The Direct Determination of Trace Quantities of Manganese in Blood and Serum Samples Using Selective Volatilization and Graphite Tube Reservoir Atomic Absorption Spectrophotometry

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Manganese is an important biological trace element (COMENS 1956, EVERSON and DANIELS 1934) and is known to be essential in the nutrition of both animal and man (COTZIAS 1958). Even though the element has significance biologically, its exact mode of action is not completely understood (MAHONEY and FERRANTE 1964, FERRANTE 1961). Chronic exposure to the metal results in locura mangania or the madness of acute manganic intoxication which is more frequently found in mining areas where occupational exposure is more common than in other parts of the world (ROSENSTOCK, et al. 1971). Presenting signs of this disease are disorders of mentation, disorientation, impairment of memory and judgement, acute anxiety, emotional liability, compulsive acts, hallucinations, illusions and delusions (ROSENSTOCK, et al. 1971).

In view of the very large number of trace metals for manufacturing of materials such as paints, plastics, rubbers and paper, it is likely that injestion either directly or indirectly of these metals, particularly by children, may occur, and the probability of the accumulation in body tissue beyond tolerance levels would increase. The total concentration of manganese in man is approximately 10-20 mg (MAHONEY, et al., 1969), and because of its low concentration in man, there has been difficulty in developing an analytical procedure for monitoring occupational exposure to the element.

There are a number of analytical methods available for trace element determination in body tissue. Included are emission spectroscopy, spark-source mass spectrometry and neutron activation analysis. These techniques, although sensitive, are expensive, difficult to apply to routine analysis where large number of samples are to be analyzed, and usually require pre-concentration from large sample sizes.

At present the most widely used analytical method for trace element determination is atomic absorption spectrophotometry (AA). Although simultaneous multi-element determinations are not yet feasible by AA, the technique has good sensitivity for single element determinations and is easily adaptable to routine analysis.

A recent development in AA has been the introduction of the flameless graphite tube atomizers (HGA) which has resulted in a significant increase in the absolute sensitivity attainable by the technique. This improvement in sensitivity together with the small sample requirement makes the non-flame atomizer uniquely suitable for the

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direct analyses of trace elements from environmental and biological materials where sample size is usually a limiting factor. The HGA method has been used successfully for the determination of trace elements in biological samples (HANIG and APRISON 1967) and has been used for the direct determination of chromium in urine (ROSS and GONZALEZ 1973) and for cadmium in both blood and serum (ROSS and GONZALEZ 1973).

Due to the sensitivity, specificity and simplicity of analysis of this technique, it was the purpose of this investigation to adapt the HGA technique for the direct determination of manganese in biological materials. The method is quick and adaptable to routine analyses.

EXPERIMENTAL

Apparatus

A Perkin-Elmer model 303 AA spectrophotometer equipped with a Dynatronic Instruments model Graphicorder-10 recorder, a deuterium arc background corrector and a Perkin-Elmer HGA-2000 heated graphite atomizer were employed in this investigation. The AA spectrophotometer was operated according to the standard conditions for the analysis of manganese recommended by the manufacturer. The graphite tubes are purged with 1.5 l/min of nitrogen to provide an inert atmosphere.

With the use of atomization systems that result in a transient or peak absorption signal, the utilization of a background corrector which attenuates the scattering signal produced during the atomization cycle becomes increasingly desirable. However, biological matrices produce a large amount of smoke, and only partial compensation of peak absorption is achieved; therefore, it is desirable, in order to obtain maximum peak height, to carefully align the beam from the hollow cathode lamp with respect to the deuterium arc reference beam as described by Manning (MANNING and FERNANDEZ 1970).

The temperatures reported are also given in terms of voltages which were measured directly across the atomizer terminals. Injections into the HGA were made by means of Eppendorf micropipettes with disposable plastic tips.

Reagents

Secondary working standard solutions were prepared fresh daily by diluting a 1000 µg/ml manganese reference (Fisher Scientific Co.) with deionized doubly glass distilled water which showed no manganese absorption under the conditions described in this work. Methyl isobutyl ketone (MIKB) and nitric acid were purified by distillation from a silica still. Ammonium pyrollidine dithiocarbamate (APDC) was recrystallized from alcohol-water, and its solutions were extracted with several portions of MIKB before use. All containers used were of teflon or high density polyethylene and were rinsed thoroughly with silica distilled nitric acid.

Procedure

Aliquots of twenty microliters of 50% urine or serum aqueous solutions were introduced into the graphite tube using disposable micropipettes. Samples of cerebral spinal fluid were not diluted. Calibration curves were obtained by injecting manganese working standard solutions and by fortifying serum or spinal fluids with manganese concentrations equal to those added to the aqueous calibrating solutions.

The graphite tube reservoir power supply was programmed as follows:

Drying	30 seconds	at	150° C	0.77v
Charring	50 seconds	at	1350° C	4.2 v
Atomizing	8 seconds	at	2150° C	7.2 v

The charring temperature and time were found to be critical since the salts present in the biological fluids must be selectively removed prior to the atomization step of the manganese.

Samples were also analyzed by chelating and extracting manganese with APDC and MIKB as described by Christian (CHRISTIAN and ROBINSON 1971). Manganese chelates decompose on standing in MIKB, therefore, extracted solutions of the metal chelates are aspirated immediately.

Results and Discussion

The concentration of manganese in blood serum is very near or at the detection limit of the AA using the conventional flame technique, and the levels found in urine and other biological fluids are below the limits of detection of the instrument. In the case of assays at or near the detection limit of an instrument, considerable work is required to demonstrate that the small signals are, in fact, from the material under study. With the increased sensitivity of the flameless AA-HGA technique by several orders of magnitude, this problem has been improved considerably. The authenticity of the manganese signal employing this method was established by observing the energy meter of the spectrophotometer during the atomization cycle with the deuterium arc background corrector on. The reading on the energy meter indicates deuterium emission instead of hollow cathode lamp energy. If any broad band absorption takes place during the atomization cycle, the needle deflects to indicate attenuation. The charring temperature and time employed in the investigation afforded a distinctive manganese signal without noticeable matrix interference or detectable losses of manganese during the ashing cycle.

A unique feature of the HGA is the fact that trace metals in biological fluids may be determined directly without prior sample workup thus eliminating changes of error which result from reagent contamination and sampling handling. The accuracy of the above method was established by analyzing the samples directly and comparing the results by the extraction method. These data are illustrated in Table 1. The data obtained

TABLE 1

The Determination of Manganese in Blood Serum by the Direct Method and by APDC-MIKB Extraction

Sample	Direct (µg/100 ml)	Extraction (µg/100 ml)
1	1.48	1.61
2	1.82	1.96
3	2.03	2.13
4	0.850	1.23
5	2.85	2.71
6	4.05	4.10

by the two procedures are in close agreement. Recovery data listed in Table 2 was obtained by spiking urine samples which afforded no manganese absorption at the limits of detectability of the prescribed procedure. The mean recovery for these samples is 99.3% (range 90.0-107%).

The relative standard deviations (RSD) for peak signals calculated from ten injections of each serum, urine and aqueous standard solutions containing 2.0 µg/100 ml of manganese were 2.3, 4.8 and 2.4 percent respectively. These data are in good agreement with those cited in the literature (CHRISTIAN and ROBINSON 1971). No manganese was detected in cerebral spinal fluid by the direct or the extraction method. However, the levels of manganese found in urine ranged from non detectable quantities to 2.5 µg/100 ml with a mean manganese concentration of 1.26 µg/100 ml.

TABLE 2

Recovery of Manganese from Serum and Cerebral Spinal Fluid

Sample	Mn Added (µg/100 ml)	Mn Found (µg/100 ml)	% Recovery
Serum			
A	1.5	1.4	90
B	2.0	1.8	90
C	3.0	2.9	97
D	3.5	3.6	101
E	4.0	4.1	103
F	4.5	4.6	101
G	6.0	6.0	100
Spinal Fluid			
A	1.5	1.5	100
B	3.0	3.2	105
C	4.5	4.9	108

The concentrations of urine manganese reported in the literature vary greatly. The detection limit obtained for manganese in body fluids in this investigation is 0.1 µg/100 ml based on 20 µl injections of 1:1 serum/water samples into the HGA. Injections of up to 100 µl samples are possible using this technique, and larger injections increases the sensitivity; however, direct injections of larger sample volumes into the HGA would increase the amount of non-specific absorption encountered from the salts present in the biological media affording incorrect manganese readings.

In summary, the HGA method for the determination of manganese is attractive because of the increased sensitivity over the conventional flame technique. The method allows samples to be analyzed directly thus eliminating errors incurred in sample handling and reagent contamination. The method is simple, quick and adaptable to routine analysis.

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