THE DETERMINATION OF TRACE ELEMENTS IN HUMAN HAIR BY ATOMIC ABSORPTION SPECTROSCOPY

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SUMMARY

The concentrations of copper, iron, magnesium, and zinc in the hair of eighteen different adult male subjects have been determined by atomic absorption spectroscopy over periods ranging from 4 to 10 months. The mean for each of the four analysis elements is shown for each subject. A mean, median, and range are given for each subject. A mean, median, and range are given for each element taking all subjects into consideration. A study was made of the pre-treatment of hair, including a comparison of detergent washed and organic solvent washed hair and an investigation of other wash parameters. Recovery studies were made for each analysis element.

INTRODUCTION

Although methods have been developed for the determination of copper, iron, magnesium, and zinc in biological materials by atomic absorption spectroscopy, little work has been done to establish a similar procedure applicable to the analysis of these elements in human hair, a biological sample of unique promise and problems. The analysis of human hair affords the opportunity to monitor conveniently trace metal concentrations which may reflect abnormal levels of such elements in the body. It has been shown that zinc levels in the hair of individuals known to have a zinc deficiency are significantly lower than normal values[1,2], and that the zinc levels increased following oral zinc sulfate therapy. However, Rice and Goldstein[3] and Martin[5] have shown that there is no abnormal deposition of copper in the hair of patients with Wilson's disease in contrast to most other organs and tissues. Schneider and Anke[4] have investigated the mineral content in human hair in different pathological cases.

Prior to the development of neutron activation analysis most hair analysis was conducted spectrophotometrically[3,5,6]. Kopito *et al.*[7] used atomic absorption to analyze for lead in hair as an aid in the diagnosis of chronic, mild, or subacute lead poisoning in children, but did not expand its applicability to other elements. The majority of methods established for the analysis of trace metals in human hair have

been of a forensic nature using neutron activation analysis. Perkons and Jervis[8] reported the presence of 18 different trace elements and their concentrations in human hair. Bate and Dyer[9] have also investigated the mean concentrations and ranges of trace elements in human hair using neutron activation analysis. Schneider and Anke[10] have conducted an investigation of the dependence of sex, age, hair color, and type of hair on the concentrations of calcium, magnesium, phosphorus, zinc, manganese, copper, and molybdenum in human hair. An emission spectrographic study by Goldblum *et al.*[11] covered 14 different elements in human hair.

The present study was initiated to extend our biological trace metal investigations[12] to include human hair, a convenient, readily available, and relatively unused sample. The object was to determine the range of concentrations of the analysis elements in human hair to establish a baseline which would allow the use of hair analysis as a diagnostic aid. As a sensitive, accurate, and relatively interference-free technique, atomic absorption allows rapid analysis for elements which might be of significance.

EXPERIMENTAL

Reagents

Working standards for each element were prepared by dilution of 1000 μg/ml standard solutions (Fisher Scientific Company, Pittsburgh, Pa.). The Fisher standards were determined to be satisfactory when checked against standards prepared from spectrographically pure reagents. Dilutions were made with distilled water passed through a research grade ion exchange column (Type R-2 Cartridge, Illinois Water Treatment Company, Rockford, Ill.).

The nitric acid (redistilled, G. F. Smith Chemical Company, Columbus, Ohio) and perchloric acid (doubly vacuum distilled, G. F. Smith Chemical Company) used for the wet digestions were selected because of very low trace metal content.

Apparatus

Analyses were performed using a Perkin–Elmer Model 303 atomic absorption spectrophotometer with a DCR readout unit. A Boling burner head replaced the standard slot burner for greater sensitivity. Hollow cathode current, slit settings, and gas flow rates were set as suggested by the manufacturer.

Method

Special precautions were taken to avoid trace element contamination, as previously described[12]. The hair analyses were made on approximately 0.5 g hair samples obtained from the nape of the neck in most cases. Each sample was minced with a stainless steel surgical scalpel until the individual hairs were less than 1 cm in length. After cutting, the samples were thoroughly mixed to ensure homogeneity. Surface contamination was removed by washing the samples in 500 ml polyethylene bottles with 150 ml of a 1% non-ionic detergent solution, 7X O-Matic*, which contained negligible amounts of the analysis elements. The bottles were agitated on a mechanical shaker for 30 min at room temperature. Upon completion of the washing cycle,

* Linbro Chemical Co. Inc., 681 Dixwell Ave., New Haven, Conn. 06511 (U.S.A.).

the samples were transferred to a polyethylene filter crucible and rinsed with a total of 1 l of deionized water to remove the detergent. The samples were dried overnight at 110° (average weight loss 6–8%), weighed and transferred to 50-ml Erlenmeyer flasks for digestion. The dry weight of the samples ranged from 0.3 to 0.8 g.

The wet digestion involved the addition to the sample of 6 ml of nitric acid which was allowed to react slowly at room temperature to prevent excessive foaming. After then warming the nitric acid digest, 1 ml of perchloric acid was added and the digestion continued on a hot plate at about 200° until dense white fumes of perchloric acid were evolved. At this point the mixture was water clear and less than 1 ml of solution remained. Each sample was transferred to a 5-ml volumetric flask and diluted to volume for copper, iron, and magnesium. Further dilutions were required for zinc and in some cases, magnesium, as determined by the range of concentrations encountered.

RESULTS AND DISCUSSION

Pre-treatment of hair

Sample preparation has been one of the major problems in hair analysis. Surface contaminations should be completely removed from the hair prior to analyzing to ensure that the trace element concentrations obtained reflect only that present within the hair structure. Methods for removing surface contaminations are numerous but fall generally into two categories: (1) use of organic solvents and (2) use of detergents or shampoos.

Coleman[13] has suggested that the quantity of trace elements remaining in the hair may vary widely depending on the solvent used and the particular trace element. A 2-h Soxhlet extraction with diethyl ether was said to minimize concentration variations between washed and unwashed hair.

Perkons and Jervis[14] removed surface contamination from hair prior to irradiation for activation analysis by brief washing in alternate baths of distilled water and a 50/50 mixture of acetone and alcohol for a total duration of several minutes. It was shown that soaking of hair in distilled water, ionic detergent, or a 50/50 mixture of acetone and alcohol for 40 h results in a change in the concentration of trace elements in the hair. It was suggested that some elements (*e.g.*, sodium, bromine, and gallium) might be partially removed from the hair, while others (*e.g.*, copper, iron, and manganese) might be deposited in or on the hair during soaking.

Bate[15] investigated the relative effectiveness of several solvents and washing procedures for removing surface contamination from the hair. He concluded that detergent washing is more efficient for activation analysis because sodium and bromine are almost completely removed from the hair. These elements when present in the irradiated samples interfere with trace elements of similar half-lives such as vanadium, aluminum, and manganese.

Bate and Dyer[9] compared two techniques of removing surface contamination. One was a 2-min organic solvent wash, followed by several rinses with distilled water. The other consisted of several rinsings with a non-ionic detergent (Kyro EO)* in distilled water. A comparison of the gamma ray spectra of duplicate hair samples

* Procter and Gamble, Industrial Soap and Chemical Products Division, P.O. Box 599, Cincinnati, Ohio (U.S.A.).

washed using the above procedures showed that for zinc, copper, and manganese, the concentration in each sample was essentially the same. However, they found that the organic solvents were inefficient for removing inorganic contamination and also felt it was more reasonable from a forensic standpoint to wash a hair sample with a detergent or shampoo, because it is normally washed in this manner.

Goldblum *et al.*[11] removed surface contamination from hair by washing samples with a 10% Tween 80 solution (nature of the cleansing agent was not indicated). The samples were shaken for 30 min on a mechanical shaker and rinsed three times with doubly distilled water. Reinhold *et al.*[1] washed hair samples with approximately 100 ml of 0.1% solution of soap (Ivory Flakes) at room temperature for 20 min. After a duplicate washing and thorough rinsing with deionized water the samples were immersed in three successive organic solvent baths, for 5 to 10 min each (the first two containing 95% ethanol and the third diethyl ether).

Rice and Goldstein[3] removed surface contamination from hair by soaking the samples in several changes of isopropyl alcohol for 24 to 36 h. Martin[5] prepared hair samples for analysis by thoroughly washing them successively with deionized water, a 50/50 mixture of alcohol–ether, and acetone. Upon completion of the washing cycle the samples were rinsed three times with deionized water.

Wash comparisons

Since reliable results have been claimed for each washing procedure outlined, our first study was to compare an organic solvent wash with a non-ionic detergent wash. A large homogeneous hair sample was divided into two equal parts, each weighing approximately 1 g. One part was washed with 150 ml of the 1% 7X O-Matic solution, as previously described. The other part was washed using a 2-h Soxhlet extraction with 150 ml of a 50/50 mixture of absolute ethanol and acetone. Once the washing cycle was complete, the samples were analyzed in duplicate.

A comparison was made on three different homogeneous samples, the results of which are shown in Table I. In each case there was no significant difference in the

TABLE I

COMPARISON OF NON-IONIC DETERGENT WASH AND ORGANIC SOLVENT WASH ON HOMOGENEOUS HAIR SAMPLES

Sample	*Wash method*	*Concentration (μg/g) dry weight*			
		Copper	*Iron*	*Magnesium*	*Zinc*
1	Detergent	37.3	13.3	6.5	191.5
	Organic	37.1	25.7	66.4	196.7
2	Detergent	26.3	9.2	120.8	250.8
	Organic	27.9	23.6	259.8	262.5
3	Detergent	14.9	11.0	18.2	164.8
	Organic	13.4	17.3	27.2	171.5

copper concentrations present in detergent-washed and solvent-washed hair. The zinc concentrations appear to be slightly, but consistently, lower in the detergent wash method. However, the differences are small. The magnesium and iron concentrations are quite dependent upon the washing method. The results indicated that iron and magnesium were removed in the course of the non-ionic detergent wash. Table II shows that there were only minor differences between the unwashed and the organic

TABLE II

TRACE ELEMENT CONCENTRATIONS IN UNWASHED AND ORGANIC SOLVENT-WASHED HOMOGENEOUS HAIR SAMPLES

Sample	*Wash method*	*Concentration (μg/g) dry weight*			
		Copper	*Iron*	*Magnesium*	*Zinc*
1	Unwashed	39.1	32.9	74.6	218.0
	Organic	37.1	25.7	66.4	196.7
2	Unwashed	16.6	50.0	46.1	207.3
	Organic	15.1	44.5	40.2	213.1

solvent-washed samples. The differences between the organic and detergent washes may possibly be attributed to: (1) the failure of the organic wash to remove surface iron and magnesium contaminants, or (2) the leaching action of the non-ionic detergent which removes iron and magnesium from the inner structure of the hair, or (3) a combination of these effects.

Since this work was initiated to establish normal mean values for certain trace metals in the hair with the hope of using significant deviations from these norms as diagnostic aids in future work, the washing procedure must yield reproducible results to be satisfactory. Both washing techniques met this criterion, but the non-ionic detergent method was chosen as the better washing procedure due to the ease in handling, speed, safety, and the relative ionic free nature of the detergent. As suggested by Bate and Dyer[9], a non-ionic detergent should produce no greater (or less) changes in hair trace element composition than *in situ* washing and would therefore be more valuable for diagnostic purposes. The time interval since the subject last washed his hair would be less important. Results from iron and magnesium analysis in hair samples following an organic wash might be greatly dependent upon any prior detergent wash.

To determine the effects of commercial shampoos *versus* the non-ionic detergent used in these studies, a homogeneous hair sample was divided into three parts, one of which was washed with the 7X O-Matic, the other two were washed with different popular commercial shampoos. The results shown in Table III, indicate that commer-

TABLE III

COMPARISON OF NON-IONIC DETERGENT AND COMMERCIAL SHAMPOOS ON A HOMOGENEOUS HAIR SAMPLE

Type of wash	*Concentration (μg/g) dry weight*			
	Copper	*Iron*	*Magnesium*	*Zinc*
7X O-Matic	12.4	16.2	21.5	162.4
Comm. Shampoo 1	9.6	13.7	21.7	193.6
Comm. Shampoo 2	10.4	11.9	22.0	153.5

cial shampoos are equivalent to the 7X O-Matic in their effect on iron and magnesium concentrations. Therefore, for the shampoos tested, which are taken to be indicative of commercial detergent shampoos, a subject who had recently shampooed his hair prior to submitting a sample for analysis might greatly influence the iron and magnesium results if an organic wash were used routinely to prepare the hair samples for analysis.

Effect of wash parameters

Several other variables in the wash procedure were studied in order to determine the degree of control required over each to guard against their influencing the analytical results. The standard wash period was 30 min. A series of homogeneous hair samples were allowed to wash for additional 30-min increments up to a total wash period of 3.0 hours for one of the samples. Only zinc seemed to be affected by the extended wash times and then showing only a gradual concentration reduction which after 180 min reached a 10% loss compared to the 30-min standard wash.

The amount of detergent used for a hair was also varied. 50,100, 150 and 200 ml of a 1% 7X O-Matic solution were used to wash separate, homogeneous portions of a hair sample. No systematic differences were seen in the concentrations of the four elements. Comparison of the elemental concentration values from each of the wash solutions showed them to be within experimental error.

Recovery studies

Recovery experiments were performed on each of the four analysis elements in order to determine the effect, if any, of the digest matrix on absorbance. None of the elements are known to be generally subject to interferences with the possible exception of magnesium, which forms refractory spinels with aluminum, titanium, and zirconium. However, by proper selection of experimental conditions, even these may be eliminated[16]. For the recovery studies, a homogeneous hair sample was divided into four equal, weighed portions. Prior to digestion, the analysis elements were introduced in increasing amounts to each portion by pipet addition of standard aqueous solutions. Thus, the recovery study reflects not only the effect of matrix but any possible loss during digestion as well. The concentration readout increase due to each addition, with reference to the blank sample which had no addition, was compared to the actual amount added to each sample. Table IV shows that the recovery is essentially complete for each element.

TABLE IV

RECOVERY OF COPPER, IRON, MAGNESIUM, AND ZINC IN HOMOGENEOUS HAIR SAMPLES

Sample	*Recovery*	*Concentration (μg/g) dry weight*							
		Copper		*Iron*		*Magnesium*		*Zinc*	
Blank		22.1		13.3		23.0		236.1	
	Expected	28.9		20.1		29.8		251.2	
Addition 1			(92.5%)		(95.0%)		(95.8%)		(99.5%)
	Received	26.7		19.1		28.5		251.0	
	Expected	35.9		27.1		36.8		263.3	
Addition 2			(100%)		(94.5%)		(100.5%)		(102.1%)
	Received	35.9		25.6		37.0		269.0	
	Expected	42.8		34.0		43.7		278.7	
Addition 3			(102.0%)		(107.5%)		(100.8%)		(99.1%)
	Received	43.5		36.6		44.0		276.2	

Comparison of results with literature values

Table V shows our results for copper, iron, magnesium, and zinc concentrations in human hair. Eighteen different male subjects were monitored for periods ranging from 4 to 10 months. Samples were requested once a month except for Case 1, where

TABLE V

TRACE ELEMENT CONCENTRATIONS IN HUMAN HAIR (MALE ADULT)

Case	*No. of samples*	*Mean concentration (μg/g) dry weight*			
		Copper	*Iron*	*Magnesium*	*Zinc*
1	19	18.0	13.7	39.3	204.1
2	9	11.8	13.3	16.6	161.9
3	9	11.5	9.7	20.2	205.8
4	8	—*	17.6	21.5	201.9
5	7	9.7	12.5	24.6	194.0
6	7	19.6	38.6	36.6	192.6
7	6	14.5	9.3	17.3	166.0
8	6	12.1	11.5	30.9	194.1
9	6	13.5	13.5	21.2	195.2
10	6	18.1	17.1	75.1	203.0
11	5	11.9	16.4	49.6	152.9
12	5	24.2	30.1	28.6	197.3
13	5	16.4	17.9	25.1	169.9
14	5	13.5	10.0	16.7	171.3
15	5	11.6	14.1	18.5	159.7
16	5	14.8	10.1	11.8	159.3
17	5	13.3	10.1	10.2	144.7
18	4	12.1	9.2	15.2	143.2
Mean		14.5	15.3	26.6	178.7
Median		13.5	13.5	21.5	171.3
High		24.2	38.6	75.2	205.8
Low		9.7	9.2	10.2	143.2

* See text.

two samples were taken weekly. Specific written instructions were given to each person regarding sample collection. A mean value for each of the four elements was determined for each case from the respective number of hair samples submitted. Then a mean value for each element was obtained by averaging the individual means from each case. The range and medianare also shown to indicate the concentration spread during the study.

Data are available in the literature for copper, iron, magnesium, and zinc levels in human hair. However, it is often difficult to compare values due to different sample treatment; also, there seem to be different norms for males and females, the latter showing markedly higher trace element concentrations. Unfortunately, some of the analytical hair data in the literature are not designated as to sex. Due to the inherent variability of trace elements in human hair, a concentration range is quite often reported rather than a mean or median value. The inclusion of far outlying results may produce such a large range as to limit the value of the analytical result, particularly for comparison purposes.

For zinc, mean values (μg/g of dry hair) of 177 by neutron activation analysis[9], 181 and 163 by colorimetric methods[1,17], and 119.6 by emission spectroscopy[2] have been reported. Values of 99 and 212 are also reported[18,23]. Our mean of 178.7 and median of 171.3 μg/g compare quite favorably with most of these values. This is to be expected since zinc is not particularly affected by sample pre-treatment. Ranges reported for zinc are 64–562 by emission spectroscopy[11], 150–210 by X-ray fluorescence[19], and both 300–1100 and 51–602 by neutron activation analysis[8,9]. Our range in Table V is of mean values for each subject, which would account for the

more restrictive 143 to 206 μg/g. A total range for the 122 individual analyses for zinc was 81 to 314 μg/g.

For copper in human hair, mean values of 34.1 μg/g by neutron activation analysis[9], and 15, 9.8, 16.0, 8.9, 18.5, and 11.7 μg/g by colorimetric methods[3,17,5,20,21,1] have been reported. Our mean of 14.5 and median of 13.5 μg/g agree well with the colorimetric data. Copper like zinc, is not influenced by the type of sample wash used. Ranges reported for copper are 14–40 and 31–128 μg/g by emission spectroscopy[18,11], 8–234 and 8–250 μg/g by neutron activation analysis[9,8]. Values of 63–108, 15–47, and 22–52 μg/g are also found[22,23,6]. The range of mean values from our studies was 9.7 to 24.2 μg/g. For 114 individual analyses of copper in human hair the range was 6.9 to 35.5 μg/g. It should be noted in Table V that the copper mean for Case 4 is excluded due to its very high mean of 348.1 μg/g. The range in this individual case is 187.3 to 739.5 μg/g, extraordinarily high compared to the other 17 cases. A brief investigation into his environment revealed a copper concentration in his drinking water (from a deep well) of about three times that from the city water supply.

There is little information in the literature concerning iron and magnesium concentrations in human hair. For magnesium in human hair, a value of 109 μg/g is reported[10] in male hair and as well a range of 10 to 101 determined by emission spectroscopy[11]. This is to be compared to our mean of 26.6, median of 21.5, and a range of means spanning 10.2 to 75.2 μg/g. A total range for 122 analyses was 4.2 to 88.8 μg/g. For iron, a literature value of 141 μg/g[24] and ranges of 9–132 and 0.8–11 μg/g are reported[6,11]. Our mean was 15.3, the median 13.5, range of means 9.2 to 38.6, and the total range of 122 analyses was 5.9 to 82.1 μg/g.

Our data agree reasonably well with previous literature values for zinc and copper, with the exception of the Egyptian control group which reported 99 μg/g for zinc against our 178.7, and the neutron activation analysis value for copper of 34 μg/g *versus* our 14.5, which was close to the other literature values. For iron and magnesium, a comparison is difficult to make against the very limited literature data, but our means are clearly below the one value listed for each element. However, our ranges compare more favorably. The reduction of iron and magnesium concentrations which showed up in the detergent washing procedure probably accounts for our lower mean values.

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