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Atomic Absorption Analysis of Some Trace Metals of Toxicological Interest

Introduction

Scope of Review

The continuing interest in the chronic toxicology of lead, mercury, cadmium, and other metals points to the need for examining recent analytical methodology applied to the more toxic heavy metals in animal tissue samples. This review emphasizes atomic absorption spectrometry (AAS), and includes the period 1971–1974, although some listings are dated shortly before or after this four-year period. The large number of articles demanded selectivity in listing. The main criterion was a description of AAS showing some optimization for blood, urine, or mammalian tissues. Articles of special interest are generally those which indicate examination of interferences, matrix effects, sample treatment, or instrumental optimization. Atomic fluorescence spectrometry (AFS) and atomic emission spectrometry (AES) are mentioned for those cases in which a certain advantage or potential for future development is indicated. The author realizes that worthy reports may have been overlooked; he would be glad to hear of specific contributions which should have been listed.

When feasible, it is desirable to use alternative, independent techniques as a check on accuracy. Thus, colorimetric, electrometric, or chromatographic alternatives should be considered as possibilities for confirmation. Some analysts may favor one of the modern voltammetric techniques, such as stripping analysis, over the flameless AAS methods for elements such as lead, cadmium, thallium, or arsenic. Osteryoung and Osteryoung [*1*] have discussed the pulse polarographic analysis of toxic heavy metals. Coulometric titrations have been less frequently described for biosamples [*2*].

For bioapplications of AAS the text by Christian and Feldman [*3*] should be a starting point. The review by Winefordner and Vickers [*4*] on flame spectrometry discusses fundamental developments and gives authoritative advice in finding background information for bioapplications. Reviews on the application of AAS to biological materials include those by Willis [*5*], Schroeder and Nason [*6*], Dawson [*7*], and Rousselet [*8*]. Wilson [*9*] discusses performance of analytical methods, and Tölg [*10*] reviews sample treatment for extreme trace analysis.

Horncastle [*11*] has reported the determination of several metals in postmortem urine. For drug-related deaths he has obtained changes in urine trace metal content which suggest a need for confirmatory investigation.

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Berman [*12*] reviewed methodology of the middle 1960's for application of AAS to trace metal analysis of toxicological materials. She presented conventional techniques for 17 metals, and states that the trace metals of most frequent concern in toxicological investigations are probably lead, cadmium, thallium, mercury, and arsenic. Berman included the flameless techniques in her recent critical review [*13*] of biochemical applications of AES and AAS to 21 elements. For step-by-step procedures of analysis the reader is referred to the instrument manufacturers' instruction manuals, as well as to the following discussions. In addition, a summary of published AAS applications to analytical toxicology (with a review of atomic absorption instrumentation) appears in Ref *14*.

The very narrow absorption peaks of atoms contrast with the generally broad absorption bands of molecules. Thus, the selectivity of AAS is vastly superior to that of colorimetry. The sensitivity of conventional flame AAS, however, is not generally better than the sensitivity of colorimetry or of the newer voltammetric techniques. Matrix effects, chemical interferences, band absorption, and scattering interferences can be significant in AAS. These interference problems must always be considered by the AAS trace analyst. They are generally reduced by proper use of standard additions (or matrix-matched standards) and by proper background correction. The use of releasing agents, protective agents, or matrix modifiers has been of less interest to the trace analyst, whose primary problem is sensitivity. Thus, this review will emphasize separation methods for matrix removal and analyte preconcentration.

Before individually discussing the application of AAS to the toxicological analysis of eight metals, we will consider the principal types of flameless atomizers, some other instrumental innovations, and the enduring problems of sample treatment and trace analytical techniques.

Flameless Atomizers

General Comparisons—Recent work [*15*] with the flameless AAS techniques shows detection limits 10 to 100 times below the ordinary range of flame methods. Woodriff [*16*] has listed the principal devices for flameless atomization. He states that boats, rods, and wires are subject to greater signal variation due to effects on analyte vaporization caused by air currents, matrix materials, and differences in sample placement. Among the tube furnaces, the Massmann and the carbon rod atomizer (CRA or "mini-Massmann") have attained wide commercial application. Matoušek and Stevens [*17*] described use of the carbon rod atomizer for determining lead and several other metals in blood. These authors mentioned the problem of chemical interferences, requiring either a standard addition technique or matrix matching of standards. Bratzel and Chakrabarti [*18*] concluded that the carbon rod gives performance superior to the West type of carbon filament atomizer. Clark et al [*19*], in turn, have critically surveyed the Delves sampling cup technique [*20*]. In the absence of any possible analyte interferences, they conclude that the Delves technique is useful for only lead, silver, selenium, bismuth, and gallium, of the 14 elements tested.

Syty [*21*] has provided an extensive and excellent discussion of the several flameless atomizers including design, optimization, sensitivities, applications, advantages, limitations, interferences and their correction, and comparison to flame techniques or to other flameless devices. Also discussed are hydride generation techniques for arsenic, etc.; the cold vapor method for mercury; and other recent sample introduction methods, such as modulated injection and ultrasonic nebulization. Adaptation by commercial suppliers is included. The Syty article should be consulted before purchase or use of a commercial flameless device.

The three principal new commercial adaptations of atomization devices for the period of this review are the graphite atomizer (Massmann furnace), carbon rod atomizer, and tantalum ribbon. Sampling boats, cups, and cuvets are advocated by many workers,

but some of these devices had been introduced commercially before the period reviewed here.

Massman Atomizer—The Massmann tube furnace (heated graphite atomizer or HGA) consists of a 55-mm graphite tube with an 8-mm inside diameter. The sample is added to the cold tube with a pipet or syringe through a hole in the wall at the midlength of the tube. An initial gentle heating to dry the sample is followed by a stronger heating for carbonization before the final heating for analyte atomization with a current of about 400 A. The water-cooled electrodes supporting the ends of the tube permit rapid cooling for subsequent sample introduction. Ideally, no other sample ashing or treatment procedure is required. Woodriff [*16*] notes that use of peak area rather than peak height should reduce errors caused by matrix material. This expected difference in accuracy is explained by the greater effect of matrix material on rate of analyte atom formation compared to the matrix effect on analyte residence time in the light path. When peak area is measured, the relative sensitivity ideally increases with the cube of atomizer path length. Matrix materials in flameless atomization also cause interferences by broadband absorption and by scattering of source light. Except when excessive, both these errors can be corrected by the method of Koirtyohann and Pickett [*22*]. This correction involves an optical balancing as performed by the deuterium arc or xenon arc background corrector.

As a rule, however, interferences in flameless methods, although largely uninvestigated, appear to be more severe than in flame methods. Almost every element appears to slow the analyte vaporization, giving a peak height depression. Thus, the techniques of flame spectrometry—preconcentration, standard addition, and matrix matching—may still be required and the flame methods should not be neglected unless sample size and sensitivity provide definite constraints.

Carbon Rod Atomizer—Matoušek and Stevens [*17*] described the carbon rod atomizer (CRA) applied to bio-trace-metal determinations. The carbon rod, clamped between two water-cooled terminals, was made of Ringsdorff spectroscopic-grade graphite having dimensions similar to those of the graphite tube. The rod is solid, however, except for a transverse bore (at midlength and perpendicular to the longitudinal axis) for sample placement and an access hole connecting to the bore for sample injection. A power unit provides operating current for the rod in a preselected sequence of drying, ashing, and atomization. To minimize oxidation of the graphite and to reduce analyte interferences, a sheath of nitrogen or hydrogen flows around the rod from a chimney below. The unit contains conduits for electrical power, gas, and cooling water. Nonatomic absorption is corrected by a deuterium lamp technique.

Due to the problem of chemical interferences (as in all flameless methods), use of either standard additions or matrix-matched standards is recommended. Application of 2 ml of xylene into the rod before injection of samples or standards may improve reproducibility of measurements. The relative standard deviation (RSD) or coefficient of variation in the absorption mode was 5 to 12% at the subnanogram level. The CRA is sometimes reported to be more sensitive in the fluorescence mode.

Tantalum Ribbon—Hwang et al [*23*] discuss the optimization of flameless AAS based on an enclosed atomization chamber with a tantalum ribbon as heating element. Modified after the device of Donega and Burgess [*24*], the ribbon (about 14 by 58 mm) contains an indentation at midlength which can hold in the range of 100 ml of sample. The ribbon is clamped to electrodes inside the absorption cell, which is fitted with end windows of quartz. Connections to the cell lead to the power supply and to inert gas for purging. As with the HGA and CRA devices, a cycle of sample drying, ashing, and atomization temperatures can be preset. Automatic background correction is with a continuum source.

According to Hwang et al the critical parameters for maximizing sensitivity with the tantalum ribbon are the temperature, the distance between optical path and ribbon, and

the properties and flow rate of the purge gas. At the nanogram level the RSD is about 2 to 4%.

Principal Atomization Devices Available—At present the following atomization devices are supplied in the United States mainly by the instrument companies listed below.

Device	Company
Graphite tube	Perkin-Elmer Corp. (HGA); Fisher Scientific Co.
Graphite cuvet (in pressure chamber)	Instrumentation Laboratory, Inc.
Carbon rod	Varian Associates (CRA)
Tantalum ribbon	Instrumentation Laboratory, Inc.; Fisher Scientific Co.
Cuvets of tungsten or graphite (in pressure chamber) containing microboats of tungsten or graphite	Instrumentation Laboratory, Inc.
Sampling boat (tantalum) and Delves cup (nickel)	Perkin-Elmer Corp.

Other Instrumental Innovations

Some recent instrumental applications may increase the efficiency of trace metal analyses on toxicological samples. Elser and Winefordner [*25*] used a continuum source with double modulation: chopper modulation of source radiation and wavelength modulation of transmitted radiation. The wavelength modulation enhanced signal-to-noise ratio (by yielding a derivative signal) while minimizing Rayleigh scattering and molecular absorption. The chopper modulation eliminated flame emission. The elements cadmium, chromium, iron, and nickel showed detection limits of about 0.1 ppm without preconcentration. Thus, the continuum source, with its supporting modules, may be able to reduce the number of source lamps required by a laboratory needing only occasional metallic analyses in a wide range of analyte capability.

In the important area of nebulization for flame applications, Copeland et al [*26*] showed that ultrasonic nebulization improves sensitivity, sometimes by a factor of three to four, over pneumatic aspiration.

Advances of potential interest to bioapplicators of atomic spectrometry are contributed from the areas of fluorescence and emission. Investigations [*27*] by atomic fluorescence with a graphite rod atomizer and with thermostated multiple-element electrodeless discharge lamps (EDL's) showed detection limits at 0.01 to 100 picograms (pg). These limits for lead, mercury, cadmium, and thallium, among others, are equal to or better than reported limits by any other atomic spectrometric method. Spectral and chemical interferences were minimal.

In atomic emission the inductively coupled plasma (ICP) is a promising excitation source for the simultaneous multielement determination of trace elements in biological fluids [*28*]. A multichannel direct-reading spectrometer allows simultaneous analyses on microlitre samples with essentially no sample preparation. The ICP excitation for atomic emission is expected to gain importance in analysis of real biological samples. Precision is ± 3 to 5%. Detection limits for the elements tested are in the ppb range (5 μg of lead per 100 ml).

Webb et al [*29*] used the d-c arc with direct reading emission spectrometer to determine trace metals in biological tissues. The RSD at 0.1 ppm was about 10%. They described a method for adjusting sample macroelement composition to that of a common standard matrix.

Matrix Adjustment

Techniques of matrix adjustment or matrix removal for improving the utility of flame methods are continually reported. Thus, Maktovich and Christian [*30*] have observed for several elements an increase in signal which is linear with increasing acetone content of the aqueous solution. Lemonds and McClellan [*31*] showed that for various alcohols, ketones, and esters the viscosities and boiling points were linearly related with signal enhancement. Articles on the use of chelation-solvent extraction [*32,33*], the comparison of ketone extracting solvents [*34*], and the discussion [*35*] of solubility characteristics of chelates of ammonium pyrrolidinedithiocarbamate (APDC), a ligand frequently used by AAS workers, all appeared in a single, rather recent journal issue. These four articles are of the type which should closely interest those applying AAS to toxicological trace analysis. Ellis [*36*] has reviewed preconcentration and sample preparation as applied to flame spectrometry.

With flameless atomization devices, "matrix modification" has recently gained interest for separation of the matrix from a volatile analyte during the charring step in the atomizer device. The addition of ammonium nitrate, for instance, facilitates removal (or decreases the interference) of sodium chloride in seawater before analyte volatilization [*37*].

Sample Dissolution

Several recent studies concern sample decomposition or solubilization prior to AAS measurement. Decomposition of biological materials in a pressure device [*38*] and with activated oxygen [*39*] has been described. Soluene-100®, a quaternary ammonium hydroxide, is recommended [*40*] as a tissue solubilizer prior to AAS measurement. Gorsuch [*41*] authoritatively reviewed destruction of biological samples, and his article is a firm starting point in this area. For macroelement analyses Ducharme and Pena [*42*] prescribe a glass tube, with thermometer fitting, for HNO_3-$HClO_4$ digestion of 1-g samples of biological homogenate. They did not mention use of an oxidation catalyst, nor have these authors applied the technique to volatile or other trace elements. Pekarek et al [*43*] claim no sample treatment is necessary when analyzing chromium in serum by a graphite tube atomizer with a deuterium arc background corrector.

For wet digestions of samples prior to flameless methods, sulfuric and perchloric acids are to be avoided in the final solution, as these acids cause more light scatter than does nitric acid. By using a diluted H_2SO_4-50% H_2O_2 tissue digest, Schramel [*44*] found no interferences for manganese and five other elements, although lead and vanadium suffered matrix interference. A standard addition technique [*3*] should be used, at least on a periodic basis, as a monitor of accuracy if an independent analytical method is not available. For use of the carbon rod (mini-Massmann) atomizer, formation of a pyrolytic coating (by flowing methane through the heated chamber) is recommended to improve reproducibility. A recent, promising technique for blood lead using the Massmann tube involves analysis after dilution of the blood 1:4 with a surfactant such as 1% Triton X-100®. For AAS determination of beryllium, the Massmann tube gives a sensitivity 10 to 100 times improved over flame methods.

Contamination and Loss

With the help of neutron activation, Versieck et al [*45*] showed that steel surgical blades and, particularly, Menghini needles were unsatisfactory collectors for determination of some trace elements. Taylor and Marks [*46*] described contamination by rubber end caps on pistons of disposable syringes. Using radioactive tracers, Doshi and Patel [*47*] showed dramatic losses (as might be expected) when dry ashing for several elements

from marine tissue. But wet ashing under reflux gave greater than 90% recovery for mercury and selenium, and 100% recovery for chromium, antimony, and arsenic. Losses of lead, cadmium, and several other metals by adsorption on containers have been investigated by Struempler [*48*]. No single container type proved satisfactory for all ions. Polyethylene did not adsorb cadmium, and acidification to pH 2 with nitric acid prevented lead or cadmium adsorption on borosilicate.

Qualitative Screening

In forensic cases, for screening prior to quantitation, one might attempt thin-layer chromatography (TLC) of the metal dithizonates, as suggested by Tewari and Bhatt [*49*]. Embellishments, in fact, appear even in more familiar areas. Thus, Maiti et al [*50*] describe limits, interferences, and optimization of the classical Reinsch test for arsenic, antimony, bismuth, and mercury. The metals deposited on copper foil were sublimated for analysis by TLC.

Lead

Introduction

Lead, mercury, and cadmium generally dominate the attention of those interested in chronic toxicology of metals. Lambie [*51*] has discussed acute lead poisoning. Absorbed slowly from the gastrointestinal tract, lead can also be absorbed from the respiratory tract when inhaled. Lead storage depots even larger than the liver and kidney are the adrenal, thyroid, jejunum, and especially the skeleton [*3*, p. 407]. The accumulation of lead is indicated by the results of Kehoe et al [*52*]. The critical blood lead level has generally been considered to be 80 μg/100 ml. But recent evidence suggests [*53*] that a level above 40 μg/100 ml may cause central nervous system impairment in children. Most of the blood lead is found in the erythrocytes. Toxic symptoms can occur when conditions force release of lead from depots into the circulation unless the blood contains strong ligands, such as ethylenediaminetetraacetic acid (EDTA). Most absorbed lead is excreted by the kidneys. Urinary concentrations greater than 50 ppb have generally indicated lead poisoning. Urinary lead excretion following intravenous administration of $CaNa_2EDTA$ can be used to demonstrate abnormal lead burdens.

Many of the recent AAS applications to lead analysis in toxicology have been in pediatrics, where the problem of lead ingestion in children will remain critical for many years. The restriction to small samples from children has provided much of the impetus to flameless atomization techniques for blood lead determination. Other biochemical parameters, such as δ-aminolevulinic acid excretion or blood prophyrin-hematocrit determination, have been correlated with blood lead; these parameters are frequently important in the determination of the toxicological thresholds for lead [*53*].

Flame Atomization

When using conventional AAS without preconcentration, the useful limit for lead is about 5 ppm. Lead absorption can be enhanced sixfold by extracting H_2PbI_4 into methyl isobutyl ketone (MIBK) [*54*]. Dithizone or one of the dithiocarbamates have been the most frequently cited complexing agents for lead.

The first reported determination of lead in urine by Willis [*55*] involved direct extraction of undigested urine at pH 3 with APDC into methyl *n*-amyl ketone. Using standard additions, this direct extraction proved satisfactory compared with a colorimetric procedure for ashed urines containing pathological levels. Using a similar procedure, Pierce and Cholak [*56*] found significant light scattering, correctable by subtracting the absorption at 220.4 nm from the absorption at the lead resonance line at 217.0 nm.

Among the modifications of the Willis procedure [*55*] for urine lead is the one by Yamauchi [*57*], who treated the urine with trichloroacetic acid (TCA) and added Triton X-100® to prevent emulsion formation. (In the flameless techniques for lead, the surfactant Triton X-100® serves as a frequently mentioned matrix modifier.)

For blood lead analysis, sample treatment usually has involved TCA precipitation of proteins. Murphy et al [*58*] reviewed over 20 atomic absorption techniques reported through 1970 for lead analysis in urine and blood, including the Delves cup and sampling boat systems. For blood lead these authors prefer a technique (modification of Selander and Cramer [*59*]) involving TCA precipitation, chelation with APDC, and extraction into MIBK. EDTA did not interfere with lead extraction, and data confirmed recommendations of other workers that the "upper normal limit" for children and adults is 50 μg/100 ml.

Some workers, however, find that EDTA as an anticoagulant or therapeutic agent interferes with lead extraction. Zinterhofer et al [*60*] required addition of $CaCl_2$ to displace lead from EDTA in the blood or urine of patients receiving EDTA therapy.

Rather than using TCA as a releasing agent, Mitchell et al [*61*] utilized Triton X-100® to induce hemolysis as the only treatment prior to APDC-MIBK extraction. These authors found no interference by EDTA, but they state that storage of blood at 20°C for 15 days caused a loss of about 67% in the apparent lead concentration. Cold storage in the presence of phosphate also caused significant lead loss.

With an air-acetylene flame, Iida et al [*62*] found no lead interference from aqueous physiological concentrations of sodium, potassium, calcium, iron, silicon, or phosphorus. At concentrations greater than 2.5%, however, magnesium and aluminum interfere. In some tissues (red cells, kidney, etc.) some trace metals may occur in milligram quantities which precipitate with APDC and cause erratic extraction of lead. Yeager et al [*63*] recommend extraction at pH 8.5 and use of cyanide to mask iron, copper, and zinc. Neither bismuth nor cadmium are sequestered, but bismuth would probably appear only in feces. In any case, aliquot adjustment or direct analysis of digests may be advised.

Kopito et al [*64*], using lead-203, investigated sample preparation techniques used in conventional AAS methods for blood lead. A single TCA precipitation followed by extraction with APDC-MIBK, when properly performed, gave a recovery of 94% of the blood lead with excellent precision. A combination of TCA and $HClO_4$ gave less efficient extraction. Acid digestion with HNO_3-$HClO_4$ at 150°C to near dryness caused no loss of lead even from uncovered beakers. Complete digestion was necessary for efficient coprecipitation with bismuth. The authors provided evidence confirming the lack of validity of standard additions used with undigested biological samples; a difference in the mode of in vivo and in vitro lead binding was indicated.

To solubilize liver, kidney, and hair samples for AAS analysis of lead, cadmium, zinc, and copper, Murthy et al [*65*] employed aqueous tetramethylammonium hydroxide. Tissue levels, reproducibility, and recovery compared well with nitric acid digestion. These authors also used the standard-addition technique.

Searle et al [*66*] evaluated an anodic stripping voltammetry (ASV) lead analysis against an AAS procedure that uses APDC extraction. Both blood and urine were digested with perchloric acid before ASV analysis. Urine was extracted directly for AAS analysis. Precision for the extraction flame AAS was somewhat superior to the ASV precision. The AAS analysis was less time-consuming and tended to give slightly higher results. "Normal" urine lead levels ranged from 0.7 to 4.7 μg/100 ml by both methods.

Flameless Atomization

Delves Cup—For blood lead determinations the sensitivity and sample size requirements of pediatrics have recently made flameless AAS procedures dominant. Of wide popularity is the Delves nickel cup technique [*20*], developed from the tantalum boat of

Kahn et al [67] and the nickel absorption tube of White [68]. Hicks et al [69] found good correlation between conventional extraction AAS (5 ml of blood) and the Delves method (10 μl of blood). The RSD of the Delves method was 8% at a level of 46 μg/100 ml. For matrix matching, blood of known lead content was flamed in the cup together with the aqueous standards. Accuracy was further checked on an interlaboratory basis.

Several modifications of the Delves technique are reported to improve this type of flameless AAS. Attachment of a time-delay circuit [70] eliminates nonspecific absorption on instruments lacking a continuum background corrector. Precoating of the nickel cups with albumin [71] obviates the need for standard additions. Use of aqua regia [72] with fused silica cups, rather than hydrogen peroxide for sample treatment, improves precision and reduces background. Total freedom from background absorption is recently reported by Jackson and Mitchell [73] for a microsampling cup procedure determining lead in diluted tissue homogenates.

A type of matrix modification deserving further characterization and optimization with the Delves cup is the "punched disk" technique. A sample from a capillary prick is spotted onto filter paper, which is punched out as a standard-sized disk for flaming in the cup. Several workers [74,75] have found good correlation of this disk method with the more time-consuming procedures, including cathode ray polarography [76].

Carbon Rod Atomizer—Another widely discussed flameless atomizer for lead analysis is the carbon rod atomizer (CRA). This device consists basically of a graphite rod with a transverse bore for sample containment. Kubasik and co-workers performed blood lead determinations with the CRA both on 0.5 μl of undiluted, heparinized blood [77] and on 1.0 μl of blood diluted with two volumes of Triton X-100® [78]. Precision of measurements (RSD = 11% at 38 μg/100 ml) for untreated blood was about half that for Triton®-diluted blood, but was considered adequate for clinically significant amounts of lead. Analyses on untreated blood compared well with analyses on Triton®-diluted blood, and the latter analyses compared favorably to conventional AAS with sodium diethyldithiocarbamate (NaDDC)-MIBK extraction. Standards for both methods were prepared in a whole blood matrix. The authors found no matrix effect from lead standards in blood relative to standards in Triton X-100®. Dilution with Triton X-100® was superior to dilution with physiological saline, deionized water, or concentrated nitric acid. Sensitivity of the direct method was 1.0 μg/100 ml. Individual chemical interferences were not examined.

For analysis of lead, cadmium, and thallium in undigested urine, however, Kubasik and Volosin [79] found extraction (NaDDC-MIBK) necessary to reduce background to a range correctable by a continuum source. Standard additions were used to further reduce matrix effects on extraction and atomization. The effect of nine foreign ions was studied, and the pH range for extraction was optimized. Addition of calcium chloride reduced extraction interference produced by EDTA. According to the authors' thorough investigation, cadmium posed a contamination problem and thallium required care to achieve proper atomization.

Rosen and Trinidad [80] injected into the CRA samples of heparinized blood sandwiched as a bolus in the syringe between segments of xylene. Preventing the samples from soaking into the carbon rod, the xylene also served as a "flush out" mechanism. Blood containing EDTA and about 1.0% Triton X-100® yielded complete recovery of lead. The CRA technique permitted about 35 determinations per hour. Since nonatomic absorption was rated at about 1.0%, background correction was not used.

Graphite Furnace—Using the Massmann graphite furnace in conjunction with the deuterium arc background corrector, Evenson and Pendergast [81] showed the desirability of analyzing lead in diluted erythrocytes. The sampling of erythrocytes, rather than whole blood, both improved precision and reduced absorption inhibition by potassium and sodium. The method involved a 1:1 dilution of the erythrocytes with 5% Triton X-100®.

Charring time was 40 seconds at 525 °C. The method compared well with Triton®-diluted red cells analyzed by conventional AAS with APDC-MIBK extraction. At expected anticoagulant concentrations, neither heparin, oxalate, nor EDTA affected lead absorbance. Potassium showed a 15% lead depression, while magnesium and iron showed slight enhancements. Chloride, phosphate, and bicarbonate showed no effect individually, but lead absorption was depressed in media above pH 3. The RSD of the method was 4%. Sample-matrix effects, relative to the acidified aqueous standards, were not described.

For the graphite furnace, Norval and Butler [*82*] found that integrated absorbance provided a more satisfactory calibration curve for blood than did peak height measurement. Since analysis by standard additions compared well with the use of aqueous standards, the standard additions were not used routinely. Pre-ashing of blood with hydrogen peroxide was required prior to the ashing step in the tube; otherwise an accumulating ash buildup constricted the optical path. The optimum final furnace temperature of 1840 °C was determined in the presence separately of chloride, sulfate, and nitrate. The chloride medium provided the most sensitive analysis. For normal samples the RSD was 11%. The detection limit was 0.5 ng.

Iron in blood samples depressed lead absorption for Machata and Binder [*83*]. The resulting lack of sensitivity and precision with the graphite furnace (for lead, thallium, cadmium, and zinc) could be avoided by addition of lanthanum salts to the matrix. Precise determinations called for the use of a low temperature asher (oxygen plasma produced in a high frequency induction furnace).

Tantalum Ribbon—The tantalum ribbon flameless atomizer has been promoted for blood lead determination by Hwang and co-workers [*84*]. Using 25 μl of 1:10 water-diluted whole blood, these investigators confirmed the accuracy of their device by splitting samples with an independent laboratory, which analyzed by flame AAS and by dithizone colorimetry. The tantalum ribbon procedure exhibited an RSD of about 7% at the lead level of 40 μg/100 ml. Fifteen to twenty samples could be analyzed per hour. Standardization was by lead addition to whole blood of known lead content. Background from the water-diluted whole blood samples depended on both source line and sample. Although three times greater than lead absorption, the background could be compensated for with a hydrogen continuum source operated at twice the frequency of the hollow cathode lamp.

On the other hand, with ketone extracts of blood Hwang et al [*85*] showed negligible background by tantalum ribbon atomization for blood lead. These APDC-MIBK extracts were produced from 0.1 ml of whole blood containing saponin for hemolysis and formamide to reduce emulsion.

Comparison of Flameless Techniques for Lead—From the above discussions it might be assumed that lead analysis in biological materials is currently a task requiring some consideration, experience, and care. Indeed, Keppler et al [*86*], studying the reliability of blood lead determination in 60 laboratories, found less than half the reported results to be within acceptable limits of accuracy.

Anderson et al [*87*] experimentally reviewed accuracy and precision of blood lead analysis for three flameless devices: the Delves nickel cup in an air-acetylene flame, an electrically heated graphite crucible (developed from the graphite rod), and the electrically heated tantalum ribbon. For each atomization technique both liquid and impregnated disk samples were tested. Liquid samples for the Delves procedure received the Delves peroxide treatment, while liquid samples for the graphite crucible were prediluted with Triton X-100® and for the tantalum ribbon with ammoniacal *n*-butanol containing a silicone antifoaming fluid.

Precision using the Delves cup or tantalum ribbon was superior to the precision when using the graphite crucible. Precision of systems using liquid blood was slightly better than counterpart systems using the impregnated paper disks.

Accuracy was not tested by comparison with an independent technique. Yet, by assigning the most precise of the Delves cup analyses as the reference, the tantalum ribbon results showed less background interference than did results obtained with either the graphite furnace or, in many instances, with the Delves cup.

Four commercial instruments were tested by the same protocol with the above atomization devices and techniques. Complete analysis time for batches of 100 samples varied from 4.5 to 7.0 minutes per sample. For single samples, however, time ranged from 48 to 80 minutes. These time frames confirm the recognized fact that flameless methods are routinely slower than conventional AAS methods.

Lead Content of Tissues

The recent literature contains many reports and discussions on lead values in the various soft and hard tissues. As a result of human exposure to lead, tissue values (which may not correlate well with blood values) sometimes show a larger enhancement factor for lead content than does blood. Unfortunately, space does not permit a listing here of these pathological findings, many of which would have forensic interest. Kehoe [*88*] reviewed occupational lead poisoning.

Mercury

Introduction

The toxicological and environmental concern for mercury analysis is complemented, fortunately, by the very sensitive determination of total mercury with recent, routine techniques. The high volatility of mercury metal and the early availability of low pressure mercury vapor lamps allowed atomic absorption measurements of mercury vapor in air at least as early as 1939 [*89*], without use of a monochromator. The mercury vapor concentration meters (mercury analyzers) employed for these vapor determinations are still used for sensitive determinations of mercury volatilized from biodigests.

The general tendency of mercury to combine with sulfur groups has been implicated with many of its biological activities. In rats subjected to long-term exposure to inorganic mercury by injection or inhalation, the metal accumulates predominantly in the kidney complexed to metallothionein. Well supplied with sulfhydryl groups, the low molecular weight protein metallothionein also binds cadmium and zinc in the renal cortex [*90*].

The urine level of mercury is not always related to the appearance or severity of clinical symptoms [*91*], but once symptoms appear, the kidney usually excretes at least 150 ppb. Recent data [*92*] show that the maximum normal urine mercury is probably about 20 μg per 24-hour sample.

Reviewers of total mercury determination in biomaterials [*93–97*] frequently suggest that for mercury at the 5-ppb level the technique preferred for routine determination is flameless (cold vapor) AAS, preceded by wet oxidation with permanganate-persulfate and by mercury reduction with tin(II) chloride. Preconcentration of the mercury vapor on gold foil, etc., is recognized as a worthwhile improvement and oxygen combustion (as with the Schöniger flask) is sometimes favored for dry samples. Sample decomposition methods continually receive attention, and some recent improvements are noted below. The following discussions will emphasize "biosamples" rather than "environmental" analysis, although there is, of course, some overlap in methodology.

The formerly popular dithizone colorimetry was complicated, even in the relatively favorable case of mercury, by formation of dithizone adducts with undigested organomercurials, and variation in analyte speciation. Even with careful work, mercury concentrations below 100 ppb cannot be determined accurately by colorimetry.

Conventional flame AAS is not particularly sensitive for mercury; it is a factor of 10^5 less sensitive (without preconcentration) than the now familiar cold vapor methods. Early use of APDC-MIBK extraction of blood and urine digests gave detection limits only at about 10 ppb [*98*]. Vickers and Merrick [*99*] stated the detection limit for mercury in aqueous solution by flame AFS as 2 ppb. Taking advantage of the superior sensitivity of flame AAS for zinc as compared to mercury, Yamamoto et al [*100*] described an interesting indirect flame method for mercury. The mercury(II) bromo anion was selectively extracted by the bipyridyl-zinc chelate in 1,2-dichloroethane. Several cations and anions showed either positive or negative interferences. Thus, a biodigest, containing compensatory interferences, might be expected to have an easily corrected total specific interference.

Cold Vapor Techniques for Total Mercury Determination by AAS

Historical Notes—The great majority of mercury analyses are now conducted by the cold vapor method originally used with AAS by Poluektov et al [*101*]. These workers observed an increase in sensitivity when aspirating a mercury solution containing tin(II) chloride. They also observed that a flame is not necessary and that air bubbled through the solution after addition of the tin(II) would carry the mercury vapor through a drying tube and into a quartz gas absorption cell. Their study included optimization of parameters and demonstration of lack of specific interference among 15 cations. The detection limit of 0.5 ng has been difficult to surpass.

This cold vapor technique is often associated with Hatch and Ott [*102*], who utilized recirculation of the carrier air in a closed system for metallurgical, rock, and soil samples. Most recent cold vapor methods utilize a one-shot entrainment of the mercury from the reduction vessel, rather than recirculating the carrier air. Tin(II) chloride appears to be an efficient reductant, although hydroxylamine salts [*103,104*] or hydrazine hydrate [*105*] have been recommended either in conjunction with the tin(II) or alone. Lopez-Escobar and Hume [*106*] employed sodium borohydride after organodecomposition with ozone.

During the 1960's AAS determinations of mercury in blood [*107*] and in urine and hepatic and renal tissue [*108*] involved heated vaporization of the mercury from dithizone extracts of the sample digests. These systems included three or more traps for collecting interferences, and were lengthy procedures. One determination [*109*] even required a hydrochloric acid back-extraction of the dithizone extract as preparation for cold vapor aeration. However, the use of mercury vapor concentration meters in some of these determinations, even after the availability of atomic absorption spectrometers, was not necessarily a drawback in itself.

Representative Techniques—Representative of the techniques for urine mercury is the method of Lindstedt [*110*]. Digestion of a 1-ml sample (or standard) proceeded in a loosely stoppered vessel overnight at room temperature in a mixture of H_2SO_4-$KMnO_4$. Excess permanganate was decolorized with hydroxylamine hydrochloride. Then, immediately after introduction of tin(II) chloride solution, the digestion tube was fastened to the air bubbler. Absorption is recorded within a few seconds. Air was prefiltered through a tube containing active carbon and gold sand, and a cotton-wool plug in front of the absorption cell kept water from condensing in the cell. Lindstedt used a calibration curve for the range from 10 to 300 ppb. Factors influencing sensitivity were air velocity, sample volume, and temperature. Foaming in urine digests when aerating with a gas dispersion tube indicated incomplete digestion. Among about 25 substances tested, bromide and iodide were severe interferences. The detection limit was about 2 ppb with an RSD of 7% at 40 ppb. The blank was about 4 ng. Bubbling required only a minute per sample.

For total mercury in either urine or heparinized plasma, Kubasik et al [*92*] digested 0.5-ml samples with H_2SO_4-$KMnO_4$ overnight in an ice bath. Tin(II) chloride was then added to an aliquot of digest supernatant fluid. To reduce water vapor carry-out, the mercury vapor was swept from the reaction vessel by air passed over the reduction fluid rather than bubbling through it. Passage of the mercury-containing air through a tube of magnesium perchlorate further lessened water collection in the absorption cell. Absorbance was read at 253.7 nm. The authors state there was no background at this wavelength since absorbance could not be observed with a magnesium lamp at 285.2 nm. Recovery tests were performed on organomercury compounds labeled with ^{203}Hg.

"Normal" urines averaged 1.4 μg/24 h (with a range from 0 to 20). These urines all measured less than 20 ppb Hg. Normal plasmas averaged 1.1 μg/100 ml (range from 0.7 to 1.8). For individuals exposed to increased mercury the plasmas averaged 2.1 μg/100 ml (range from 0 to 9.4) and for urines, 81 ppb (range from 0 to 342).

Rains and Menis [*111*] found modifications were necessary in order to obtain satisfactory precision and accuracy in analysis of standard reference materials by the cold vapor technique. Factors studied included digestion, drying agents and traps, reductants, reaction volume, purge gas, interferences, and contamination.

A sample of about 2.5 g of liver was digested at 150°C in HNO_3-H_2SO_4 under reflux from a water-cooled Allihn condenser. After cooling and addition of an HNO_3-$HClO_4$ mixture, the digestion continued, this time with the condenser air-cooled, until dense fumes of perchlorate appeared. Cooling and rinsing of the condenser was followed by a second heating to perchlorate fumes. Use of either the Schöniger flask or of a sealed-tube technique was less satisfactory than acid digestion.

To trap vapors which could cause background absorption the authors tested several recommendations. Cold traps or drying agents were satisfactory only for short periods. The best technique was to pass the mercury vapor through Teflon® tubing directly to the absorption cell heated to 200°C. Correction for any remaining background absorption was by a ratio method using the aluminum line from the aluminum sleeve of the mercury hollow cathode lamp.

Additional studies showed that tin(II) chloride as a reductant was superior to either hydroxylamine hydrochloride or hypophosphorous acid. Furthermore, with a 125-ml reduction vessel, absorbance decreased for sample volumes over 30 ml. All eight anions investigated (especially iodide, bromide, sulfate, and phosphate) reduced the mercury absorption. Minor quantities of cations, however, had no effect.

At the 15-ppb level, mercury values in liver averaged 10% different from values obtained by neutron activation analysis (NAA). The RSD was 5% against 12% by NAA.

Background Correction—One of the primary concerns when using any of the vapor absorption techniques has been to eliminate or to correct for background absorption at the mercury line. It may be necessary to use both a physical separation of the nonspecific interferences and an optical compensation correction for the residual background. Removal of the background-causing particles or gases is attempted using cold traps, drying tubes, and absorbers. Alternatively, the mercury can be preconcentrated from the gas stream interferents before vaporization into the absorption cell. Mercury absorbers have included impregnated charcoal [*112*], palladium chloride on glass wool [*113*], a gold filter [*114*], or copper wire [*115*]. Palladium chloride was used by Windham [*113*] to remove mercury from the sample vapor prior to measuring background absorption. In the method of Lidums [*114*], combustion gases from dry-combusted organic samples are passed through a heated column of oxides of silver, manganese, and cobalt together with Na_2CO_3. This column completes the oxidation of gases from the initial combustion and also decomposes mercury compounds. Mercury is then collected by a gold filter attached to the column before being heat evaporated for cold vapor determination at 253.7 nm. The reported detection limit is 0.5 ppb for organic mercurials. No systematic difference was observed when compared with NAA.

Mercury also has been electrolytically deposited from solution to form an amalgam with copper [*116*], and it has been chemically reduced with hydroxylamine hydrochloride to form an amalgam with silver wire [*117*]. Detection limits for these techniques are in the low ppb range.

From fish digests, Cumont [*118*] compared electrodeposition of mercury on a platinum wire (followed by electric heating) with the $SnCl_2$ cold vapor technique. Sample oxidation was by HNO_3-H_2SO_4 with V_2O_5 catalyst at -75°C. In both cases liberated mercury was carried to the absorption tube by a nitrogen stream. The detection limit of the cold vapor method (8 ppb) was superior to that of the electrolysis method (12 ppb). Precision and analysis time also favored the chemical reduction procedure.

In order to obviate the need for a gas purging system, Deitz et al [*119*], after addition of the tin(II) chloride, extracted a 10-ml aliquot of head space vapor with a gas syringe for transfer to the absorption cell. The RSD was 47% at the 4-ppb level in beef.

Sample Decomposition—The major difficulty in trace mercury analysis is not a limitation of sensitivity, but rather the choice of procedure for sample decomposition and separation of mercury from interferences. Biosample decomposition methods for mercury analysis comprise a rather wide range of techniques. Dry ashing in either a closed system or in a stream of oxygen is recommended, as is acid digestion either in a tube or under reflux. Uthe et al [*104*] claim that carbon-mercury bonds are cleaved by treatment with H_2SO_4 at 55°C, followed by addition of $KMnO_4$. Some workers add persulfate for more complete oxidation. Recent wet ashing combinations include the catalyst V_2O_5 added to mixtures of HNO_3 and H_2SO_4 [*118,119*] or to mixtures of HNO_3 and $HClO_4$ [*120,121*]. Reduced temperatures are sometimes specified; Cumont [*118*] suggests -75°C. Deitz et al [*119*] state that digestion at 140°C in the HNO_3-H_2SO_4-V_2O_5 mixture is required for equally efficient recovery of methylmercury and "inorganic" mercury. These authors claim that covering or saturating the samples with HNO_3 before heating allows catalytic wet oxidation of biological materials with negligible loss of mercury. Also frequently suggested as preparation for mercury analysis of biomaterials is wet digestion in a closed Teflon® [*122,123*] or polyethylene tube [*124*]. Other recent wet digestions include HNO_3-HBr [*125*] and H_3PO_4-H_2O_2 [*126*]. Hydrogen peroxide is infrequently suggested, and use of 1*N* HCl for extraction of hair was found unsatisfactory [*127*]. Also unworkable was TCA precipitation of homogenized tissue followed by chelate-MIBK extraction [*128*]. Several literature statements note the inability of the particular acid treatments utilized to decompose fatty material.

Dry combustion has been favored by several workers. These techniques include furnace combustion at 800 to 1000°C [*128–130*] and use of an oxygen-filled Schöniger flask [*103*].

There would appear to be lack of agreement as to whether freeze-drying of biological matter is accompanied by less than 3% loss [*131*] or by more significant mercury loss [*132*].

In summarizing biodecomposition methods for mercury determination, Lamm and Růžička [*93*] briefly discuss wet decomposition of solid samples using an HNO_3-H_2SO_4 mixture in a modified Bethge apparatus. For liquid samples they suggest use of H_2SO_4-$KMnO_4$. For solid samples, however, the wet digestion drawbacks (difficulty in decomposing fat and possible high blank values) led the authors to prefer Schöniger flask combustion. Ultrapure oxygen is cheaper than ultrapure liquid reagents, and studies showed that mercury could be completely removed from the flask, even from the rubber expansion balloon.

There is room, of course, for personal bias in the art of sample decomposition. Liquid samples containing organomercury or functional groups (possibly remaining after standard wet digestion) which would reduce the volatility of sample mercury can be submitted to the digestion refinement of Lopez-Escobar and Hume [*106*]. In this procedure a stream of oxygen-ozone is bubbled through the sample prior to cold vapor determination of the mercury with sodium borohydride as the reductant. More than a dozen "organomercurials," including solutions of $HgCl_2$ in cysteine, cystine, or domestic beer, gave

essentially complete recovery of mercury by this ozonization-borohydride treatment. (There was no detectable mercury in beer.)

Stability of Mercury(II) Solutions—Nearly all mercury can be volatilized, even at room temperature, from solutions containing only traces of reducing species. This is due to evaporation of metallic mercury, formed either by stepwise reduction of mercury(II), or by disproportionation of mercury(I) complexes. Volatility losses can be reduced by the presence of an appropriate oxidant from among those having a greater oxidation potential than mercury(II). Losses of mercury from solution may also be due to volatility of $HgCl_2$, precipitation of basic salts, or adsorption onto vessel walls or onto undigested, refractory, or insoluble colloidal particles (for example, fat, silica, or $CaSO_4$). Retention by undigested complexing agents cannot be overlooked.

Syty [*21*] discussed the instability of aqueous standard solutions of inorganic mercury. "Unpreserved" solutions of mercury(II) in the ppb range show hourly decreases in concentration, whereas the presence of 5% nitric acid and 0.01% dichromate is reported to maintain full strength for several months [*133*]. For these solutions glass containers are much superior to vessels of either polyvinyl chloride or polyethylene.

Addition of known amounts of mercuric sulfate to dry gelatin produces a stable standard for flameless AAS of biological materials [*134*]. The gelatin standard might best be treated by the same decomposition method applied to the samples.

Auxiliary Equipment and Automated Methods—The commercially available flameless atomic absorption accessories and instruments for mercury analysis are listed in Ref *135*. Alternatively, several authors [*136,137*] describe construction from inexpensive and easily available equipment of attachments for commercial AAS units. Gilbert and Hume [*138*] describe an attachment providing one of the more favorable sensitivities reported recently for mercury analysis. Improvements consist of use of a reduction cell, the entire bottom of which is fritted for efficient gas dispersion; use of the rapidly acting reductant tin(II) chloride; and minimizing dead volume between the reduction vessel and absorption cell. An anhydrous magnesium perchlorate drying tube keeps water from condensing in the absorption cell. The detection limit is 0.04 ppb, with a standard deviation half that for the recirculation method of Hatch and Ott [*102*].

Methods for automated sample changing and purging have been described recently [*139-141*]. In the methods of Lindstedt and Skare [*140*] and of Skare [*141*], digests of urine (H_2SO_4-$KMnO_4$) or of blood (HNO_3-$HClO_4$) are analyzed in an apparatus consisting of an automatic sample changer, peristaltic pumps, and a spectrophotometer (or a simple mercury monitor). After completion of the purging, the mercury-free sample solution is pumped back from the reduction vessel to its original tube in the sample changer by reversal of the pumps. Thirty samples per hour can be analyzed without supervision. The detection limits are in the low nanogram range, and the RSD at the 100-ng level is about 2%.

Other Atomic Spectrometric Techniques

At least two considerations will encourage development of atomic fluorescence techniques for mercury determination. As with other emission measurements, fluorescence measurement enjoys the "emission advantage" [*94*, p. 44] in trace analysis. In addition, under some conditions the relative effect of molecular absorption interference is expected to be much lower in atomic fluorescence than in atomic absorption [*142*]. Thus, Thompson [*143*] presents AFS measurement of mercury using a solar-blind photomultiplier, which obviates the need for a monochromator. Thompson notes the effect of background due to organic solvents as less in the fluorescence mode than with absorption. Neither an enclosing cell nor a drying column is required. For a wide range of samples Muscat et al [*144*] used either reduction-aeration or combustion techniques for mercury

vapor generation, and a silver amalgamator for collection prior to AFS. The determination limit was 0.6 ng of mercury.

For significant improvement (compared to flameless AAS) in mercury detection limits at the 253.7 nm intercombination line, both Dagnall et al [*145*] and Lichte and Skogerboe [*146*] employed AES techniques. In the latter method, an obvious candidate for the mercury detection limit "prize," the authors use their microwave-induced plasma as an excitation medium for sample vapor purged with argon from a commercial cold vapor system. Conduction of the mercury as a vapor from the treated sample provides a somewhat fortuitously optimal sampling rate for maintaining a stable plasma. Problems with variation of background were reduced by a derivative spectrometric technique. With digests of blood and hair the detection limit is 60 pg; the RSD at 0.01 ppb of mercury is about 11%. Due to the need for excitation energy, AES methods are not, of course, cold vapor techniques.

Organomercury Determination

Selective Chemical Treatment—The organic mercury compounds, especially the alkylmercurials, are more toxic than other mercury species. The human body has an affinity for the alkylmercurials, and they easily pass the blood-brain and placental barriers. Since almost any form of mercury entering the aquatic environment can be biologically converted to methylmercury by either enzymatic or nonenzymatic methyl transfer [*94*], it may become necessary ultimately to control all forms of mercury pollution.

In order to separately determine inorganic mercury (including sulfhydryl mercury) and organomercury, Magos [*147*] and Magos and Clarkson [*148*] employed a differential reduction with the cold vapor technique. Reduction by tin(II) chloride in basic solution (probably stannite) was shown to release sulfhydryl mercury together with less than 5% of the methylmercury present. On the other hand, basic reduction with tin(II)-cadmium(II) chloride gave the total mercury determination due to the much more rapid mercury-carbon bond heterolysis. Determination of both inorganic and organic mercury could be performed on the same sample of 10% liver homogenate without prior digestive treatment. Cysteine, added to the acidified sample, retained mercury until addition of sodium hydroxide after addition of the appropriate reductant. In this method, however, ethylmercury underwent about 30% reduction, even by tin(II) alone.

Magos and Clarkson [*148*] report average blood mercury for ten hospital employees as 5.4 ppb (standard error or SE of 0.3) with organic mercury (probably methylmercury) accounting for about two thirds of the total. Among a group of Eskimos with moderate exposure to mercury in food, the total and organic mercury were both 40% higher. For the Eskimo group about 90% of the blood mercury was in the red blood cells (RBC's), with 85% of that being methylmercury (organic). A standard-addition technique was used; blank values ranged from 2.5 to 3.0 ng. The RSD's at the 4-ppb level were as follows: total mercury 0.6%, inorganic mercury 8.7%, and organic mercury 5.6%. Use of duplicates for comparison analysis with NAA showed a mean deviation between the two methods of 3.6% for whole blood at a concentration range from 10 to 50 ppb.

Gage and Warren [*149*] found the use of cysteine unnecessary for determining inorganic mercury if the tin(II) was added in basic solution before aeration. For three fungicides, however, they observed that for methoxyethylmercury and ethylmercury chlorides, prior treatment with acid cysteine solution achieved over 95% recovery of mercury when using basic tin(II) chloride as the sole reductant. Apparently, formation of a cysteine adduct brings organomercury into the aqueous phase of the homogenate for ready attack by the reducing agent. Phenylmercury, however, was more resistant to breakdown by the cysteine-tin(II) treatments and, according to Magos [*147*], methylmercury by this treatment is quite refractory to liberation.

Chromatographic Determinations—The reader is referred to Fishbein [*150*] for a review of various chromatographic techniques for biomercury. Westöö [*151*] optimized recovery of methylmercury from liver by the "cysteine acetate method" for gas chromatography (GC) determination. By her initial procedure the supernate of liver homogenate, after protein precipitation with molybdic acid, was mixed with an HCl-NaCl solution prior to benzene extraction. Then back-extraction with cysteine acetate in saturated Na_2SO_4 was followed by reextraction with benzene after acidifying with HCl.

Giovanoli-Jakubczak et al [*152*] presented a modification of the GC procedure of Westöö to validate the determination of organomercury in hair by the cold vapor AAS method of Magos and Clarkson [*148*]. Methylmercury was determined by electron capture after separation on a column of 2% polyethylene glycol succinate on Chromosorb G® (a diatomaceous-earth GC support). The first 6 cm of the column were packed with finely powdered potassium iodide. The authors state that digestion of hair by heating at 125°C in saturated NaOH liberates but does not decompose methylmercury. Cysteine, however, was required in standard solutions of methylmercury to prevent decomposition of the organomercury during the alkaline digestion. Organic mercury calculated from AAS data compared well with methylmercury determined on the same samples by GC. The RSD by GC was 4%.

The investigation of hair-washing procedures as well as the performance of recovery studies for the digestion and extraction steps utilized mercury-203. Increase of mercury concentration from the base to the tip of the hair was interpreted as showing the importance of mercury contamination on hair surface. Thus, samples were washed with a commercial non-ionic detergent before digestion.

In one individual a segment analysis of hair correlated well with personal exposure of the individual to excess mercury. The total and inorganic mercury changed in parallel.

According to Hartung [*153*], under the acidic conditions of the Westöö procedure [*151*], any dimethylmercury present will dissociate. If, however, one initially adds cysteine-borate buffer at pH 8.2, dimethylmercury is stabilized and available for extraction by toluene. Monomethylmercury, on the other hand, forms a water-soluble cysteine adduct, from which monomethylmercuric chloride can be released for toluene extraction by addition of hydrochloric acid. Conversion of dimethylmercury to monomethylmercuric bromide by reaction with $HgBr_2$ improves the sensitivity of the electron capture detection, due to the strong electron affinity of the halide.

Ealy et al [*154*] provide a GC identification of methoxyethylmercury, methylmercury, and ethylmercury chlorides from biological material leached for 24 hours with 1*M* NaI. An aliquot of the benzene extract was injected onto a column of 5% cyclohexanedimethanol succinate on Anakrom ABS® (a diatomaceous-earth GC support).

Tanaka et al [*155*] employed papain or pepsin digestion of fish homogenates, followed by continuous extraction of methylmercury with benzene in a reflux extractor. Continuation with the acetate method was followed by GC determination with 93% recovery.

Gonzalez and Ross [*156*] combined GC with mercury vapor detection by atomic absorption after furnace evaporation. This approach appeared faster and simpler to the authors than the extensive cleanup procedures used with electron capture.

Jensen and Jernelöv [*157*] utilized gas chromatography/mass spectrometry (GC/MS) to determine methylmercury halides at the 50-pg level.

For TLC analysis Tatton and Wagstaffe [*158*] optimized parameters for separation of various organomercurial fungicide residues as dithizonates. Best resolution was in hexane:acetone (93:7) on silica gel. As little as 2 μg could be detected, with about 90% recovery claimed for the 0.01 to 5-ppm range.

Alternative Techniques for Total Mercury Determination

Several alternatives to NAA and colorimetry are available for comparison with AAS

and other atomic spectrometric methods for mercury. Allen and Johnson [*159*] used stripping voltammetry at a rotating ring-disk electrode. The electrode consisted of a platinum ring and a glassy carbon disk. An electroplated layer of gold on the electrode provided best results: a detection limit of 0.01 ppb with an RSD of 7.5% at 0.1 ppb.

Kinetic methods have attracted attention. Ke and Thibert [*160*] took advantage of the mercury inhibition of the iodide catalysis of the ceric-arsenite system. Rohm et al [*161*] developed for biodigests a coulometric titration to monitor a ligand-exchange reaction labilized by trace mercury. Cyanide in ferrocyanide is replaced by orthophenanthroline in the presence of microgram quantities of mercury. Liberated cyanide is then titrated with electrogenerated iodine by constant-current coulometry. The authors suggest a practical range from 0.1 to 1.0 μg of mercury.

For samples down to 0.1 ppb of mercury, Růžička and Lamm [*162*] recommend their own automated substoichiometric radioisotope dilution method with either Schöniger or permanganate decomposition and with preconcentration by dithizone extraction.

Mercury Content of Tissues

The recent literature on the clinical toxicology and pharmacokinetics of mercury contains much of forensic interest. Friberg and Vostal [*163*] discuss transport, metabolism, toxicology, and normal tissue values of mercury. Iverson et al [*164*] present mercury decay profiles in the guinea pig. Norseth [*165*] shows that the rat biliary excretion of mercury is independent of the mercury dose, even for doses differing by several orders of magnitude. Dinman and Hecker [*166*] attempt to correlate human blood and hair values for mercury intoxication.

Space permits only a brief discussion of several recent normal values, all obtained by AAS methods. Yamamura et al [*167*] list normal total mercury of blood and urine as 9.0 ppb (±6.0) and 3.5 ppb (±2.3), respectively. In a thermometer factory, 26% of the workers had a blood level greater than 300 ppb. Two of these subjects showed chronic mercurial poisoning symptoms. Sumari et al [*168*] state that the "critical zone" for whole blood is 200 ppb and for hair 60 ppm. One tenth of these values, they claim, should consitute the "safety limits." In Americans, however, Gutenmann et al [*169*] obtained a normal hair range from 0.01 to 2.5 ppm. Of the dentists' hair samples tested, 89% were above this range.

For human autopsy tissue values Mottet and Body [*170*] used a typical cold vapor AAS technique, preceded by investigation of digestion methods with care in obtaining proper recovery values. Most tissues contained about 250 ppb of mercury, with liver and kidney containing several times higher amounts. The whole blood content was about 25 ppb.

Cadmium

Recent reviews of cadmium in human health include those by Friberg et al [*171*] and Lee [*172*]. Ullucci and Hwang [*173*] have summarized some toxicological problems and recent analytical methods for biocadmium analysis.

Factors giving cadmium one of the higher sensitivities by conventional flame AAS are the intense, stable, hollow cathode lamps available and the relatively efficient cadmium atomization in the air-acetylene flame. Sensitivity in aqueous solution by flame aspiration with ordinary equipment is in the low ppb range. An oxidizing, nonluminous flame should be used for cadmium. Direct aspiration of urine may be satisfactory for monitoring workers exposed to cadmium dust since levels may be 600 ppb.

A toxicological survey of cadmium by flame AAS, including anion exchange preconcentration from blood or urine, is described by Vens and Lauwerys [*174*]. Urinary

cadmium from workers in a cadmium plating factory showed a fourfold increase over normal.

The concentration of "normal" urine cadmium in Japan by flame AAS is about 2 ppb, as determined after HNO_3-H_2SO_4 digestion and extraction into MIBK as the chelate of APDC [*175*]. In the hands of Holroyd and Snodin [*176*], employment of APDC at pH 4.5 (but without digestion) gave a urine cadmium detection limit of 1 ppb.

For flameless atomization of biological samples, the literature shows competition among several techniques for cadmium, a somewhat volatile metal. Kubasik and Volosin [*79*] thoroughly investigated the carbon rod atomizer for cadmium, lead, and thallium determinations on biological fluids. Use of diethyldithiocarbamate-MIBK preconcentration reduced both nonatomic absorption and specific interferences. Standard additions were employed to further minimize interferences.

For the Massmann graphite furnace, Ediger [*177*] suggests "matrix modification" for cadmium analysis. Thus, a higher charring temperature is permitted if the cadmium is stabilized by addition of either $(NH_4)_2HPO_4$ or $(NH_4)_2SO_4$. Furthermore, addition of ammonium nitrate reduces the scattering by sodium chloride to the level correctable by a background corrector.

Robinson et al [*178*] demonstrate a carbon bed atomizer on which 4 μl of a 1:10 dilution of urine is heated to about 1400°C by a radiofrequency generator. The detection limit was 0.1 pg of cadmium. Metallic interferences, however, were not tested.

Delves cup and sample boat techniques satisfy the needs of some workers for cadmium analysis. Ulander and Axelson [*179*], using the Delves cup, found a difference in blood cadmium between smokers and nonsmokers. Variability in the technique required obtaining a mean of five determinations on each sample. Nonsmokers' blood levels were at about 0.8 ppb, while smokers' values ranged up to seven times that amount. Interferences were not examined.

Hauser et al [*180*] used vacuum-oven drying and low temperature ashing prior to analyzing cadmium and lead in blood by tantalum boat. For cadmium a background corrector was essential. No data were presented for the variation of absorption with varying interferences.

For both cadmium and lead, Newton et al [*181*] use a tungsten alloy wire loop soaked in the sample solution for a specified time. After accumulating analyte, possibly by ion exchange, the loop functions as an electrically heated atomizer. The detection limit was 0.01 pg of cadmium.

Ullucci and Hwang [*173*] took advantage of both conventional flame and flameless AAS to determine cadmium in human biosamples. Acidified urines were aspirated directly into the flame using standard additions. Automatic background correction, scale expansion, and signal integration made possible direct determination of cadmium in urine. "Normal" urine cadmium was 4 ppb.

Blood and other tissues were analyzed with the tantalum ribbon flameless sampler after sample treatment. One tenth of a millilitre of heparinized blood, containing additions of formamide and saponin, was extracted by APDC-MIBK in a procedure similar to that used for lead at the same laboratory [*85*]. "Normal" blood averaged 3 ppb. Hair, nails, and tissue were digested by successive additions of nitric acid and 10% hydrogen peroxide at 85°C. In hair thoroughly washed with Triton X-100® the cadmium ranged from 1 to 5 ppm. The kidney and liver values were in the ppm range and varied significantly within the same organ sample, depending on the section of organ sampled.

Sensitivity by tantalum ribbon was 4 pg. This posed some contamination problems. Polypropylene tubes were far less contaminating than glass, but nevertheless required cleaning, preferably in 5% Triton X-100® solution rather than in nitric acid.

Colorimetric determination of cadmium by dithizone chelation has been optimized by Yamamoto et al [*182*] with respect to solvent extraction into nitrobenzene. Another colorimetric procedure [*183*] involving pyridilazonaphthol and requiring much effort at masking interferences, points to one of the well-known advantages of AAS techniques.

Cadmium is analyzed favorably by ASV at about 10 ppb. Sinko and Gomiscek [*184*] report its determination in 0.5 ml of blood serum after digestion with 0.5 ml of HNO_3:$HClO_4$ (3:1) in a Teflon® autoclave. Cadmium in bovine liver also yields results by ASV in the ppb range [*185*].

Other tissue cadmium studies include analysis in human autopsy tissue, classified by age and disease category [*186*]; analyses on kidney [*187*]; and analyses on hair in 13 U.S. cities [*188*].

Storage in formol of kidney or liver from cadmium-treated rabbits is stated [*189*] to cause elution in one to ten weeks of up to 10% of the tissue cadmium.

Thallium

Although formerly used in dermatology, thallium enters toxicology mainly as a rat bait (thallous sulfate). Curry et al [*190*] reviewed analytical methods for thallium in biomaterials.

For whole blood, Berman [*98*] preferred extraction of a TCA centrifugate with NaDDC-MIBK at pH 6.0 to 7.5. Standards were extracted similarly for comparison in an oxidizing air-acetylene flame at 276.7 nm. The detection limit was 5 ppb. With urine the extraction was performed after pH adjustment, while tissue and hair received HNO_3-$HClO_4$ digestion prior to extraction.

Several workers [*190–193*] utilized the selective extractability of thallium(III) halide for preconcentration prior to flame photometry. From a biodigest (HNO_3-H_2SO_4-$HClO_4$) Miniussi [*193*] extracted the bromo complex into ethyl amyl ketone. The extraction was not pH dependent and produced an extract stable for 125 h.

By flame AAS, Curry et al [*190*] report a detection limit of 40 ppb in NaDDC-ketone extracts of biodigests or TCA supernates. These workers used the 276.8-nm line with an air-acetylene flame.

The literature notes [*190,194*] that several metals and anions give thallium an enhancement interference, which is suggested as being physical in nature.

With AES, Rains [*195*] reports a detection limit for thallium of about 2 ppb without preconcentration. By flame AFS (argon-hydrogen-entrained air), the detection limit for thallium at 8 ppb [*196*] is exceeded significantly only by cadmium and zinc.

In the area of flameless AAS, Curry [*190*] was one of the first workers in toxicology. His work with the tantalum microsampling boat for thallium analysis showed the advantage of the small samples required. With a heated graphite atomizer Machata and Binder [*83*] found interference from iron in blood samples when analyzing thallium, lead, and cadmium.

Using the CRA, Kubasik and Volosin [*79*] found extraction of urine necessary to reduce nonatomic absorption to acceptable levels. A 3-ml sample was adjusted to pH 3 to 7 for extraction with NaDDC-MIBK. The authors state that sensitivities for thallium, lead, and cadmium using either direct urine or ketone extracts are lower than when vaporizing aqueous standards. The standard-addition method was used. From any of nine excess normal urinary constituents, thallium was recovered at 98 to 105%. Thallium added to urine at 30 ppb was recovered in the range from 80 to 123% (mean 99.5%). The RSD was 3.4%. Blank values were not reported; thallium was not detected in normal urine. A response could not be obtained for thallium using a "fresh" carbon rod.

Employing a graphite rod atomizer and thermostatted EDL's, Patel et al [*197*] could find neither spectral nor chemical interferences for thallium by flameless AFS.

Recent interest in colorimetric determinations of thallium involves Victoria Blue B [*198*] and malachite green, etc. [*199*] complexed with thallium(III).

Christian and Purdy [*192*] reported a coulometric titration of thallium from evaporated extracts of biodigests. Reduction with sulfite is followed by titration with coulometrically generated bromine. The RSD was 7% at 80 μg of thallium in blood.

The direct determination of thallium in urine by a-c ASV at the hanging mercury drop electrode is reported by Levit [*200*]. Reproducibility was claimed as 2.5% for 50 ppb in urine.

Berman [*98*] relates several toxicological cases. A two-year-old male with marked ataxia and hair loss had a blood thallium level of 300 ppb and a urine thallium level of 1240 ppb. His three-year-old sibling had a similar blood level, with about 300 ppb in the urine. A 9-year-old female with ataxia and marked hair loss had a blood thallium level of 900 ppb and a urinary excretion of 700 ppb. Another patient complaining of excess hair loss exhibited a urine thallium level of about 35 ppb.

Arsenic

Arsenic is probably the most extensively distributed elemental poison. It has had much agricultural, industrial, and medicinal use as an insecticide, rodenticide, herbicide, or defoliant; a constituent of alloys, glasses, pigments, and dyes; a medicinal for humans and animals; and a growth promoter in livestock. Organic arsenic, excreted largely in the bile, is generally less toxic than inorganic arsenic, excreted in both the feces and urine. Arsenic(III), by blocking sulfhydryl groups, has generally been considered more acutely toxic than arsenic(V). Both oxidation states are found in biological material. Most human food contains less than 1 ppm of arsenic; but the Crustacea (lobsters, crayfish, etc.) can concentrate the element to 100 ppm or more. The carcinogenic hazard of industrial exposure to inorganic arsenic is gradually being recognized [*201*].

The resonance lines of arsenic occur in the far ultraviolet (UV) region, where much of the source radiation is absorbed by the air and by flame gases. In addition, many photomultiplier tubes are less sensitive in the far UV region. To overcome these difficulties Menis and Rains [*202*] recommend using an argon-hydrogen-entrained air flame, and an EDL as the source. In this way they detected 0.1 ppm at the 193.7-nm line.

Although arsenic(III) can be extracted by APDC for either flame or flameless atomization, much recent analysis of arsenic, antimony, bismuth, selenium, etc. involves generation of the volatile hydrides. Syty [*21*] gives an extensive review of the hydride generation technique. Holak [*203*] was apparently the first to use HCl-KI-$SnCl_2$-Zn for generation of arsine in analysis. After its formation in the generation vessel the arsine (or other volatile hydride) can be concentrated by freezing [*203*], swept directly into the flame, precollected in a balloon, or chemically trapped for solution-aspiration into the argon-hydrogen-entrained air flame. Chu et al [*204*] carried the generated arsine by a flow of argon into an electrically heated absorption cell in which atomization occurred at 700°C. This allowed an increase in sensitivity over the argon-hydrogen-entrained air aspiration by a factor of two.

For blood and hair, Lichte and Skogerboe [*205*] used the microwave-induced plasma for emission analysis. Their generator consists of a column of granular zinc through which the carrier gas flows and into which the sample is injected after pretreatment with $SnCl_2$. This reduction technique saves considerable time. The detection limit was reported as 5 ng of arsenic.

For speedier analysis and for lower blanks, many analysts are now reducing the arsenic with sodium borohydride [*206*]. Use of borohydride generation, balloon reservoir collection, and argon flushing of the arsine to an argon-hydrogen-entrained air flame is reported to give detection limits as follows: arsenic 0.15, antimony 0.25, and bismuth 0.25 ppb, with RSD less than 7% [*207*].

In the field of flameless atomizers a tantalum boat was recently used [*208*] for AAS analysis of several elements, including arsenic, bismuth, cadmium, and thallium. Fernandez et al [*209*] suggest for flameless atomization that addition of salts of cadmium, copper, cobalt, nickel, or zinc to the sample constitutes a matrix modification which decreases the volatility of arsenic by forming arsenides.

Investigators continue to recommend colorimetric or nonoptical methods for arsenic. Myers and Osteryoung [*210*] determined arsenic(III) by differential pulse polarography. The detection limit was 0.3 ppb with 1*M* HCl as the supporting electrolyte. Arsenic is determined kinetically [*211*] by oxidation with bromine. With a visual or potentiometric end point the detection limit is 5 ppb of arsenic(III).

For colorimetric determination Kauffman and Sunderman [*212*] have digested with a $MgO\text{-}Mg(NO_3)_2$ slurry and added hydrochloric acid to liberate arsine, which is absorbed into silver diethyldithiocarbamate solution to form a red complex. The practical range was from 0.25 to 1.0 ppm.

It should be remembered that significant amounts of arsenic may be lost by dry ashing, even below 500°C [*213*]. Simon et al [*214*] have shown that use of molybdenum(VI) as a catalyst in the $HNO_3\text{-}H_2SO_4\text{-}HClO_4$ digestion mixture for urine maintains strongly oxidizing conditions to prevent loss of volatile $AsCl_3$. In addition, digestions are much speedier when using the catalyst. Griffin et al [*201*] provide suggestions for performing perchloric acid digestions. They also list references to the statistical evidence for the carcinogenic character of inorganic arsenic.

Christian and Feldman [*3*, p. 418] discuss normal arsenic ranges for human tissues.

Of therapeutic and forensic interest, arsenic in hair was correlated with known therapeutic amounts of Fowler's solution (potassium arsenite) [*215*].

Bismuth

Inorganic bismuth is occasionally used as an antacid. Bismuth compounds are still used extensively in veterinary medicine. Although bismuth is not present in newborns, Tipton [*216*] found small amounts in some samples of human tissue. Values of bismuth in the kidney and lung ranged up to several hundred ppm, but the median for all tissues examined was less than 1 ppm.

For bismuth the flame absorption is critically dependent on flame type and ratio of fuel to oxidant [*3*]. Use of an air/coal-gas flame, aspiration from solutions containing acetone, and an EDL source have all been stated to increase bismuth absorption.

Nakahara et al [*217*] showed that when using an inert gas-hydrogen-entrained air flame, most of the elemental interferences for bismuth could be eliminated by addition of 2000 ppm of magnesium (as the chloride) or 0.1*M* ammonium salts (chloride or perchlorate).

Hall and Farber [*218*] determined bismuth residues in dog tissues and fluids by AAS after acid digestion. The greatest accumulation of administered doses was in the kidney.

Woodward [*219*] described use of an EDL in AAS determination of bismuth and arsenic.

Volatile hydride generation for bismuth by AAS has employed either $TiCl_3$-Mg [*220*] or sodium borohydride [*206*] for reduction.

Berman [*13*] uses dithiocarbamate extraction to attain with the graphite furnace a detection limit of 10 ppb in blood or urine. Blood is precipitated by TCA, while urine is extracted directly.

Several determinations for bismuth by ASV have appeared recently. Mal'kov et al [*221*] analyzed bismuth in 1 ml of ashed blood using a mercury-plated graphite electrode. Greater than 50-fold excess of copper required dithizone extraction. Sensitivity was 10 ppb with an error of ±15%.

Florence [*222*] studied ashing procedures for oysters, etc. prior to determination of bismuth, antimony, lead, cadmium, thallium, etc. by ASV. Volatility losses were not

observed when ashing was performed in either a muffle furnace at 450°C, in an oxygen-plasma furnace at 200°C, or in HNO_3-$HClO_4$.

Recent colorimetric methods for bismuth include the use of thiothenoyltrifluoroacetone [*223*] or trihydroxyfluorones [*224*].

Beryllium

Toxic in extremely small amounts, beryllium is of concern to workers in manufacturing processes using this metal. Berylliosis, tumorogenicity, rickets, and enzyme inhibition have resulted from exposure to the metal.

Due to formation of refractory oxides, beryllium gives little absorption in the conventional air-acetylene flame. Ramakrishna et al [*225*], working with the N_2O-C_2H_2 flame and with solutions containing 10% diethylene glycol diethyl ether, obtained a sensitivity of 25 ppb. The determination was essentially interference-free.

Beryllium, however, is an element demonstrating the advantage of the Massmann tube. In biological samples the sensitivity is 2 to 10 ng [*226*], a significant improvement over conventional air-C_2H_2 flame aspiration. Even lower detections have been reported using extraction techniques and the graphite furnace.

A nitrogen-sheathed N_2O-C_2H_2 flame has been useful for determination by AFS of metals, such as beryllium, which form refractory oxides and have resonance lines below 300 nm [*227*].

From blood, tissue, etc., beryllium has been determined by gas chromatography as the trifluoroacetylacetonate chelate using electron capture detection [*228*]. Samples were subjected to either low temperature ashing or to pressure decomposition with an HNO_3-HF mixture in a Teflon® tube. The detection limit was stated as 0.01 ppb. Interfering elements were masked with EDTA.

A molecular fluorometric determination of beryllium can proceed from complex formation with hydroxy derivatives of chromone in CCl_4. The practical range is from 0.01 to 0.25 μg of beryllium [*229*].

Boron

For boron with a high temperature flame (N_2O-C_2H_2) the sensitivity is about 100 ppm. Using flame absorption, Luecke [*230*] noted improved sensitivity and noise levels with the NO-C_2H_2 flame Alder et al [*231*] recommend AES in the secondary interconal region of a fuel-rich O-Ar-C_2H_2 flame for boron and elements forming refractory oxides.

Hayashi et al [*232*] performed an indirect determination of boron by extracting tetrafluoroborate into nitrobenzene as an ion pair with tris(1,10-phenanthroline)cadmium(II). Boron is measured at the cadmium absorption line with a sensitivity of 5 ppb.

In animal tissue the colorimetry of boron, according to Mair and Day [*233*] should start with combustion in a low temperature, radiofrequency-excited oxygen plasma (low temperature asher). Extraction of the acidified digest with $CHCl_3$ containing 10% 2-ethyl-1,3-hexanediol is followed by formation of the curcumin complex. The error is about 10% in the range from 2 to 20 ppb.

Fisher [*234*] has reviewed the toxicology of boron.

Summary

A selective and critical review, mainly from 1971 to 1974, of atomic absorption applied to the determination of eight toxic metals (lead, mercury, cadmium, thallium, arsenic, bismuth, beryllium, and boron) in blood, urine, and tissue is presented. Discussion involves both flame and flameless atomization, sample preparation, matrix modification,

background correction, and contamination. Advantages of other atomic spectrometric techniques and use of recent confirmatory instrumental methods are included. Some normal and toxic levels are mentioned.

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