A Direct Method For The Determination of Manganese in Whole Blood: Patients With Seizure Activity Have Low Blood Levels

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Summary

A direct method is presented for the determination of manganese in whole blood or serum by atomic absorption spectroscopy, using a graphite furnace with a temperature ramp controller. Blood or serum is diluted with an equal volume of a five percent solution of Triton-X100 containing heparin. This prevents the blood from coagulating and produces a solution with a viscosity suitable for injection into the furnace. The determination is linear in the range of 0-20 ppb with recoveries of around 100 percent.

Blood levels from control subjects have a mean of 14.8 ppb, while serum levels are 1.2 ppb. A clinical application of the method is presented in a comparison of blood manganese levels in a group of patients with seizure activity as compared to a control group. The manganese levels were significantly lower in the patients with seizure activity, 9.9±4.9 ppb as compared to 14.8±3.9 for the control group. Dietary supplements of manganese aid in the control of seizures.

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Introduction

Manganese either excess or deficiency has been known to have an important role in cerebral function. Chronic manganese toxicity has been observed in manganese miners due to the inhalation of ore dust. This leads to a psychotic condition called loco manganica which is characterized by a profound psychosis; later, neurological disturbances occur which are similar to those observed in Parkinson's disease. Experimental evidence gives indications of an interaction between manganese, dopamine, and cyclic AMP (Papavasiliou et al., 1965; Cotzias et al., 1971). Mena et al. (1970)

reported that the administration of L-dopa to patients suffering from chronic manganese toxicity resulted in the disappearance of rigidity and hypokinesia.

Manganese deficiency has been demonstrated in a number of animal species. Manganese is essential for normal growth, reproduction, and skeletal development. Manganese deficiency also affects cerebral function. Hurley et al. (1963) demonstrated a relationship between seizure activity and manganese deficiency in rats. The seizure threshold was found to be significantly lower in manganese deficient animals. Recently Tanaka (1977) has presented a preliminary report on low blood manganese levels in epileptic patients.

Blood manganese levels in man may be a useful index for the determination of a deficiency or excess. Manganese is normally present in human blood in a minute amount, usually below 20 ppb. Contamination is a serious problem in the determination of trace elements at this level, particularly if the element in question is fairly ubiquitous in the environment, as is the case with manganese. The determination of manganese in blood by atomic absorption spectrometry requires the use of the graphite furnace technique if reasonable sample volumes are to be employed.

The procedure developed in this study for the determination of manganese in blood involves the direct determination on a blood sample which has been merely diluted with an equal volume of five percent Triton-X-solution. The Triton-X-solution provides a homogeneous sample of low viscosity which can be readily injected into the furnace. The sample preparation is simple and minimizes the risk of sample contamination. The study also presents the practical application of the method for the determination of manganese blood levels in patients with seizure activity and makes a comparison with a control group.

Methods

Reagents

1. Stock Standard

Commercial manganese standard (Fisher Scientific, Pittsburg, Pa.) 1000 ppm, which had been prepared by dissolving manganese metal in dilute nitric acid was diluted 1:100 with ultrapure deionized water to give a 10 ppm stock standard.

2. Working Standard

Working standard was prepared immediately before use by diluting the 10 ppm stock standard 1:100 with ultrapure deionized water to give 10 ppb. This standard was further diluted serially to give 50, 25,12.5 and 6.25 ppb standards.

3. Five percent Triton-X Solution

Ten g Triton-X 100 (Sigma Chemical Co., St. Louis, Missouri) was weighed into a 200 ml volumetric flask. One hundred ml of deionized water was added, as well as 2 ml of sodium heparin 10,000 units/ml (Riker Labs, Northridge, Cal.). Deionized water was then added to volume. All reagents were checked for manganese contamination. No significant levels were found.

4. Preparation of Glass and Plastic Ware

All glassware was cleaned with 50 percent nitric acid and rinsed with ultrapure deionized water.

Polystyrene tubes were used wherever possible. These were cleaned by soaking overnight in a 0.4 percent solution of disodium ethylene diamine tetraacetate, EDTA (Fisher). The tubes are then rinsed with deionized water and dried by inversion at room temperature.

5. Analytical Procedure

Blood was collected with plastic disposable syringes and emptied into a polystyrene tube. A 1 ml acid cleaned Ost-wald Folin pipet was used to transfer the blood to a polystyrene tube containing 1 ml of Triton-X reagent. The sample was mixed on a vortex mixer and stored at 4°C until analyzed. Samples were analyzed in triplicate by injecting *25M* samples into the furnace.

The analyses were carried out with a Perkin Elmer 503 atomic absorption spectrophotometer with an HCA 2000 graphite furnace which was equipped with a temperature ramp accessory. Peaks were recorded with a model 056 recorder. The operating conditions were listed in Table 1. The furnace used was a straight graphite tube.

6. Clinical Material

Manganese determinations were carried out on blood samples which are routinely collected on outpatients of the Brain Bio Center. Manganese determinations were run on a group of patients who have seizure activity diagnosed as being epileptic and an appropriate control group. 7. Statistical Analysis

A comparison of means was carried out using Student's t test.

TABLE 1 OPERATING PARAMETERS FOR MANGANESE DETERMINATION

Wavelength			**279.5**
Slit			3(0.2 **mm)**
Deuterium background correction			on
Argon Gas Flow			3
Gas Interrupt			on
Lamp			15 **ma**
Furnace			
	Dry	100°	**60"**
	Char	**100-1100[D]**	**90" ramp**
		1100»	30"
Atomuation		**2400"**	**8"**
Sample 26*il			

Results and Discussion

Manganese with the procedure outlined can be determined to a level of about one ppb. Figure 1 is a standard curve up to 25 ppb. Over this range the analysis is linear.

FIGURE 1

Standard curve for the determination of manganese

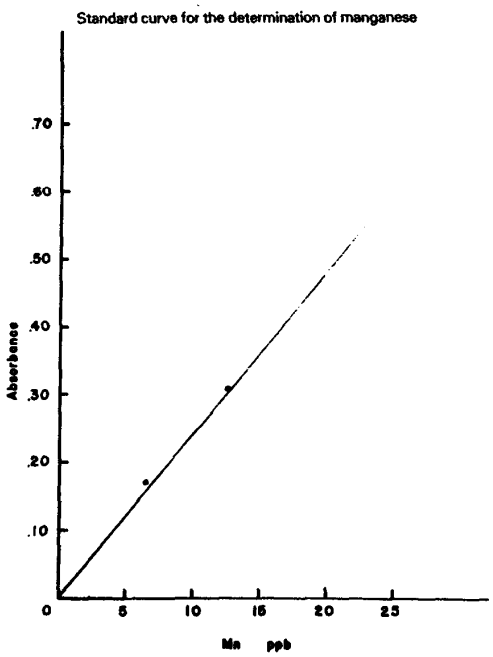
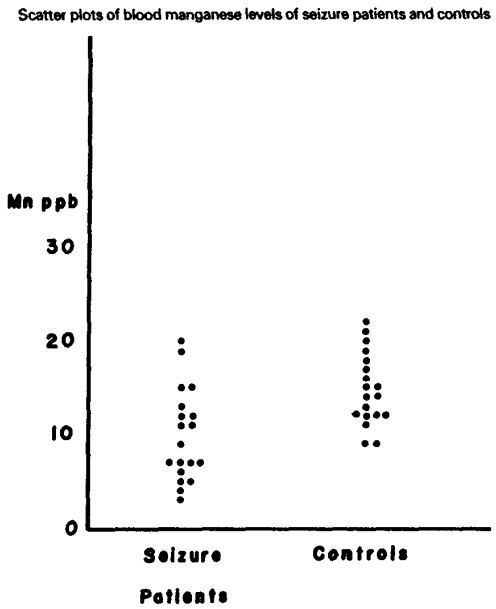


FIGURE 2

Scatter plots of blood manganese levels of seizure patients and controls



An analysis of ten replicate samples indicated that the relative coefficient of variation was 2.05 percent. Table 2 presents the results of recovery studies. The recovery obtained is satisfactory at the levels of addition employed.

TABLE 2

MANGANESE RECOVERY STUDY

LOODMn	MnADDED	TOTAL Mn	% RECOVERY
8.0 ppb	6.25 ppb	14.0 ppb	96
8.0 ppb	12.5 ppb	23.0 ppb	104
8.0 ppb	25 ppb	34.5 ppb	106

A comparison of the values obtained in this study with literature values, Table 3, indicates a consensus that normal whole blood manganese levels are between 10 and 20 ppb. Serum levels are between one and two ppb so the bulk of manganese is found in the red cell.

TABLE3

COMPARISON WITH RECENT LITERATURE VALUES

Reference	Method Whole Blood	n	Mn x± SD (ppb)
This Study	Flameless AA	10	14.9±4.2
(7)1977	Flameless AA	5	9.8±2.4
(8) 1976	Flameless of chelate AA	20	12.2±3.9
(9)1975	Flameless AA Serum		11.0±4.2
This Study	Flameless AA	10	1.20±0.99
(10)1976	Flameless AA	19	1.02±0.19
(11)1974	Flameless AA		1.94
(12) 1974	Flameless AA		13.4
(13)1974	Flameless AA		21.8
(14)1974	Neutron Activation		0.57

The method described has been applied to an examination of blood manganese levels in patients who are suffering from seizure activity. Table 4 presents the manganese levels of these patients as compared with a control group.

TABLE 4

MANGANESE LEVELS IN PATIENTS WITH SEIZURE ACTIVITY COMPARED TO A CONTROL GROUP

	Patients	control
n	19	19
Age	28.0±13.3yrs	38.0±14.6yrs-
Mn	9.9± 4.9 ppb	14.8± 3.9 ppb
	t - 3.395 P - .005	

In each group, 19 individuals were examined who were fairly well matched for age. In the group with seizure activity, the mean manganese level was 9.9±4.9 ppb as compared to 14.8±3.9 ppb for the control group. The values for the seizure group ranged from 3-20 ppb while the control group ranged from 9-22 ppb.

Mean hematocrit values for the seizure patients and the control group were 42.2±4.0 and 41.2±3.4 respectively. The difference was not statistically significant. The lower manganese level in the seizure patients is not an artifact due to fewer erythrocytes.

The whole blood manganese levels were significantly lower in the patients with seizure activity ($P<.005$). The clinical significance of the low manganese levels remains to be evaluated. Is there a connection between seizure activity and low manganese levels or are the low levels an artifact due to the effect of medication? Nearly all of the patients with seizure activity were on dilantin, barbiturates or both. Therefore, the effect of medication cannot be ruled out. However, examination of a limited number of patients who do not suffer from seizure activity but are on dilantin for other reasons were found not to have low manganese levels. In uncontrolled trials we find that manganese is helpful in controlling seizures of both the minor and major types.

Manganese ions have been shown to have a significant role in cerebral function. Kostial and Juricic (1956) have shown that manganese ions block synaptic transmission. Several studies have shown a connection between manganese ions and neurotransmitters. Manganese has been shown to stimulate adenyl cyclase in brain tissues (Johnson and Sutherland, 1973). Manganese is believed to react with the catalytic unit of adenyl cyclase in-tracellularly. Activation of adenyl cyclase by manganese results in an increase in cyclic AMP which stimulates dopamine synthesis.

Manganese ion is also known to decrease the acetylcholine output from preganglionic nerve endings (Kostial et al., 1974). Manganese ion may also share with other divalent cations the property of raising the threshold voltage for impulse propagation. Both manganese and choline deficiencies are believed to interfere with membrane stability and this could be responsible for facilitating the propagation of seizure activity.

One model of epilepsy (Barbeau and Donaldson, 1974) extensively studied is the topical application of cobalt or aluminum to cerebral tissue. These metals could be antagonists to the function of manganese. Another model involves the intraventricular administration of ouabain, an agent known to be a powerful inhibitor of membrane transport mechanism.

Manganese deficiency could conceivably play a role in seizure susceptability because it appears to influence membrane stability and may therefore act as a neuromodulator of transmitter activity.

The findings of the present study we believe warrant further investigation of the relationship of manganese ion to seizure activity and the possible use of manganese dietary supplements for the control of seizure activity.

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