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Zinc and Copper in Human Cerebrospinal Fluid

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Abstract

Zinc and copper have been estimated in CSF of 14 normal volunteers, nine men and five women. Zinc was analyzed by limited-aspiration flame atomic absorption spectrophotometry using a deuterium continuum light source. Copper was analyzed in 0.1% HNO_3 by flameless atomic absorption spectrophotometry with a graphite cuvette on a flameless atomizer. Recovery of added zinc varied less than 5% and that of the added copper varied less than 8%. CSF zinc was 31.5 ± 19.8 μg/L (mean ± 1 SD); CSF copper, 7.5 ± 3.1 μ/L. Values obtained for CSF zinc are about ½ those we and others obtained previously, the decrease related almost exclusively to removal of interference by the CSF matrix, which produced spuriously elevated values without use of the deuterium light source. Values obtained for CSF copper were approximately one-tenth those we and others had obtained previously. The decrease related, in part, to the removal of matrix effects, but also to improvement of the signal-to-noise ratio present in other techniques.

Index Entries: Trace metals, in human CSF; zinc, in human CSF; copper, in human CSF; cerebrospinal fluid, zinc and copper in.

Introduction

Zinc and copper are trace elements found in brain and involved in cerebral function. Zinc is the fourth most prevalent element found in brain (*1*, *2*) and acute zinc depletion has been associated with severe alterations in mental function in both

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adults (*3*) and infants (*4*). Copper is involved in myelination of neural tissue and changes in mental function associated with copper excess in Wilson's disease (*5*) or depletion in trichopoliodystrophy (*6*) are well known.

For these and other reasons it would be extremely useful to estimate zinc and copper concentrations in brain tissue of patients with symptoms of various aberrant mental processes. Since this is not practical, measurements of these metals in cerebrospinal fluid (CSF) offer data that might be useful in understanding some of these processes and we (*7*) and others (*8–20*) have measured the concentration of zinc and copper in human CSF. However, several problems limited the usefulness of these estimates. First, measurement techniques, until recently, yielded what now appear to be spuriously elevated results; thus, data obtained with spectroscopy (*8–12*), activation analysis (*13, 14*), spark-source mass spectroscopy (*15*), or conventional flame-aspiration atomic absorption spectroscopy (*7, 16–20*) have limited value. Indeed, many of these values can now be considered to be elevated at least 10-fold above present estimates for CSF copper and twofold for CSF zinc. Second, most studies were carried out in patients with neurological or psychiatric disease processes and little data were collected from normal volunteers, so that comparison between normals and ill patients could not be systematically made.

It is the purpose of the present study to measure zinc and copper concentrations in CSF of normal volunteers and to define the validity of these measurements by systematic methodological studies.

Materials and Methods

Subjects

The subjects of this study were 14 normal volunteers, 9 men, 5 women, 13 Black, one Caucasian, aged 21–76 years (mean 33 years). Each of these subjects was in good health and was taking no prescribed medication or mineral supplements at the time of study. Each subject participated in the study after signing an informed consent which was part of a protocol approved by the Human Use Committee, St. Elizabeth's Hospital, Washington, DC.

CSF was obtained in the morning from each subject lying in the prone position following insertion of a 22-gage stainless steel needle into the L4-5 interspace. Fluid was visually clear in each case; microscopic examination of the fluid from the initial tube collected revealed fewer than three red blood cells per low power microscope field. After removal of 10 mL, a 0.5 mL sample was placed into a plastic tube and stored at −40°C until assayed. Samples were coded in a series that included patients with various psychiatric disorders, the code unknown to the investigators performing the studies. In this manner, samples were evaluated in a double blind study.

Apparatus

An atomic absorption spectrophotometer (Instrumentation Laboratory Inc., Wilmington, MA, Model 951) equipped with a deuterium background corrector and a graphite furnace (Instrumentation Laboratory Model 555) were used. Wavelengths of 213.9 and 324.7 nm were used for zinc and copper, respectively, with spectral bandwidth of 1 nm for both metals. All instrumental adjustments were made according to the manufacturer's instruction manual. A deuterium continuum light source was used in all analyses. This source covered the range from 200 to 330 nm although at 330 nm activity was only one-quarter peak activity. This light source was designed to minimize the matrix effects that contribute to background spectroscopic activity and can be considered a background correction lamp.

Reagents

Deionized distilled water (Millipore Corp., Bedford, MA) was used throughout. Analytical grade nitric acid (Fisher Scientific Co.) was used in preparing solutions. Acid solutions up to 1% HNO_3 contained neither zinc nor copper as detected by flameless atomic absorption spectrophotometry.

Standards

An intermediate standard containing 200 μg/L (200 ppb) zinc and copper was prepared from a commercial stock solution (Alfa Division, Danvers, MA) containing 1000 μg zinc or copper/mL (1000 ppm) solution. Aqueous working standards of 5, 10, 25, 50, and 100 ppb of zinc and copper were made in 0.1% HNO_3 from the intermediate standard.

Glassware

All glassware was soaked overnight in 6*N* HCl and thoroughly rinsed with deionized water. Plastic vials and caps, used for dilutions, and plastic micropipet tips were washed with 5% HNO_3, rinsed with water, and dried before use.

Estimation of CSF Copper

CSF samples were diluted 1 : 1 with 0.2% HNO_3 in plastic vials (resulting in a 0.1% solution). The instrument was calibrated by injecting 20 μL aqueous copper standard into a graphite cuvette with an Eppendorf micropipet and the drying cycle initiated. The time–temperature control program of the furnace is shown in Table 1. After all copper standards (5, 10, 25, 50 ppb) were analyzed, the calibration curve was computed by the built-in minicomputer in the instrument. The curve was linear in the concentration range of the standards used. Samples were then analyzed under identical conditions using argon gas. Results were printed by a computer controlled printer and simultaneously displayed on a CRT screen. Samples were measured in duplicate or triplicate with variation of less than 6%.

Recovery of copper was studied by adding known quantities of copper to a pooled CSF sample. These experiments were performed at various concentrations of HNO_3 ranging from 0.05 to 5%. Recoveries of copper were poor when concentrations of HNO_3 $< 0.1\%$ or $> 0.5\%$ were used. Quantitative recoveries were

TABLE 1
Graphite Furnice Program for Estimates of Human CSF Copper

Mode of operation;	Single beam (peak height)
Purge gas:	Argon
Purge gas flow rate:	12
Volume of sample injected:	20 μL
Deuterium continuum lightsource:	(range, 200–330 mm)

	Time, s × 5	Temperature, °C
Drying:	7[a]	25–75
	7[a]	75–125
Ashing	9	500–750
	9	750–900
Atomization:	0	2400
	2	2400–2600
Integration:	2[b]	

[a] IL flameless atomizer Model 555 is equipped with drying, ashing, and atomization cycles in two stages.

[b] Occurs over entire atomization cycle.

obtained in the HNO_3 concentration range of 0.1–0.5% and varied from 4 to 8% (Table 2).

A series of dilutions of a CSF sample were made with HNO_3 such that HNO_3 concentrations in diluted samples were 0.1% and copper concentrations were determined as described above. Results of standard dilutions are shown in Fig. 1 and are essentially linear except when undiluted samples were determined.

Estimation of CSF Zinc

Zinc in CSF was analyzed by a limited aspiration flame technique (*21, 22*). For this technique 100 μL of sample was aspirated from a small teflon cup attached to the stainless-steel capillary tube of the nebulizer (nebulizer intake) and the signal (peak height) of the sample recorded. The instrument was calibrated by aspiration of 100

TABLE 2
Recovery of Copper Added to Human CSF

Cu added, μg/L	Cu found, μg/L	Cu expected, μg/L	Cu recovered, %
0	12	—	—
5	17	17	100
12.5	23.5	24.5	96
25	40	37	108

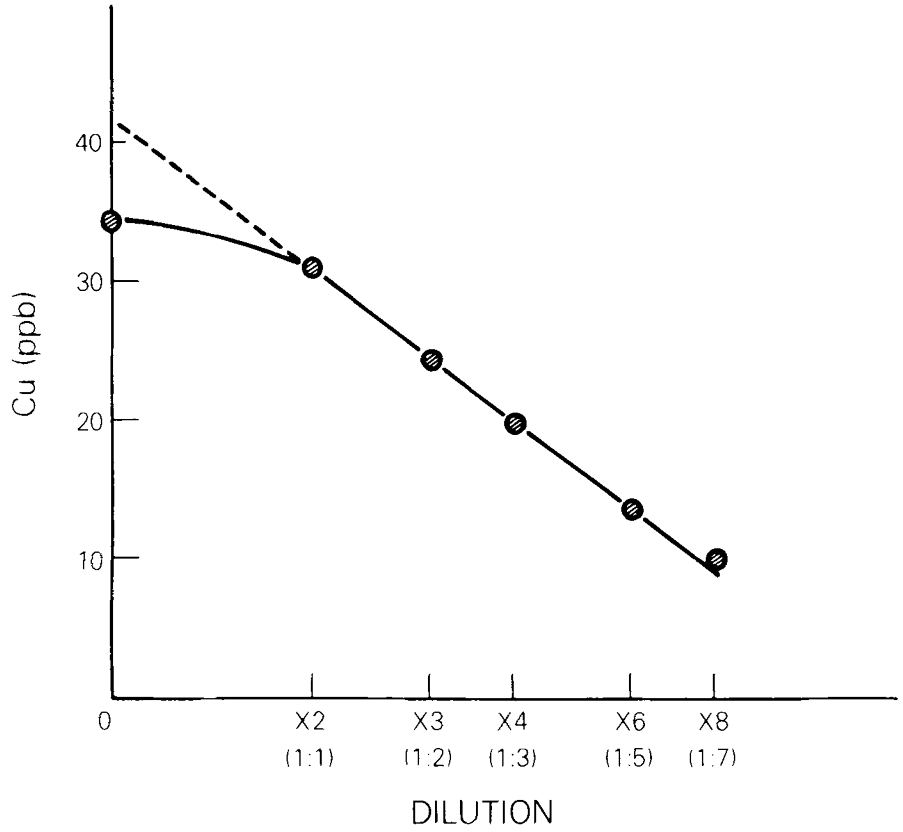

Fig. 1. Estimation of copper in human CSF following dilution with 0.2% HNO_3 (final dilution, 0.1%). Copper in ppb was estimated by flameless atomic absorption spectrophotometry (see Methods). The dotted line is the extrapolation of the dilution curve to the ordinate. Dilutions, on the ordinate, were plotted on a logarithmic scale.

μl of various zinc standards (10, 25, 50, and 100 ppb). Calibration curves were then obtained as described in the method for copper analysis. CSF samples were analyzed by flame aspiration, as described, using a single-slot Boling burner with an air–acetylene flame. Signals were displayed on both a CRT screen and on a computer-controlled paper tape operated by a high speed printer. Duplicate sample variation was ± 3%. Dilution of CSF samples with either 0.2% HNO_3 or deionized water revealed a linear response; e.g., 1 : 1 dilution resulted in a 50% decrease in the measured estimates of CSF zinc. Quantitative recoveries were always obtained when known amounts of zinc were added to a CSF sample and did not vary by more than 5%.

In order to determine the contribution of nonspecific absorption to estimates of CSF zinc several CSF samples were analyzed with and without the use of background correction. This was carried out by estimating zinc concentration using zinc lamps (at 213.9 nm) in each of the two channels of the atomic absorption spectrophotometer and comparing these results with those of the same sample measured with one zinc lamp and a background correction deuterium lamp in each of the two channels. With the latter mode of operation nonspecific absorbance was electronically subtracted from the total absorbance of the sample. Both channels were calibrated by the limited aspiration technique with aqueous zinc standards. Results of these analyses are shown in Table 3 and indicate that, without background correction, estimates are approximately twice those obtained with background correction in place.

Results

Concentrations of zinc and copper in CSF in normal volunteers are shown in Table 4. The concentration of zinc in CSF is approximately four times higher than copper. The concentration of zinc in CSF is about 3% of zinc in serum or plasma whereas CSF copper is less than 1% of copper in serum or plasma.

Discussion

The data obtained in the present study reflect estimates of zinc and copper in CSF from normal subjects. Previous investigators have used a variety of subjects to obtain what was considered "normative" data including patients undergoing surgery in which spinal anesthesia was used, patients undergoing procedures for evaluation of degenerative disc disease, patients with neuropsychiatric disorders, or patients with seizure disorders. The subjects in this study were normal volunteers who had neither acute nor chronic illness of any kind and who were compensated financially to participate in the study.

Estimates of CSF zinc concentration with the present method are approximately one-half values obtained previously in our own laboratory (*7*) and in others (*16, 18, 19*) using flame-aspiration atomic absorption spectrophotometry in which background correction was not applied. Use of this latter technique afforded little difference between signal and noise and resulted in variable and consistently elevated estimates of CSF zinc. Indeed, the mean difference between values obtained with and without background correction in the present study is 48%, essentially accounting for the elevation in estimates obtained with each of the previous studies. These results are consistent with the presence of CSF matrix effects that are ameliorated with the use of background correction. The limited aspiration technique affords better sample control than unlimited aspiration and allows more efficient use of the oftimes small CSF samples available for analysis.

Estimates of CSF copper concentration by the present method are approximately one-tenth values obtained previously in our own laboratory (*7*) and in others (*17–20*) using unlimited flame aspiration atomic absorption spectrophotometry by methods similar to those used previously for zinc. These differences can be related to the low signal-to-noise ratio obtained by the previously used technique, which was also obtained without the benefit of background correction. However, unlike zinc, it is not possible to obtain reliable measurements of CSF copper using any

TABLE 3
Estimates of Zinc Concentration in 14 Human CSF Samples Determined with and without Background Correction by Flame Limited-Aspiration Atomic Absorption Spectrophotometry by the Present Method

	Uncorrected for background	Corrected for background	Difference	
			μg/L	%
	39	18	21	54
	80	50	30	38
	87	57	30	34
	43	23	20	47
	36	19	17	47
	49	25	24	49
	37	20	17	46
	38	19	19	50
	58	29	29	50
	45	23	22	49
	46	19	27	59
	42	20	22	53
	65	35	30	46
	74	40	34	36
Means:	53	28	24	48
SD:	7.3	12.5	5.5	
SEM:	2.0	3.5	1.5	

TABLE 4
Estimates of Zinc and Copper in CSF of 14 Normal Volunteers

Subject	Age	Sex	Race	Zn, μg/L	Cu, μg/L
1	41	F	B	72	8
2	27	M	B	72	17
3	31	M	B	46	5
4	29	M	B	45	7
5	34	F	B	28	10
6	25	M	B	25	4
7	76	M	B	25	7
8	26	M	C	25	7
9	28	M	B	20	7
10	45	F	B	19	7
11	33	F	B	18	7
12	25	M	B	18	7
13	21	F	B	14	6
14	21	M	B	14	6
			M ± SD:	31.5 ± 19.8	7.5 ± 3.1

modifications of flame aspiration atomic absorption spectrophotometry with present technology. Tyrer et al. (*20*) have used a limited aspiration technique for copper similar to that which we used here for zinc, but the mean values they obtained were about twice those we obtained; they did not report on their ability to recover added copper or whether dilutions yielded linear results.

The present data indicate that flameless atomic absorption spectrophotometry with carbon cuvette usage affords reliable and reproducible estimates of CSF copper in which added copper can be recovered quantitatively and dilutions yield linear results.

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Note Added in Proof

To estimate CSF copper, values determined at 1 : 1 dilution in 0.1% HNO_3 were multiplied by a factor of 1.4 as defined by the standard curve.