Direct Atomic Absorption Spectrometric Determination of Manganese in Whole Blood of Unexposed Individuals and Exposed Workers in a Norwegian Manganese Alloy Plant

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Manganese is an essential trace element to mammals; however, exposure to high concentrations may result in adverse human health effects. The mining of manganese ores, and the exposure to dust and fumes in manganese alloy plants represent potential toxic hazards. The toxicity of manganese may be grouped into two major categories, viz. a chronic disorder of the central nervous system, and a manganic pneumonia.

Recent reviews (TOLONEN 1972; ANONYMOUS 1973; ANONYMOUS 1975) discuss the toxicological and environmental effects of manganese, and survey the sampling and analysis of the element.

Today the most widely used analytical method for trace element determination in whole blood and serum is atomic absorption spectrometry (AAS). For about a decade the universally employed atomizer in AAS has been the flame; however, by replacing this atomizer with a graphite or metal furnace, an important increase of sensitivity and a reduction of the requirements to sample size is obtained.

The present work was carried out as a subproject under a program with the purpose of elucidating the state of health of workers employed in a Norwegian manganese alloy plant.

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EXPERIMENTAL

Apparatus

The measurements were made with a Perkin-Elmer 400 S spectrophotometer, the cup version of the Varian-Techtron CRA 63 graphite furnace and the Perkin-Elmer 159 single channel recorder. The furnace was heated with a power supply of own construction. The instrument was equipped with an arc source deuterium lamp for background correction.

A temperature calibration curve for the furnace was plotted with the use of an Ircon Radiation Thermometer focused on the inner wall of the graphite cup.

Sample and standard solutions were introduced into the atomizer with a 2 µl Oxford Labs. Intern. Corp. Ultra-Micro pipette, and a 5-50 µl adjustable Finnpipette (Kemistien OY, Finland).

Reagent and standard solutions

A primary 1000 p.p.m. manganese standard solution was prepared by dissolving 1 g of high-purity metal in 50 ml (1+9) sulfuric acid ("Suprapur" Merck) and diluting the solution to 1000 ml with water. Working standard solutions were prepared daily by dilution with water.

The furnace was purged with argon (purity 99.9% by volume).

Preliminary work

The results of and experiences from some introductory experiments are summarized below.

The maximum volume of whole blood that could be ashed in the graphite cup without the risk of obstructing the optical path was 2 µl.

With the high scale expansion employed in the present measurements it was found very important to align the cup correctly in the optical path, incorrect alignment gave a varying base line.

Experiments demonstrated that manganese was not lost during the present two ashing steps, and that the analysis could be made without the use of background corrector.

It was found necessary to base the analysis upon using samples of whole blood as the standards, the main reason for this being that the same amount of manganese in a sample of whole blood and in aqueous solution did not give the same peak height. The concentration of manganese in the whole blood standards was established with the standard addition method.

Procedure

Before the start of the measurements, the hollow-cathode lamp was heated for about 15 min. The flow of argon was adjusted to 3.5 l min^{-1}. The measurements were made at the wavelength 279.5 nm, with slit 2 nm, and with about 20 times scale expansion. The analyses were based upon measuring peak heights.

2 µl of undiluted heparinized, haemolyzed whole blood were transferred to the graphite cup. In order to avoid the blood from creeping up along the wall of the cup, the pipette tip should not touch the wall during the introduction of the sample. The heating program was as follows: drying at 90 °C for 40 s, first ashing 530 °C for 25 s, second ashing at 1200 °C for 40 s and atomization at 2200-2250 °C for 3.5 s.

During the first 20 atomizations, the sensitivity decreased by about 30 percent; in this period a standard was run after every second sample. After 20 to 25 runs, the sensitivity of the cups was found to be approximately constant, with the use of these cups a standard was atomized after every fifth sample.

Three of the samples listed in Table 1 (sample No. 1, 20 and 25) were employed as standards; the concentration of manganese in these samples was established as follows. From each of the three samples a volume of 1 ml blood was transferred into three sample bottles, to one of the bottles 10 µl of a 1 p.p.m. manganese standard solution were added, to a second bottle 10 µl of a 2 p.p.m. manganese standard solution were transferred, the concentration of manganese added corresponding to 10 and 20 p.p.b., respectively. The content of the metal in the three

samples was then established by replicate (6-9) determinations by the standard addition technique.

RESULTS

Table 1 gives the concentration of manganese in whole blood from workers exposed for different periods in a Norwegian manganese alloy plant mainly producing ferromanganese and silikomanganese; for comparison samples from unexposed persons working within the plant or living in the vicinity of the plant were also analyzed. (From analyses of dust in the plant during the past five years, the exposure is approximately 1 mg Mn/m^3.) Table 1 also contains some medical data on the exposed persons.

The present analytical results were checked by analyzing 13 of the 25 samples in a laboratory of clinical chemistry. In this laboratory a Varian-Techtron AAS equipped with the tube version of the present furnace and a deuterium background corrector was used. 0.5 ml of the samples were diluted with the same volume of a 0.4% solution of Triton X-100. The graphite tube of the furnace was heated to about 100 °C, 1 µl of the mixture was introduced into the hot tube, the sample was ashed by 25 s heating at temperature setting 8, and atomized by 1.5 s heating at temperature setting 9. The results from these analyses are included in Table 1.

As apparent from Table 1 and a calculation of the correlation ($r = 0.90$), the agreement between the data from the two laboratories is satisfactory.

It was further considered of interest to obtain some information on the normal levels of manganese in the Norwegian population. For this purpose samples were collected from a control group of 32 unexposed persons from various parts of the country. The group consisted of persons of both sexes, the age varied from 24 to 60 years. The contents of manganese in whole blood from the group varied from 6 p.p.b. to 30 p.p.b., the mean value was 10 p.p.b., and the standard deviation 4 p.p.b.

The mean value of the control group compares favourably with the average given in Table 1 of unexposed individuals.

The furnace technique of AAS was applied by BUTTGEREIT (1973), BEK et al. (1974), GRAFFLAGE et al.

TABLE 1

Analytical results for manganese in whole blood from unexposed individuals and from exposed workers in a manganese alloy plant

Group	Age	Sample No.	Conc. in p.p.b. of Mn		
			present lab.		external lab.
			$\bar{x}$[a]	$\bar{x}_g$[b]	x[c]
I	24	1[d]	8		8
	52	2	9		9
Unexposed	19	3	8	10	-
individuals	20	4	14		14
	21	5	10		-
II	35	6	15		14
Workers	41	7	15		-
exposed	46	8	20	16	22
for 1 year	43	9	14		-
	43	10	16		16
III	55	11	10		-
Workers	34	12	8		7
exposed	31	13	16	11	-
for 5 years	31	14	12		10
	27	15	9		-
IV	40	16	12		8
Workers	48	17	9		-
exposed	31	18	11	13	12
for 10	30	19[d]	9		-
years	28	20[d]	22		23
V	55	21	10		-
Workers	67	22	9		8
exposed	49	23	6	13	-
for more	60	24[d]	19		15
than 10	56	25[d]	23		-
years					

a, mean value of two or three determinations; b, group mean value; c, analyses made in Dr. Fürst's clinical laboratory, Oslo, Norway; d, employed as standard.

(1974), ROSS and GONZALEZ (1974), BOURDON et al. (1974), and MUZZARELLI and ROCCHETTI (1975) to the determination of manganese in whole blood and/or serum, the latter authors being the only to analyze whole blood from healthy donors by the furnace technique of AAS. Their data (mean value 11 p.p.b., standard deviation 4.4 p.p.b., and range 3-21 p.p.b.) are in very good agreement with the present results for unexposed persons.

The present study showed that the concentration of manganese in whole blood from workers in a manganese alloy plant did not increase with the time of employment; this result is in agreement with previous conclusions (CHANDRA et al. 1974), viz. that estimation of manganese levels in serum has no significance in detecting manganese poisoning.

Accuracy, precision and detection limit

When carefully analyzed whole blood samples are employed as standards, the present method can be assumed to be accurate.

The precision - as obtained by the present equipment and technique - is approximately 1 p.p.b. .

The detection limit is about 2 p.p.b.

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