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DETERMINATION OF MANGANESE IN BIOLOGICAL MATERIALS†

Prepared for publication by

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Determination of manganese in biological materials

Summary - Although the essential nature of manganese in humans has yet to be firmly established, it is clearly an important constituent of a number of enzyme systems, and is clearly implicated in a number of deficiency conditions in animals. The accurate determination of manganese in biological materials is therefore of considerable importance for both metabolic and toxicological studies in humans and animals. Much of the earlier literature on manganese determinations, in particular in serum and urine, is marred by errors in the analytical procedures due principally to a failure by analytical chemists to fully appreciate the requirements for stringent control of contamination during sampling and sample handling prior to analysis. The wider availability of electrothermal atomic absorption instruments, particularly those incorporating the most recent advances in technology has brought the determination of manganese in serum and urine within the scope of many routine hospital laboratories. The application of this technique, coupled with the availability of centres providing activation analysis as a reference method, could have a significant impact on our knowledge of the biochemical functions of manganese in the next ten years.

CLINICAL BACKGROUND

The essential nature of manganese has been known for more than 50 years, but even today there is still very little evidence with respect to humans and most published information relates to animal studies (ref. 1). Despite fairly extensive animal investigations, the precise biochemical and nutritional functions of manganese remain poorly understood and the scope for future research efforts is very wide indeed. Until recently, analytical techniques of satisfactory sensitivity and accuracy were not widely available and attempts by many researchers to use unsatisfactory procedures have led to the publication of much erroneous data and also to biochemical conclusions of dubious value (ref. 2). Clearly, the major problems which have given rise to this situation are the very low concentrations of manganese in biological samples of all types from humans and animals, and a lack of awareness of the need to exercise control over extraneous contamination during all the steps of an analytical procedure.

Knowledge of the effects caused by manganese deficiency and of the biochemical functions of the element were recently reviewed by Hurley (ref. 1). Some typical examples of manganese deficiency in animals are the skeletal disorder perosis or "slipped tendon disease" in chickens (ref. 3) and the development of a genetic mutant in mice known as the "pallid mouse" in the offspring of manganese deficient animals (ref. 4). Both these problems can be completely prevented by diets supplemented with manganese. As indicated by these examples, manganese deficiency in animals generally results in skeletal abnormalities and impaired reproduction, but abnormalities in carbohydrate and lipid metabolism have also been noted (ref. 5).

In humans, the only case of manganese deficiency was reported by Doisy (ref. 6). The manganese deficiency in this case was observed in a patient under study for vitamin K deficiency and symptoms included a decrease in plasma cholesterol and slowed growth of hair and nails. The liver appears to be a key tissue in manganese metabolism, where it acts as the co-factor for many liver enzymes such as hepatic mitochondrial superoxide dismutase and pyruvate carboxylase (ref. 7). A number of workers have found an increase in serum manganese during liver metastases and hepatobiliary diseases such as acute hepatitis, chronic hepatitis, post hepatitis cirrhosis, cirrhosis and extra-hepatic biliary obstruction (refs. 7-9). Versieck et al. (ref. 9) also found a correlation between serum albumin and serum manganese concentrations in acute, chronic and post necrotic cirrhosis and between serum aminotransferase activities and serum manganese concentration, and postulated that increased serum manganese levels could be used as an index of liver cell damage.

Although less common than with other elements, toxicological effects caused by excessive exposure to manganese are known (ref. 10). Workers exposed to high levels of manganese oxide dust in mines or ore-processing plants have an increased incidence of pneumonia and bronchitis. Once absorbed, manganese can affect the brain, leading to a disease resembling Parkinson's Disease. It has been suggested that the measurement of manganese in blood and urine may not be very helpful in assessing exposure (ref. 11), as manganese blood levels showed no correlation with the degree of exposure of workers in a Norwegian manganese alloy plant.

Although manganese is considered to be essential in humans, no manganese deficiencies have been observed in patients on total parenteral nutrition. Nevertheless, manganese is considered sufficiently important that it is often added to trace element supplements. One such supplement, supplying 2.2 mg Mn day^{-1}, appeared excessive as both elevated serum manganese and increased urinary excretion were observed in patients subjected to this treatment (ref. 12).

Knowledge about the important metabolic functions of manganese has undoubtedly been limited by the lack of suitable and widely available instrumentation for its measurement in biological fluids and tissues. Whilst there are undoubtedly some valuable research efforts recorded in the literature, there is also a plethora of work which relies on manganese determinations which can now be judged erroneous. The wider utilisation of electrothermal atomisation atomic absorption instruments in recent years has in particular made the possibility of studying the biochemical roles of manganese much more feasible for the general hospital biochemist. It is essential that the use of such methods is associated with a full or increased awareness of the analytical problems of contamination during sampling and sample handling, and those related also to the measurement of very low levels of elements like manganese in biological samples.

MANGANESE CONCENTRATIONS OF INTEREST

The levels of manganese in rat tissues are very low and lie in the range from about 2.0 to 0.1 μg g^{-1} (ref. 1). In human tissues, concentrations are of a similar order (ref. 13), with levels about 1.0-1.5 μg g^{-1} in liver, 0.5-1.0 μg g^{-1} in kidney and around 0.05-0.15 μg g^{-1} in muscle, all calculated on the basis of wet weight. These levels are low but not extremely low and provided adequate precautions are taken during sampling and digestion (ref. 13), the resulting solutions should be amenable to measurement by a wide range of analytical techniques.

Concentrations of manganese in red cells and consequently whole blood are substantially higher than in serum (refs. 9,13,14). Versieck et al. (ref. 9) first reported a mean value of 15.0 μg g^{-1} wet weight for 46 subjects with a range between 8.1 and 36.9 μg g^{-1}, and other groups have obtained results in close agreement (ref. 13). Mean levels of manganese in whole blood have been found to lie between 8.4 and 12.2 μg l^{-1} for a number of population studies (ref. 14), with typical individual ranges between 3 and 30 μg l^{-1}. Once again, these levels are amenable to measurement using a number of techniques and provided elementary precautions are taken, hospital laboratories should find the use of electrothermal atomic absorption instrumentation both adequate and convenient for these determinations.

Levels of manganese in both serum and urine are however substantially lower in normal healthy subjects and have caused much more serious problems for the analytical chemist (refs. 2,13,14). Several different independent groups have now recorded mean serum manganese levels of between 0.54 and 0.63 μg l^{-1} for normal populations of various sizes and in various parts of the world. An acceptable consensus is therefore available and leads to the conclusion that higher levels recorded elsewhere must have been subject to contamination during sampling or to other sources of positive error. Of the studies represented in this group, that by Fernandez et al. (ref. 15) was the earliest and is interesting in that a spectrophotometric catalytic method was used for the final manganese determination, which was based on manganese(II) catalysis of the periodate oxidation of leuchomalachite green. A mean value of 0.63 μg l^{-1} with a range of 0.36 to 0.90 μg l^{-1} (n=12) was reported by these workers in 1963. Three other groups (refs. 9,16,17) reported very similar results using neutron activation analysis. The means and ranges were:-

Mean, μg l^{-1}	Range, μg l^{-1}		
0.59	± 0.18	(n=14)	(ref. 16)
0.54	0.36-0.78	(n=11)	(ref. 17)
0.57	0.38-1.04	(n=50)	(ref. 9)

Very similar results were also reported by Halls and Fell (ref. 12) using an electrothermal atomic absorption spectrometric procedure which yielded a mean of 0.58 μg l^{-1} and a range of 0.36-0.96 μg l^{-1} (n=9). Possible sources of error which may have afflicted the many reported studies of serum manganese levels have been reviewed by Versieck and Cornelis (ref. 2). Contamination is thought to be by far the most common problem, one group indicating themselves that an earlier study had been subject to a systematic error through contamination (ref. 16). Versieck and Cornelis (refs. 2,9) in addition point out that the substantially higher levels of manganese in packed blood cells requires that the separation of serum from whole blood must be carried out with extreme care.

A similar consensus now exists around a mean value for manganese in urine (refs. 13,14). Three atomic absorption studies have all yielded mean values below 1 μg l^{-1} (refs. 12,18,19). The results, 0.65±0.53 μg l^{-1} (n=20) (ref. 18), mean 0.54 μg l^{-1}, range 0.08-2.67 μg l^{-1} (n=126) (ref. 19), and mean 0.70 μg l^{-1}, range 0.1-1.5 μg l^{-1} (n=16) (ref. 12) are remarkably similar, given the variability of urine output itself. Some interesting results on manganese excretion via urine were reported by Cornelis et al. (ref. 20). These indicated a remarkable drop in manganese excretion, as well as total urine volume, when the donor changed to a low sodium chloride diet. Although these measurements were limited to a single subject, they do indicate the potential dependence of urine manganese levels on dietary factors.

Analysis of the current literature therefore supports the general conclusions that mean serum manganese levels for a reasonable sized population are around 0.5 to 0.6 μg l^{-1}, with individual values lying between 0.3 and about 1.0 μg l^{-1}. For urine, mean values should be of the order of 0.5 to 0.7 μg l^{-1} with a wider range from 0.08 to 2.5 μg l^{-1}, depending on the urinary output and other individual factors. Matched against these figures, much published data appears erroneously high and must be subject to considerable doubt. It is quite clear that for these sample types, analytical techniques capable of detection below 0.1 μg l^{-1} manganese are required. In the absence at present of a range of adequate standard reference materials, these normal ranges should themselves be used by analytical chemists for the evaluation of new techniques and procedures.

SAMPLE HANDLING PRIOR TO MEASUREMENT

As in the case of chromium (ref. 21), sample contamination before measurement must be the major cause of errors in many reports on the determination of manganese in biological samples. It is easy to criticise earlier workers from the comfort of hindsight. In the authors' opinion, such criticism is often misplaced since in many cases, scientists were working at the limits of knowledge and expertise available at the time. It is also still not clear if the accepted normal values for many elements have reached their final values, or have merely stopped falling at a point consistent with the degree to which contamination can currently be prevented. However, scientists today should be capable of learning from errors identified in the past and should ensure that their results are at least consistent with current knowledge, exemplified in this case by the manganese levels for normal subjects discussed in the preceding section.

It is clear that many earlier workers did not realise that far more stringent exclusion of contamination was essential for trace element analysis at or below the 1 μg l^{-1} level, compared to the 1 μg ml^{-1} level. One of the first groups to appreciate this in the case of manganese was Cotzias and co-workers (refs. 16,22). In their earlier study published in 1961 (ref. 22) they reported a mean serum manganese level of 2.50 μg l^{-1}. Subsequent re-examination of their own work revealed a systematic contamination of the samples by manganese and later analysis allowed them to obtain a more acceptable figure of 0.59 μg l^{-1} (ref. 16). Versieck et al. (ref. 23) also demonstrated the improvement in performance that can be obtained by paying close attention to the sample handling procedure. In an initial stage of their investigations, a mean value of 6.7-6.9 μg l^{-1} was obtained for the mean serum manganese concentration of 12 healthy adults. After the blood sampling procedure was improved and rigorous care taken to avoid airborne contamination, the mean values dropped by an order of magnitude to 0.63-0.64 μg l^{-1}. It should be noted that the same radiochemical procedure was used in both cases. The influence of airborne contamination on precision and accuracy was also clearly demonstrated in the work of Heydorn and Nørgard (ref. 24).

One of the main sources of contamination in blood analysis undoubtedly stems from the use of stainless steel needles for sampling (refs. 13,23). As would be expected from the composition of stainless steel and the lower analyte value in serum, this problem is much more serious for chromium (refs. 21,23). In the experiments of Versieck et al. (ref. 23) blood was sampled with a radioactivated needle and successive 20 ml aliquots drawn from the same sample pool. Contamination recorded in the first aliquot varied from 0.077 to 0.45 μg l^{-1} manganese for four separate needles. This was reduced significantly in subsequent aliquots but the overall contribution to the level of manganese in the resulting blood sample remained

substantial. Similar experiments carried out with a polypropylene catheter significantly reduced contamination levels, the highest recorded being an addition of 0.025 μg l^{-1}. Thus, it is essential that a polypropylene over-the-needle catheter (all-plastic cannula) be used for sampling blood for trace element analysis of manganese and chromium, as well as for other elements such as nickel. In addition, it is recommended that the initial portion of the sample drawn should be rejected or used for other analyses. Most important, it is critical that staff responsible for taking samples are fully aware of the different requirements necessary for sampling for trace element analysis compared to other hospital procedures. If the correct procedures are not followed at the sampling stage, the efforts of laboratory staff responsible for the analysis of the sample will be completely wasted.

The container into which the sample is transferred should also be carefully evaluated. Versieck et al. (ref. 23) showed that the stoppers of one commercial product made specially for trace element analysis, could contribute substantially to manganese contamination of blood samples. The manganese addition was found to be between 0.046 and 0.292 μg l^{-1} after 30 min. shaking inside the stoppered container. Polyethylene containers were also shown to introduce manganese contamination, which although still significant was substantially reduced by even a simple rinse with quartz distilled water. The importance of selecting and evaluating the correct container for the sample and of cleaning it thoroughly before use can not be overemphasised. Stringent precautions must also be taken against airborne contamination when the sample is collected and wherever or whenever it is opened for analysis. Obviously, clean-room conditions are ideal for such purposes but acceptable results have been obtained in normal biochemical laboratories provided containers are cleaned thoroughly and sensible precautions are taken to avoid environmental contamination.

Two procedures which have been used successfully for the determination of manganese in serum will serve to indicate the type of precautions required:

Procedure 1. Intended for neutron activation analysis (ref. 25)

"Venous blood samples were taken with a polypropylene over-the-needle catheter (Intranule 110 16; Vygon) and collected in high-purity quartz tubes (Spectrosil, length 120 mm, o.d. 16 mm, wall thickness 1.1-1.5 mm), previously cleaned with twice distilled water, boiled for two successive periods of 2 hours in a mixture of equal volumes of nitric and sulphuric acids (Suprapur), rinsed again, and finally steam cleaned for 3 hours with distilled-in-quartz water. The samples for the determination of manganese were collected after withdrawal of approximately 50 ml of blood for routine clinical laboratory investigations."

Procedure 2. Intended for routine hospital use for serum manganese determinations (ref. 12)

Blood was collected through a plastic cannula (Venflon, Viggo AB, Sweden). This is inserted with an inner steel needle which is then withdrawn leaving the cannula in place. Since the first 10 ml of blood withdrawn is contaminated by the steel needle, it is rejected or used for other determinations. The following 10 ml is transferred to an acid-washed polypropylene centrifuge tube. Acid washing of all containers is achieved by standing overnight in 20% V/V AristaR nitric acid followed by thorough rinsing in deionised/distilled water. After clotting and centrifuging, the serum sample is transferred to an acid-washed sample vial and stored at 4-10°C.

Although the levels of manganese in tissues and whole blood are higher and steel blades and needles have been used successfully because the relative effect of contamination is less, contamination will still occur (ref. 23), and sampling using quartz or titanium knives for tissues, and the above procedures for whole blood are strongly recommended.

The collection or urine samples presents different but no less serious hazards. A 24 hour collection regime requires good housekeeping by hospital nursing staff, and the use of special acid washed containers (refs. 12,20). Halls and Fell (ref. 12) have reported that the use of disposable fibre bottles, now common in hospitals for urine disposal, leads to extreme manganese contamination. A suitable procedure for urine collection established at Glasgow Royal Infirmary is:

Procedure 3. Intended for routine hospital use for urine (ref. 12)

Random urine samples were voided into acid washed 25 ml Sterilin Universal containers and 0.5 ml of concentrated sulphuric acid (AristaR) added. Twenty four hour urine samples were collected in 2 litre polyethylene containers and 5 ml glacial acetic acid (AristaR) was added. Aliquots were transferred to Sterilin Universal containers for storage at 4°C.

The Sterilin containers were shown to be free of manganese contamination. All 2 litre containers were acid washed before use by standing overnight in 20% V/V AristaR nitric acid.

The problems of contamination will obviously also occur during any sample pretreatment procedures. The sensitivity of electrothermal atomic absorption spectrometry has meant that most workers prefer a direct method of analysis. However, extraction of manganese from urine with cupferron into methyl isobutyl ketone was developed by Van Ormer and Purdy for determination by flame atomic absorption (ref. 26). Blanks were high at 1.1 μg l^{-1} manganese. When corrected for the blank, ten urine samples from one subject gave a mean value of 3.0 ± 0.6 μg l^{-1} which is high by today's standards. The same extraction procedure was used by Buchet et al. (ref. 18) for the ETA-AAS determination of manganese in whole blood and urine. In this case, results which are consistent with current literature were obtained, viz. 12.2 ± 3.9 μg l^{-1} (n=20) for whole blood, and 0.65 ± 0.53 μg l^{-1} (n=20) for urine samples from non-occupationally exposed persons.

The levels of manganese in blood and urine are such that it is possible with correct precautions to carry out pretreatment procedures such as ashing or chelation/extraction. The procedure should be fully evaluated for purity of reagents, sources of contamination from glassware and other utensils and clean room conditions should be used if possible. In addition, regular blank determinations should be carried out.

ANALYTICAL TECHNIQUES

A wide range of analytical techniques is available for the determination of manganese in standard reference materials such as Bowen's Kale (ref. 13), and in most tissue samples. Such methods include spectrophotometry, arc emission spectrometry, flame atomic absorption spectrometry, and proton induced X-ray emission spectrometry. Such methods are rarely convenient for the lower levels of manganese found in serum and urine, and when they have been used have often provided erroneous or at best ambiguous data (refs. 2,26). Although neutron activation analysis and electrothermal atomic absorption spectrometry are the most popular methods, some others have been used successfully if infrequently.

Catalytic analysis

One of the earliest papers to report acceptable results for the determination of manganese in human serum utilised a catalytic procedure which involved spectrophotometric monitoring of the reaction (ref. 15). The procedure takes advantage of the catalytic effect of manganese (II) on the reaction at pH3 between periodate and leuchomalachite green (p,p'-tetramethyldiaminotriphenylmethane). The serum samples were dried at 116°C for 2 hours in Vycor test tubes, and the residues were then ashed in a muffle furnace at 540°C for 1 hour. At the end of this period, the cooled residue was twice moistened with one drop of water, redried and reashed. Finally, the residues were dissolved in 0.3 ml 1MHCl and heated at 70°C to dissolve the salts. The description of the procedure is remarkable for the care with which possibilities of contamination were excluded. Thus Vycor tubes were used as borosilicate glass contains "too much manganese", all glassware was thoroughly washed, and the ashing procedure was carried out with a minimum of reagent additions. For its time, this strikes one as a remarkable piece of work, and it is perhaps disappointing that the procedure has not been used more widely.

Electrothermal atomic absorption spectrometry (ETA-AAS)

Since the main atomic absorption line for manganese is at 279.5 nm, the determination of this element has not suffered from the same serious difficulties as chromium with regard to background correction (ref. 21). However, the maximum ashing temperature that is recommended with manganese in biological materials is 1100°C (refs. 12, 27-30). At this char temperature, most of the organic matrix will be removed, but the major fraction of the inorganic salts will remain at the atomisation stage. Since these contribute substantial background signals at the manganese wavelength, an accurate means of compensating for this is essential. Although one group has suggested that better background correction can be achieved at this wavelength with the Zeeman effect technique (ref. 31), most authors have used instruments incorporating D_2-arc background correction systems with considerable success (refs. 7,12,27-30). However, D_2-arc based systems may not be able to compensate accurately for the large background signals and steps should be taken to reduce the magnitude of the background signal during atomisation of manganese (refs. 12,14,30). Addition of nitric acid, use of a low flow of argon during atomisation, and the use of fast heating rates all contribute to a reduction in background, and enable accurate analysis to be carried out with instruments based on D_2-arc compensation. The highest possible ashing temperature should be used for longer times to assist in the removal of matrix salts. Deproteinization with 5% v/v nitric acid is also recommended as a means of reducing the build up of carbonaceous residue inside the graphite tube (ref. 30).

Recent studies have shown that magnesium nitrate is an effective matrix modifier for manganese, and when used with a platform allows ashing temperatures up to 1400°C to be used (refs. 31,32), but application to the determination of manganese in biological materials has not been reported to date.

Alternative approaches to the accurate compensation of high background signals include the use of background correction systems based on a single light source, such as the Zeeman effect (refs. 31,33) or the pulsed hollow cathode lamp (ref. 34). An alternative approach developed in recent years by O'Haver and his colleagues uses a continuum light source with a high resolution spectrometer to carry out simultaneous multi-element analysis by atomic absorption spectrometry (ref. 35). In a recent paper, Lewis et al. (ref. 36) described the use of this instrument system for the simultaneous determination of manganese, chromium, aluminium and nickel in blood serum. Efficient background correction is achieved in this system using wavelength modulation procedures based on an oscillating quartz refractor plate mounted at the entrance slit of the monochromator. Measurement of the absorption of several elements simultaneously is achieved with the continuum source with multi-channel detection using the echelle grating in a polychromator configuration. Samples of serum (2 ml) are placed in silanised quartz test tubes with 20 µl of 2% m/v magnesium nitrate, frozen at -20°C and freeze dried. The freeze dried samples are then ashed in a muffle furnace up to a maximum temperature of 480°C. The ashed sera are then dissolved in 0.5 ml of Ultrex 5% V/V nitric acid. The magnesium nitrate acts as both an ashing aid and matrix modifier and allowed an ashing temperature of 1300°C to be adopted for all the above elements. Atomisation from a platform gave a mean method detection limit of 0.11 µg l^{-1} manganese in serum. A result of 2.2 µg l^{-1} for a bovine serum pool, now issued as National Bureau of Standards Standard Reference Material 8419 (refs. 37,38), was in good agreement with values obtained by a variety of other techniques. Analysis of 30 adult human sera gave a mean value of 0.48 µg l^{-1} with a range of 0.15-1.49 µg l^{-1} in excellent agreement with current literature values. Although this technique is not commercially available, the ability to determine several trace elements simultaneously in biological samples could be of intense future interest in the biochemical laboratory.

Electrothermal atomic absorption spectrometry is clearly the most important current technique used for the determination of manganese at very low levels in biological materials. Recent developments in technology have significantly improved the performance of most instrument systems, so that results of acceptable accuracy and precision can be achieved at the levels of manganese of most interest. Typical recommended procedures are given by Halls (ref. 14). The ability to obtain the most accurate results still however requires considerable skill on behalf of the analyst, and also a detailed knowledge of the procedures required to avoid sample contamination and to achieve optimum performance from the analytical instrument.

Carbon furnace atomic emission spectrometry (CF–AES)

In recent years, it has been demonstrated that sensitive atomic emission measurements can be made with commercially available electrothermal atomic absorption instruments (ref. 39). A measurement carried out with such an instrument does not however incorporate automatic background correction in the emission mode. Wavelength modulation, as also used for continuum source atomic absorption (ref. 35), is however a convenient and straightforward means of correcting for the continuum radiation which constitutes the major source of background in carbon furnace atomic emission spectrometry (ref. 40-42). The recent development of a purpose built single channel instrument for carbon furnace atomic emission spectrometry has been described (ref. 40). The system incorporates a high resolution echelle monochromator, a commercial graphite furnace atomiser with platform atomisation, and a wavelength modulation system for automatic background correction. A detection limit of 0.029 µg l^{-1} for manganese has been achieved with this instrument, and application to the determination of manganese (as well as a number of other elements) in urine has been described (refs. 43,44). Good agreement with an ETA-AAS procedure (ref. 12) was obtained for samples from patients on an intravenous feeding regime who had urinary manganese levels between 0.98 and 42.2 µg l^{-1}. The higher than normal levels were associated with a rather excessive addition of manganese through the trace element supplement given to the patients (ref. 12). Analysis of urine samples from nine normal subjects gave a mean of 1.09 µg l^{-1} in reasonable agreement with other literature values.

Carbon furnace atomic emission spectrometry has the potential to offer a convenient route to simultaneous multi-element trace analysis of biological materials (ref. 45), and to provide sensitive analysis at relatively low cost (ref. 46). At present, however, there are no purpose built commercial instruments available for this technique, and its application is limited to specialised laboratories prepared to make the time and effort available to assemble their own instrument systems.

Inductively coupled plasma emission spectrometry (IC–PES)

Detection limits for manganese in the ICP lie in the region of 1 μg l^{-1} (refs. 47,48). Whilst this makes the method suitable for the determination of the higher concentrations of manganese found in foods (including standard reference materials) (refs. 48,49), the direct determination of manganese in serum and urine is not possible. In addition, such samples will usually require dilution before introduction through typical nebulisation systems, and the dilution factor will further degrade detection limits.

An ion-exchange preconcentration procedure has been described for the determination of manganese in sea water (ref. 47), which might also be suitable for urine analysis, but the only work which has attempted urinary manganese analysis with the ICP involved preconcentration of manganese on a poly(dithiocarbamate) resin (ref. 50). For manganese, the resin can be mixed directly with the urine sample, taking about 100 mg to 500 ml urine. After complexation, the resin is digested in a mixture of nitric acid and hydrogen peroxide and the solution reduced to 5 ml, providing a concentration factor of 125. Two samples of urine from a normal subject gave values of 1.02 ± 0.28 μg l^{-1} (n=5), and on a separate occasion 0.93 ± 0.13 μg l^{-1} (n=10), which are in agreement with accepted literature values. The detection limit of the method appears to be close to 0.24 μg l^{-1}.

A technique which is growing in popularity for biological samples involves the use of electrothermal atomisation for sample introduction into the ICP. When using an ETA system, the organic part of the matrix can be removed as usual in a preliminary ashing stage, leaving the manganese and other trace elements to be transported to the ICP with the major salts present in the matrix. Camara-Rica et al. (ref. 51) described such a technique for the determination of manganese in whole blood samples, and more recently two groups have investigated similar systems for the determination of manganese in NBS SRM orchard leaves and bovine liver and serum and urine (refs. 52,53). The detection limit in one system, 0.4 μg l^{-1}, was reasonably low using 50 μl of sample injected into an HGA 74 electrothermal atomiser, but serious matrix interferences were encountered and the authors recommended the use of the standard additions procedure (ref. 52). No interferences were observed on manganese in the second system using either urine or digested resin from the above preconcentration procedure and a detection limit of 0.18 μg l^{-1} was achieved (ref. 53). Application to real sample analysis was not reported. Further studies of the combination of resin preconcentration, electrothermal vapourisation, and ICP determination may yield interesting results.

Neutron activation analysis

Neutron activation is an important reference method for the determination of manganese in biological materials, and has performed a significant role in helping to establish the normal levels of the element in serum (ref. 2). Reactor irradiation produces the isotope ^{56}Mn by the reaction $^{55}Mn\ (n,\gamma)\ ^{56}Mn$. As ^{56}Mn has a half life of only 2.587 hours, the determination must be carried out rapidly in a laboratory close to the reactor. Interference comes from the presence of iron in the sample due to the reaction $^{56}Fe\ (n, p)\ ^{56}Mn$, but is mainly a problem in the analysis of packed cells or whole blood which have high iron concentrations. Versieck et al. (ref. 9) overcame this problem by using a reactor with a high ratio of thermal to fast neutron flux. This reduced the interference to about 7% which could be easily corrected for.

A number of different separation procedures have been used following irradiation and cooling. Cotzias et al. (ref. 16) used precipitation as tetra phenylarsonium permanganate, Versieck et al. (ref. 9) preferred extraction with oxine at pH8 into chloroform, and Damsgaard et al. (ref. 17) utilised a similar extraction procedure with diethylammonium diethyldithiocarbamate. Some of the more difficult problems associated with the determination of manganese in urine have been discussed by Cornelis et al. (ref. 20).

Excellent results can be achieved by neutron activation analysis, although the procedures are clearly lengthy and time-consuming and beyond the scope of typical hospital laboratories. The provision of facilities for this type of analysis performs an essential back-up function for the hospital biochemist and it is to be hoped that studies of the metabolism and speciation of manganese in biological systems will continue to be a significant contribution from those centres endowed with nuclear reactors, and the facilities to perform high quality trace element analysis.

CONCLUSIONS

Despite the fact that there is now a consensus around normal values of manganese in serum and urine, publications still appear containing results at variance with these values. It is vital that the major contributions made by Versieck and Cornelis (refs. 2,13) and others to the establishment of normal values for manganese and other elements are disseminated as widely as possible, and that those responsible for the refereeing of scientific papers ensure that only work of acceptable quality appears in the scientific literature.

Recent technological developments in the design and operation of electrothermal atomisers have made it possible for many laboratories to carry out regular determinations of manganese in biological fluids. The recent provision of a standard reference material bovine serum pool with a recommended value of 2.6 ± 0.5 μg l^{-1} manganese (refs. 37,38) will give such laboratories a means of evaluating their analytical performance. The manganese content of this material is clearly higher (by a factor of 4-5) than the level in human serum. Whilst being a major step forward, it will not allow the evaluation of accuracy at the lowest levels required, nor will it of course allow the evaluation of contamination during sampling. Rigorous elimination of all sources of contamination remains the major requirement of all analytical procedures. Scientists intending to contribute to our knowledge of the role of manganese should ensure that their esults are consistent with the accepted normal ranges given in this publication and elsewhere, as otherwise they may waste the efforts of themselves and others in the collection and evaluation of useless data. It should, however, be possible for some of the gaps in our knowledge of the biochemical functions of manganese in humans to be filled in, in the course of the next 5 to 10 years. To achieve valid and worthwhile results, it will be essential that workers ensure that the levels of contamination of their samples are reduced to a minimum, and that the procedures outlined in this paper are adopted or improved upon. Provided that correct care and attention to detail are undertaken, the study of manganese as an essential element could take a significant step forward in the immediate future.

REFERENCES

1. L.S. Hurley, in P. Bratter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, Walter de Gruyter & Co., Berlin, 3, 239 (1984).
2. J. Versieck, R. Cornelis, Anal. Chim. Acta, 116, 217 (1980).
3. L.S. Hurley, Physiol. Rev., 61, 249 (1982).
4. G.C. Cotzias, L.C. Tang, S.T. Miller, D. Sladic-Simic, L.S. Hurley, Science, 176, 410 (1972).
5. R.M. Leach, in W.G. Hoekstra, J.W. Suttie, H.E. Ganther, W. Mertz (Eds.), Trace Element Metabolism in Animals, University Park Press, Baltimore, 2, 51 (1974).
6. E.A. Doisy, in W.G. Hoekstra, J.W. Suttie, H.E. Ganther, W. Mertz (Eds.), Trace Element Metabolism in Animals, University Park Press, Baltimore, 2, 668 (1974).
7. A. Favier, J. Arnaud, L. Dumolard, J-P. Zarski, in P. Bratter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, Walter de Gruyter & Co., Berlin, 3, 719 (1984).
8. J. Versieck, J. Hoste, F. Barbier, Acta Gastra-enterol.Belg., 39, 340 (1976).
9. J. Versieck, F. Barbier, A. Speecke, J. Hoste, Clin. Chem., 20, 1141 (1974).
10. M. Piscator, in L. Friberg, G.F. Nordberg, V.B. Vouk (Eds.), Handbook on the Toxicology of Metals, Elsevier, Amsterdam, 485 (1979).
11. D.L. Tsalev, F.J. Langmyhr, N. Gunderson, Bull Environ. Contam. Toxicol., 17, 660 (1977).
12. D.J. Halls, G.S. Fell, Anal. Chim. Acta, 129, 205 (1981).
13. J. Versieck, CRC Critical Reviews in Clinical Laboratory Sciences, CRC Press Inc., 22, 97 (1985).
14. D.J. Halls, in M. Stoeppler, Trace Metal Analysis in Biological Specimens, in the press.
15. A.A. Fernandez, C. Sobel, S.L. Jacobs, Anal. Chem., 35, 1721 (1963).
16. G.C. Cotzias, S.T. Miller, J. Edwards, J. Lab. Clin. Med., 67, 836 (1966).
17. E. Damsgaard, K. Heydorn, N.A. Larsen, B. Nielson, Riso Report, 271, Danish Atomic Energy Commission, Roskilde.
18. J.P. Buchet, R. Lauwerys, H. Roels, C. de Vos, Clin. Chim. Acta, 73, 481 (1976).
19. T. Watanabe, R. Tokunaga, T. Iwahana, M. Tati, M. Ikeda, Brit. J. Ind. Med., 35, 73 (1978).
20. R. Cornelis, A. Speecke, J. Hoste, Anal. Chim. Acta, 78, 317 (1975).
21. J.M. Ottaway, G.S. Fell, Part IV of this series, Pure Appl. Chem., in the press.
22. P.S. Papavasiliou, G.C. Cotzias, J. Biol. Chem., 236, 2365 (1961).
23. J. Versieck, F. Barbier, R. Cornelis, J. Hoste, Talanta, 29, 973 (1982).
24. K. Heydorn, K. Nørgard, Talanta, 20, 835 (1973).
25. J. Versieck, J. de Rudder, J. Hoste, F. Barbier, G. Lemey, C. Vanballenberghe, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 59 (1979).
26. D.G. Van Ormer, W.C. Purdy, Anal. Chim. Acta, 64, 93 (1973).

27. D.J. Halls, Anal. Proc., 18, 344 (1981).
28. A. Favier, D. Ruffieux, A. Alcaraz, B. Maljournal, Clin. Chim. Acta, 124, 239 (1982).
29. V. Hudnik, M. Marolt-Gomiščck, S. Gomiščck, Anal. Chim. Acta, 157, 143 (1984).
30. K.S. Subramanian, J.-C. Meranger, Anal. Chem., 57, 2478 (1985).
31. W. Slavin, G.R. Carnrick, D.C. Manning, E. Pruszkowska, At. Spectrosc., 4, 69 (1983).
32. W. Slavin, G.R. Carnrick, D.C. Manning, Anal Chem., 54, 621 (1982).
33. P.A. Pleban, K.H. Pearson, Clin. Chem., 25, 1915 (1979).
34. S.B. Smith, G.A. Hieftje, Appl. Spectrosc., 37, 419 (1983).
35. T.C. O'Haver, Analyst, 109, 211 (1984).
36. S.A. Lewis, T.C. O'Haver, J.M. Harnly, Anal. Chem., 57, 2 (1985).
37. U.S. National Bureau of Standards, Standard Reference Material 8419, U.S. Department of Commerce, Gaithersburg, Spring 1985.
38. C. Veillon, S.A. Lewis, K.Y. Patterson, W.R. Wolf, J.M. Harnly, J. Versieck, L. Vanballenberghe, R. Cornelis, T.C. O'Haver, Anal. Chem., 57, 2106 (1985).
39. J.M. Ottaway, R.C. Hutton, D. Littlejohn, F. Shaw, Wiss. Z. Karl-Marx-Univ., Leipzig, Math.-Naturwiss. R., 28, 357 (1979).
40. M.S. Epstein, T.C. Rains, T.C. O'Haver, Appl. Spectrosc., 30, 324 (1976).
41. L. Bezur, J. Marshall, J.M. Ottaway, R. Fakhrul-Aldeen, Analyst, 108, 553 (1983).
42. L. Bezur, J. Marshall, J.M. Ottaway, Spectrochim. Acta, 39B, 787 (1984).
43. J.M. Ottaway, L. Bezur, R. Fakhrul-Aldeen, W. Frech, J. Marshall, in P. Bratter, P. Schramel, Trace Element Analytical Chemistry in Medicine and Biology, Walter de Gruyter & Co., Berlin, 575 (1980).
44. W. Frech, J.M. Ottaway, L. Bezur, J. Marshall, Can. J. Spectrosc., 30, 7 (1985).
45. J. Marshall, D. Littlejohn, J.M. Ottaway, J.M. Harnly, N.J. Miller-Ihli, T.C. O'Haver, Analyst, 108, 178 (1983).
46. D.C. Baxter, I.S. Duncan, D. Littlejohn, J. Marshall, J.M. Ottaway, J. Anal. At. Spect., 1, 29 (1986).
47. S. S. Berman, J.W. McLaren, S.N. Willie, Anal. Chem., 52, 488 (1980).
48. P. Schramel, Spectrochim. Acta, 38B, 199 (1983).
49. R.L. Dahlquist, J.W. Knoll, Appl. Spectrosc., 32, 1 (1978).
50. R.M. Barnes, P. Fodor, K. Inagaki, M. Fodor, Spectrochim. Acta. 38B, 245 (1983).
51. C. Camara-Rica, G.F. Kirkbright, R.D. Snook, At. Spectrosc., 2, 172 (1981).
52. A. Aziz, J.A.C. Brockaert, F. Leis, Spectrochim. Acta, 37B, 369 (1982).
53. R.M. Barnes, P. Fodor, Spectrochim. Acta, 38B, 1191 (1983).