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DETERMINATION OF MOLYBDENUM IN BIOLOGICAL MATERIALS

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Determination of molybdenum in biological materials

Abstract - The accurate determination of molybdenum in biological materials is of considerable importance because of the essential role played by this element in human metabolism. However, data on the Mo content in biological samples and especially in clinical materials are often contradictory due to the inadequate sensitivity and reliability of the instrumental techniques used and to insufficient control of contamination during the analytical procedure. Despite recent developments in Mo determination by electrothermal atomic absorption and plasma atomic emission spectrometry, measurements in human blood and serum remain beyond the scope of most hospital laboratories involved in the usual routine testing. Further development of atomic spectrometric methods or a wider availability of neutron activation analysis may provide more data which is required for a better understanding of the nutritional role of Mo and its metabolism in humans. Advantages and limitations of the analytical techniques used for the determination of Mo in biological materials are critically discussed with particular attention given to clinical samples. The most common sources of analytical error are discussed and methods for their reduction are given.

BIOCHEMICAL AND CLINICAL BACKGROUND

As early as 1953 (ref. 1) molybdenum was recognized to be an essential trace element for many species including man. It is a component of many enzymes responsible for the initial stages of nitrogen, carbon and sulfur metabolism of plants, animals and man and participates in a large number of enzymatic reactions (ref. 2,3). There is an absolute dependence by plants on Mo as it plays a vital role in the earth nitrogen cycle where it is involved both in nitrate reduction and nitrogen fixation.

Although molybdenum is essential for animals it shows evidence of toxicity at high levels. General symptoms of excess doses of Mo for ruminants are: retarded growth, decreased milk and meat production, osteoporosis, severe gastroenteritis and coma (ref. 4). Cattle receiving feed containing high levels of available Mo develop copper deficiency diseases (Cu-Mo antagonisme) which may become lethal (ref. 5). Molybdenum intoxication depends on its speciation and is also influenced by the uptake of other elements such as S^{VI}, W^{VI}, Cu, Pb and Zn.

In humans, a deficiency of Mo in the diet may lead to some healths deffects. Diets with a low Mo content (20 µg/kg) adversely effect growth and may lead to neurogical disorders and even early death (ref. 6). Molybdenum also seems to play a positive role in the prevention of tooth decay (ref. 7). On the other hand, excess doses of Mo can be detrimental, persons having a diet rich in Mo being susceptible to gout, uricemia and xanthinuria and often suffer from an inflammation of extremities due to excess uric acid deposition (ref. 8,9).

The practice of supplementing human diets with trace elements has stimulated studies of Mo as an essential nutrient. Several studies using diets providing between 1 and 14 µg of Mo per kg body weight per day report that an optimal diet should provide about 2 µg Mo per kg body weight per day (ref. 7). This result is comparable with a daily intake of 128 µg Mo proposed recently for a mixed diet composite (USDIET-I) (ref. 10). The Mo requirement for infants is closely reflected in the Mo content of breast milk (5-63 ng/g) with the level depending on the time after parturition (ref. 11-14).

It must be emphasized that studies of Mo metabolism in man have often be hampered by the lack of sufficiently sensitive analytical methods for monitoring Mo in human tissues and body fluids. For this reason in many cases it is difficult to determine whether the symptoms attributed to Mo defficiency or excess are due to biological variations or simply to experimental error. A better understanding of the role of Mo in human nutrition depends on improving the sensitivity and accuracy of the analytical methods involved. Only then will the biological function of Mo and the role of Mo as an essential trace nutrient be fully understood.

MOLYBDENUM CONCENTRATIONS OF INTEREST

The level of Mo in plants depends on the soil content and soil conditions (ref. 15). As Mo is taken up by plants in the form of the MoO_4^{2-} anion, plants grown on acidic soil accumulate very little Mo. The reported values of Mo in plants vary from 0.1 μg/g to several μg/g depending on the material analyzed and the Mo content of animal tissues do not differ significantly from these values. Early reported values for bovine liver were in the range 2.7-4.9 μg/g (ref. 7) while the more recently reported values are 2.3-3.9 μg/g for bovine (ref. 16-19) and 0.23 μg/g for canine liver (ref. 20).

The results reported for the urinary Mo concentration of normal healthy human subjects vary between 33 and 113 μg/l. The early values of 33 μg/l from a normal subject and 57 μg/l from subjects living in a molybdenoferrous area (ref. 7), agree with those reported recently by Barnes et al. (ref. 21) - 54-58 μg/l and by Wester (ref. 22) - 54 μg/l. Other workers, however, have found higher Mo concentrations with levels varying from 80 to 113 μg/l (ref. 23-25). The above data are difficult to compare as there are other factors to be taken into consideration but the ratio Mo:creatinine would be more useful. Nevertheless the disparities may not be significant and probably depend on individual biochemical factors. Wandt and Pougnet (ref. 26) determined the Mo content in urinari calculi as 2.2 μg/g.

The Mo contents reported for human milk are low. However, although most of the values reported (5-25 ng/g (ref. 12), 8-22 ng/g (ref. 13) and 5-63 ng/g (ref. 14)) are in a quite good agreement with each other. One publication (ref. 27) gives the much lower level of less than 0.26 ng/g. Molybdenum has also been determined in human lung tissue the values being 8-740 ng/g (ref. 28) and 71 ng/g (ref. 25), nail - 0.15-5 μg/g and 0.2 μg/g (ref. 25,28), brain - 16-29 ng/g (ref. 29) and liver - 0.57 μg/g (ref. 30).

Many biochemical laboratories can now determine Mo at the above levels owing to an increased use of electrothermal atomic absorption and inductively coupled plasma emission spectrometry. However difficulties arise in the determination of Mo at the sub-ng level. They are best illustrated by the disparate values for Mo content in human serum and whole blood obtained by different workers. The results reported vary by about two orders of magnitude (from 0.55 to 257 ng/ml for serum (ref. 31-33) and from 0.6 to 13.1 ng/ml for the whole blood (ref. 25, 34-36)). Certain environmental and individual differences may of course exist, but such large discrepancies are more likely to be caused by the use of analytical techniques of insufficient accuracy as well as by contamination factors. The more probable values seem to be the lower ones obtained by Versieck et al. (ref. 31,33) and confirmed by other investigators (ref. 37-39). These workers were quite aware of the risk of contamination and introduced quality control into their procedures. However, it is possible, that the serum Mo content is even lower and the present level is dictated by the limitations of current analytical technology. The establishment of the true serum Mo level is also hampered by the lack of suitable reference materials. Until a human serum Certified Reference Material is available for Mo, workers are advised to institute rigorous quality control procedure and be cautious with any data not consistent with the currently accepted level i.e. 0.5-0.6 ng/ml (ref. 31,33,37).

SAMPLE HANDLING PRIOR TO MEASUREMENT

Although Mo is not a common element in the environment contamination represents a great hazard, when analysing samples where Mo is present at ng/g (human milk and tissues) or sub-ng/ml (human blood and serum) levels. Particularly at the sub-ng/g level contamination is likely to eclipse the original Mo content in the sample (ref. 32) and this is the main reason for large discrepancies in the results obtained. Since many errors arise at an early stage of the analytical procedure (sample collection and storage), information on the history of the sample must be available to the analyst.

One of the sources of contamination is the use of Mo-containing stainless steel needles, scalpels, scissors and other surgical tools for sampling. The Mo release from these instruments was reported (ref. 25) to be lower than 1 ng but this is nevertheless too great at sub-ng/g levels. Recently, Mianzhi and Barnes (ref. 40), analyzing commercial serum, found a Mo content of 20.7±2.6 ng/ml, much higher than the currently accepted value, probably because of contamination during sampling. Pietra et al. (ref. 25) found it impossible to estimate the Mo contamination in the blood collection process and their values of 8.1 ng/ml and 11.3 ng/ml are much higher than currently accepted ones (ref. 34,36). For these reasons needles made of Pt-Ir, Pt-Ru (ref. 41), siliconized metal needles, titanium surgical tools as well as plastic catheters and cannula (ref. 42) are recommended and to avoid contamination the first portion of the sample should be discarded (ref. 32). Another possible source of contamination is the container used for sample collection and storage (ref. 42). Teflon, quartz and polyethylene are the recommended materials and thorough cleaning of the containers is very important. High purity quartz tubes (Spectrosil) used by Versieck et al.

(ref. 31-33) for blood collection were cleaned with twice distilled water, boiled in a mixture of equal volumes of nitric acid (min. 65%) and sulfuric acid (96%) (Suprapure; Merck), rinsed again and finally steam cleaned with triple quartz distilled water. Clean room conditions are usually not necessary for Mo determination but the analyst should be aware that considerable airborne contamination may arise when samples are taken in places of high Mo exposure.

The procedures for the determination of Mo usually involve sample drying, digestion and/or a separation step. The analyst should always bear in mind that all manipulations at the sample preparation stage may result either in contamination and/or the loss of the analyte. Freeze drying (8 h), drying (100 °C, 8 h) of mouse serum, liver and human hair were found not to cause any Mo losses (ref. 43). However, in the case of mouse serum dry-ashed at 600 °C for 12 h some losses of Mo occurred. Low temperature ashing in a stream of nascent oxygen was find to give quantitative Mo recoveries during the decomposition of serum (ref. 43) and blood (ref. 44). Wet mineralization procedures are usually considered to be susceptible to contamination but this risk may be reduced by the use of Suprapure or sub-boiled acids. The mineralization of plant material with mixtures of HNO_3-H_2O_2, and HNO_3-$HClO_4$ is recommended (ref. 45). Serum and blood samples are usually decomposed with concd. HNO_3 (ref. 39) or a mixture of HNO_3-$HClO_4$ (ref. 40). The procedure for wet ashing of urine involves the use of HNO_3-H_2O_2 (ref. 21) mixtures. PTFE pressure bombs have considerable advantages reducing the time for mineralization and avoiding airborne contamination (ref. 21,40). The necessity for cleaning all vessels in acid cannot be overemphasized. The vessels used by Ericson et al. (ref. 39) for reagent and sample preparation were cleaned by soaking for at least 2 h in a solution of NRS-250 detergent, rinsing with distilled water and soaking again for at least 2 h in a polyethylene bin containing a 10% v/v solution of reagent grade nitric acid in distilled water. Then they were thoroughly rinsed with demineralized distilled water (DDW), dried face down on rubber mating and stored in plastic bags. A final DDW rinse was performed immediately before use. The Teflon digestion vessels were cleaned by refluxing for at least 1 h with 1 ml of concd. Ultrex nitric acid, followed by a thorough DDW rinse. The Teflon caps for the vessels were cleaned by soaking overnight in a 10% v/v solution of Ultrex nitric acid in DDW and thoroughly rinsed with DDW.

The whole procedure for sample preparation and Mo preconcentration should always be carefully evaluated with respect to various sources of contamination such as reagents, glassware used and airborne particles and it is mandatory to include blank experiments in parallel.

ANALYTICAL TECHNIQUES FOR Mo DETERMINATION: GENERAL

As for all analyses, the determination of Mo in biological materials should be subject to careful statistical evaluation (ref. 46,47). Wherever possible Mo should be determined in duplicate by at least two independent analytical techniques. Analysts should use Certified Reference Materials (CRMs) for procedure validation (ref. 48). However, in the case of Mo serious difficulties arise as the most suitable method for its determination at the sub-ng/g levels is neutron activation analysis (ref. 49) which is still inaccessible to many laboratories and also the number of biological CRMs with a certified Mo content remains small (ref. 42). Moreover, most of these materials have a certified Mo content at the μg/g level, relevant to the analysis of plants: NBS SRM 1571 orchard leaves (0.3 μg/g), NBS SRM 1572 citrus leaves (0.17 μg/g), Bowen's kale (2.3 μg/g) or animal tissue: NBS SRM 1577a bovine liver (3.5 μg/g). To date, there is no CRM available for Mo in human serum or blood. The recently introduced NBS SRM 8419 bovine serum (12 ng/ml) has only partially filled the lack of a CRM for this matrix as the Mo concentration in human serum is an order of magnitude lower that in animal serum. At present, trace elements (including Mo) are being determined in the new NBS SRM 909 human serum (ref. 38) but until it appears on the market, workers determining the Mo content in human blood and serum must institute a rigorous regime of analytical quality control and check that they are consistent with currently accepted Mo levels in human body fluids.

General criteria for the selection of a method suitable for trace analysis are: sensitivity, accuracy, precision, speed, minimum sample pretreatment as well as cost and availability. Many analytical methods meet relevant criteria for the determination of Mo at the μg/g level in plants, most tissue samples and foodstuffs. They include a variety of techniques such as spectrophotometry, polarography, voltammetry, emission spectrography, X-ray fluorescence as well as flame atomic absorption spectrometry but they usually require a Mo separation and preconcentration step. The most widely used spectrophotometric methods were recently reviewed by Schwedt and Dunemann (ref. 23) and applied to the determination of Mo in urine (80-96 ng/ml), wine (5.5-5.9 ng/ml) and plants (2.7-3.0 μg/g). Applications of other of the above methods can be found in the publication of Parker (ref. 50) and references therein.

The most suitable techniques for the determination of Mo in biological samples at the ng/g level include electrothermal atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry and neutron activation analysis. They are discussed in detail below. However, it can be expected that the growing popularity of inductively coupled plasma mass spectrometry (ICP-MS), simultaneous multielement atomic absorption spectrometry (ref. 51,52), epithermal neutron activation analysis (ref. 53), proton induced X-ray excitation spectrometry (ref. 54,55), direct current plasma atomic emision spectrometry (ref. 56), spark-source mass spectrometry (ref. 57) and catalytic methods (ref. 58,59), will generate some alternative approaches in near future.

ELECTROTHERMAL ATOMIZATION ATOMIC ABSORPTION SPECTROMETRY (ETA AAS)

Very low level determination of Mo by flame AAS is impossible because of the inability of the flame to provide suitable reducing conditions for efficient atomization. Typical detection limits attained in a N_2O-C_2H_2 flame, 0.2-0.5 mg/l (ref. 60,61), are not sufficiently low for the Mo determination at the ng/g level in biological samples. Electrothermal atomization is therefore necessary. The sensitivity of the technique depends on the heating rate (ref. 39) and hence, atomization from a L'vov platform is less efficient (due to a lower heating rate) than the direct atomization from the tube wall (ref. 62). Tubes newly coated with pyrolytic graphite are usually recommended but some workers prefer the uncoated graphite tubes and find them better for the determination of Mo in NaCl containing matrices (ref. 63). The detection limits reached using commercial graphite furnaces are reported to be 6-10 pg of Mo (ref. 60,62,64).

A big problem in the determination of Mo by ETA AAS is carbide formation (ref. 60,63,65). Carbides formed on the tube surface enhance memory effects resulting in increased and variable blanks and loss of sensitivity. Also carbon deposits from undigested biological samples may magnify the above effects even further (ref. 60). An inconvenience in the use of pyrolytically coated graphite tubes is their relatively short lifetime (40-300 firings (ref. 38,39,60)) leading to a progressive decrease in sensitivity and this slow decrease of the Mo signal during the tube lifetime should be corrected for by a periodic recalibration (ref. 60).

The application of ETA AAS to the direct determination of Mo is hampered by the susceptibility of this technique to matrix interferences. The most common of these are Mo signal suppresion by sodium and calcium chlorides, ammonium salts as well as by phosphoric and hydrochloric acids (ref. 66) and Mo signal enhancement due to volatile organomolybdenum compounds and carbonyls (ref. 67). The above effects can be avoided by sample digestion and separation of Mo from the acid digest by extraction of its complex with a chelating agent, e.g., pyrrolidinyldithioformate (ref. 64), benzohydroxamic acid (ref. 39,68) and toluene-3,4-dithiol (ref. 56), or by the use of various matrix modifiers such as ascorbic acid (ref. 69), $Mg(NO_3)_2$ (ref. 51), NaF (ref. 38), $(NH_4)_3PO_4$ and EDTA (ref. 66). Extraction procedures are lengthy, they require the use of additional reagents and are therefore prone to contamination.

Another means of overcoming matrix interferences is selective thermal removal of the matrix before the atomization step. An atomization temperature of 1860 °C at which the NaCl matrix seems to have been totally volatilized was reported (ref. 64). Complete destruction of organic compounds and selective volatilization of any matrix present were obtained by following temperature program involving drying at 100 °C, carbonization at 600 °C, charring at 1700 °C and atomization at 2700 °C (ref. 70). This procedure, which was applied to the analysis of mineral water, also seems to be suitable for urine analysis. The method developed by Ericson et al. (ref. 38) for the direct determination of Mo in human serum makes use of a seven step graphite furnace temperature program and of effective background correction.

Some matrix effects can be also corrected for by measuring the peak area and not the peak height. The peak height may vary with the matrix composition since the analyte vaporisation rate is controlled by the matrix whereas the integrated absorbance remains constant (ref. 60). On the other hand, the peak height method facilitates observation of lower detection limits (ref. 62) and determinations near the blank level are more reliable because they are less affected by the base line alteration (ref. 71). A better sensitivity obtained by the peak height method should be matched against better precision with the peak area measurement. Background correction is often necessary due to non atomic absorption arising from the formation of molecular compounds during the ashing and atomisation steps. Most workers report the use of D_2-background correction (ref. 64) but the Zeeman method appears to lead to a better sensitivity (ref. 14,38,39). ETA AAS has been used succesfully for determining Mo in human milk, milk powder (ref. 14,27), plants (ref. 56,60,66, 71,72), rat organs (ref. 68), bovine and human serum (ref. 38,39).

Recently, O'Haver (ref. 73) developed a new atomic absorption spectrometer which applies a continuum source. When coupled to a carbon furnace atomizer, it enables a multielement determination of trace elements (incl. Mo) in biological samples. Microliter samples can be used. Atomization at 2700 °C, a fast heating rate, the use of matrix modifiers such as $Mg(NO_3)_2$ (ref. 51) or HNO_3 (ref. 52) and efficient background correction allowed the direct Mo determination in bovine serum. However, difficulties were encountered when this method was applied to human serum because the blanks were very high and variable (3-14 ng/ml) and the detection limit (ca 2 ng/ml) was larger than the Mo level in human serum (ref. 51) by a factor of about 3. Although the sensitivity of this technique for Mo is still not very high and the instrument is not commercially available, its many advantages such as direct, fast multielement determination promise well for the future.

ETA AAS is the most readily available technique for the determination of Mo at the ng/g level in biological samples and recent reports (ref. 38,39) on its application to the determination of Mo in human serum suggest that the time has come for the introduction of ETA AAS into routine determinations of Mo in hospitals.

INDUCTIVELY COUPLED PLASMA EMISSION SPECTROMETRY (ICP AES)

Although ICP AES was widely used for the determination of many metals in biological materials in the late seventies, Mo seems to have been excluded from the multielement array of simultaneous ICP instruments. The main reasons are probably the lack of sensitivity and spectral interferences occuring in ICP AES determination of Mo. Typical detection limits quoted, 8-15 ng/ml (ref. 74,75) are rather high in comparison with other metals and the sensitivity is often reduced even further by a dilution factor arising from sample pretreatment and/or dilution to minimize transport and nebulization effects. When determining Mo in human or bovine serum in particular but also in plants and animal tissues preliminary preconcentration of Mo is necessary in order to ensure reasonable accuracy.

The application of ICP AES to the determination of Mo in biological materials is also hampered by matrix effects (ref. 74,75). Although this technique is relatively free from many chemical interferences often encountered in AAS, spectral effects due to the presence of Ca, Mg, Fe and Al are much more common and occur at all intensive Mo lines (ref. 76). Moreover, alkali and alkaline earth metals at the level present in biological materials adversely affect nebulization efficiency and cause stray light and background effects (ref. 77). To avoid the above effects calibration with a matrix matched solution and/or the separation of Mo from the matrix are necessary. The most commonly used method for this purpose is to complex Mo on a [ref. poly(dithioformate)] chelating resin (ref. 21,40,75,77,78) and follow with elution or (more often) ashing of the resin. Other techniques such as extraction of the Mo complexes with 8-hydroxyquinoline have also been reported (ref. 76).

Background correction is important for the determination of Mo in biological materials. As the variation in background for different samples is considerable, measurements made without adequate background correction lead to incorrect results (ref. 74). There are two principal methods of background correction in ICP AES (the peak height and the peak area methods), each having advantages and disadvantages which should be carefully evaluated before selection. Generally, for biological samples the peak height method with its shorter measuring time, low sample consumption and moderate reproducibility is preferred to the peak area method with its longer measuring time, higher sample consumption though better precision (ref. 74).

ICP AES has so far found application to the determination of Mo in urine (ref. 21,74), bone (ref. 75,78), animal diets and faeces (ref. 79), plants (ref. 45,76,77,80), urinari calculi (ref. 26), animal tissues (ref. 40,45,77), milk powder (ref. 74) *and serum (ref. 40).*

Recently, a technique involving the use of graphite rod electrothermal vaporisation for sample introduction into an ICP discharge has been developed (ref. 81). The use of the system allows the removal of the organic part of the matrix in the preliminary ashing step but unfortunately the major alkali and alkaline earth metal salts remain resulting in Mo signal enhancement. Separation of Mo is necessary to avoid spectral interferences. A procedure for the determination of Mo in biological samples in a multielement array has been developed but application to real sample analysis has not been reported (ref. 81). The detection limits claimed: 70 pg (peak height method) and 160 pg (peak area method) are considerably lower than in classical ICP AES and permit the use of microvolume samples. Argon-halocarbon mixtures are required for Mo to obtain a high sensitivity but addition of a halocarbon introduces band emission

similar to that observed with organic solvents. However, the electrode lifetime is long and 300-400 analyses can be performed with *in situ* pyrolysis.

ICP AES will be applied to the analysis of human fluids without pretreatment with greater success if more sensitive nebulization systems are developed and spectral interferences from the matrix are avoided. Some methods such as ultrasonic nebulizers, heated spray chambers, hydraulic high pressure nebulizers (ref. 82) and pneumatic crossflow nebulizers working at 80 °C (ref. 83) have been proposed to improve the detection limit.

NEUTRON ACTIVATION ANALYSIS (NAA)

Neutron activation analysis has played an important role in establishing the true Mo level in human serum and is recommended by the National Bureau of Standards for the determination of Mo in biological CRMs (ref. 84). All values published up to 1980 for the concentration of Mo in human serum which are still considered to be reliable were obtained by means of NAA (ref. 49).

The procedures involving NAA are less susceptible to contamination as the sample dissolution and chemical separations are carried out after activation. However, although generally NAA is said to be a blank free technique, special precautions should be taken to avoid contamination during all sample pretreatment steps such as lyophilization or ashing prior to irradiation. A frequent source of error is the Mo contribution from the wall of the quartz irradiation vials. Typical blanks found for Vitrosil and Spectrosil quartz were: 1.0 ± 0.6 and 0.4 ± 0.2 ng, respectively (ref. 43), and these are significant taking into account the Mo level in human serum. Spectrosil quartz vials, bought in large batches from the supplier are recommended. As the Mo content in polyethylene is generally lower (ref. 85) than in quartz, the use of polyethene bags is advised for the irradiation (ref. 86).

Reactor irradiation produces the radio-isotope ^{99}Mo (by the reaction: ^{98}Mo (n,γ) ^{99}Mo) which has a half-life of 67 h and decays through β-emission to a radioactive daughter isotope ^{99m}Tc with a half-life of 6 h. Instrumental NAA is based on the spectrometric measurement of the 140.5 keV photopeak of ^{99m}Tc. The measurements should be carried out after at least 30 h following irradiation when equilibrium between ^{99}Mo and ^{99m}Tc is established and both activities decay with the parent's half life. The samples are usually irradiated for 16-100 h in a thermal neutron flux of about 10^{12}-10^{13} cm^{-2}s^{-1}. The irradiation time is limited by pressure build-up in the sealed quartz vials and explosion risk caused by radiation damage of biological samples. Samples should be lyophilized or ashed (ref. 36) prior to activation in order to reduce this increase in pressure. After the irradiation but before opening the vials are usually cooled in liquid nitrogen to minimize the loss of volatile irradiated material.

Direct measurement of low levels of Mo in biological samples by instrumental NAA is difficult. The strong Compton continuum of high energy γ-rays ^{24}Na and ^{42}K and *bremsstrahlung* due to high energy β –radiation of ^{32}P (always present in body fluids) render it difficult to measure the activity of the ^{99m}Tc γ-photopeak. Moreover, as biological samples, especially blood, have a relatively high iron content, spectral interferences from the 142.5 keV γ-ray photopeak of ^{59}Fe may create a problem for Mo determination when the ratio Fe:Mo exceeds 1000 (ref. 17). For the above reasons very low Mo concentrations can be determined only after a radiochemical separation of Mo involving extraction of Mo complexes with chloride (ref. 11), pyrrolidine-DTC (ref. 17), DDTC (ref. 17,29), BPHA (ref. 16,18), 8-hydroxyquinoline (ref. 31), substoichiometric extraction with tetraphenylarsonium ion using rhenium(VII) as carrier (ref. 87), separation of Mo on ion-exchange (ref. 25,36,88,89) or MnO_2aq (ref. 86,90) columns and distillation of ^{99m}Tc, daughter isotope of ^{99}Mo (ref. 37).

Molybdenum has been determined by NAA in a variety of samples such as blood and serum (ref. 22,25,34,35,92-94), human lung (ref. 25,95), nails (ref. 25), brain (ref. 29), liver (ref. 30), urine (ref. 22,24,25), milk powder and human milk (ref. 11-13,16,18,86,87), bovine liver (ref. 11,16,18,29,86), plant material (ref. 11,17,18,88,90,91) and foodstuffs (ref. 16,17,29,36,87).

NAA is an excellent reference technique to check on the accuracy of other methods and to establish the Mo content in CRMs. However, it is not appropriate for routine analysis as it is time consuming and requires instrumentation which is not widely available.

Recently, a new technique of instrumental NAA using epithermal neutrons for trace Mo content has been reported (ref. 53). Its main advantage over the classical NAA is that interfering elements are activated to a lesser extent thus rendering a post-irradiation separation unnecessary and therefore allowing a rapid

instrumental determination with better accuracy. The possibility of reducing analysis time is interesting especially for busy routine hospital laboratories where it is necessary to analyse a large number of samples and to obtain accurate and reliable results. However, some technical problems arising from the necessity for neutron filters and lower neutron flux as well as high cost of the instrumentation reduce the scope of its potential applications.

CONCLUSIONS

The results of Versieck et al. (ref. 32,33) obtained using techniques with a stringent contamination control are in agreement with those of other workers who claim the normal Mo serum level to be 0.5-0.6 ng/ml. However, many reports which have appeared since that time would suggest a higher value. Although the value of 0.8 ng/ml reported by Ericson et al. (ref. 38,39) is within the tolerance limits, serious doubt must be placed on other considerably higher values. The probable reason for this is that some workers still analyze samples without knowing their history because it rarely hapens that the analyst himself is involved in the sample collection. It is essential to make hospital staff who are responsible for sampling aware of the stringest requirements of sample collection.

The currently accepted values of Mo levels in serum and the whole blood are similar. Does this mean that this element accumulates only in serum and not in red cells? This question remains to be elucidated.

ETA AAS and ICP AES are expected to be widely used in the near future for the routine hospital laboratory determination of Mo in urine but there remains a need for a method of appropriate sensitivity and simplicity for use in the determination of Mo in blood and serum. Supplementation of diets with trace elements is of growing popularity and AAS instruments with multi-step furnace temperature programs, Zeeman background correctors and autosamplers are required in the hospital laboratories for the monitoring required for Mo control. It must be emphasized, however, that the use of sophisticated analytical techniques will not exempt the analyst from rigorous contamination control, careful calibration and related measures required to maintain a high standard of laboratory practice. Experience and laboratory skill of the analyst are also of primary importance.

In order to improve the state of the art and to assure a good level of quality assurance CRMs for biological materials at the ng/g level are urgently needed. The issue of a CRM for human serum with a certified Mo content would be of enormous value. Also interlaboratory surveys of the Mo determination in human blood and serum should be undertaken.

Studies on the form of the molybdenum species in human fluids would also be of interest but with the current difficulties in the determination of total molybdenum, studies on its speciation appear to be very distant.

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