysis of such standard reference materials that will put interlaboratory comparisions on a firm footing. Although there are only a few such materials available, we would encourage their use where appropriate.

In situations in which there are not appropriate standard reference materials, we encourage individual investigators to make their own. The results of precision and recovery studies on the standard reference material should be known before any large-scale analysis is done. This procedure would serve to improve the basis for comparison of data obtained in different laboratories with regard to observed differences.

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Je Anne Burg and Elizabeth Hallstein did the statistical analysis.

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