MANGANESE TOXICATION IN THE HUMAN BODY DETERMINED BY ACTIVATION ANALYSIS

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This paper reports the first application of neutron activation analysis to a medical problem in Thailand. In November 1964, a cooperative program was started between physicians from the Toxicological Department of Siriraj Hospital and scientists from the Office of Thai Atomic Energy Commission. Because a number of employees of a Bangkok dry-cell battery factory had been hospitalized with manganese poisoning, it was proposed that samples of blood, urine and cerebrospinal fluid (CSF) from these people be analyzed for manganese by neutron activation with the ultimate objective of using these analyses for diagnosis and correlative study of the disease. Manganese is known to accumulate in CSF where it results in loss of muscle control, hysteria and eventual death (*1*). Neutron activation has previously been used to determine manganese in several biological systems (*2–7*) because manganese in very low concentrations is an essential element. In early work Reiman and Minot (*8*) reported normal blood levels of manganese of 0.005–0.02 mg/100 gm; the concentration in urine is much smaller. Using neutron activation analysis of CSF from normal humans, Cortzias and Papavasiliou (*9*) found manganese-concentration ranges from 0.00008 to 0.00015 mg/100 ml. Moreover, Kanabrocki *et al* (*10*) used neutron activation analysis to determine average manganese concentrations in pooled human serum (0.001 mg/100 ml), CSF (0.00026 mg/100 ml) and urine (0.00091 mg/100 ml).

SAMPLES AND SAMPLING TECHNIQUES

Samples were supplied by the Toxicological Department, Siriraj Hospital, from patients known to be suffering from manganese poisoning, from suspected patients and from controls.

Blood. 1 ml of blood was drawn from the arm and inserted in a clean prewashed polyethylene vial which was heat-sealed and stored in a refrigerator until ready for analysis.

Urine. 24-hr specimens were collected under conditions which minimized external contamination.

CSF. Samples were withdrawn by sterile technique using a stainless-steel puncture needle. They were stored in vials in a similar manner to that used for blood.

TABLE 1. Mn CONTENT OF BODY FLUIDS FROM NORMAL HUMANS

Sample number	Blood (mg/100 ml)	Urine (mg/100 ml)	CSF (mg/100 ml)
1	0.0022	0.0008	0.0012
2	0.0076	0.0003	0.0005
3	0.0055	0.0007	0.0008
4	0.0034	0.0007	0.0006
5	0.0070	0.0007	0.0004
6	0.0024	0.0006	0.0010
7	0.0043	0.0001	0.0008
8	0.0069	0.0006	0.0004
9	0.0016	0.0007	0.0006

TABLE 2. Mn CONTENT OF BODY FLUIDS FROM WORKERS EXPOSED TO Mn-ORE DUST BUT WHO SHOW NO SYMPTOMS

Sample number	Blood (mg/100 ml)	Urine (mg/100 ml)	CSF (mg/100 ml)
1	0.0029	0.0057	0.0005
2	0.0022	0.0053	0.0004
3	0.0069	0.0025	0.0005
4	0.0036	0.0027	0.0005
5	0.0046	0.0035	0.0008
6	0.0126	0.0068	—
7	0.0023	0.0124	—
8	0.0025	0.0056	—
9	0.0112	0.0047	—

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TABLE 3. Mn CONTENT OF BODY FLUIDS FROM PATIENTS SUFFERING FROM Mn POISONING

Sample number	Blood (mg/100 ml)	Urine (mg/100 ml)	CSF (mg/100 ml)
1	0.0097	0.0017	0.0105
2	0.0016	0.0260	0.0077
3	0.0067	0.0048	0.0016
4	0.0059	0.0075	0.0095
5	0.0022	0.0045	0.0045
6	0.0051	0.0007	0.0019
7	0.0040	0.0005	0.0040
8	0.0053	0.0012	0.0015
9	0.0057	0.0005	0.0084

TABLE 4. COMPARATIVE RESULTS OF Mn CONTENT IN HUMAN BLOOD (mg/100ml)

Determined in this laboratory	by Butt *et al* (6)	by H. J. M. Bowen (1)	by Bethard *et al* (5)
0.00454 ± 0.00075	0.004 ± 0.001	0.00245 ± 0.0009	0.003

TABLE 5. ACCURACY TESTS

Standard Mn in μg	Counts at 0.84-Mev peak	cpm for 1 μg Mn
3.0	66,656	22,215
5.0	111,027	22,205
10.0	229,886	22,988

IRRADIATION AND ANALYSIS

The nuclear reaction ^{55}Mn (n, γ)^{56}Mn (2.56-hr half-life) was used for activation. Samples in plastic vials together with manganese standards were irradiated in the core-access element of the Thai Research Reactor (TRR-1) at a neutron flux of about 10^{31}n/cm²/sec for 10 min. Upon removal the sample was dissolved in conc. HNO_3 and 10 mg of manganese carrier added. The mixture was then wet ashed with fuming nitric acid and hydrogen peroxide to destroy organic matter. MnO_2 was precipitated by adding $KClO_3$. After the precipitate was filtered, dried and weighed to determine the chemical yield, the activity of induced ^{56}Mn was found using gamma-ray spectrometry by comparing the area of the 0.85-Mev and 1.8-Mev gamma-ray photopeaks with those from the induced standards. Figure 1 shows the gamma-ray spectra of typical samples before chemical separation of manganese. Figure 2 shows gamma-ray spectra of the separated manganese precipitate.

RESULTS

The results of manganese content in blood urine and cerebrospinal fluid are given in Tables 1–3. The amounts of manganese found in these experiments for normal human blood and urine are in good agreement with those found by conventional methods (*11*) and activation analysis (Table 4). Differences in manganese levels are probably due to differences in diet or other environmental conditions.

It can be seen in Fig. 1 that instrumental neutron activation analysis could hardly be performed on blood, urine and CSF because the gamma-ray emission of ^{24}Na tends to dominate the gamma-ray spectrum of ^{56}Mn (*12*) in the neutron irradiated samples even though the samples were cooled 1–2 hr. Therefore techniques for separating ^{24}Na and other radioisotopes in the sample after irradiation are necessary (*13*). The purification procedure diminished the contaminating ^{24}Na as shown in Fig. 2. In most cases ^{24}Na could not be detected in the precipitate. The agreement is good between these results and those of other workers as shown in Table 4.

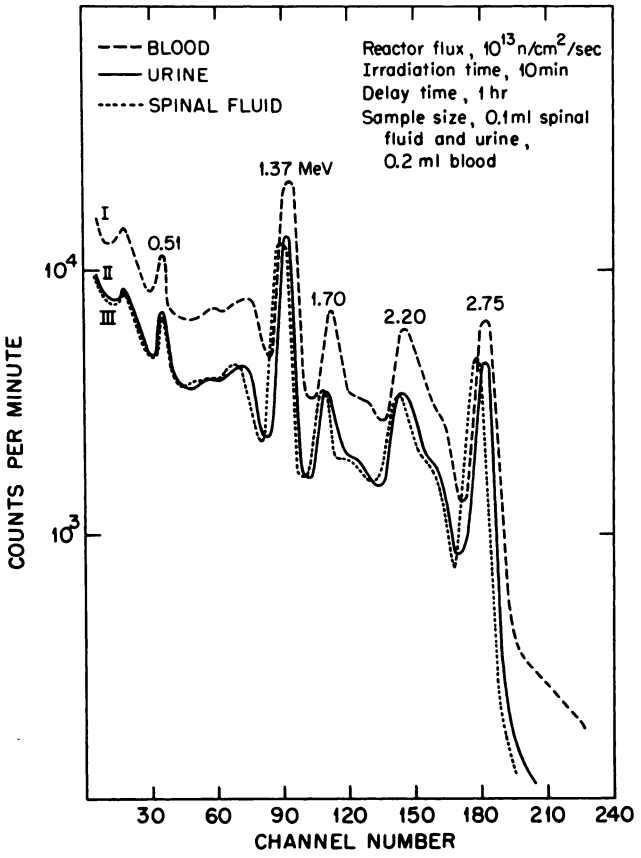

FIG. 1. Gamma-ray spectra of typical samples before chemical separation of manganese.

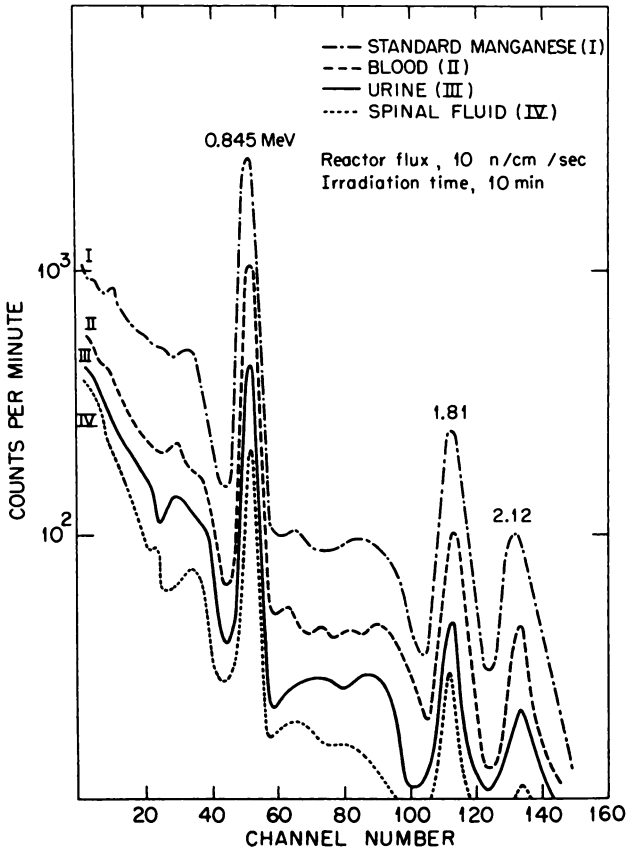

FIG. 2. Gamma-ray spectra of the separated manganese precipitate.

ERRORS AND INTERFERENCES

Table 5 shows tests of accuracy made for the method. The precision of this method is ± 1.5% which is quite satisfactory.

Some error may be introduced by variations in irradiation time caused by manual insertion and withdrawal of samples from the core-access element. Irradiation of sample and standard together minimize this.

The fast neutron reaction ^{56}Fe (n,p)^{56}Mn results in interference from iron in blood only. This is calculated to be about 15% or ± 0.007 μg ^{56}Mn. Irradiation under cadmium could minimize this, but cadmium irradiations were not possible with the operational procedures of TRR-1.

CONCLUSION

An increase of the concentration of manganese in body fluid from persons exposed to large amounts of manganese ore dust has been detected. Neutron activation analysis has been used to detect very low levels of manganese in body fluids of normal and exposed persons. The destructive method must be used because of large amounts of interfering ^{24}Na, but the separation method is rapid and simple.

From these results normal manganese levels (in mg/100 ml) appear to vary between 0.002 and 0.008 for blood, 0.0001 and 0.0008 for urine and 0.0004 and 0.0012 for CSF. Definite increases in the amount of manganese present in the body fluids of persons suffering from manganese poisoning were noted. The results indicate that the use of neutron activation analysis as a diagnostic tool for this condition is clearly feasible.

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