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Editorial

Trace Element Estimation – Methods & Clinical Context

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Abstract: Understanding the effects of trace metals on human health is as complex as it is fascinating. As mentioned earlier, the high concentrations may prove toxic, as also, depletion in the concentration of the essential trace elements may cause various metabolic instabilities due to enzyme dysfunction. In the era of rapid industrialization and technological advances, it is imperative to watch keenly for contamination of the environment and its vital composition from heavy metal wastes emanating out of industries. Many metabolic disorders in man are accompanied by alterations in the concentration of one or more trace elements in some body fluid, especially blood serum or plasma It is thus important to update ourselves with various techniques available for such determinations, their operational aspects, advantages / disadvantages etc. More recently, element analysis from hair and nail has been stated as the best indices of such contamination and has also been discussed in this article.

Key Words: Atomic Absorption Spectrophotometer, ICP-MS, Stripping Voltameter, Colorimetry, Electro Thermal Vaporization, Neutron Activation Analysis, Laser Microprobe Mass Analysis

Introduction

Several metal ions such as sodium, potassium, magnesium, and calcium are essential to sustain biological life. At least six additional metals, chiefly transition metals are also essential for optimal growth, development, and reproduction, i.e. manganese, iron, cobalt, copper, zinc, and molybdenum.

An element, which is required in amounts smaller than 0.01% of the mass of the organism, is called a trace element. Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all essential trace metals become toxic when their concentration becomes excessive. Usually this happens when

the levels exceed by 40-200 fold those required for correct nutritional response. In addition to the metals essential for human life, our diet including the water we drink and the air we breathe may contain toxic metals like mercury, lead, cadmium, chromium, silver, selenium, aluminium, arsenic, and barium. These metals can cause chronic or acute poisoning and should be eliminated as much as possible from the living environment.

Understanding the effects of trace metals on human health is as complex as it is fascinating. As mentioned earlier, the high concentrations may prove toxic, as also, depletion in the concentration of the essential trace elements may cause various metabolic instabilities due to enzyme dysfunction. Equally, industrial-based metallic contamination of the air, soil, and water supplies can have a dramatic impact on our well-being. Added on to this is the toxic accumulation of these elements due to the intake of various drugs.

In recent years, awareness that trace elements play a very important role, either beneficial or harmful, in human health has increased. Many metabolic disorders in man are accompanied by alterations in the concentration of one or more trace elements in some body fluid, especially blood serum or plasma.(1) Interest in trace-element research in clinical medicine, biology, environmental studies, toxicology, and nutrition has become an exciting frontier, and during the last two decades the number of publications on this subject has progressively increased. Recent developments in instrumentation have lowered the limits for determining many trace elements to the low nanogram or even picogram range, thus enabling determination of parts per billion (ng/g) and, in some cases, even less.(2) The present needs for trace measurements and techniques for micro- and sub microanalysis are already substantial, and recent reports indicate that the requirements and demands for such capabilities will increase considerably.(3-6)

Because most essential trace metals are present in biological specimens in very low concentrations(7), precise and accurate analysis is most essential if meaningful results are to be obtained. Trace-element supplementation is becoming widely used for patients undergoing total parenteral nutrition therapy. Monitoring these patients for the elements has been recommended, but resource restrictions and analytical problems, particularly those related to contamination, prevent adoption of such programs.

Specimen Collection: Analyses for trace elements in biological fluids are uniquely susceptible to extreme errors unless special precautions are taken during collection, storage, and analysis. The integrity of the specimen may be compromised before it is analyzed, by contamination during collection and processing or by attenuation of the analyte concentration during storage. If this happens, determined values are not valid even though the method of analysis is extremely sensitive and highly accurate. Obstacles to obtaining precise and accurate analytical data arising from these factors are discussed. Control procedures applicable at all stages for ascertaining the sources of error and eliminating them should be considered.

In recent years, awareness that trace elements play a very important role, either beneficial or harmful, in human health has increased. Many metabolic disorders in man are accompanied by alterations in the concentration of one or more trace elements in some body fluid, especially blood serum or plasma. Interest in trace-element research in clinical medicine, biology, environmental studies, toxicology, and nutrition has become an exciting frontier, and during the last two decades the number of publications on this subject has progressively increased. Recent developments in instrumentation have lowered the limits for determining many trace elements to the low nanogram or even picogram range, thus enabling determination of parts per billion (ng/g) and, in some cases, even less. The present needs for trace measurements and techniques for micro- and sub microanalysis are already substantial, and recent reports indicate that the requirements and demands for such capabilities will increase considerably. Because most essential trace metals are present in biological specimens in very low concentrations, precise and accurate analysis is most essential if meaningful results are to be obtained. In this context, the various factors that influence the precision and accuracy of trace-metal analysis must be identified and controlled. Contamination and the stability of standards and controls are among the more important of these factors.

The reliability of any analyte determination is affected by the extent of contamination during collection, containment, processing, and analysis of the specimen. It is also influenced to a considerable extent by the accuracy with which a value may be assigned to the standards and (or)

controls and the stability of the specimens, standards, and controls during containment. A general discussion of the techniques and methods for preventing contamination may be found in the literature. Most practical information and the descriptions of useful techniques, however, are widely scattered and not readily accessible.(8)

Methods and Estimations: The development of analytical instrumentation over the past 30–40 years has allowed us not only to detect trace metals at the parts per quadrillion (ppq) level, but also to know its valency state, biomolecular form, elemental species, and isotopic structure. Lead was the most commonly studied of all the trace elements and the techniques that developed early in time are mostly described on the basis of their lead estimation capacities. As recently as the early 1960s, trace elemental determinations were predominantly carried out by traditional wet chemical methods such as volumetric, gravimetric, or colorimetric assays. It wasn't until the development of atomic spectroscopic (AS) techniques, in the early to mid-1960s, that the clinical community realized that they had a highly sensitive and diverse trace element technique that could be automated. Every time there was a major development in AS, trace element detection capability, sample throughput, and automation dramatically improved.(9) The developments and recent breakthroughs in atomic spectroscopy have directly affected our understanding of the way trace metals interact with the human body. The major element studied in humans was lead for a long time and various methods were devised for this purpose.

Lead assays were initially carried out using the dithizone colorimetric method, which was sufficient for the time (late 1950s, early 1960s), but was very slow and labor-intensive. It became more automated with the development of anodic stripping voltammetry (9), but blood lead analysis was not considered a truly routine method until AS techniques became available.

When flame atomic absorption (FAA) was first developed, an elevated blood lead level was considered to be 60 µg/dL (600 ppb), well above the FAA detection limit of 2 µg/dL (20 ppb) at the time. But when preparation of the blood samples was taken into consideration, FAA struggled to meet this level. Preparation typically involved either dilution with a weak acid followed by centrifuging and filtering, acid digestion followed by dilution and centrifuging and filtering, or, more recently, dilution with a strong base such as tetramethylammonium hydroxide (TMAH). When sample preparation was factored into the equation, the elevated blood lead concentration of 60 µg/dL was reduced to 2–5 µg/dL (20–50 ppb)—virtually identical to the FAA detection limit.

An accessory called the Delves Cup was developed in the late 1960s to improve the detection limit of FAA.(9) The Delves Cup approach used a metal crucible or boat usually made from nickel or tantalum, which was positioned over the flame. The 10-100µL sample was pipetted into the cup and the heated sample vapor passed into a quartz tube, which was also heated by the flame. The ground state atoms generated from the heated vapor were concentrated in the tube and therefore remained in the optical path for a longer period of time. This resulted in much higher sensitivity and lower detection capability, which meant that the elevated blood lead level of 60 µg/dL could be detected with comparative ease. Because of its relative simplicity and low cost of operation, the Delves Cup became the standard method for carrying out blood lead determinations for many years.

Unfortunately, the Delves Cup approach was found to be very operator-dependent and not very reproducible; sometimes, it involved complicated sample preparation and required calibration with blood matrix standards. For these reasons, the approach became less attractive after the commercialization of Electro Thermal Atomization (ETA) in the early 1970s. This new approach offered detection capability for lead of ~0.1 ppb — 200 fold better than FAA. However, its major benefit for the analysis of blood samples was the ability to dilute and inject the sample automatically into the graphite tube with very little off-line sample preparation. In addition, because the majority of the matrix components were "driven-off" before atomization at 2700°C, interferences were generally less than with the Delves Cup, which used a much cooler acetylene flame to generate the atoms. This breakthrough meant that blood lead determinations, even at extremely low levels, could now be carried out routinely in an automated fashion.

The next major milestone in AS was the development of Zeeman background correction (ZBGC) in 1981, which compensated for nonspecific absorption and structured background produced by complex biological matrices like blood and urine. In conjunction

with the stabilized temperature platform furnace (STPF) concept, ZBGC allowed for virtually interference-free graphite furnace atomic analysis (GFAA) of blood samples.(9) The success of the ZBGC/STPF approach, primarily due to the fact that it could analyze many different kinds of samples using simple aqueous standards, launched it as a method for analyzing most types of complex matrices by GFAA.

Although GFAA had been the accepted way of doing blood lead determinations for more than 15 years, the commercialization of inductively coupled plasma–mass spectrometry (ICP–MS) in 1983, gave analysts a tool that was not only 50–100 times more sensitive, but suffered from less severe matrix-induced interferences than GFAA. In addition, ICP–MS offered multielement capability and much higher sample throughput, making it very attractive to the clinical community. It must be emphasized that these are approximate aqueous lead detection limits and are shown for comparative purposes. They do not represent detection levels achievable directly in the blood.

An added benefit of the ICP–MS technique is that it also offers isotopic measurement capability. This is a very attractive feature to many clinical labs, because it gives them the ability to carry out isotopic tracer, dilution and ratio (9) measurements, which are beyond the realms of the other traditional AS techniques. In fact, the isotopic measurement capability allows researchers to actually pinpoint the source of lead poisoning by comparing the isotope ratios of blood lead samples with those of possible sources of lead contamination. The principle behind this approach, known as isotopic fingerprinting, is based on the fact that lead has four naturally occurring isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. Thus, when lead is ionized in the plasma, it generates four ions, all with different atomic masses. All the lead isotopes, with the exception of ^{204}Pb, are radiogenic—the products of radioactive decay of either uranium or thorium. Therefore, their relative abundance varies depending on the rock type and geological area. This means that in all lead-based materials and systems, ^{204}Pb is the only isotope that has remained essentially unchanged at 1.4% since the earth was first formed. The ratios of the isotopic concentrations of ^{208}Pb, ^{207}Pb, and ^{206}Pb to that of ^{204}Pb will therefore vary depending on the source of lead. This fundamental principle is used to match lead isotope ratios in someone's blood to a particular environmental source of lead contamination.

The lead isotope ratios can be measured using an Electro Thermal Vaporization (ETV) sampling accessory coupled to the ICP–MS. An ETV system uses a heated graphite tube (similar to that used in a GFAA), to thermally pretreat the sample. But instead of using the tube to produce ground state atoms, its main function is to drive off the bulk of the matrix before the analytes are vaporized into the plasma for ionization and measurement by the mass spectrometer. The major benefit of ETV-ICP–MS for this application is that complex matrices like blood, gasoline, and pottery clay material can be analyzed with very little interference from the matrix components. An additional benefit with regard to taking blood samples is that typically only a 20-50µL aliquot is required for analysis.

A rapid survey of the elements in biological materials, covering most of the elements in the periodic table, is possible by using available software for semi-quantitative analysis (SEMI-QUANT) by inductively coupled plasma mass spectrometry. The procedure takes 5 mm after sample preparation and gives results with a precision (CV) of '20%. At a 10 fold dilution, 13 elements can be consistently and reliably detected in serum and 15 elements in whole-blood samples. At present the most important limitation of this method is mass overlap by polyatomic species for some elements of interest (e.g., Cr, Mn, and V). However, for the set of elements that can be reliably determined at endogenous concentrations, including Li, B, Mg, Fe, Cu, Zn, Rb, and Sr, the rapid scanning capability may be useful. Although matrix effects limit the direct interpretation of the semi-quantitative output, reasonable estimates of concentration are attainable by using matrix-matched standards or by adding a multielement standard to an aliquot from one sample in the set. We also present an example of determination of 25 elements in saliva from a patient with extensive dental work: Components of many of his dental alloys were readily identified. The method may also prove useful for screening multiple toxic exposures to heavier elements, such as Pb, Ti, Cd, and Hg.

Determination of a growing number of elements in body fluids places increasing demands on clinical laboratories. In part, this demand reflects an increasing range of occupational and environmental exposures. To some extent, an increasing awareness of multielement interactions, and their potential clinical impact, underscores the need for comprehensive analysis of human body fluids. Although determinations

of elements such as Mg, Fe, Cu, and Zn are unquestionably important, much of the current interest in multielement techniques is predicated on the belief that a longer list of elements will gain importance, as their biological significance becomes known. However, the elaboration of this potentially important area of medicine currently awaits the development of technologies that facilitate acquisition of reliable data in the clinical setting. The most common method for quantitative trace element analysis of biological materials is atomic absorption spectroscopy. Good results are obtained at relatively low cost with this method, which is, however, incapable of a rapid multielement survey. Several atomic emission and mass spectrometric techniques have offered some degree of multielement capability, but none has proven sufficiently flexible and robust for the clinical laboratory.

Inductively coupled plasma-mass spectrometry (ICPMS) has emerged as a promising and versatile means of providing rapid, multielemental profiles of a wide variety of samples. This technique has been applied to serum and blood with emphasis on quantitative analysis of selected elements of biological interest. Such analyses at present require careful attention to matrix matching and spectral interpretation, and are improved by differential optimization of instrumental settings in selected mass ranges. However, ICP-MS also has the capability of rapidly scanning masses across the periodic table.

AAS in Comparison with NAA (Neutron Activation Analysis) and its adaptation to overcome the pitfalls:(10)

The method most frequently used to determine zinc concentrations in plasma and erythrocytes is flame atomic absorption spectrometry (AAS). However, accuracy comparable with that with neutron activation analysis (NAA) can be achieved with AAS only if sources of error peculiar to this method are taken into account. The major disadvantage of conventional AAS is the need for a relatively large sample volume: 0.5-1 mL of serum or plasma for each determination. An automated micromethod of AAS that obviates this objection has been available for sometime as an alternative. It is especially advantageous in analysis for trace metals when the sample volume is limited- e.g., in dialysis patients-because very little of sample suffices. Plasma zinc is determined in a sample diluted with an equal volume of demineralized water. Because blood plasma is relatively viscous, results for such samples cannot be accurately compared with those for aqueous calibration solutions. In fact, the most substantial measuring error in AAS is ascribable to differences in viscosity between samples and standard.(11) To correct for this, it is therefore necessary to add some relatively viscous substance such as glycerol to the calibration solutions. Because no data on the micromethod have been reported, we investigated such procedural correction for viscosity. The current method for determining erythrocyte zinc concentration is a direct measurement, after lysis and dilution of the erythrocytes with demineralized water (12) or other lysing agents such as Triton X-100 surfactant.(13) Owing to the inhomogeneity of sample fluids, a measuring error may occur. Gorsucht (14) found that dry-ashing is not the best technique for preparing samples for zinc analysis, because the high temperatures cause volatilization and residue problems. When erythrocytes are decomposed by use of traditional wet-chemistry techniques, losses or contamination can easily contribute to systematic. Only wet-ashing in a closed system, with use of a pressure decomposition device, gave AAS results that agreed with the relative values determined by direct NAA.

Applications In Forensic Medicine:

Current means of detection of facts from forensic specimens rely heavily upon the `experience' of the investigating pathologist, with corroborating evidence often absent. This brings in a lot of subjectivity to the calculations and analysis of forensic specimens. If radioactive isotope tracing is to be of forensic interest, then it should meet a few criteria:

1. To have a half-life commensurate with the time scale of investigation that is required (i.e. <40Years);

2. To be abundant enough to be detected easily by conventional analytical techniques and

3. To have some biological function, so as to be incorporated into the human bone.

The nuclides lead-210 ($_{210}$Pb) and polonium-210 ($_{210}$Po) are radioactive members of the $_{238}$U series and are widely distributed within the environment. They enter the human body from two main sources, direct ingestion in foodstuffs and the decay of ingested 226Ra which is retained in the bone and bony tissue.(15) Direct

inhalation of these nuclides is almost negligible, although inhalation of a short-lived parent isotope ($_{222}$Rn) can result in elevated concentrations of $_{210}$Pb and $_{210}$Po in human tissue.(16)

Consequently, concentrations of $_{210}$Pb and $_{210}$Po have been determined in human bone mainly to evaluate the contribution to internal radiation doses and little investigation has been undertaken into the potential for using these isotopes as detection tools for dating human skeletal remains. This is despite concentrations of $_{210}$Pb and $_{210}$Po in bone and tissue samples having been reported from a number of countries.

However, to date there remains an incomplete database and no indication that the method is applicable to forensic investigations, although its possibilities had been previously suggested by Swift.(17) Studies have been conducted to evaluate the potential of using $_{210}$Pb and $_{210}$Po nuclides in conjunction with trace elements to provide a meaningful estimate of the post-mortem interval. Any anatomical site can be standardized for this purpose. Trabecular bone has a large surface area to volume ratio and multiple cavities, which provides ideal conditions for adherence of soil particles and heteroionic exchange. It is thus highly susceptible to diagenetic changes. Therefore bones with a higher ratio of trabecular to cortical bone have an increased risk of diagenesis, for example, ribs and calvaria. Cortical tissue from a long bone is durable (18,19), hence improves the likelihood of recovery, and is less vulnerable to heteroionic exchange. Classically long bones, such as the femur, are used within analytical studies.(20) For these reasons, samples of diaphyseal compact bone ideal for this purpose, with care taken to ensure that the trabecular component is removed before analysis of the sections.

Reference value for concentrations of some elements have been detailed in a paper by Margaret et al.(21)

AAS for Pb; AAS, fluorometry, and NAA for Se; and NAA for I are the most frequently used analytical techniques. ICP-AES and polarography have also been successfully used for determination of Pb. Except for Pb in blood serum; in most other tissues and body fluids these elements are present in concentrations exceeding 10 pg/kg or 10 ug/L. There still are some unresolved problems in determining these elements in some clinical specimens. Generally, these elements occur at pg/kg or pg/L concentrations in most tissues and body fluids; therefore, their determination in most clinical specimens presents a considerable challenge.

Manganese: Mn is another element for which there is a modest amount of documentation of results. Mn is a component of stainless-steel devices, occurs in air dust, and is present in most plastics. Because natural Mn concentrations in human tissues and body fluids are generally low, efforts to determine this element without clean-room facilities suitable for "ultra" trace-element analysis is a waste of time and resources.

Nickel: Values of Ni are poorly documented. The values reported mainly refer to milk, liver, and hair. Nickel has been extensively investigated in blood and urine in the context of occupational exposure. According to recent investigations, median concentrations of Ni in serum and urine could be as low as 0.2 (range <0.05 to 1.3) and 1.3 (range0.3 to 4.6) pg/L, respectively. Because the values are on the microgram per liter or per kilogram level, the risk of contamination is serious. More investigations are needed to be able to formulate reference values for Ni in all the clinical specimens in unexposed adults. Values for hair show wider scatter, a clear indicator of analytical problems. According to a recent investigation, the Ni content of hair is about 1.25 ±0.46 mg/kg in healthy control subjects.

Chromium, manganese, molybdenum, and fluorine. Frequently used analytical methods for this group of elements are AAS and NAA for Cr and Mn, NAA for Mo, and the ion-selective electrode technique for F. Except for F, the remaining three elements have been determined in a number of tissues and body fluids and show a wide range of concentrations. Cr is high in hair and low in almost all the remaining specimens; Mn and Mo are more concentrated in hair, kidney, and liver than in the fluids; and F is high in bone, with moderate concentrations, 100 pg/kg or less, in many other sorts of specimens. This group of elements is very difficult to determine in biological systems, because of their both low concentrations and unresolved methodological problems.

Aluminum: Al is very sensitive to external contamination. Graphite-furnace AAS is the technique preferred for its determination. Many erroneous results have been published in the literature.

Boron: B is very poorly documented for almost all human tissues and body fluids. Earlier

investigations in which colorimetry was used suffered from contamination and other methodological problems, and very high values were reported. In contrast, a very recent study by NAA and MS reports31 ±5.6 pg/kg in whole blood, 22 ±5 pg/kg in blood serum, and 2.6 ±2 pg/kg in RBC.

Mercury: Hg is reasonably well documented in several specimens. Because Hg is ubiquitous in the environment, precautions against contamination of the sample are mandatory. The dietary intake of Hg is reflected in whole blood rather than in serum, because Hg readily binds to RBC. Thus, in populations consuming large quantities of fish and other sea foods, median values for RBC may be as high as 16 pg/kg (range 9 to 34 pg/kg). Corresponding findings in whole blood median value of 9.5 pg/L (range 1-59 pg/L). In serum, Hg concentrations may be as low as 1 pgf/L, depending on the exposure. Accumulation in hair is one way that Hg is eliminated. Also, hair is the main target for external exposure.

In analysis for trace elements in erythrocytes, sample treatment can represent a substantial source of error, in addition to that in the sampling process. When the erythrocytes are separated from the plasma, adherent (trapped) plasma must be taken into account by either:

a)Determining it by adding a tracer substance to the blood sample before centrifugation; even so, depending on the molecular weight of the tracer, different values are measured *o*r

b)Washing the erythrocytes: It has been shown that, with triple washing, 99.5% of the trapped plasma can be removed without changing the intra-erythrocyte concentration; thus this procedure should be used exclusively.

Analysis in hair: A method called Flow injection-inductively coupled plasma mass spectrometry has been evaluated for determining the distribution profile of trace elements along a single strand of hair. In this method, hair is cut into several mm long sections from follicle to the distal end. Scalp hair is considered a suitable biological sample for estimating the intake of, and (or) exposure to, some trace elements, e.g., Hg and As; external contamination and failure to correlate with body burden limit its usefulness for other trace elements. Hair is formed in the matrix cell, where it incorporates various elements from the blood at a relatively constant rate. After formation, the hair is separated from the body's internal metabolism; therefore, its composition reflects the concentration of elements in blood at the time of formation.

Hair grows at 1 cm per month. Researchers attempting to trace the intake/exposure history of trace elements in individuals have analyzed the distribution profile of trace elements along the length of the hair strand. The methods used so far to profile elemental concentration along a single strand of hair include x-ray fluorescence spectrometry and proton-induced x-ray emission spectrometry. Inductively coupled plasma mass spectrometry (ICPMS) spectrometry is another potential method for such analyses.

The U.S. Environmental Protection Agency provides a concise, well-researched answer to this question with the following summary:

The milk, urine, saliva and sweat measure the component that is absorbed but excreted; the blood measures the component absorbed and temporarily in circulation before excretion and/or storage; the hair nails and teeth are tissues in which trace minerals are sequestered and/or stored.

This same study concluded that human hair could be used effectively for biological monitoring of the highest priority toxic trace metals.

"...Human hair has been selected as one of the important monitoring materials for worldwide biological monitoring in the Global Environmental Monitoring System (GEMS) of the United Nations Environmental Program."

Concentrations of Lead and other heavy metals in the hair provide an accurate and relatively permanent record of exposure, and there is a strong correlation between concentrations in hair and concentrations in internal organs. Hair analysis appears to be a reliable, simple, and atraumatic method of assessing body Zinc stores. Furthermore, blood is not a suitable material to analyze for Cadmium, since the metal remains in blood only very briefly and, in consequence, the levels are always extremely low. Therefore, studies of Cadmium and Lead in blood would be inadequate to demonstrate associated exposure, while analysis of hair might lead to important findings. Hair is better than blood in reflecting long-term exposure of Cadmium. Hair analysis appears to be a reliable, simple, and atraumatic method of assessing body Zinc stores.

The present sample preparation procedure, together with introduction of a small sample into

the ICP-MS detector, provides a highly sensitive method for determining trace elements (1 ig/g or less) in small segments of human hair. Nutritionally and toxicologically important trace elements, e.g., Zn, Cu, Hg, and Pb, can be determined in a hair segment at their normal or unexposed level. Some toxic trace elements at lower concentrations, e.g., Ti, can be determined when the segment is from an exposed individual.

Laser Microprobe Mass Analysis

Histochemical staining is well known to be a useful procedure for localizing trace elements in tissues. Such methods contribute substantially to the diagnosis of (e.g.) overload with aluminum, iron, or lead and associated toxicity.

The aluminon technique is widely used to monitor or follow aluminum in undecalcified bone sections of dialysin patients with bone disease. In this way, the demonstration of aluminum at the osteoid/calcified-bone boundary and in histiocytic bone marrow cells in patients with dialysis-associated osteomalacia is indeed relevant. Stain specificity is limited, however, and there may be undesired interferences by other elements. Moreover, solutions coming into contact with tissue sections should be controlled for contamination and leaching out of the element being studied. Sophisticated micro analytical techniques may delineate the specificity of routinely used histochemical methods.

Laser microprobe mass analysis (LAMMA), a recent mass-spectrometric procedure, is by its nature highly element specific, and has a spatial resolution of analysis in the micrometer range. It may offer lower detection limits as compared with the histochemical aluminon staining, and it is much more sensitive than conventional electron probe x-ray microanalysis. In contrast to some other micro analytical equipment, LAMMA gives a light-microscopic view of the tissue section to be analyzed. We therefore considered it worthwhile to investigate its use in evaluating the sensitivity and specificity of histochemical staining of inorganic substances. **Conclusion:** There is no question that developments in measurement techniques have helped us to better understand the toxicity effects of metals over the past 30 years. AS has allowed us to lower the actual level of lead considered dangerous in young children from 60 to 10 µg/dL, helped to reduce elevated blood levels of children in the United States from 88.2 to 4.4%, and allowed to pinpoint with a high degree of certainty the environmental sources of lead contamination. However, such is the power and versatility of modern instrumentation and its accessories that it has also dramatically improved our understanding of other trace metal-related human diseases. The toxic effects of arsenic and hexavalent chromium or the nutritional benefits of iron and selenium would still be relatively unknown if it were not for the continual improvements in instrumentation. Although the techniques have been successfully applied to many other application areas, there is no question that its use as a biomedical and environmental research tool has had a direct impact on the quality of many people's lives.

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