Pure & Appl. Chem., Vol. 58, No. 12, pp. 1707–1720, 1986.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON MICROCHEMICAL TECHNIQUES AND TRACE ANALYSIS*

DETERMINATION OF CHROMIUM IN BIOLOGICAL MATERIALS†

Prepared for publication by

J. M. OTTAWAY[1] and G. S. FELL[2]

[1]Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, G1 1XL, UK
[2]Trace Metals Unit, Biochemistry Department, Glasgow Royal Infirmary, Glasgow, G4 0SF, UK

*Membership of the Commission during the period (1983–85) when this report was prepared was as follows:

Chairman: B. Griepink (Netherlands); *Secretary*: A. Townshend (UK); *Titular Members*: K. Biemann (USA); E. Jackwerth (FRG); A. Lamotte (France); Z. Marczenko (Poland); Yu. A. Zolotov (USSR); *Associate Members*: K. Ballschmitter (FRG); K. Beyermann (FRG); R. Dams (Belgium); K. Fuwa (Japan); M. Grasserbauer (Austria); W. Gries (S. Africa); K. Heinrich (USA); A. Mizuike (Japan); J. M. Ottaway (UK); G. Tölg (FRG); D. E. Wells (UK); D. G. Westmoreland (USA); *National Representatives*: R. Gijbels (Belgium); H. Thoma (Brazil); J. Janák (Czechoslovakia); M. J.-F. Leroy (France); Z. Horváth (Hungary); M. Sankar Das (India); R. C. H. Hsia (Malaysia); A. D. Campbell (New Zealand); B. Salbu (Norway); W. Kemula (Poland); A. Cedergren (Sweden); E. A. Terent'eva (USSR).

†Series title: Critical Evaluation of Analytical Methods for Determination of Trace Elements in Various Matrices

Determination of chromium in biological materials

Summary - The determination of chromium in biological materials is of considerable importance because it has been shown to be essential by animal experiments and has potential importance in human medicine. At higher levels it is also toxic and symptoms of chromium toxicity are well established. Numerous errors have appeared in earlier analytical measurements of chromium because analysts have paid too little attention to potential sources of contamination, and to the stringent requirements of particular instrumental measurements. As a result of recent studies, a clearer understanding of the nutritional requirements for chromium, and of the levels of chromium in the serum and urine of normal subjects is available. Recent developments in technology have provided analytical techniques with sufficient sensitivity for both these determinations, although serum chromium at the lowest levels is still a difficult determination for routine hospital laboratories without specialised facilities. Recent developments in analytical techniques are reviewed in the light of clinical requirements both now and in the foreseeable future.

CLINICAL BACKGROUND

Chromium in common with other biologically active metals can pose a toxic hazard at high dosage or exposure, but when deficient in diet it can be associated with reversible biochemical and clinical effects.

The toxic nature of chromium(VI) is well established and industrial workers exposed to dichromates or chromic acid can suffer skin lesions, lung disease, and an increased incidence of various forms of cancer (ref. 1). Improved industrial hygiene can reduce these problems, and monitoring of absorbed water soluble Cr is possible by measuring urinary Cr output (the main pathway of Cr excretion). When respired air contains more than 0.05 mgCr m^{-3} (the threshhold limiting value) then urinary chromium can exceed 30 µg Cr(g creatinine)$^{-1}$ (approx. 30 µg l^{-1}) (ref. 2). Measurement of chromium at this level presents few serious analytical problems, although the proportions of chromium(VI) and chromium(III) in various materials are of considerable interest, and the nature of the carcinogenic action of chromium is still unknown.

The greatest challenge to the analytical scientist lies in the study of chromium as an essential nutrient for animals and man. It is now over 25 years since Mertz and coworkers (refs. 3-5) showed that brewers' yeast was able to reverse the glucose intolerance found in rats fed certain stock diets, and that the active principle in the yeast contained chromium(III). Current knowledge of the nutritional role of chromium has been reviewed in excellent recent articles by Anderson in 1981 and 1983 (refs. 6,7). It is now known that an organo-Cr complex of uncertain structure is synthesised in tissue from absorbed chromium(III), and that this complex potentiates the actions of insulin. Deficiency of this complex either through absolute dietary lack of chromium(III) or reduced ability to synthesise the specific complex results in glucose intolerance and reduced efficiency of insulin, affecting carbohydrate, amino acid and lipid biochemistry. Symptomatic chromium(III) deficiency has been clearly demonstrated in man during prolonged intravenous feeding when purified nutrients (amino acids and carbohydrates) do not contain sufficient chromium(III) to meet requirements. In two studies, supplementation of the intravenous fluid with 250 µg Cr day^{-1} (ref. 8) or intravenous supplementation with 150 µg Cr day^{-1} (ref. 9) reversed severe glucose intolerance, improved nitrogen balance and weight gain, and reduced signs of peripheral neuropathy.

Studies of the metabolism of chromium in man have been seriously hampered by the lack of satisfactory analytical procedures for the determination of chromium(III) in diet, tissues and body fluids. Indeed much erroneous data has been published, and ambiguous conclusions

drawn on such data, due to the use of inadequate or inappropriate methods (refs. 7,10). A favourable clinical and biochemical response to chromium supplementation has not been consistently demonstrated in diabetic patients. Improvements in glucose tolerance and changes in lipoprotein biochemistry have been reported but these biochemical markers are subject to significant variations quite independently of chromium status. The chromium concentrations observed in blood, serum and urine of diabetics and other categories of patients vary enormously (ref. 10) making the prior selection of a chromium deficient group impossible. Many workers have relied upon the differences between chromium concentrations in various patient groups or after some treatment such as glucose loading or chromium supplementation (ref. 11), but the fact that the absolute values of the chromium determinations are often in serious doubt destroys confidence in the clinical conclusions (refs. 10,12). Only recent awareness of the limitations and problems associated with analytical techniques for the determination of chromium, has allowed rigorous studies of the normal levels of chromium, and the effects of both chromium supplementation and glucose loads to be established (ref. 13).

An accurate estimate of normal urinary chromium output is also of importance in assessing dietary chromium requirement. Assuming an intestinal absorption efficiency of around 1% (established by radiotracer studies) (ref. 14), then an average urinary output of 1 μg Cr day^{-1}, suggests a dietary requirement of between 50-200 μg Cr day^{-1}, since urinary excretion is the main route of chromium loss (ref. 15). It appears that many western diets based on refined carbohydrate and other processed foodstuffs low in chromium may give rise to marginal deficiency in large segments of the population. The risk of inadequate chromium intake may be of particular significance for pregnant and lactating women, since the concentration of chromium is highest in the organs of the newborn and declines rapidly during the first few years of life (ref. 16). The developing foetus may therefore deplete maternal tissue chromium reserves unless the dietary chromium intake is adequate. One study (ref. 16) showed that the average dietary chromium intake was only about 29 μg Cr day^{-1}, which was much lower than that for any other world population studied at that time. These results have recently been amply confirmed in a U.S. study by Anderson and Kozlovsky (ref. 17) whose paper also includes data from a number of other studies. The average daily intake of chromium for 10 males over a 7 day period was 33 μg with a range from 22-48 μg, whilst for 22 females studied at the same time, a mean of 25 μg and a range of 13-36 μg was reported (ref. 17). Chromium absorption was found to be inversely related to dietary intake, absorption at a dietary intake of 10 μg day^{-1} was as high as 2%, but at an intake of 40 μg day^{-1} this was decreased to 0.5%. More than 90% of the diets tested were below the minimum suggested safe and adequate daily intake of 50 μg Cr (ref. 17).

It seems clear that much of the published clinical work on chromium requires, at the very least, confirmation using a truly specific and accurate analytical technique for total chromium. Even this may not be sufficient to reach firm clinical conclusions in all cases. An improved bioassay for the organo-Cr complex or Glucose Tolerance Factor (GTF) has been proposed (ref. 18) and may give more relevant information. Although chromium is required physiologically as special organo-Cr complexes, it now seems clear that normal individuals do not need to ingest biologically active chromium directly from foodstuffs (ref. 6), as inorganic chromium can be converted to a useable form in vivo. The use of accurate procedures for total chromium determinations therefore remains of fundamental importance to the clinical biochemist.

It is essential that analytical chemists working on this problem should provide methods which are sufficiently sensitive, which give accurate results, and that proper methods of validation are made available and are used whenever low level chromium determinations (<10 μg l^{-1}) are carried out. Where such ideal facilities are not available, the analyst should ensure that his results are at least consistent with currently accepted levels of chromium in body fluids and tissues. One of the more important attributes required in a good analytical chemist is scepticism, and this should be applied to his own results first, particularly if they are not consistent with other literature. The purpose of this paper is to review current knowledge of the levels of chromium in the more important biological materials, to assess probable sources of error which have led and still lead to the publication of erroneous data and to discuss analytical techniques used for this type of chromium analysis. In this latter section, the limitations of some procedures are described and the more satisfactory approaches are highlighted.

CHROMIUM CONCENTRATIONS OF INTEREST

Analysis of the higher levels of chromium found in problems of toxicity rarely presents great difficulties, but the determination of levels of chromium in samples from normal subjects, and in deficiency states is much more difficult. Values published over the past 25-30 years show a wide variation between 0.14 and 185 μg l^{-1} of chromium in blood serum for example (ref. 12). The wider availability of electrothermal atomic absorption instruments in the late seventies and eighties has meant that chromium determinations at these levels are within the reach of many more biochemical laboratories. Unfortunately, the more recent establishment of the fact that chromium concentrations in blood serum and urine are

well below 1 μg l^{-1} in normal subjects is still not fully appreciated by all workers in this field, and is sometimes even misinterpreted in standard texts (ref. 19). The sharp differences between currently accepted values for chromium in the two principal biological materials and most of the older data, means that many earlier analytical studies and procedures must be ignored or considered inadequate, and many of their conclusions challenged or reinvestigated.

The literature of measured values for blood serum and urine has been reviewed elsewhere (refs. 10,12), and although there remains scope for geographical variations, it seems clear that chromium values in normal subjects in both cases lie below 1 μg l^{-1}. Five studies (refs. 20-24) have now produced values for the mean chromium concentrations in blood serum of normal subjects in the range 0.075-0.16 μg l^{-1}. Both neutron activation analysis and electrothermal atomic absorption spectrometric methods were used in these studies. It should be noted that chromium levels in individuals show considerable variations (ref. 20) and are likely to reflect recent intake. In most cases it is not yet clear whether this represents a real difference in serum chromium between one subject and another or is caused by random errors in the analytical procedure (ref. 25). However, a remarkable agreement in mean values is indicated by the five studies based on variable population sizes and different analytical techniques. Three examples will serve to illustrate these features. In 1978, Versieck et al. (ref. 20) obtained a range of normal human serum values for 20 subjects between 0.0382 and 0.351 μg l^{-1} with a mean value of 0.160 μg l^{-1}. Neutron activation analysis was the technique used. More recently Kumpulainen et al. (ref. 23) used electrothermal atomic absorption spectrometry and obtained very similar results with a range from 0.08 to 0.20 μg l^{-1} (6 subjects), and a mean value of 0.12 μg l^{-1}. In addition, Veillon et al. (ref. 24) also using electrothermal atomic absorption reported a normal mean value of serum chromium of 0.11 μg l^{-1} for 15 healthy adults, in close agreement with the earlier data. Some doubt must be placed on the value of 0.075 $\pm$ 0.069 μg l^{-1} reported for 14 normal subjects by Vanderlinde et al. (ref. 22). This value was below the detection limit of the electrothermal atomic absorption method used, and was only derived by subtraction of a blank value which falls above the detection limit.

The level of chromium in the urine of normal healthy subjects varies between 0.2 and 1.0 μg l^{-1} (ref. 10). Thus Vanderlinde et al. (ref. 22) obtained a recalculated value (ref. 10) of 0.56 $\pm$ 0.43 μg l^{-1} for 14 healthy subjects and several other groups have reported values in a similar range (refs. 11, 23,26). Clinical studies indicate that chromium output increases in for example insulin dependent patients (ref. 22), or after strenuous exercise such as a 6 mile run (refs.27,28), and in a number of other conditions (ref. 10). Since urinary output itself is variable, chromium concentrations in urine will undoubtedly exhibit a wide concentration range.

In their recent lecture paper (ref. 10) Cornelis and Wallaeys concluded that normal serum levels were in the range 0.1-0.2 μg l^{-1}, and that normal urine values were below 1 μg l^{-1} with a published range of 0.2 to 1 μg l^{-1}, and a daily excretion of 0.3 to 2.0 μg. Analysis of the current literature supports these conclusions, and suggests that data which does not conform to these criteria must be subject to considerable doubt, which also applies to any conclusions drawn from such data. Much of the published literature prior to 1978 which relies on chromium determinations suffers from this deficiency. These figures also provide a benchmark for the development and evaluation of new analytical techniques and procedures, and should be used by analytical chemists in the absence of a wide range of adequate standard reference materials. It is to be hoped that the recent introduction by the U.S. National Bureau of Standards of RM 8419 bovine serum (ref. 29), with a recommended chromium content of 0.30 $\pm$ 0.05 μg l^{-1} will provide laboratories with an important new method for the self validation of analytical results and procedures.

CAUSES OF ANALYTICAL ERROR

One cause of low results which has been put forward persistently over the years, is loss of chromium by volatilization during sample drying and/or dry ashing. Such a hypothesis would suggest that the normal values indicated above are too low. A review of the extensive literature on this subject (ref. 12) confirms that this argument can not be sustained. Several careful studies (in for example refs. 25,30-33) have shown that losses by volatilization during dry ashing at temperatures as high as 450°C are insignificant. All the other sources of analytical error relevant to the determination of chromium will produce high results and provide a pattern which is entirely consistent with the literature. As scientists have become more aware of the problems associated with trace analysis of metals, so the reported concentration of chromium in biological samples has fallen (ref. 12). Improvements in sensitivity of analytical techniques have also contributed to this trend. Amongst the more easily identified sources of error are the following:

Sampling and storage prior to analysis

Since the total levels of chromium to be determined in blood serum, urine or other materials are at the $\mu g\ l^{-1}$ level or below, accidental contamination prior to the actual analytical measurement will invalidate the result. Such problems are much less noticeable for elements such as copper or zinc which are present at around the $\mu g\ ml^{-1}$ level, but for manganese, nickel, chromium, aluminium, etc. where the normal levels are around 1 $\mu g\ l^{-1}$ or below, contamination during sampling and storage represents probably the greatest hazard to an accurate analysis (refs. 34,35).

Disposable steel needles used widely in medical practice for the taking of blood samples, can not be used for sampling blood intended for trace metal analysis of elements such as chromium, manganese and nickel (refs. 25,35,36). It has been clearly shown by sampling blood with a radioactivated needle that a substantial transfer of chromium (as ^{51}Cr) takes place from the needle. Four 20 ml aliquots of blood were drawn through a stainless steel needle and additions of 89.9, 12.7, 10.4 and 15.9 $\mu g\ l^{-1}$ chromium were found in the samples. Since these levels are between about 100 and 1000 times the level of chromium in the sample, they are clearly useless for analysis. Similar contamination levels have been found from the use of stainless steel surgical blades for the preparation of tissue biopsies (ref. 35). It is essential that hospital staff responsible for sampling are fully aware of the different requirements necessary for sampling for trace metal analysis compared to other hospital procedures. A polypropylene over-the-needle catheter or an all plastic cannula should be used and the initial portion of the sample drawn should be rejected or used for other analyses (see below for typical procedures).

The vessels used to separate and store blood samples and to collect urine samples are a further source of contamination. Stabilisers used in plastics, anticoagulants used to obtain plasma and any preservatives or acids used to stabilise urine samples need careful evaluation. The increasing usage of disposable compressed paper urine bottles for bed-ridden patients has been found to seriously contaminate urine with metals (Fe, Mn, Zn and Cr) released from the scrap paper used in manufacture (ref. 37). The stoppers of containers often add to trace metal contents of samples kept in them (ref. 35), although this problem is generally more noticeable for elements such as zinc and manganese.

It is quite clear that sample collection and storage represents the most important part of an analytical procedure for chromium in biological samples. Some typical procedures adopted are:

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 chromium were collected after withdrawal of approximately 50 ml of blood for routine clinical laboratory investigations."

Procedure 2. Intended for routine hospital use for serum chromium concentrations above 0.5 $\mu g\ l^{-1}$ (ref. 38).

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.

Procedure 3. Intended for electrothermal atomic absorption spectrometric determination of serum chromium at normal levels (ref. 24).

Serum samples (1-2 ml) were collected in all plastic syringes (Safety-Monovette, Sarstedt, Princeton, NJ, USA) using needles of the "butterfly" type, with a short, siliconized needle attached to a length of small bore poly(vinyl)chloride tubing (Minicath, Deseret Medical, Sandy, UT, USA). Samples were ashed in specially fabricated quartz test tubes (10 mm diameter, 100 mm length). The tubing was cleaned in 3%(V/V) hydrofluoric acid and water before fabrication and similarly afterwards. Before use, the tubes were immersed in 10%(V/V) nitric acid at 100 °C overnight, and soaked in the same acid at room temperature for one week. The tubes are siliconized by filling with 5%(V/V) dichlorodimethylsilane in toluene and rinsed with methanol (once) and water (six times). Samples were then weighed into the tubes, with

10 µl of 0.186 g ml^{-1} solution of $Mg(NO_3)_2 6H_2O$ per ml of serum taken, and then frozen at -20°C prior to lyophilisation. All operations were carried out in a class 100 clean room and lyophilization was carried out in an apparatus of special design which was free of stainless steel. Samples are lyophilized overnight, and then ashed at increasing temperatures up to a limit of 480 °C in a muffle furnace with a chamber made of light-weight ceramic material with completely embedded heating elements. The ash was dissolved in 1 ml of 0.1M hydrochloric acid, and 25 µl aliquots of this solution used directly in the graphite furnace.

Procedure 4. Intended for routine hospital use for urine (ref. 39).

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

These procedures have all been used successfully, although Procedure 2 has not been used for the determination of serum chromium at the very lowest levels (< 0.5 µg l^{-1}) as available analytical instrumentation has not permitted this. The importance of acid cleaning all containers can not be overemphasised. Alternative samplers or containers should always be fully evaluated before use for this type of analysis.

Sample preparation for analysis

The problems of contamination obviously extend to any sample pretreatment procedures. Adoption of analytical techniques which are sufficiently sensitive and free of interferences from the matrix of biological samples, thus obviating the need for complex sample preparation steps, is the obvious approach for a busy routine hospital laboratory. The increasing use of electrothermal atomic absorption instruments has done much to make this a reality. If sample pretreatment is necessary or considered appropriate, then great care must be taken to ensure that chromium from the reagents or containers used, or indeed from the environment does not contaminate the sample and invalidate the results. Destruction of the organic matrix by wet or dry ashing methods or solvent extraction/chelation methods of preconcentration are all liable to cause contamination and the reagents and apparatus employed should be carefully evaluated before use. Where such operations are used, they should be carried out under the best "clean room" conditions.

As a result of differences in the matrix and chromium concentrations, the requirements for sample preparation are much greater for serum chromium analysis than for the determination of chromium in urine. Several groups of workers (refs. 23,26,39-42) have now demonstrated that urinary chromium analysis is feasible using direct injection of samples into a graphite furnace for atomic absorption spectrometry. Thus sample pretreatment can be completely avoided and provided precautions are taken during sampling and storage, results of acceptable accuracy may be obtained in many routine hospital laboratories. Since urinary excretion provides a suitable means of monitoring chromium ingestion, electrothermal atomisation atomic absorption spectrometry (ETA AAS) has become extremely useful to the clinical toxicologist.

The determination of serum chromium is much less satisfactory. Not only does the sample matrix demand sample pretreatment before most instrumental measurements can be made, but the lower concentration of chromium in serum means that reagent and apparatus blanks are more significant, and the possibility of random contamination during pretreatment steps is also likely to impair accuracy. Versieck et al. (ref. 25) found that serum samples required dry ashing before irradiation for neutron activation analysis. Blank values were quoted to vary between 0.0262 and 0.0704 µg l^{-1} (mean of four determinations 0.0478 µg l^{-1}) and will obviously have a significant influence on serum chromium concentrations reported, after mean blank subtraction, to be in the range 0.0381 to 0.351 µg l^{-1}. In neutron activation analysis such blank levels will depend critically on the material of the vessels used and their pretreatment (refs. 43,44). Vanderlinde et al. (ref. 22) found it necessary to employ wet oxidation of serum samples with nitric acid and hydrogen peroxide for electrothermal atomic absorption spectrometry. As mentioned earlier, only the contribution of the blank in their method raised the chromium measurement above the detection limit.

Silanization of quartz tubes used for dry-ashing of serum samples prior to electrothermal atomic absorption measurement was found to considerably improve the recovery of ^{51}Cr added to rat serum (ref. 24). Addition of magnesium nitrate as a matrix modifier also aided recovery of chromium (see Procedure 3 earlier). Many authors have been concerned about possible losses of chromium during wet ashing procedures, particularly when perchloric acid is used. Shapcott (ref. 45) however showed, using additions of $^{51}CrCl_3$ to serum, hair and tissue samples, that quantitative recoveries were obtained using either $HNO_3/HClO_4$ or $HNO_3/HClO_4/H_2SO_4$ mixtures. For electrothermal atomic absorption spectrometry the latter mixture was preferred, as the final product which is a mixture of sulphates and a few percent

of sulphuric acid has a less deleterious effect on tube lifetimes than the mixture of perchlorates from the former. The author stresses the need to use Suprapur (Merck) or Ultrex (J.T. Baker) reagents for serum or urine digestions.

It seems clear that serum chromium determinations on normal subjects should not be attempted by laboratories unless the best clean room conditions are available. Reagents of the highest purity, and materials and apparatus with the lowest chromium contents must be used. Even under the most rigorous conditions, repeated blank determinations should be carried out.

Background absorption in electrothermal atomisation atomic absorption spectrometry

Since ETA-AAS has been by far the most common technique used for the determination of chromium in biological materials, it follows that a high proportion of the inaccurate data has been produced by this method. Whilst it is rarely possible to identify sources of error in a particular procedure, and many errors must have been caused by contamination during sampling, the difficulty of achieving accurate background correction at the chromium wavelength must have contributed to errors in much of the earlier literature data (ref. 46). Guthrie et al. (ref. 46) were the first to identify and clarify the nature of this problem with respect to the determination of chromium in urine. The special features which make this a particularly difficult ETA-AAS determination are:

(i) The maximum charring temperature which can be used for chromium either in aqueous solution or in urine is about 1200 °C (but see later comments on the use of a matrix modifier). This was demonstrated beyond all doubt by some careful experimentation using samples spiked with ^{51}Cr radiotracer (ref. 41). At this temperature, most if not all the organic matrix is removed but a large proportion of the major biological salts remain even after long charring times (ref. 46), and give a substantial background signal during the atomisation stage. High quality background correction is therefore essential.

(ii) Most commercially available instruments rely on deuterium arc background correction. The intensity of these lamps at the chromium wavelength of 357.9 nm is low and deteriorates rapidly with use. In order to achieve accurate background correction, it is necessary to match the intensity of the D_2 arc with that of the hollow cathode lamp (HCL), and this is normally achieved by operating the HCL at reduced current, or by using a light baffle in the HCL beam. Although the latter method is generally superior to the first, both will give increased noise on the output signal of the atomic absorption instrument compared to operation at wavelengths below 320 nm.

(iii) The chromium concentrations of interest in the urine of normal subjects are close to the detection limit of the method, and signals will often be commensurate or not much larger that the typical errors of all background correction systems caused by the non-simultaneous measurement of the D_2-arc and HCL signals (refs. 47,48).

There is no doubt that poor background correction has contributed to errors in chromium determinations in this field. However, developments in instrumentation over the past seven or eight years have significantly improved the performance of both electrothermal atomisers and atomic absorption instruments. One of the more important developments was the introduction of a tungsten-halogen background corrector lamp as an alternative to the D_2-lamp (refs. 21,24,42). This has much greater intensity in the visible region of the spectrum, and more specifically at the chromium wavelength and considerably improves background correction performance. The use of pyrolytic graphite coated tubes and faster furnace heating rates and lower internal gas flow rates during the atomisation stage have all improved sensitivity (refs. 24,42). Several groups of workers have now also shown that D_2-arc background correction may be used effectively in more modern instruments for the determination of chromium in urine and serum, provided the atomisation conditions are correctly optimised (refs. 39,40,49), and one group has shown that valid measurements could be made with an instrument without the use of background correction facilities (ref. 26). Most authors would, however, agree that background correction should be used and some recent alternative approaches offering efficient background correction at the chromium wavelength are reviewed below.

Inadequate sensitivity or detection capability

Item (iii) above highlighted the limitations of ETA-AAS when signals for the analyte are too close to the detection limit, and results for chromium in serum now widely quoted in the literature were actually obtained from measurements below the detection limit of the method (ref. 22). It remains true, particularly in the case of serum analysis, that normal chromium levels are close to the detection power of most analytical techniques in use. The authors believe that this limitation is often overlooked. Errors from variations in blank levels, extraneous contamination, instrument noise, etc. are likely to be increasingly ignored as the sophistication of computer controlled instrumentation grows, and the analyst's confidence in the answers displayed gains momentum.

It is always preferable to use an analytical procedure which gives a detection limit substantially (ideally > 10 times) lower than the levels to be measured. The detection limit referred to here is that for the whole procedure used and not that for the final instrumental measurement. Thus a method capable of reaching a detection limit of 0.01 μg l^{-1} is required for both serum and urine analysis. Since few analytical techniques in use for chromium analysis offer such ideal characteristics, the limitations of the methods and of the data produced should always be carefully evaluated.

ANALYTICAL TECHNIQUES

Analytical techniques suitable for the determination of chromium in biological materials have been reviewed by a number of authors (refs. 6,50-52). A wide range of techniques may be used for the determination of the higher levels of chromium found in hair, bones and most foods and diets. Great care still needs to be taken during sampling and/or digestion of such samples if accurate results are to be obtained (refs. 45,50), but the problems are less severe than for serum and urine. The problem of selecting the most appropriate washing procedure to remove exogenous chromium from hair samples before analysis has been highlighted elsewhere (ref. 53). In addition a number of standard reference materials are available with chromium contents relevant to the analysis of foods, e.g. NBS SRM 1571 Orchard Leaves (2.6 μg g^{-1}), NBS SRM 1569 Brewers' Yeast (2.12 μg g^{-1}), NBS SRM 1577 Bovine Liver (0.088 μg g^{-1}), NBS SRM 1570 Spinach (4.67 μg g^{-1}), and more recently NBS SRM milk powder (2.6 ng g^{-1}), and should be used to evaluate the accuracy of digestion procedures and analytical techniques used for higher chromium concentrations (refs. 50,51). Instrumental methods suitable for such sample types and for serum and urine chromium concentrations above about 10 μg l^{-1} include spectrophotometry, voltammetry, instrumental neutron activation analysis, X-ray fluorescence, flame atomic absorption spectrometry, spark source mass spectrometry and arc emission spectrometry (refs. 6,52). If required, information should be sought from the cited references, and details from references therein.

For the determination of chromium at levels below 10 μg l^{-1}, the range of analytical techniques is much more limited, although some recent developments have suggested potential alternatives to electrothermal atomic absorption spectrometry and destructive neutron activation analysis, the most commonly used methods to date.

Electrothermal atomisation atomic absorption spectrometry (ETA-AAS)

The problems of background correction in ETA-AAS have been discussed earlier. Using modern commercial graphite furnaces detection limits between 0.05 and 0.15 μg l^{-1} can be reached. To achieve such levels advantage must be taken of facilities such as fast heating rates and reduced internal gas flows during atomisation (ref. 42). In addition, it has been shown that graphite tubes coated with pyrolytic graphite give improved sensitivity and detection limits for the determination of chromium (refs. 26,42), and the use of total pyrolytic graphite tubes may have significant advantages in the future (refs. 54,55).

Current state-of-the-art operation of most commercial ETA-AAS systems requires the use of a platform (ref. 56). Due to the lower heating rate of a platform placed inside the graphite tube, the sensitivity for involatile elements such as molybdenum and vanadium is actually inferior to direct atomisation from the tube wall, which is usually the recommended procedure for these elements (ref. 56). With chromium, which is only slightly less volatile, the advantage to be gained from using a platform is at best marginal. Addition of magnesium nitrate as a matrix modifier extends the range of available charring temperatures to 1600 °C, which is useful for ensuring more complete removal of the inorganic salts in biological samples prior to the atomisation stage (ref. 56). The purity of the magnesium nitrate used must however be carefully evaluated. Using this reagent and a Zeeman 5000 instrument, Slavin et al. (ref. 56) have reported a detection limit of 0.1 μg l^{-1} using a 20 μl injection of 1+1 diluted urine. Using five sequential 20 μl aliquots with intermediate charring allowed them to reach a detection limit of 0.02 μg l^{-1}. Application to sample analysis has not so far been reported. Using the same instrument system, a similar detection limit of 0.14 μg l^{-1} (20 μl) was recorded for urine chromium using a pyrolytic graphite coated tube with no platform, and 0.16 μg l^{-1} was obtained with a platform, both measurements being based on peak area (ref. 57).

Using a pyrolytic graphite coated tube with a platform, but with magnesium nitrate as matrix modifier, Veillon et al. (ref. 24) recommended an ashing temperature of 1350 °C in their method for serum analysis. Using peak height measurements, a detection limit of 0.03 μg l^{-1} was achieved using a single 25 μl aliquot of the final ashed sample solution produced by their pretreatment procedure (see Procedure 3 earlier). Calibration graphs were prepared from a bovine serum pool.

Although one group have recently suggested that direct analysis of chromium in urine may be achieved without background correction (ref. 26), most authors including the present ones recommend the use of an efficient and highly accurate background correction procedure. Acceptable results can undoubtedly be achieved using D_2-arc systems (refs. 39,40), but three recommended methods (refs. 24,41,52) suitable for determinations at the very lowest levels have utilised the tungsten-halogen lamp based correction method first investigated by Kayne et al. (refs. 21,22). In a recent article, Halls and Fell (ref. 49) have shown that the interference observed with D_2-arc background correction can be removed by the use of a lower atomisation temperature than would be considered normal for chromium viz. 2400 °C. The interference effect in the determination of chromium in urine is ascribed to the effect of excessive emission of radiation by chromium in the presence of sodium and particularly potassium. Although the effect is increased by the use of the high gain settings required when D_2-arc background correction is used because of the need to balance the energies between the two light sources, it is still observed at normal gain settings. Thus it may remain a significant source of interference in other instrumental systems such as that based on the use of a tungsten-halogen lamp for background correction. The potential advantages in accuracy to be gained from methods based on a single light source in conventional atomic absorption instruments, viz. Zeeman effect (ref. 56), and pulsed hollow cathode lamps (ref. 58) have yet to be fully documented for chromium analysis. The loss of sensitivity implicit in the latter method may be of considerable significance in determinations such as chromium in biological fluids.

All commercial atomic absorption instruments are based on the measurement of atomic absorption signals by narrow line sources. An alternative developed by O'Haver and his colleagues uses a continuum source and has proved particularly successful for the determination of chromium in biological materials. The instrument system, which was recently reviewed (ref. 59), is based on a high resolution echelle spectrometer and a high intensity 300W Xenon short-arc continuum source. Efficient background correction (up to 3.0 absorbance units of static background has been claimed (ref. 60)), is achieved by wavelength modulation using an oscillating refractor plate at the entrance slit of the monochromator. The use of this system to obtain accurate results for the determination of chromium in urine has been reported in a number of publications (refs. 42,46,61), and a detection limit of 0.09 µg l^{-1} consistent with normal line-source AAS measurements has been reported (ref. 61). A recent publication has described the use of this instrument for the simultaneous determination of Al, Cr, Mn and Ni in blood serum (ref. 62). Samples of serum (2 ml) were placed into silanized 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. Atomisation, which was from a platform, gave a mean method detection limit of 0.09 µg l^{-1} of serum. Continuum source atomic absorption results were in excellent agreement with those obtained by other techniques for a bovine serum pool containing 0.3 µg l^{-1} chromium (refs. 29,62,63). Although this technique is not yet commercially available, the sensitivity and background correction capability of the system, offer exciting potential for future application.

Electrothermal atomic absorption is clearly the most widely used technique for chromium analysis at low concentrations in biological materials. The quality of a number of different background correction methods now available mean that it can be used with increasing confidence. Detection limits of around 0.1 µg l^{-1} in all the systems make it reasonably convenient for most urine samples. The levels of chromium in serum are however close to or below current detection capability, and the successful application of ETA-AAS to this determination remains difficult and has been described in very few reports.

Carbon furnace atomic emission spectrometry (CFAES)

Although commercial electrothermal atomisers are intended for use in atomic absorption instruments, it is possible to make very sensitive measurements using the same instrumentation in atomic emission spectrometry (ref. 64). The development of a single channel instrument based on a high resolution echelle monochromator and a commercial graphite furnace atomiser, in which efficient background correction is achieved by wavelength modulation as in continuum source atomic absorption has been described (ref. 65), and its application to the determination of a number of trace elements of clinical interest evaluated (refs. 66-68). A detection limit for chromium of about 0.02 µg l^{-1}, following direct injection of 1+1 diluted urine onto a pyrolytic graphite coated platform, has been reported in a number of publications (refs. 61,65,66). Using the same spectrometer/background correction system for both continuum source atomic absorption and furnace atomic emission showed a CFAES detection limit 4.5 times better than for continuum source atomic absorption spectrometry (ref. 61). It should be noted that an HGA 500 furnace without a platform was used for atomic absorption measurements, but an open-ended furnace, the HGA 2000 (or in Europe HGA 72) was used with a platform for emission. The higher vapour phase temperatures experienced by the analyte under platform atomisation, provide a significant improvement in emission detection limits (ref. 65). Results for the analysis of a urine standard containing approx. 0.5 µg l^{-1} chromium by both

furnace emission and continuum source atomic absorption were in excellent agreement with figures obtained by a gas chromatography/IDMS method described below (ref. 61).

In recent studies (refs. 69,70), a carbon furnace atomic emission instrument based on a low resolution monochromator has been described and its application to the determination of chromium in urine evaluated. Background correction is again achieved by wavelength modulation, and atomisation is from a probe inserted into a HGA 72 atomiser fitted with a totally pyrolytic graphite tube. No significant spectral or chemical interferences from the urine matrix were encountered and urine samples were analysed against aqueous standards either directly or after 1+1 dilution with water. The detection limit of the low resolution monochromator system was 0.3 $\mu g\ l^{-1}$, but it is predicted that lower detection limits will be achieved by further optimisation of the spectrometer and optical system. The potential for highly sensitive, accurate, low cost chromium analysis is considerable.

Inductively coupled plasma emission spectrometry (ICPES)

Whilst this technique has made great strides forward in the past ten years and is now used widely in a variety of fields, applications in clinical analysis are few. Detection limits for chromium are generally quoted in the range 1-5 $\mu g\ l^{-1}$ (ref. 71), and whilst this is satisfactory for the analysis of biological materials such as foods and soils, it is not sufficiently sensitive for materials such as serum or urine (ref. 72). Although chemical interferences are uncommon, spectral interferences are much more common than in atomic absorption, and samples of urine and serum generally require substantial dilution before they can be introduced into a plasma using conventional nebulisation techniques. The dilution factor will further degrade practical detection limits. Although nebulisers are now available for solutions of high solid content, the improvement is unlikely to be sufficient to allow the direct determination of chromium in serum or urine.

The use of electrothermal atomisation as a sample introduction device for ICPES has offered some interesting possibilities, and Barnes et al. (ref. 73) have described the use of a carbon rod atomiser coupled to an ICP for the determination of a number of elements in urine. Although the detection limit for chromium was still only 4 $\mu g\ l^{-1}$, excellent results were obtained for the NIOSH-NBS freeze dried urine standard containing 50 $\mu g\ l^{-1}$. Mianzhi and Barnes (ref. 74) have demonstrated a resin preconcentration procedure which has allowed the determination of lower levels of chromium in urine samples. Samples of 500 ml of urine are adjusted to pH 5.0 and shaken with 0.2g of a specially prepared poly-(acrylamidoxime) resin which quantitatively extracts chromium(III). After shaking for 24 hours, the resin is isolated and dissolved in 5 ml of 1+1 nitric acid, and analysed by a standard additions procedure using a standard ICP with a Babington nebuliser. Although the procedure requires further evaluation at the lowest levels, acceptable results were achieved for samples containing between 1 and 50 $\mu g\ l^{-1}$ chromium, and the reported detection limit was 0.028 $\mu g\ l^{-1}$ for a 500 ml urine sample.

Destructive neutron activation analysis

Although a number of groups have reported on the use of neutron activation analysis for the determination of chromium in serum (ref. 12), only one has reported results which are consistent with accepted levels of chromium in serum and their publications should be consulted for details of the method used (refs. 20,25).

Reactor irradiation produces the radio-isotope ^{51}Cr by the reaction $^{50}Cr\ (n, \gamma)\ ^{51}Cr$, and this isotope has a half life of 27.8 days and a photopeak at 320.0 keV. Large, lyophilised serum samples can not be irradiated at a high neutron flux. The danger of explosion due to the increase in pressure caused by radiation damage is too great. Samples (100 mg) were therefore dry ashed over a period of 24 hours prior to irradiation, using successively increasing ash temperatures of 100, 200, 350, and 450 °C. Although the effects of contamination after irradiation can be ignored in neutron activation analysis, extreme care must be taken during all sample pretreatment steps. Versieck et al. (refs. 20,25) irradiated ashed samples for 12 days at a flux of $\sim 10^{14}$ neutrons $cm^{-2} s^{-1}$. The samples were allowed to decay for 30 days before mineralisation in a mixture of perchloric and nitric acids. Selective separation of chromium as chromyl chloride was then carried out in 10 ml of perchloric acid with three successive portions of 5 mg Cr as carrier, before radioactive measurement using a Ge(Li) detector.

Whilst excellent results have been achieved with this procedure, it is clearly lengthy and time consuming. The expensive instrumentation required also precludes the use of this method for routine analysis, but it does provide a useful reference method. This work has also performed the vital role of confirming data obtained by other techniques, and established the need for improved sensitivity and accuracy in chromium analysis in biological materials. Some of the problems associated with the determination of chromium in urine (ref. 75) and

red blood cells (ref. 43) by neutron activation analysis have been discussed by Cornelis and coworkers.

Gas chromatography/isotope dilution mass spectrometry

Chromium can be converted into a volatile complex, trifluoroacetylacetonate chromium(III), which can be separated from other similar metal complexes by gas chromatography and detected with considerable sensitivity using electron capture, microwave excitation, atomic absorption and mass spectrometry for detection (ref. 52). Unfortunately, the complicated procedures required for the quantitative formation of the complex from chromium in biological samples like serum or urine, produce high blanks and make chromium determinations impossible (ref. 76). An exception to this is the method developed by Veillon et al. (ref. 77) based on stable isotope dilution mass spectrometry and dual ion monitoring. Before the chelation/extraction procedure, the sample of urine (mainly ^{52}Cr) is spiked with Cr_2O_3 which contains 96.79% ^{50}Cr. After freeze drying, the mixture of urine and spike, are subjected to overnight ashing in a plasma oxygen asher, and subsequently ashed with hydrogen peroxide. Double distilled trifluoroacetylacetone is added and the chelation reaction carried out in sealed tubes for 2 hours at 100 °C. The chelate is extracted into hexane and excess ligand which is also extracted, removed by washing with 1M sodium hydroxide. Samples in hexane were separated on a glass column packed with 1% SE-30 on chromasorb-W at 125 °C.

The accuracy of this method has been confirmed by the analysis of NBS RM 1569 Brewers' Yeast and by comparison of results for normal urine samples with those obtained by other techniques (refs. 24,42,61,77). As with neutron activation analysis, the complexity and cost of this method render its widespread use impractical, but it has performed and will continue to perform an essential role as a reference method. Application to serum analysis could provide a significant future application area.

SPECIATION OF CHROMIUM

The exact form of chromium present in glucose tolerance factor has still to be established, although a bioassay for its determination has been proposed (ref. 18). Several groups have addressed themselves to other aspects of the speciation of chromium in biological samples. It has been shown that chromium exists entirely as chromium(III) in urine (refs. 74,78,79). In addition if chromium(VI) is added to urine, it is rapidly reduced to chromium(III). A study of the distribution of chromium among the serum proteins in a lyophilized reference animal serum has also been reported by Graf-Harsanyi and Langmyhr (ref. 80). The total concentration of chromium in this sample was reported to be 61 $\mu g\ l^{-1}$ which is more than 100 times higher than normal human serum, but the authors were able to use gel filtration combined with ETA-AAS to show that the chromium existed in two main fractions, one in the high molecular weight or macroglobulin group, and a low molecular weight fraction associated with albumin and transferrin. The majority of the chromium was bound to the transferrin component.

For most analysts the problems of determining total chromium at the levels found in normal serum or urine are sufficiently complex to preclude active studies of chromium species in these materials. In view of the current state of knowledge of the metabolism of chromium, more information on the species of chromium present in biological samples remains a desirable if distant goal.

CONCLUSIONS

The paper by Versieck and Cornelis in 1980 (ref. 12) provides a landmark in the development of analytical procedures for the determination of chromium (and other elements) in biological materials. Although many analytical chemists were fully aware of the errors in the earlier literature and the need for careful control of contamination etc. before then, it has not been possible since that date for anyone to ignore the lessons learnt from the strenuous efforts of early workers in this field. Unfortunately, it is clear that much of the pre-1980 data on chromium is invalid and that biochemical conclusions drawn from it are subject to doubt.

Fortunately, it should now be possible for many hospital laboratories to deploy commercial instrumentation with sufficient sensitivity for the determination of chromium in urine. The same cannot be said for serum analysis, especially for samples from normal or healthy subjects. There remains a need for analytical techniques of improved sensitivity for both serum and urine, but particularly the former. A method with a detection limit lower than 0.01 $\mu g\ l^{-1}$ which would allow serum analysis after a moderate dilution would be valuable in the elucidation of many clinical questions. Even with such a method, it would be necessary to ensure strict control of all forms of contamination, particularly at the sampling stage.

The recent preparation and characterisation of a bovine serum reference material, NBS RM 8419, with a recommended chromium concentration of 0.30 ± 0.05 μg l^{-1} should make an important contribution to the quality of reported serum chromium values (refs. 29,63). The usefulness of this material is enhanced because the chromium concentration has been confirmed by a wide range of techniques used in several different laboratories and is close to that of normal human serum levels (ref. 10). It should be used as a test of analytical accuracy whenever the measurement of normal or deficiency state populations is undertaken.

The recent paper by Cornelis and Wallaeys (ref. 10) has highlighted some areas in which clinical conclusions are in doubt. For our part, we believe that a number of specific questions of biological interest require re-evaluation or renewed examination with analytical techniques and procedures of established accuracy. Amongst these questions are:

(i) What is the dietary requirement for chromium in a normal healthy person? How is this altered in diseases such as diabetes? Do average diets supply this requirement? Recent evidence suggests they may not (ref. 17).

(ii) What is the structure of GTF and how is this synthesised and then transported to potentiate insulin at peripheral tissue sites?

(iii) Is there a real change in serum and urinary chromium after glucose loading? Can lower than normal (i.e. <0.1 μg l^{-1}) levels of serum chromium be demonstrated in diabetic patients or those with cardiovascular disease?

(iv) Should chromium(III) be added to the intravenous regimes or other nutritional support given to various categories of patient? Is so, what is an adequate yet safe dosage?

These and other questions concerning our general understanding of chromium metabolism in health and disease await the more widespread availability of commercial instrumentation of adequate sensitivity and of analysts able to apply new methodology in an appropriately careful manner.

REFERENCES

1. S. Langård, T. Norseth, in Handbook of the Toxicology of Metals, L. Friberg, G.F. Nordberg, V.B. Vouk (Eds), Elsevier, Amsterdam, 179 (1979).
2. S. Tola, J. Kilpio, M. Virtamo, K. Haapa, Scand. J. Work Environ. and Health, 3, 192 (1977).
3. W. Mertz, K. Schwarz, Arch. Biochem. Biophys., 58, 504 (1955).
4. K. Schwarz, W. Mertz, Arch. Biochem. Biophys., 85, 292 (1959).
5. W. Mertz, K. Schwarz, Am. J. Physiol., 196, 614 (1959).
6. R.A. Anderson, Sci. Total Environ., 17, 13 (1981).
7. J.S. Borel, R.A. Anderson, in E. Frieden (Ed.), Biochemistry of the Essential Ultratrace Elements, Plenum, New York, 175 (1984).
8. K.N. Jeejeebhoy, R.C. Chu, E.B. Marliss, G.R. Greenberg, A. Bruce-Robertson, Am. J. Clin. Nutr., 30, 531 (1975).
9. H. Freund, S. Atamian, J.E. Fischer, J. Am. Med. Assoc., 241, 496 (1979).
10. R. Cornelis, B. Wallaeys, in P. Brätter, P. Schramel (Eds.), Proceedings of the 1984 International Workshop on Trace Element Analytical Chemistry in Medicine and Biology, Neuherberg, Munich, West Germany, Walter de Gruyter, Berlin, 219 (1984).
11. G. Saner, V. Yüzbaşiyan, O. Neyzi, H. Gunoz, N. Saka, S. Çiğdem, Am. J. Clin. Med., 34, 1676 (1981).
12. J. Versieck, R. Cornelis, Anal. Chim. Acta, 116, 217 (1980).
13. R.A. Anderson, N.A. Bryden, M.M. Polansky, Am. J. Clin. Nutr., 41, 571 (1985).
14. R.J. Doisy, D.H.P. Streeton, J.M. Freiberg, A.J. Schneider, in A.S. Prasad (Ed.), Trace Elements in Human Health and Disease, Academic Press, New York, 79 (1976).
15. W. Mertz, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 1 (1979).
16. J. Kumpulainen, E. Vuori, S. Mäkinen, R. Kara, Br. J. Nutr., 44, 257 (1980).
17. R.A. Anderson, A.S. Kozlovsky, Am. J. Clin. Nutr., 41, 1177 (1985).
18. R.A. Anderson, M.M. Polansky, E.E. Roginski, W. Mertz, J. Agric. Food Chem., 26, 858 (1978).
19. E. Berman, Toxic Metals and Their Analysis, Heyden, London, 1980.
20. J. Versieck, J. Hoste, F. Barbier, H. Steyaert, J. De Rudder, H. Michels, Clin. Chem., 24, 303 (1978).
21. F.J. Kayne, G. Komar, H. Laboda, R.E. Vanderlinde, Clin. Chem., 24, 2151 (1978).
22. R.E. Vanderlinde, F.J. Kayne, G. Komar, M.J. Simmons, J.Y. Tsou, R.L. Lavine, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 49 (1979).
23. J. Kumpulainen, J. Lehto, P. Koivistoinen, M. Uusitupa, E. Vuori, Sci. Total Environ., 31, 71 (1983).

24. C. Veillon, K.Y. Patterson, N.A. Bryden, Anal. Chim. Acta, 164, 67 (1984).
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. L. Ping, K. Matsumoto, K. Fuwa, Anal. Chim. Acta, 147, 205 (1983).
27. R.A. Anderson, M.M. Polansky, N.A. Bryden, E.E. Roginski, K.Y. Patterson, D.C. Reamer, Diabetes, 31, 212 (1982).
28. R.A. Anderson, M.M. Polansky, N.A. Bryden, Biol. Trace Elem. Res., 6, 327 (1984).
29. U.S. National Bureau of Standards, Standard Reference Material 8419, U.S. Department of Commerce, Gaithersburg, Spring 1985.
30. T.T. Gorsuch, Analyst, 87, 112 (1962).
31. G.B. Jones, R.A. Buckley, C.S. Chandler, Anal. Chim. Acta, 80, 389 (1975).
32. S.R. Koirtyohann, C.A. Hopkins, Analyst, 91, 403 (1977).
33. J. Versieck, J. Hoste, J. de Rudder, F. Barbier, L. Vanballenberghe, Anal. Lett., 12, 555 (1979).
34. D. Behne, J. Clin. Chem. Clin. Biochem., 19, 115 (1981).
35. J. Versieck, F. Barbier, R. Cornelis, J. Hoste, Talanta, 29, 973 (1982).
36. J. Versieck, A. Speecke, in Nuclear Activation Techniques in the Life Sciences, IAEA, Vienna, 39 (1972).
37. G.S. Fell, A. Shenkin, D.J. Halls, in P. Bratter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, W. de Gruyter, Berlin, 217 (1980).
38. D.J. Halls, G.S. Fell, Anal. Chim. Acta, 129, 205 (1981).
39. D.J. Halls, G.S. Fell, in P. Bratter, P. Schramel (Eds.), Proceedings of the 1982 International Workshop on Trace Element Analytical Chemistry in Medicine and Biology, Neuherberg, Munich, West Germany, Walter de Gruyter, Berlin, 667 (1983).
40. M.W. Routh, Anal. Chem., 52, 182 (1980).
41. C. Veillon, K.Y. Patterson, N.A. Bryden, Clin. Chem., 28, 2309 (1982).
42. C. Veillon, K.Y. Patterson, N.A. Bryden, Anal. Chim. Acta, 136, 233 (1982).
43. R. Cornelis, J. Hoste, in P. Bratter, P. Schramel (Eds.), Proceedings of the 1982 International Workshop on Trace Element Analytical Chemistry in Medicine and Biology, Neuherberg, Munich, West Germany, Walter de Gruyter, Berlin, 766 (1983).
44. R. Cornelis, J. Hoste, J. Versieck, Talanta, 29, 1029 (1982).
45. D. Shapcott, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 43 (1979).
46. B.E. Guthrie, W.R. Wolf, C. Veillon, Anal. Chem., 50, 1900 (1978).
47. R.A. Newstead, W.J. Price, P.J. Whiteside, Prog. Analyt. Atom Spectrosc., 1, 267 (1978).
48. J.M. Harnly, J.A. Holcombe, Anal. Chem., 57, 1983 (1985).
49. D.J. Halls, G.S. Fell, J. Anal. At. Spect., 1, 135 (1986).
50. J. Hubert, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 15 (1979).
51. R.G.V. Hancock, in D. Shapcott, J. Hubert (Eds.), Chromium in Nutrition and Metabolism, Elsevier, Amsterdam, 31 (1979).
52. J. Kumpulainen, in A. Vercruysse (Ed.), Techniques and Instrumentation in Analytical Chemistry, Evaluation of Analytical Methods in Biological Systems, Part B, Hazardous Metals in Human Toxicology, Elsevier, Amsterdam, 253 (1984).
53. J. Kumpulainen, S. Salmela, E. Vuori, J. Lehto, Anal. Chim. Acta, 138, 361 (1982).
54. D. Littlejohn, I. Duncan, J. Marshall, J.M. Ottaway, Anal. Chim. Acta, 157, 291 (1984).
55. T.C. Dymott, M.P. Wassall, P.J. Whiteside, Analyst, 110, 467 (1985).
56. W. Slavin, G.R. Carnrick, D.C. Manning, E. Pruszkowska, At. Spectrosc., 4, 69 (1983).
57. J. Egila, D. Littlejohn, J.M. Ottaway, to be published.
58. S.B. Smith, G.A. Hieftje, Appl. Spectrosc., 37, 419 (1983).
59. T.C. O'Haver, Analyst, 109, 211 (1984).
60. J.M. Harnly, T.C. O'Haver, Anal. Chem., 49, 2187 (1977).
61. J.M. Harnly, K.Y. Patterson, C. Veillon, W.R. Wolf, J. Marshall, D. Littlejohn, J.M. Ottaway, N.J. Miller-Ihli, T.C. O'Haver, Anal. Chem., 55, 1419 (1983).
62. S.A. Lewis, T.C. O'Haver, J.M. Harnly, Anal. Chem., 57, 2 (1985).
63. 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).
64. J.M. Ottaway, R.C. Hutton, D. Littlejohn, F. Shaw, Z. Karl-Marx-Univ., Leipzig, 28, 357 (1979).
65. L. Bezur, J. Marshall, J.M. Ottaway, R. Fakhrul-Aldeen, Analyst, 108, 553 (1983).
66. J.M. Ottaway, L. Bezur, R. Fakhrul-Aldeen, W. Frech, J. Marshall, in P. Bratter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, Walter de Gruyter, Berlin, 575 (1980).
67. J. Marshall, J.M. Ottaway, Talanta, 30, 571 (1983).
68. W. Frech, J.M. Ottaway, L. Bezur, J. Marshall, Can. J. Spectrosc., 30, 7 (1985).
69. D.C. Baxter, I.S. Duncan, D. Littlejohn, J. Marshall, J.M. Ottaway, J. Anal. At. Spect., 1, 29 (1986).
70. D.C. Baxter, D. Littlejohn, J.M. Ottaway, G.S. Fell, D.J. Halls, J. Anal. At. Spect., 1, 35 (1986).
71. S.S. Berman, J.W. McLaren, S.N. Willie, Anal. Chem., 52, 488 (1980).
72. R.L. Dahlquist, J.W. Knoll, App. Spectrosc., 32, 1 (1978).

73. R.M. Barnes, P. Fodor, Spectrochim. Acta, 38B, 1191 (1983).
74. Z. Mianzhi, R.M. Barnes, Spectrochim. Acta, 38B, 259 (1983).
75. R. Cornelis, A. Speecke, J. Hoste, Anal. Chim. Acta, 78, 317 (1975).
76. T.R. Ryan, C.R. Hastings Hogt, J. Chromatog., 130, 351 (1977).
77. C. Veillon, W.R. Wolf, B.E. Guthrie, Anal. Chem., 51, 1022 (1979).
78. C. Minoia, M.M. Colli, L. Pozzoli, At. Spectrosc., 2, 163 (1981).
79. C. Minoia, A. Mazzucotelli, A. Cavalleri, V. Minganti, Analyst, 108, 481 (1983).
80. E. Graf-Harsányi, F.J. Langmyhr, Anal. Chim. Acta, 116, 105 (1980).