ATOMIC ABSORPTION SPECTROMETRY OF TRACE METALS IN CLINICAL PATHOLOGY*

F. William Sunderman, Jr., M.D.†

Abstract

Atomic absorption spectrometry provides a convenient and versatile instrumental method for measurements of trace metals in biological materials. An important innovation is the recent development of nonflame atomization devices, which have substantially improved the analytical sensitivity of atomic absorption spectrometry. Applications of atomic absorption spectrometry of trace metals in clinical pathology have been expanded by the recent recognition of clinical syndromes associated with copper and zinc deficiency, and the discovery of abnormalities of chromium and nickel metabolism in various human diseases.

This review summarizes the current status of measurements of trace metals in biological materials by atomic absorption spectrometry. The topic is discussed from the viewpoint of a clinical pathologist who is concerned with applications of trace metal analyses to the diagnosis of human diseases. In the first section consideration is given to the basic principles of atomic absorption spectrometry, with emphasis upon the recent development of nonflame atomization techniques. In the second section attention is focused upon atomic absorption of six specific trace metals (i.e., copper, zinc, lead, iron, chromium, and nickel), including discussions of recent clinical applications of analyses of these metals. Consideration of atomic absorption spectrometry of the more abundant metals (i.e., calcium, magnesium, sodium, potassium) is specifically excluded from this review. In the third section a résumé is provided of the applications of atomic absorption spectrometry to measurements of a wide variety of trace metals in biological materials. This section is designed as a source of reference for clinical pathologists who are occasionally requested to perform analyses of various uncommon trace metals for clinical or forensic diagnosis. This section includes a discussion of atomic absorption spectrometry of hair as an index of the body burden of trace metals. It also provides a summary of applications of atomic absorption spectrometry of trace metals for indirect

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†Professor and Head, Department of Laboratory Medicine, University of Connecticut School of Medicine. Director of Clinical Laboratories, University of Connecticut Health Center, Farmington, Connecticut.

measurements of various biochemical constituents of serum or urine that are important in clinical pathology (e.g., amino acids, fatty acids, porphyrins, and bromide).

The opinions expressed in this review are based upon ten years of experience during which the author has been engaged in studies of the clinical pathology of trace metals. Atomic absorption techniques have been developed in the author's laboratory for analysis of calcium,[1] magnesium,[1] copper,[2] nickel,[3,4] thallium,[5] and lead[6] in biological fluids. This experience has led to an understanding of the pitfalls and practical limitations of atomic absorption spectrometry. Discussions in this review are intended to be succinct and critical rather than comprehensive, inasmuch as atomic absorption spectrometry has been thoroughly reviewed in several excellent books.[7–11]

FUNDAMENTAL CONSIDERATIONS

The phenomenon of atomic absorption was first recognized in 1860, when Kirchoff and Bunsen[12] noted that the dark Fraunhofer absorption lines in the solar spectrum coincided with the wavelengths of elemental lines in various emission spectrums. On the assumption that atoms absorb light at the same wavelengths as they emit light, Kirchoff and Bunsen[12] deduced the presence of several elements in the solar atmosphere. In 1955 Walsh[13] demonstrated that the phenomenon of atomic absorption could serve as a spectrochemical basis for the quantitative determinations of metals. He showed that measurements of metals by flame atomic absorption spectrometry are usually more sensitive than measurements by flame emission spectrometry, and less subject to interference from other elements.

One of the first applications of atomic absorption spectrometry to clinical pathology was Willis' description[14] in 1959 of a method for analysis of serum magnesium. During the years from 1960 to 1964 atomic absorption techniques were developed for analyses of numerous metals in biological materials, stimulated by the pioneering studies of Allan,[15,16] David,[17,18] Fuwa and Vallee,[19] Malmstadt and Chambers,[20] Menzies,[21] Robinson,[22] Willis,[14,23,24] and Zettner and Seligson.[25,26] Commercial atomic absorption spectrometers began to be introduced into clinical laboratories in the United States in 1963. Since that time the use of atomic absorption analysis in clinical pathology has steadily expanded.

The principle of atomic absorption spectrometry is basically simple. Aspiration of a solution containing metallic ions or compounds into the burner of an atomic absorption spectrometer produces thermal molecular dissociation and dispersion of metal atoms throughout the flame. Small proportions of the metal atoms become excited to emit light, but the overwhelming majorities of the atoms remain in the ground state and are capable of absorbing discrete wavelengths of incident light. These specific wavelengths are provided by a lamp with a hollow cathode constructed of an alloy containing the metal to be analyzed. The beam of light is passed through the flame and is focused upon the entrance slit of a monochromator. Light at the selected wavelength is detected by a photomultiplier tube. Since the light that strikes the photomultiplier tube represents the net balance of atomic absorption and emission, any light emitted by the excited metal atoms in the flame represents a potential source of interference in atomic absorption spectrometry. Flame background emission and molecular emission bands are additional sources of interference. In order to avoid these sources of error, it is customary to modulate the incident light beam with a mechanical or electronic chopper, and to tune the photomultiplier detector circuit to the same frequency of modulation. Under these conditions the photomultiplier detector circuit responds only to the pulsed signal from the light beam and does not respond to the continuous signal produced by light emission from the flame.[27] The alternating current from the photomultiplier detector circuit is amplified and recorded.

A refinement in the design of atomic absorption spectrometers is the use of split optical beams, which are arranged so that the output of the photomultiplier circuit is the ratio of the intensity of a light that has passed through the flame to the intensity of a reference beam that

has traversed an optical path around the flame. This arrangement minimizes error resulting from fluctuation in photoemission by the hollow cathode tube. An additional refinement is the use of internal standardization spectrometry, in which the atomic absorption of the metal to be analyzed is measured as a proportion of the atomic absorption at a reference wavelength of a known amount of another metal that has been added to the sample. This arrangement minimizes errors resulting from variations in flame conditions and fluctuations in the rate of aspiration of the sample into the flame.

Flame atomic absorption spectrometry is insufficiently sensitive to permit direct measurements of various trace metals in biological fluids. For determination of such metals it is necessary to perform preliminary extraction and concentration procedures. A common technique is to prepare a complex of the metal with ammonium pyrrolidonedithiocarbamate (APDC) and to extract the metal-APDC chelate into a small volume of an organic solvent, such as methylisobutylketone (MIBK). This procedure improves the analytical sensitivity of atomic absorption spectrometry not only because of the increased concentration of the metal in the solution that is aspirated with the flame, but also because of an enhancing effect of organic solvents upon flame atomic absorption. The phenomenon of enhancement by organic solvents of the atomic absorption of trace metals is the result of (1) decreased viscosity and surface tension, relative to water, which accelerates the rate of aspiration into the flame, and (2) increased efficiency of vaporization of the aspirated solution within the flame.

An important innovation in atomic absorption spectrometry has been the development of nonflame electrothermal atomizers in lieu of the traditional burner-nebulizer systems. The use of nonflame atomic absorption as an analytical technique is still in its infancy. Until the past year the author was discouraged by his observations that nonflame atomization techniques using commercially available instrumentation were relatively imprecise, tedious, and highly subject to matrix interferences. However, the author is convinced that these instrumental limitations have recently been overcome by several instrument manufacturers, and that nonflame atomic absorption spectrometry will become established as the standard technique for trace metal determinations in clinical pathology and toxicology.

The pioneering study of nonflame atomic absorption spectrometry was reported by L'vov[28] in 1961. L'vov described a complicated atomization device in which a sample was dried on a graphite plug, which was fitted into an aperture in an electrically heated graphite tube. An alternating current arc was used to discharge the sample into an argon atmosphere within the graphite tube. Atomic recombination was retarded by the high temperature of the tube, and atomic absorption spectrometry of the atom cloud was performed along the axis of the tube. Numerous ingenious apparatuses for electrically heated nonflame atomization have been described within the past six years.[29–38] In this discussion attention is focused upon the three basic designs of electrothermal atomizers that have generally been used for analyses of trace metals in biological materials.

L'vov's original design of a graphite tube furnace was greatly simplified by Massmann.[29, 30] A diagram of an adaptation of Massmann's apparatus is illustrated in Figure 1. This design was a prototype of graphite tube furnaces manufactured by the Perkin-Elmer Corporation. A liquid sample with a volume within the range of 1 to 100 μl. is introduced into the graphite tube by means of a micropipet through the removable window. The graphite tube is heated electrically by means of electrode connections at both ends of the tube. The assembly is encased within a metal jacket, which is cooled with circulating water. During each analysis the temperature of the graphite tube is rapidly increased in a stepwise fashion so that the sample is first dried at approximately 100° C.; then the sample is pyrolyzed at a temperature in the range from 400 to 800° C. so that most of the organic constituents are destroyed. Finally the metals in the sample are vaporized and atomized at a temperature in the range from 1500 to 2500° C. During this sequence the assembly is flushed slowly with

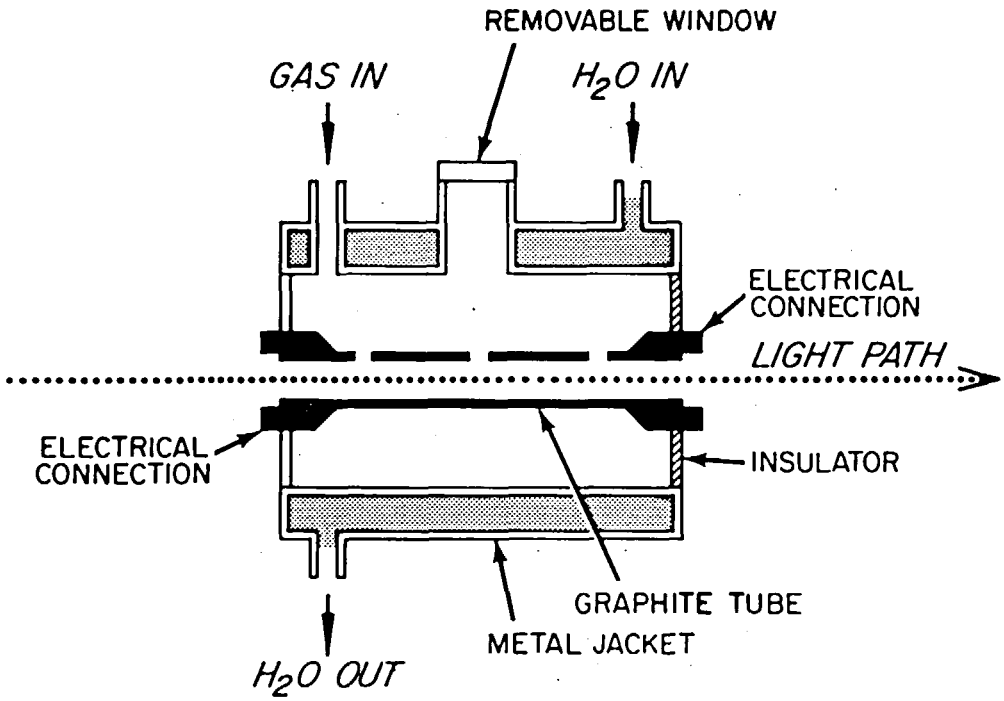
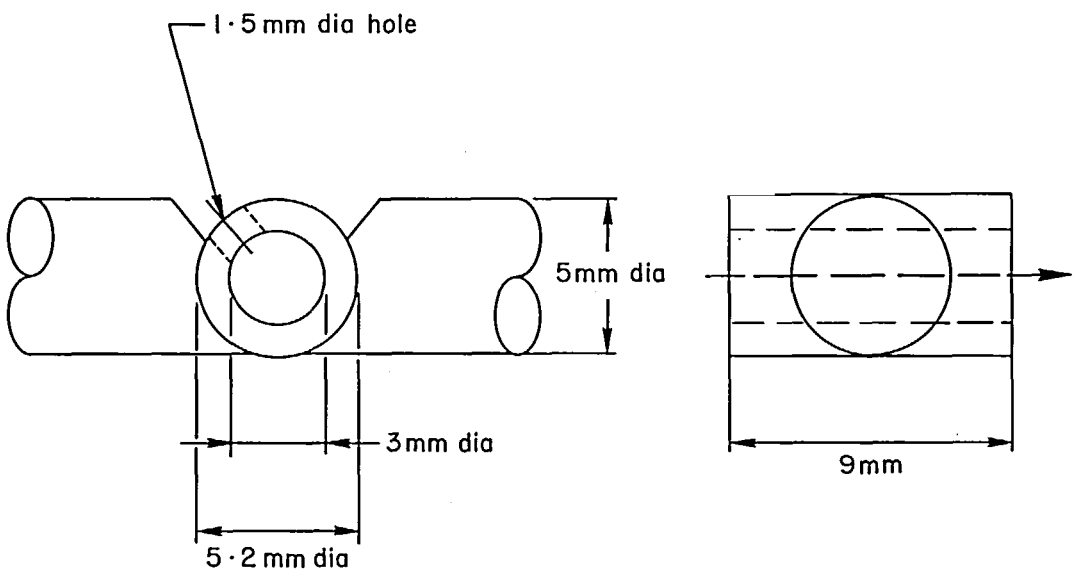


Figure 1. Cross section diagram of a graphite tube furnace (see text). (Courtesy of Perkin-Elmer Corporation, Norwalk, Connecticut.)

a stream of inert gas such as nitrogen or argon, which flows out from the ends of the graphite tube, successively sweeping away water or solvent vapors that are released during drying, smoke and fumes that are released during pyrolysis, and vaporized atoms that are released during atomization. The light path of the spec-



CARBON TUBE ATOMIZER

Figure 2. Schematic diagram of a carbon rod atomizer (see text). (Courtesy of Varian-Techtron Pty., Ltd., Palo Alto, California.)

trometer traverses the length of the graphite tube. During the atomization phase atomic absorption is measured by the spectrometer and recorded with a strip-chart recorder.

Amos and coworkers[34] described an alternative apparatus for nonflame atomization, which has served as the prototype of the carbon rod atomizers manufactured by Varian-Techtron Pty., Ltd. (Fig. 2). The sample is dried within a small cavity in a thin graphite rod, which is supported between two electrodes. The graphite rod is unenclosed but is protected by a laminar stream of an inert gas such as argon or nitrogen. Optionally the upward current of inert gas may be encircled by a concentric laminar stream of hydrogen. The elevated temperature of the heated graphite rod ignites the hydrogen, which forms a barrier that entrains atmospheric oxygen and hinders oxidation of the atom cloud that is released from the sample cavity. This simple design has the apparent disadvantages of relatively limited sample volume and relatively short path length of light through the aperture in the rod. However, Amos et al.[34] have found that this atomizer provides analytical sensitivity that rivals that of Massmann's graphite tube furnace.

A third basic design for nonflame atomization uses a tantalum boat, as described by Donega and Burgess.[33] The tantalum boat design has been adapted by Hwang and coworkers[37] in the form of tantalum ribbon atomizers, which are manufactured by Instrumentation Laboratory, Inc. (Fig. 3). The apparatus consists of a thin tantalum strip, which is shaped to serve as a combination sample boat and heating element. The ends of the tantalum strip are clamped onto the tops of two electrodes, and the assembly is enclosed within an argon-purged chamber. The electrode leads are shown at the bottom left in Figure 3, and the gas lines that carry argon in and out of the chamber are shown

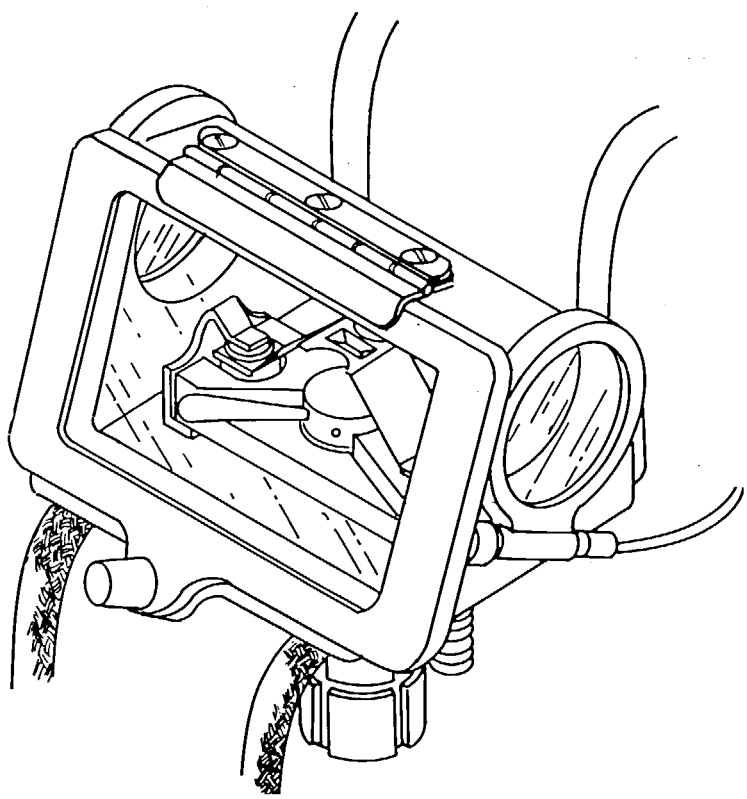


Figure 3. Sketch of a tantalum ribbon atomizer (see text). (Courtesy of Instrumentation Laboratory, Inc., Lexington, Massachusetts.)

at the top right. The optical beam traverses the two round quartz windows at the sides of the chamber and passes immediately over the rectangular sample cavity in the tantalum strip. A hinged door at the front of the chamber permits access for introduction of the sample. By use of different tantalum strips with sample cavities of various sizes, this design can accommodate sample volumes ranging from 1 to 100 μl.

When biological materials are vaporized in a nonflame atomizer, there is atomic absorption at the specific resonance wavelength of the metal being measured, and there is also nonspecific "background" continuum absorption owing to the sample matrix. For most biological measurements it is essential to make corrections for nonspecific background absorbance.[39–43] This can be done most elegantly by means of an instrument with dual monochromators (e.g., Model 810 atomic absorption spectrometer, Fisher Scientific Co.), using an internal standard or using an adjacent nonresonating line of the element to be analyzed. Alternatively correction can be accomplished by means of a hydrogen or deuterium background corrector. The hydrogen background corrector employed with the IL-Model 353 spectrometer (Instrumentation Laboratory, Inc.) is illustrated in Figure 4, but other manufacturers have designs that accomplish the same purpose. In the assembly shown in Figure 4 two discharge lamps are employed. One lamp, which contains a hollow cathode constructed of an alloy of the metal to be analyzed (e.g., lead), is electronically pulsed at a frequency of 0.5 kilohertz. The reference lamp is a hydrogen discharge tube, which is pulsed at 1 kilohertz. Coincident beams from the two lamps are passed through the nonflame atomizer, and a specific resonance wavelength of light is selected by the monochromator. The absorbance at this wavelength of light that emanates from the hydrogen lamp is electronically subtracted from that which emanates from the lead lamp, in order to generate an automatically compensated readout.[43]

The relative advantages and disadvantages of flame and nonflame atomization for atomic absorption spectrometry have been discussed by several investigators.[28, 29, 35, 44, 45] Flame atomization techniques have the following principal advantages over nonflame atomization methods:

1. Flame burners are more convenient, safe, and rapid to use.
2. Flame burners are small, durable, and inexpensive.
3. Flame burners are relatively free from sample "carry-over" or "memory" effects, whereas sample interactions may be a serious problem in nonflame atomization.
4. For flame atomization, sample solutions are simply handled by a nebulizer system.
5. Flame burners can be used with a wide variety of fuel and oxidant mixtures, permitting selection of a wide range of operating conditions.

On the other hand, nonflame atomization methods have several important advantages over flame atomization techniques:

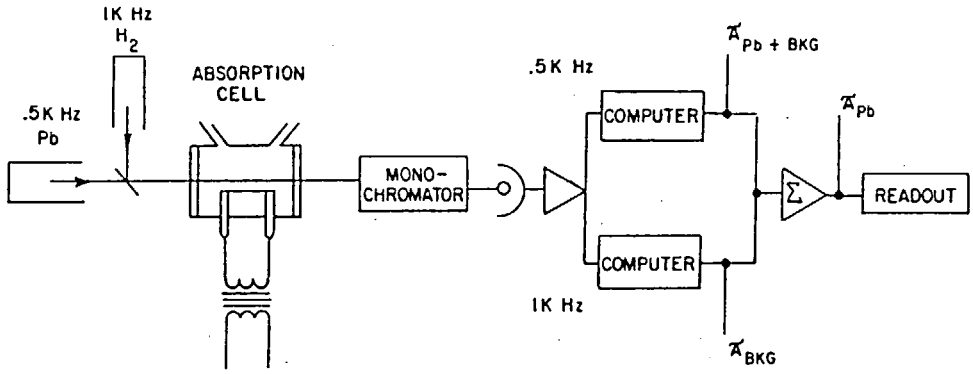


Figure 4. Schematic diagram of an automatic background corrector (see text). (Courtesy of Instrumentation Laboratory, Inc., Lexington, Massachusetts.)

1. There is less light scattering and photoemission in the nonflame absorption cell.
2. By programmed increments in temperature, organic constituents can be pyrolyzed prior to vaporization and atomization of metals.
3. Formation of refractory oxides is prevented by eliminating oxygen.
4. The chemical environment of the analyte is more precisely controlled, by avoiding fluctuations in fuel-oxidant ratios.
5. Greater analytical sensitivity is accomplished, since much smaller sample volumes can be used and since much higher concentrations of atoms per cell volume are possible with nonflame atomizers than with most burner systems.

The greater analytical sensitivity of nonflame atomization in comparison to flame atomization is attributable to three principal factors:

1. The entire sample is vaporized in nonflame atomization, whereas in most flame nebulization systems only a small fraction of the sample enters the flame.
2. The atom cloud is more slowly dispersed in nonflame atomization than in flame atomization. In flame atomization the atoms are diluted by the high flow rate of unburned gases and by the expansion of flame gases that occurs during combustion.
3. The loss of atoms by molecular recombination (e.g., with atmospheric oxygen) is more rapid in flame atomization than in nonflame atomization, since the inert atmosphere in nonflame atomization restricts chemical reactions.

In view of the efficiency of nonflame atomization techniques, it may be asked whether it is necessary to perform measurements of the atomized metals by atomic absorption spectrometry or whether atomic emission spectrometry might be equally satisfactory. In general, atomic absorption spectrometry is preferred since it has three principal advantages in comparison with atomic emission spectrometry:

1. Atomic absorption spectrometry is generally more sensitive than emission spectrometry, in part because atomic absorption spectrometry measures the more abundant ground-state atoms whereas atomic emission measures the smaller proportion of excited atoms.
2. Temperature variations have less effect upon atomic absorption spectrometry than emission spectrometry, since the absorption coefficient varies as $\sqrt{T}$, whereas the emission coefficient varies exponentially with T.[28]
3. Spectral interference is generally less serious with atomic absorption spectrometry than with emission spectrometry. Whereas emission spectrometry generates a background continuum, atomic absorption spectrometry uses hollow cathode discharge lamps that provide a small number of intense spectral lines, practically free from background. Hence, a large dispersion spectrometer is seldom essential for atomic absorption spectrometry.

A question might also be posed regarding the possible use of atomic fluorescence spectrometry in lieu of atomic absorption analysis. Several papers have suggested that flameless atomic fluorescence analysis might be even more sensitive than atomic absorption for measurements of certain trace metals.[28, 32, 34, 35] To date, the principal application of flameless atomic fluorescence spectrometry to biological materials has been in the analysis of lead.[34] Kahn[44] has recently reviewed the available data and has concluded that atomic fluorescence analyses afford little or no practical superiority over atomic absorption analyses of trace metals in biological samples.

CLINICAL APPLICATIONS

Copper

There are currently six major clinical situations in which clinical laboratories are required to measure copper concentrations in serum, urine, and other biological materials.

WILSON'S DISEASE (HEPATOLENTICULAR DEGENERATION). Analyses of copper in serum and liver biopsy specimens are essential for the diagnosis of Wilson's disease,[46] and measurements of the urinary excretion of copper are indispensable for the management of chelation therapy.[47] Estimations of nonceruloplasmin copper in serum are valuable for the diagnosis and management of acute hemolytic crises in patients with Wilson's disease.[48]

COPPER TOXICITY. Acute copper intoxication has been described as a complication of renal hemodialysis[49–52] and neonatal exchange transfusion,[53] as well as in subjects who have ingested copper salts.[54, 55] Measurements of copper in serum and urine are desirable for the diagnosis of acute copper poisoning, and are useful

in the management of the hemolytic anemia and hepatic insufficiency, which are the usual clinical manifestations.

INFANTILE COPPER DEFICIENCY. In marasmic infants who are rehabilitated on calorie-rich milk diets that are deficient in copper, a characteristic syndrome of copper deprivation frequently develops.[56–61] This syndrome is characterized by anemia, marked neutropenia, and scurvy-like bone deformities. The diagnosis of infantile copper deficiency depends upon the demonstration of hypocupremia and upon the occurrence of a favorable clinical response to dietary copper supplementation.

MENKES' KINKY HAIR SYNDROME (TRICHOPOLIODYSTROPHY). Menkes' syndrome is a progressive brain disease of male infants, which usually causes death before three years of age and which is characterized by severely retarded growth and development, "kinky" hair (pili torti), hypothermia, arterial tortuosity, scorbutic bone changes, and cerebral gliosis with cystic degeneration.[62] Menkes' syndrome has recently been shown to be associated with profound hypocupremia, hypoceruloplasminemia, and diminished concentration of copper in hair.[63–66] The copper deficiency is presumed to produce alterations in the free sulfhydryl groups of the hair, resulting in its tendency to twist.[63] Copper deficiency may also be responsible for changes in the elastic fibers of arterial walls and for scorbutic bone deformities.[63, 64] Investigations have revealed a defect in intestinal copper absorption, which may represent either a disturbance of intracellular handling of copper in the duodenal mucosa or impairment of copper transport across the serosal cell membrane.[65] According to Danks et al.,[63–65] parenteral administration of copper may possibly be therapeutically beneficial. Clinical laboratories are being requested to perform steadily increasing numbers of analyses of serum copper as screening tests for Menkes' syndrome. Potentially the screening of infants for Menkes' syndrome and Wilson's disease by analyses of serum copper may become a major application of atomic absorption spectrometry in clinical pathology laboratories.

ADULT COPPER DEFICIENCY. Copper deficiency occurs in adults as a result of defective absorption of copper, usually as a manifestation of severe disease of the small intestine.[67] The diagnosis of adult copper deficiency is supported by the presence of hypocupremia and hypocupruria.

HODGKIN'S DISEASE. Measurements of serum copper are valuable as a laboratory adjunct to monitoring relapse in patients with Hodgkin's lymphoma.[68, 69] There is apparently a close correlation between the concentration of serum copper and the clinical activity of the lymphoma. The increased serum concentration of copper is the result of hyperceruloplasminemia, which occurs as a manifestation of the "acute phase reaction." According to Hrgovcic et al.[68] and Warren et al.,[69] fluctuations in serum copper concentrations may help to guide the physician in anticipating the clinical response to treatment in patients with Hodgkin's disease.

ANALYSIS FOR COPPER. Methods of sample preparation for measurements of copper in serum or urine by flame atomic absorption are summarized in Table 1. Atomic absorption spectrometry of copper is performed at 234.7 nm. using an oxidizing (fuel poor) flame. The detection limit is 1 to 4 μg. per liter. Inasmuch as the normal concentration of copper in urine averages only 17 μg. per liter, it is advisable to perform a preliminary extraction of copper from urine in order to achieve adequate analytical precision for diagnostic purposes.[2] Chelation therapy in Wilson's disease interferes with analyses of copper in urine, unless the organic constituents have been destroyed by digestion or ashing. Dawson et al.[79] have demonstrated that inorganic salts cause interference in direct measurements of copper in urine by flame atomic absorption. Kurz et al.[83] have reported that serum viscosity causes artifactitiously low values when copper analyses are attempted by direct aspiration of undiluted serum into the flame. The various techniques listed in Table 1 have been evaluated in the author's laboratory. The author recommends the method of Sunderman and Roszel[2] for routine use in clinical laboratories for measurements of copper in serum and urine by flame atomic absorption.

Nonflame atomic absorption methods for analysis of copper in biological mate-

TABLE 1. FLAME ATOMIC ABSORPTION OF COPPER IN SERUM AND URINE

Sample Preparation	*Authors*
Serum	
1. Dry ashing	Holtzman et al.[54]
2. TCA deproteinization, DDC-MIBK extraction	Berman[70, 71]
3. HCl pretreatment, APDC-NBA extraction	Blomfield and MacMahon[72]
4. HCl pretreatment, TCA deproteinization	Sunderman and Roszel[2]
5. TCA-butanol deproteinization	Cowell[73]
6. TCA deproteinization	Parker et al.,[74] Piper and Higgins,[75] Ullucci et al.,[76] Smeyers-Verbeke et al.[77]
7. HCl dilution (1:10–1:20)	Girard,[78] Dawson et al.[79]
8. H_2O dilution (1:4)	Sinha and Gabrieli[80]
9. H_2O dilution (1:10) with 6% butanol	Meret and Henkin[81]
10. Dilution (1:1) with La_2O_3 solution	Heinemann[82]
11. Direct aspiration	Kurz et al.[83]
Urine	
1. Acid digestion, DDC-MIBK extraction	Berman[70, 71]
2. Acid digestion, APDC-MIBK extraction	Sunderman and Roszel[2]
3. H_2O oxidation, APDC-MIBK extraction	Berge and Pflaum[84]
4. Acidification, APDC-MIBK extraction	Cowell,[73] Parker et al.,[74] Piper and Higgins[75]
5. H_2O dilution (1:10) with 6% butanol	Meret and Henkin[81]
6. Acidification, direct aspiration	Holtzman et al.,[54] Dawson et al.,[79] Spector et al.[85]

List of Abbreviations Used in the Tables

AAS	Atomic absorption spectrometry
APDC	Ammonium pyrrolidonedithiocarbamate
DDC	Diethyldithiocarbamic acid
DMG	Dimethylglyoxime
IL-353	Atomic absorption spectrometer, Instrumentation Laboratory, Inc., Lexington, Massachusetts 02173 (Model 353)
MIBK	Methylisobutylketone
NBA	n-Butylacetate
PE-290B	Atomic absorption spectrometer, Perkin-Elmer Corp., Norwalk, Connecticut 06856 (Model 290B)
PE-303	Idem. (Model 303)
PE-403	Idem. (Model 403)
SDS	Sodium dodecylsulfate
TBP	Tributylphosphate
TCA	Trichloroacetic acid
TMDDC	Tetramethylenedithiocarbamate
Triton X-100	Detergent manufactured by Rohm and Haas Company, Philadelphia, Pennsylvania 19107
V-T AA-5	Atomic absorption spectrometer, Varian-Techtron Pty., Ltd., Palo Alto, California 94303 (Model AA-5)
7X-O-Matic	Detergent manufactured by Limbro Chemical Co., New Haven, Connecticut 06511

rials are summarized in Table 2. In the method of Glenn et al.[87, 88] serum (5 μl.) is dried and pyrolyzed at an unspecified temperature by use of a specially designed graphite rod atomizer in conjunction with a Perkin-Elmer Model 303 atomic absorption spectrometer. Atomization of copper is accomplished in 3 seconds at 2000° C.

TABLE 2. NONFLAME ATOMIC ABSORPTION OF COPPER IN BIOLOGICAL MATERIALS

Author	*Date*	*Atomizer*	*Spectrometer*	*Sample*	*Preparation*
Welz and Wiedeking[86]	1970	Graphite tube	PE-403	Serum	H_2O dilution (1:5)
Glenn et al.[87, 88]	1971	Graphite rod	PE-303	Serum	Direct sampling (5 μl.)
Barnett and Kahn[89]	1972	Graphite tube	PE-403	Fingernails	Direct sampling
Stevens[90]	1972	Graphite rod	V-T AA-5	Tissue	HNO_3 solubilization

Glenn et al.[87] reported that the relative standard deviation of measurements of serum copper by their nonflame atomization method was >10 per cent and that serum copper analyses by their techniques provided satisfactory correlation with flame atomic absorption by the method of Sunderman and Roszel.[2] Fuller[91] has investigated the loss of copper during preatomization heating periods in nonflame atomic absorption spectrometry. Significant loss of copper occurred when the temperature of the preatomization heating stage exceeded 600° C. For example, when the preatomization heating was 1100° C., a 50 per cent loss of copper from the sample occurred within 30 seconds. The rate of loss of copper during the preatomization heating was apparently independent of the composition of the copper solutions that were analyzed. Fuller[91] concluded that serious errors can be expected if a preatomization heating temperature above 600° C. is used for nonflame atomic absorption spectrometry of copper.

In Table 3 are summarized reported normal values for copper in serum, plasma, and urine as determined by atomic absorption spectrometry.

Zinc

The clinical significance of measurements of zinc in plasma and urine has been discussed by Davies et al.,[92] Parisi and Vallee,[93] Halsted and Smith,[94] Flynn et al.,[95,96] and Henkin and Smith.[97] Hypozincemia has been shown to be a nonspecific abnormality that is observed in a wide variety of clinical situations, including liver disease (cirrhosis, hepatitis, metastatic carcinomatosis), lung diseases (carcinoma and infections, including tuberculosis), acute myocardial infarction, chronic kidney disease with uremia, diseases with increased muscle catabolism, pregnancy, and in patients who receive corticosteroid therapy or who are taking contraceptives orally. Measurements of zinc in plasma, urine, and other biological materials currently appear to have limited importance

TABLE 3. NORMAL VALUES FOR COPPER IN SERUM (S), PLASMA (P), AND URINE (U) BY AAS

Authors	*Date*	*Sample*	*Preparation*	*No. of Healthy Subjects*	*Normal Values Mean ± S.D.*	*Range*
					μg./dl.	
Berman[71]	1965	S	TCA deproteinization, DDC-MIBK extraction	60	133	80–170
Holtzman et al.[54]	1966	S	Dry ashing	19	163 ± 40	
Parker et al.[74]	1967	S	TCA deproteinization	28	105 ± 16	
Piper and Higgins[75]	1967	S	TCA deproteinization with added butanol	40	141 ± 36	50–260
Sunderman and Roszel[2]	1967	S	HCl pretreatment, TCA deproteinization	50	119 ± 19	70–165
Dawson et al.[79]	1968	S	HCl dilution (1:20)	24	108	70–165
Blomfield and MacMahon[72]	1969	P	HCl pretreatment, APDC-NBA extraction	50	112	74–160
Sinha and Gabrieli[80]	1970	S	H_2O dilution (1:4)	200	123 ± 23	65–180
Meret and Henkin[81]	1971	S	H_2O dilution (1:10) with 6% butanol	82	106	80–147
Kurz et al.[83]	1972	S	Undiluted serum	45	125 ± 21	
Heinemann[82]	1972	S	Dilution (1:1) with La_2O_3 solution	240	104	64–184
					μg./day	
Berman[71]	1965	U	Acid digestion, DDC-MIBK extraction	10		20–50
Piper and Higgins[75]	1967	U	APDC-MIBK extraction	30	21 ± 8	2–37
Sunderman and Roszel[2]	1967	U	Acid digestion, APDC-MIBK extraction	21	18 ± 8	8–34
Dawson et al.[79]	1968	U	Direct aspiration	10	52	26–64
Spector et al.[85]	1971	U	H_2SO_4, direct aspiration	25	22 ± 16	4–63
Meret and Henkin[81]	1971	U	H_2O dilution (1:10) with 6% butanol	82	36	10–114

in clinical diagnosis or prognosis. However, four specific clinical situations will be reviewed in which zinc analyses by atomic absorption spectrometry may have particular diagnostic value.

JUVENILE ZINC DEFICIENCY. Human zinc deficiency has been recognized in Egypt and Iran for more than a decade. In these Middle Eastern countries zinc deficiency is associated with dwarfism and retardation of sexual maturation in adolescent males.[94, 98, 99] The zinc deficiency in these subjects stems from a combination of factors, including low dietary intake of zinc, high levels of phytic acid in the diet resulting in reduced zinc absorption, the habit of clay-eating, which further reduces zinc absorption, augmented loss of zinc in sweat, and increased fecal excretion of zinc owing to blood loss from chronic intestinal parasitism.[94, 99] In these subjects the concentrations of zinc in plasma, urine, and hair are usually diminished. The subjects respond favorably to zinc supplementation of the diet, with resumption of growth and sexual maturation. Recent evidence suggests that juvenile zinc deficiency also occurs in North America, although the manifestations are usually less dramatic than those seen in the Middle East. Hambidge et al.[100] have identified several children living in Denver, Colorado, who have anorexia, very short stature, hypogeusia, and diminished concentrations of zinc in hair. When these children received dietary zinc supplementation, there were prompt increases in taste acuity and in the concentrations of zinc in hair. Regrettably, measurements of zinc concentrations in plasma and zinc excretions in urine were not reported in these subjects.[100]

HYPOGEUSIA. Schechter et al.[101] have studied 91 adult patients who developed decreased taste acuity (hypogeusia) without any apparent cause. The mean serum concentration of zinc was significantly decreased in these patients. Twelve of 18 patients who were treated with oral zinc supplementation experienced diminished hypogeusia, compared with similar improvement in only three of 47 control patients who received a placebo.[101] Cohen et al.[102] have reported a significant decrease in serum zinc concentration and a significant increase in urine zinc excretion in 16 patients who developed hypogeusia following thermal burns, compared to patients with thermal burns without hypogeusia. These findings provide further support for a relationship between hypogeusia and zinc metabolism. It appears likely that measurements of zinc in body fluids may prove to be helpful in evaluating patients with hypogeusia and in monitoring their response to zinc supplementation.

CHRONIC LEG ULCERS AND BEDSORES. Recent studies have demonstrated the efficacy of oral zinc sulfate therapy in promoting the healing of chronic venous leg ulcerations that had failed to respond to conventional medical therapy.[103–106] Cohen[107] has likewise observed a therapeutic response to oral doses of zinc sulfate in subjects with bedsores. In the studies of Greaves and Skillen[104] and of Hallbook and Lanner,[106] the mean concentrations of plasma zinc were diminished in patients with leg ulcers prior to therapy. A similar observation was reported by Abbott et al.[108] in patients with bedsores.

JUVENILE ZINC TOXICITY. Chunn[109] has reported severe anemia caused by acute zinc intoxication in three children who ingested zinc from cast metal toys made from zinc alloys. In all the patients there was increased excretion of zinc in the urine, and all responded to treatment following the removal of the cast metal toys from their environment.

ANALYSIS FOR ZINC. Methods of sample preparation for flame atomic absorption spectrometry of zinc in plasma and urine are summarized in Table 4. Atomic absorption spectrometry of zinc is performed at 213.9 nm. using an oxidizing (fuel poor) flame. The detection limit for zinc is 0.5 to 1.0 μg. per liter. This sensitivity is sufficient to permit direct measurements of zinc in diluted samples of plasma or urine without prior extraction and concentration. If deproteinization of plasma with trichloroacetic acid is used, the trichloroacetic acid must be added to blank and standard samples.[117] Plasma is preferable to serum for zinc analyses, since the concentration of zinc in serum averages 16 per cent higher than in plasma, owing in part to platelet release of zinc during clotting.[118] Hemolysis must ob-

TABLE 4. FLAME ATOMIC ABSORPTION OF ZINC IN PLASMA (P) AND URINE (U)

Preparation	*Sample*	*Authors*
1. TCA deproteinization	P	Prasad et al.,[110] Parker et al.,[74] Rozner and Gorfien,[111] Davies et al.,[92] Smeyers-Verbeke et al.[77]
2. HCl dilution (1:10–1:20)	P,U	Girard et al,[78] Dawson and Walker[112]
3. H_2O dilution (1:10) with 6% butanol	P,U	Meret and Henkin[81]
4. H_2O dilution (1:1–1:10)	P,U	Fuwa et al.,[113] Helwig et al.,[114] Piper and Higgins,[75] Hackley et al.,[115] Halsted and Smith,[94] Sinha and Gabrieli,[80] Pekarek et al.[116]
5. Dilution (1:1) with La_2O_3 solution	P	Heinemann[82]
6. Direct aspiration	P	Kurz et al.[83]

viously be scrupulously avoided in measurements of plasma zinc, since erythrocytes are rich in zinc-containing enzymes.[112] Specimens of plasma for zinc analysis should be obtained at a constant time of the day in order to minimize the effects of diurnal fluctuations.[92, 112] Dawson and Walker[112] have cautioned that inorganic salts can cause interference with direct measurements of zinc in undiluted urine. The author recommends the method of Prasad et al.[110] for routine measurements of zinc in plasma and the method of Dawson and Walker[112] for zinc analyses in urine.

Nonflame atomic absorption spectrometry of zinc in biological fluids has been reported by Matousek and Stevens[119] and Kurz et al.[120] Both groups of investigators employed the Varian carbon rod atomizer. Kurz et al.[120] reported that with a 1 μl. sample of serum or urine, the coefficient of variation of replicate determinations was 4.8 per cent and the recovery of added zinc averaged 98 per cent.

In Table 5 are listed normal values

TABLE 5. NORMAL VALUES FOR ZINC IN SERUM (S), PLASMA (P), AND URINE (U), BY AAS

Authors	*Date*	*Sample*	*Preparation*	*No. of Healthy Subjects*	*Normal Values Mean ± S.D.*	*Range*
					μg./dl.	
Prasad et al.[110]	1965	P	TCA deproteinization	14	104 ± 14	
Helwig et al.[114]	1966	S	H_2O dilution (1:10)	64	91 ± 17	55–149
Parker et al.[74]	1967	S	TCA deproteinization	23	90 ± 10	
Piper and Higgins[75]	1967	P	H_2O dilution (1:4)	30	85 ± 36	10–210
Davies et al.[92]	1968	P	TCA deproteinization	67	95 ± 12	76–125
Rozner and Gorfien[111]	1968	P	TCA deproteinization	31	138 ± 29	87–234
Dawson and Walker[112]	1969	P	HCl dilution (1:20)	20	97 (9a.m.)	83–132
				20	82 (2p.m.)	66–91
Halsted and Smith[94]	1970	P	H_2O dilution (1:1)	89	96 ± 12	72–115
Sinha and Gabrieli[80]	1970	S	H_2O dilution (1:4)	200	120 ± 22	
Meret and Henkin[81]	1971	S	Dilution (1:10) with 6% butanol	82	92	63–147
Pekarek et al.[116]	1972	S	H_2O dilution (1:4)	99	102 ± 17	68–136
Kurz et al.[83]	1972	S	Undiluted serum	11	110 ± 21	
Heinemann[82]	1972	S	Dilution (1:1) with La_2O_3 solution	240	89	65–122
Kurz et al.[120]	1973	S	Nonflame atomization	25	95 ± 11	
					μg./day	
Helwig et al.[114]	1966	U	H_2O dilution (1:2)	62	525 ± 254	145–1256
Piper and Higgins[75]	1967	U	H_2O dilution (1:4)	30	517 ± 172	178–882
Dawson and Walker[112]	1969	U	H_2O dilution (1:10)	20	500	263–810
Meret and Henkin[81]	1971	U	Dilution (1:10) with 6% butanol	82	353	141–779
Kurz et al.[120]	1973	U	Nonflame atomization	4		600–1000

for zinc in serum, plasma, and urine as determined by atomic absorption spectrometry.

Lead

Atomic absorption spectrometry of lead in whole blood and urine has become a common analytical task in clinical laboratories owing to current enthusiasm for the detection of environmental intoxication. Measurements of lead concentrations in whole blood are usually employed for the diagnosis of acute lead poisoning, whereas measurements of lead excretion in urine are undertaken in order to assess the body burden of lead by means of the calcium ethylenediamine tetra-acetic acid (CaEDTA) mobilization test[121–124] or to monitor the excretion of lead during chelation therapy in lead poisoning.

There has been wide diversity among the atomic absorption methods employed in clinical pathology, public health, and industrial laboratories in regard to their accuracy, precision, convenience, and freedom from interference by chelation therapy. The multiplicity of analytical methods for lead is partly attributable to recent efforts to develop rapid direct methods for the analysis of lead in microsamples of blood from infants and children as a means of population screening for lead exposure.

The resonance lines at 217.0 and 283.3 nm. are both satisfactory for measurements of lead by atomic absorption spectrometry. The detection limit for lead at 283.3 nm. is approximately 10 μg. of lead per liter. The analytical sensitivity at 217.0 nm. is approximately twice as great as at 283.3 nm. However, baseline noise level

TABLE 6. FLAME ATOMIC ABSORPTION OF LEAD IN BLOOD AND URINE

Sample Preparation	*Authors*
Blood	
1. Dry ashing, dithizone-$CHCl_3$ extraction, TMDDC-MIBK extraction	Donovan and Feeley[124]
2. Acid digestion, APDC-MIBK extraction	Piper and Higgins,[75] Elser et al.[125]
3. Anion exchange chromatography	Lyons and Quinn[126]
4. TCA deproteinization, APDC-MIBK extraction	Berman,[127] Pierce and Cholak,[128] Selander and Cramer,[129] Murphy et al.[6]
5. TCA deproteinization, DDC-MIBK extraction	Berman et al.[130]
6. $HClO_4$ deproteinization, APDC-MIBK extraction	Devoto,[131] Cernik[132]
7. TCA deproteinization, APDC-MIBK extraction, KCN suppression of interference	Yeager et al.[133]
8. TCA deproteinization, direct aspiration	Sprague and Slavin[134]
9. TCA-$HClO_4$ deproteinization, direct aspiration	Einarsson and Lindstedt[135]
10. Triton X-100 hemolysis, APDC-MIBK extraction	Hessel,[136] Zinterhofer et al.[137]
11. Triton X-100 hemolysis, DDC-MIBK extraction	Lubran[138]
12. Saponin-Formamide hemolysis, APDC-MIBK extraction	Farrelly and Pybus,[139] Hwang et al.[140]
13. Ethyleneoxide-isononylphenol hemolysis, APDC-MIBK extraction	Lehnert et al.[141]
14. H_2O_2 oxidation, direct atomization in Delves sampling cup	Delves,[142] Fernandez and Kahn,[143] Cernik and Sayers,[144] Ediger and Coleman,[145] Olsen and Jatlow[146]
15. Direct atomization with sampling boat	Hilderbrand et al.[147]
Urine	
1. Acid digestion, APDC-MIBK extraction	Piper and Higgins,[75] Selander and Cramer[148]
2. APDC-MIBK extraction with KCN suppression of interference	Yeager et al.[133]
3. DDC-MIBK extraction	Lubran[138]
4. APDC-MIBK extraction	Willis,[149] Pierce and Cholak,[128] Farrelly and Pybus[139]
5. Dithizone-$CHCl_3$ extraction	Roosals and Vanderkeel[150]
6. Bismuth coprecipitation	Kopito and Schwachman,[151] Segal[152]
7. Thorium coprecipitation	Zurlo et al.[153]
8. Direct atomization with sampling boat	Kahn et al.,[154] Hilderbrand et al.[147]

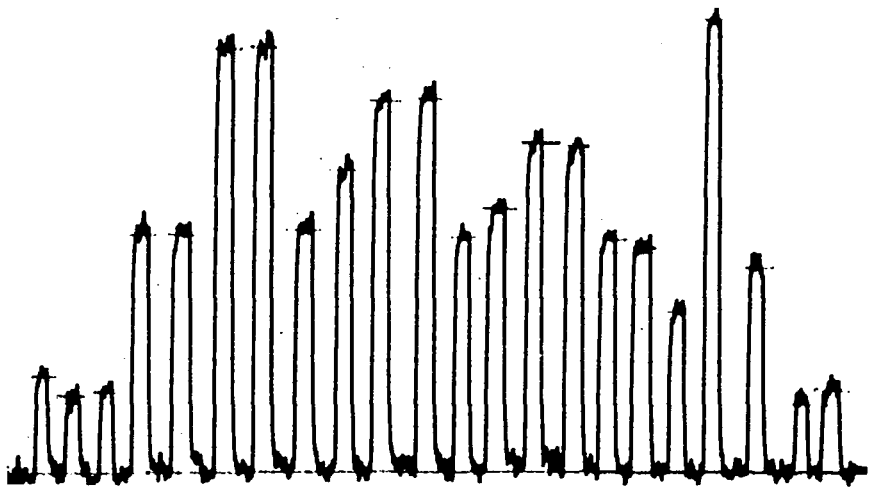


Figure 5. Typical recorder graph record for measurements of blood lead by the method of Murphy et al.[6] (see text). From left to right, peaks 1 to 3 are reagent blanks; peaks 4 and 5 are lead standards equivalent to 25 μg. Pb/dl. of blood; peaks 6 and 7 are lead standards equivalent to 50 μg. Pb/dl. of blood; peaks 8 to 20 are blood specimens from patients, and peaks 20 and 21 are reagent blanks.

is substantially less at 283.3 nm. than at 217.0 nm., the analytical precision is greater at 283.3 nm. than at 217.0 nm., and measurements at 283.3 nm. are less subject to interference by salts and organic compounds.[6, 152] For these reasons most workers have selected the resonance line at 283.3 nm. for routine use. For flame atomic absorption spectrometry of lead, an oxidizing, fuel poor flame should be used. The usual gas mixtures are acetylene:air or coal gas:air. Published methods of preliminary sample preparation for flame atomic absorption spectrometry of lead in blood and urine are summarized in Table 6. In the author's opinion the techniques developed for Selander and Cramer[129, 148] are among the most precise and dependable methods available for routine analysis of lead in blood and urine.

Murphy et al.[6] in the author's laboratory have described an adaptation of the Selander-Cramer technique[129] for blood lead that has decreased the sample volume to 2 ml. and has increased the convenience of the method. The "within the run" precision of the modified technique is 1.9 per cent (coefficient of variation), and the "week to week" precision is 2.7 per cent (coefficient of variation). The recovery of lead added to blood averages 98 per cent and the analytical results are not affected by EDTA therapy. An illustrative recorder tracing obtained by the method of Murphy et al.[6] is shown in Figure 5. Because of its precision and reliability, the method of Murphy et al.[6] is recommended for use in clinical pathology laboratories that are engaged in measurements of blood lead for routine diagnostic purposes. The method of Selander and Cramer[148] is recommended for analyses of lead in urine. In laboratories where it is essential to screen a large number of pediatric patients for lead intoxication, the Delves microsystem[143] for analysis of blood lead is recommended. Hicks et al.[155] have reported that the "within the run" precision of blood lead analyses by the Delves microtechnique is 8 per cent and that the "day to day" precision is 9 per cent. Hicks et al.[155] observed an excellent correlation between blood lead analyses by the Delves method[143] and analyses by Berman's macromethod,[127] which involves trichloroacetic acid deproteinization and APDC-MIBK extraction.

Applications of nonflame atomic absorption spectrometry to measurements of lead in biological materials are summarized in Table 7. Of the published techniques for analyses of lead in whole blood by flameless atomic absorption, the recent method of Hwang et al.[161] appears to be most promising. In this method blood is hemolyzed by 1:10 dilution with water, and 25 μl. of the diluted sample is pipetted

TABLE 7. NONFLAME ATOMIC ABSORPTION OF LEAD IN BIOLOGICAL MATERIALS

Author	*Date*	*Atomizer*	*Spectrometer*	*Sample*	*Preparation*
Hwang et al.[140]	1971	Tantalum strip	IL-353	Blood	APDC-MIBK extraction
Norval and Butler[156]	1972	Graphite tube	Unspecified	Blood	H_2O_2 oxidation
Renshaw et al.[157]	1972	Graphite tube	PE-303	Hair	Direct sampling
Kubasik et al.[158, 159]	1972	Graphite rod	V-T AA-5	Blood	Dilution (1:3) with Triton X-100
Rosen[160]	1972	Graphite rod	V-T AA-5	Blood	Diluted (1:2) with Xylene
Hwang et al.[161]	1973	Tantalum ribbon	IL-353	Blood	H_2O dilution (1:10)

directly into the sample cavity of a tantalum atomizer with a hydrogen background corrector (Instrumentation Laboratories Model 353). The drying and pyrolysis are performed simultaneously at 400° C. for 60 seconds. Atomization is performed for an unspecified period at approximately 1500° C. Hwang et al.[161] acknowledged that their method is relatively imprecise, with a "within the run" precision of <20 per cent (coefficient of variation) at a lead concentration of 17 μg. per dl. Illustrative recorder tracings of measurements of blood lead by the method of Hwang et al.[161] are shown in Figure 6. with and without use of the hydrogen background corrector. It is obvious that the background corrector is essential in order to reduce the great sample-to-sample variability and nonspecificity of measurements in the "normal" mode (i.e., without use of the background corrector).

Evenson and Pendergast[162] have critically evaluated analytical errors in measurements of blood lead by nonflame atomic absorption spectrometry. They observed quenching effects exceeding 25 per cent of the expected lead absorption when heparin or oxalate was used as the anticoagulant. They also noted significant analytical error resulting from "memory" effects in the graphite tube furnace. Sample interaction of 6 per cent was a common finding in their experience. The "within the run" precision of measurements of blood lead was 4 per cent (coefficient of variation), compared to a 15 per cent coefficient of variation for "day to day" precision.[16] In the present author's opinion nonflame atomic absorption spectrometry of blood lead holds great promise, but it is not yet sufficiently convenient and reliable to supplant conventional analyses by flame atomic absorption.

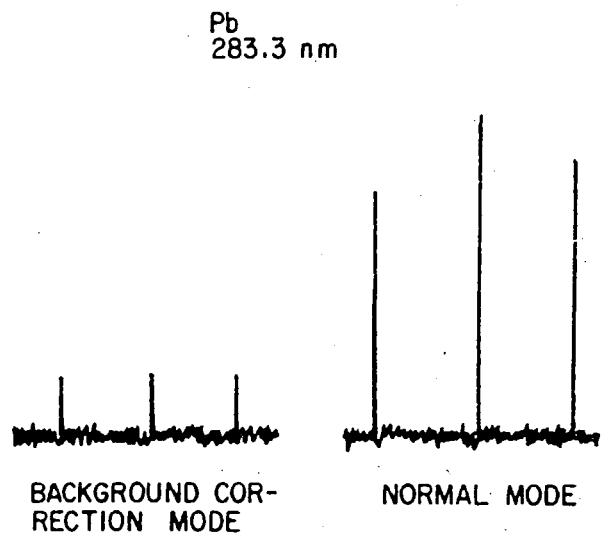


Figure 6. Recorder graph record for measurements of lead in a single specimen of blood by the method of Hwang et al.[161] (see text). (Courtesy of Instrumentation Laboratory, Inc., Lexington, Massachusetts.)

TABLE 8. NORMAL VALUES FOR LEAD IN BLOOD (B) AND URINE (U) BY AAS

Authors	*Date*	*Sample*	*Preparation*	*No. of Healthy Subjects*	*Normal Values Mean ± S.D.*	*Range*
					μg./dl.	
Piper and Higgins[75]	1967	B	Acid digestion, APDC-MIBK extraction	20	14 ± 8	0–31
Selander and Cramer[129]	1969	B	TCA deproteinization, APDC-MIBK extraction	8		7–18
Murphy et al.[6]	1970	B	TCA deproteinization, APDC-MIBK extraction	19	19 ± 4	11–19
					μg./day	
Kopito and Schwachman[151]	1967	U	Bismuth coprecipitation	18	44 ± 31	5–115
Piper and Higgins[75]	1967	U	Acid digestion, APDC-MIBK extraction	40	11 ± 8	0–21

Normal values for concentrations of lead in blood and for the excretion of lead in urine of healthy adult subjects are listed in Table 8, based upon analyses by atomic absorption spectrometry.

Iron

Measurements of serum iron and iron binding capacity are routinely performed in clinical pathology laboratories for the diagnosis of iron deficiency and iron overload. Relatively few clinical laboratories currently employ atomic absorption spectrometry for measurements of serum iron and iron binding capacity, owing to the availability of rapid and sensitive spectrophotometric methods, which are usually less susceptible to interference from hemoglobin iron. Flame atomic absorption spectrometry of iron is performed at 248.4 nm. (most sensitive line) or at 302.1 nm. (less sensitive line) with an oxidizing (fuel poor) flame. The usual burner gases are acetylene:air. The detection limit of iron is 4 μg. per liter. Flame conditions and burner height must be critically controlled. Viscosity and matrix effects cause interference in direct measurements of iron in undiluted serum.[163] According to Assendelft et al.,[165] sodium interference may introduce a systematic error in atomic absorption spectrometry of iron. Of the published methods for analysis of serum iron

TABLE 9. FLAME ATOMIC ABSORPTION OF SERUM IRON AND IRON-BINDING CAPACITY

Sample Preparation	*Authors*
Serum Iron	
1. HCl acidification, thioglycolic acid reduction, TCA deproteinization, bathophenanthroline-MIBK extraction	Zettner et al.[164]
2. HCl acidification, MIBK extraction	Dreux et al.[166]
3. HCl acidification, TCA deproteinization, ultrasonic nebulization	Uny et al.[167]
4. TCA deproteinization	Olson and Hamlin,[168] Tavenier and Hellendoorn[163]
5. H_2O dilution (1:1)	Zaino[169]
6. Dilution (1:1) with La_2O_3 solution	Heinemann[82]
7. Direct aspiration	Rodgerson and Helfer[170]
Iron Binding Capacity	
1. Fe (III) addition, unbound Fe(III) adsorbed on $MgCO_3$, then analyzed as for serum Fe	Zaino,[169] Olson and Hamlin,[168] Dreux et al.[166]
2. Fe(III) addition, unbound Fe(III) absorbed on ion-exchange resin, then analyzed as for serum Fe	Zettner et al.[164]

TABLE 10. NONFLAME ATOMIC ABSORPTION OF IRON IN BIOLOGICAL MATERIALS

Author	*Date*	*Atomizer*	*Spectrometer*	*Sample*	*Preparation*
Matousek & Stevens[119]	1971	Graphite rod	V-T AA-5	Serum	None
Glenn et al.[171]	1973	Graphite rod	PE-303	Serum	1:1 dilution (H_2O)
Olsen et al.[172]	1973	Graphite tube	PE-403	Serum	TCA deproteinization
Giridhar and Sunderman[173]	1973	Graphite tube	PE-403	Serum	None

and iron binding capacity by flame atomic absorption spectrometry (Table 9), the methods of Olson and Hamlin[168] and Tavenier and Hellendoorn[163] involve trichloroacetic acid precipitation of proteins. Trichloroacetic acid is necessary in order to remove hemoglobin and thus to prevent interference from hemoglobin iron. The method of Olson and Hamlin[168] has been employed routinely in the author's laboratory for three years, and it is recommended for use in clinical laboratories that prefer atomic absorption spectrometry to colorimetric analysis.

Nonflame atomization methods for atomic absorption spectrometry of serum iron are listed in Table 10. The method of Olsen et al.[172] is the most thoroughly documented of these procedures. In this technique hemoglobin iron is removed and serum is deproteinized by mixing 50 μl. of serum with 50 μl. of 30 per cent trichloroacetic acid, and heating at 90° C. for 15 minutes. Protein-free extract (10 μl.) is pipetted into special grooved graphite tubes for use with a new model nonflame atomizer (Model HGH-2000, Perkin-Elmer Corporation). Pyrolysis is performed during 25 seconds at 900° C. and atomization is performed during 8 seconds at 2400° C. The 302.1 nm. resonance line of iron is used for atomic absorption spectrometry. Olsen et al.[172] have achieved "within the run" precision of <5 per cent (coefficient of variation) (Fig. 7). As illustrated in Figure 8, Olsen et al.[172] have obtained an excellent correlation between analyses by their nonflame atomization method and by the flame atomic absorption procedure of Olson and Hamlin.[168] In the present author's opinion the technique of Olsen et al.[172] may be the first nonflame method for atomic absorption spectrometry of a trace metal that is sufficiently precise and reliable to be acceptable for routine diagnostic use in clinical pathology laboratories.

Normal values for serum iron con-

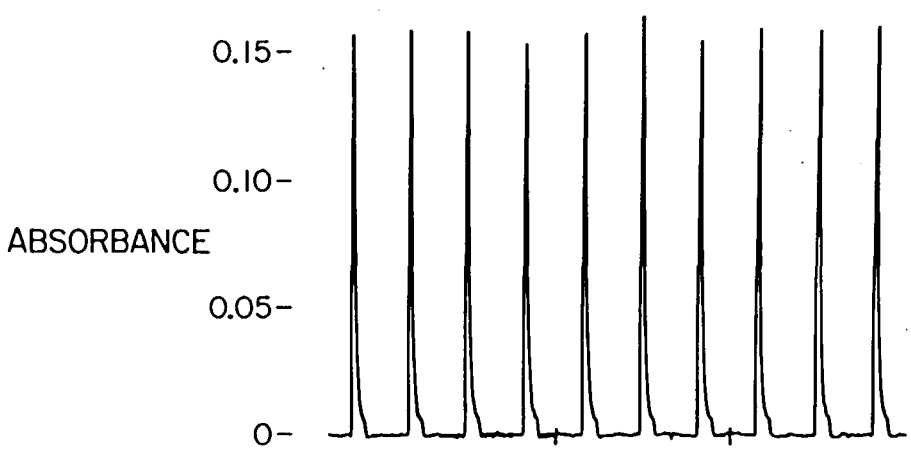


Figure 7. Recorder graph record showing the reproducibility of 10 determinations of iron in a single specimen of serum by means of the graphite tube furnace (see text). (From Olsen, E. D., et al.: Ultramicro method for determination of iron in serum with the graphite furnace. Clin. Chem., *19*:326–329, 1973.)

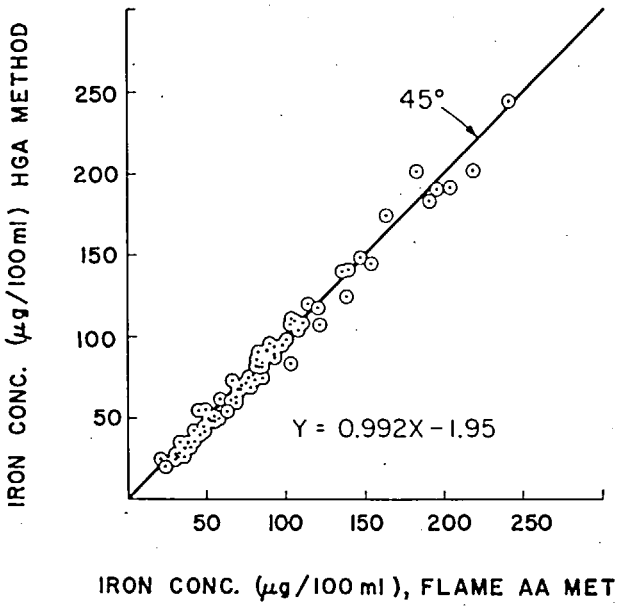


Figure 8. Comparison of results of serum iron measurements by means of the graphite tube furnace and by a flame atomic absorption method (see text). (From Olsen, E. D., et al.: Ultramicro method for determination of iron in serum with the graphite furnace. Clin. Chem., *19*:326–329, 1973.)

centrations are listed in Table 11, as reported by workers who employed atomic absorption spectrometry.

Chromium

Recent interest in the clinical pathology of chromium has been stimulated by the findings of abnormalities of chromium metabolism that may be related to glucose tolerance and diabetes mellitus. Glinsmann et al.[174] observed that oral glucose administration causes a prompt increase in plasma chromium concentrations in healthy men, but not in diabetic patients unless trace amounts of trivalent chromium are given as a dietary supplement. Levine et al.[175] reported a beneficial effect of oral chromium supplementation on glucose tolerance in elderly human subjects. Hambidge et al.[176] showed that concentrations of chromium in the hair of diabetic children were significantly lower than in the hair of normal children. Hambidge and Rodgerson[177] found that concentrations of chromium in the hair of pregnant women were significantly lower than in nonpregnant women, and they speculated that depletion of tissue chromium stores might occur during pregnancy.

Flame atomic absorption spectrometry of chromium is performed at 357.9 nm. using a reducing flame (fuel rich). The burner gases are usually acetylene:air or

TABLE 11. NORMAL VALUES FOR SERUM IRON BY AAS

Authors	*Date*	*Preparation*	*No. of Healthy Subjects*	*Normal Values* Mean ± S.D.	Range
				μg./dl.	
Rodgerson and Helfer[170]	1966	Direct aspiration	12	200 ± 49	102–298
Travenier and Hellendoorn[163]	1969	TCA deproteinization	20	122 ± 29	65–180
Olson and Hamlin[168]	1969	TCA deproteinization	57	105 ± 50	
Heinemann[82]	1972	Dilution (1:1) with La_2O_3 solution	240	98	58–157
Giridhar and Sunderman[173]	1973	Nonflame atomization	33	115 ± 18	73–151

TABLE 12. FLAME ATOMIC ABSORPTION OF CHROMIUM IN BLOOD (B), PLASMA (P), AND URINE (U)

Preparation	*Sample*	*Authors*
1. Dry ashing	P	Pierce and Cholak[128]
2. Acid digestion, $KMnO_4$ oxidation, MIBK extraction	B,P,U	Feldman et al.[178]
3. Acid digestion, $KMnO_4$ oxidation, TBP-MIBK extraction	B,U	Devoto,[179] Davis and Grossman[180]
4. H_2O dilution (1:2), with background correction at 352.0 nm.	P	Feldman[181]

hydrogen:air. The detection limit for chromium is 3 to 5 μg. per liter. Special care is essential to minimize contamination and background absorption. Methods of sample preparation for flame atomic absorption spectrometry of chromium in biological materials are summarized in Table 12. Limitations of sensitivity of flame atomic absorption have led most recent workers to employ nonflame atomization, as outlined in Table 13. The method of Davidson and Secrest[183] has been employed in the author's laboratory during the past year. In this technique plasma (0.2 ml.) is digested with perchloric acid and hydrogen peroxide and adjusted to a volume of 0.4 ml. A sample (50 μl.) of the digested sample is introduced into a graphite tube furnace, and drying is performed for 100 seconds at 100° C. Pyrolysis is performed in two steps: 60 seconds at 1100° C. and 12 seconds at 1400° C. Finally atomization is performed for 12 seconds at 2400° C. and atomic absorption is measured at 357.9 nm. The "within the run" precision of replicate analyses of chromium in plasma is <4 per cent (coefficient of variation).[183] Figure 9 illustrates the sensitivity and precision of the procedure and the independence of atomic absorption spectrometry from influence by different valence states of chromium.

Nickel

Investigations in the author's laboratory have showed that concentrations of serum nickel become increased in patients following acute myocardial infarction, stroke, burns, and septicemia.[186–188] Diminished mean concentrations of serum nickel are found in patients with hepatic cirrhosis and with chronic renal insufficiency.[187, 188] Measurements of nickel in serum, urine, feces, and hair are employed as measures of environmental and occupational exposure to nickel.[189–191]

McNeely et al.[189] measured nickel by atomic absorption spectrometry in serum and urine specimens from healthy inhabitants of Hartford, Connecticut, a city with relatively low environmental concentrations of nickel, and from healthy inhabitants of Sudbury, Ontario, the site of the largest open-pit nickel mines in North America. In the Hartford population, serum nickel concentrations averaged 0.26 ± 0.10 μg. per dl. ($N = 26$), and urine nickel excretion averaged 2.5 ± 1.4 μg. per day ($N = 20$). In comparison, in the Sudbury population serum nickel concentrations averaged 0.46 ± 0.14 μg. per dl. ($N = 25$), and urine nickel excretion averaged 7.9 ± 3.7 μg. per day ($N = 19$). These findings indicate that measurements of nickel in serum and urine are indices of

TABLE 13. NONFLAME ATOMIC ABSORPTION OF CHROMIUM IN BIOLOGICAL MATERIALS

Author	*Date*	*Atomizer*	*Spectrometer*	*Sample*	*Preparation*
Schaller et al.[182]	1972	Graphite tube	PE-305	Urine	Direct sampling (50 μl.)
Davidson and Secrest[183]	1972	Graphite tube	PE-290B	Plasma	$HClO_4$-H_2O_2 digestion
Pekarek and Hauer[184]	1972	Graphite tube	PE-403	Plasma, urine	Direct sampling (50 μl.)
Ross et al.[185]	1973	Graphite tube	PE-403	Urine	HNO_3 digestion

TABLE 14. NORMAL VALUES FOR CHROMIUM IN SERUM (S), PLASMA (P), AND URINE (U) BY AAS

Authors	*Date*	*Sample*	*Preparation*	*No. of Healthy Subjects*	*Normal Values* *Mean ± S.D.*	*Range*
					μg./dl	
Feldman et al.[178]	1967	P	Acid digestion, $KMnO_4$ oxidation, MIBK extraction	130	2.9	1.1–6.4
Feldman[181]	1969	P	H_2O dilution (1:2) with background corr. (352 nm.)	200	1.7	0.5–3.9
Davidson and Secrest[193]	1972	P	$HClO_4$ digestion, graphite furnace	7	0.51	0.31–0.72
Pekarek and Hauer[184]	1972	S	Direct analysis (50 μl.), graphite furnace	20	0.17 ± 0.06	
					μg./day	
Davidson and Secrest[193]	1972	U	$HClO_4$ digestion, graphite furnace	12	0.52	0.26–1.06
Schaller et al.[182]	1972	U	Direct analysis (50 μl.), graphite furnace	60	0.18 ± 0.11	
					μg./dl.	
Ross et al.[185]	1973	U	HNO_3 digestion (1:1), graphite furnace	20	1.2 ± 1.0	0.3–3.8

environmental exposure to nickel. Nomoto[191a] has reported that concentrations of nickel in healthy inhabitants of Matsumoto, Japan, averaged 0.21 ± 0.11 μg. per dl. (N = 23), which did not differ significantly from values for inhabitants of Hartford, Connecticut.

Szadkowski et al.[192] have noted diminutions of serum nickel in industrial workers in a steel mill who were exposed to extreme heat stress. This observation correlates with the recent report by Hohnadel et al.[193] that appreciable quantities of nickel are excreted in the sweat of healthy subjects during sauna bathing.

Flame atomic absorption spectrometry of nickel may be performed at two resonance lines, 232.0 nm. and 341.5 nm. According to Jackson and West[194] and Sundberg,[195] measurements at 232.0 nm. are two to three times more sensitive than at 341.5 nm. Adjacent nonresonating nickel lines occur at 231.0 and 338.1 nm., respectively, which are useful for background correction. Sundberg[195] has demonstrated that atomic absorption spectrometry of nickel should be performed in an oxidizing (fuel poor) flame. The usual burner gases are acetylene:air or acetylene:oxygen. The detection limit for nickel is 2 to 5 μg. per liter. Sundberg[195] has shown that Fe(III), Cu(II), Co(II), Mn(II), and Cr(II) significantly interfere with the atomic absorption of nickel when these metal ions are present in a concentration 100 times that of the nickel. The interferences are greatly influenced by the distance of the optical beam above the burner

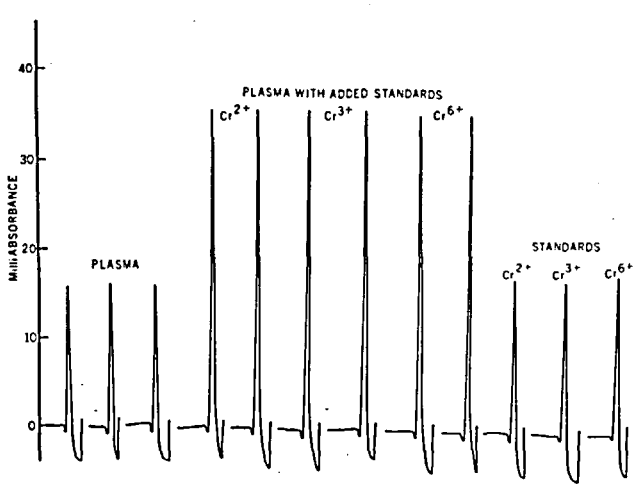


Figure 9. Tracings of recorder response showing the reproducibility of graphite tube furnace atomization of plasma chromium and aqueous chromium standards. Independence of the response to different valency states of chromium and chromium recovery after addition to plasma are demonstrated. (Plasma, wet ashed sample, 25 μl.; chromium aqueous standards, 200 pg. as $CrCl_2$, $CrK(SO_4)_2$, or K_2CrO_4.) (From Davidson, I. W. F., and Secrest, W. L.: Determination of chromium in biological materials by atomic absorption spectrometry using a graphite furnace atomizer. Anal. Chem., *44*:1808–1813, 1972.)

TABLE 15. FLAME ATOMIC ABSORPTION OF NICKEL IN BLOOD (B), PLASMA (P), SERUM (S), AND URINE (U)

Preparation	*Sample*	*Authors*
1. Acid digestion, DMG-$CHCl_3$ extraction, HCl back-extraction	U	Sunderman[3]
2. Acid digestion, APDC-MIBK extraction	B,U	Nomoto and Sunderman[4]
3. Acid digestion	B,P	Hoffmann and Fiedler[196]
4. TCA deproteinization, APDC-MIBK extraction	S	Schaller et al.,[197] Nomoto and Sunderman,[4] Lehnert et al.[198]

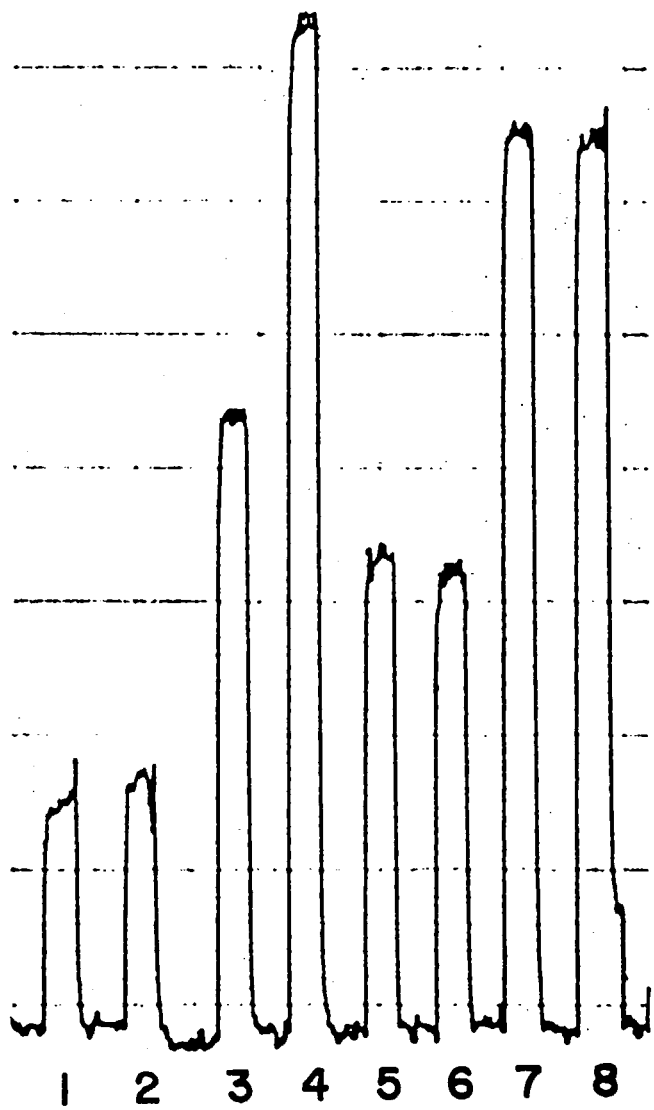


Figure 10. Typical recorder graph for atomic absorption analyses of urinary nickel. Samples 1 and 2 are reagent blanks; samples 3 and 4 are standards equivalent to 0.5 and 1.0 μg. of Ni/dl. of urine, respectively. Samples 5 and 6 are duplicate analyses of a urine sample from an inhabitant of Hartford, Connecticut, and samples 7 and 8 are duplicate analyses of a urine sample from an inhabitant of Sudbury, Ontario (see text). (From McNeely, M. D., et al.: Measurements of nickel in serum and urine as indices of environmental exposure to nickel. Clin. Chem., *18*:992–995, 1972.)

head. Maximal interference occurs at 24 mm. above the burner head. Careful focusing of the optical beam within 0 to 6 mm. above the burner head effectively eliminates these sources of interference. In atomic absorption of nickel, special care is essential to minimize nickel contamination and to compensate for background absorption.

The methods of sample preparation that have been employed for flame atomic absorption spectrometry of nickel in blood, plasma, serum, and urine are summarized in Table 15. The method of Nomoto and Sunderman[4] is recommended for analyses of nickel in serum, urine, and other biological materials. In Figure 10 is shown a typical recorder graph record for measurements of nickel in standard and urine samples.

Jackson and West[194] and Fuller[91] have investigated nonflame atomic absorption of nickel in aqueous solutions. Interference from other metals is particularly troublesome. Jackson and West[194] observed >15 per cent suppression of nonflame atomic absorption of nickel by Cr(III), Be(II), Sn(II), Fe(III), Mg(II), Mn(II), Co(II), Cu(II), Al(III), and Ca(II) when these metals were present in a concentration 100 times that of nickel. Interference by these metals could be reduced to an acceptable level by collimating the optical path by a small rectangular slit, so that the light beam passes immediately above the heated graphite rod. Nonflame atomic absorption spectrometry of serum nickel has been used by Pekarek and Hauer[184] and by the present author. Pekarek and Hauer[184] attempted to perform direct analyses of nickel in 50 μl. samples of serum by means of a graphite tube furnace, without background compensation. Their values were substantially higher than those that are obtained by flame atomic absorption spectrometry, as described by Nomoto and Sunderman.[4]

Sunderman and Nechay (unpublished observation) have recently developed a nonflame technique for serum nickel analyses that provides excellent analytical correlation with results obtained by flame atomic absorption.[4] Serum (2.5 ml.) is mixed with 3 ml. of 15 per cent (w/v) trichloroacetic acid. After 30 minutes the mixture is centrifuged to precipitate the proteins. The protein-free supernatant is decanted and saved. The precipitated proteins are washed with 2 ml. of trichloroacetic acid. After centrifugation the protein-free supernatant is decanted and combined with the initial protein-free supernatant. Phthalate buffer[4] (0.5 ml., pH 2.5) is added and the combined extracts are adjusted to pH 2.5 with concentrated ammonium hydroxide solution. Ammonium pyrrolidonedithiocarbamate solution (2 per cent w/v; 0.5 ml.) is added. The nickel-pyrrolidonedithiocarbamate complex is extracted into 1 ml. of methylisobutyl ketone. Nonflame atomic absorption spectrometry at 232.0 nm. is performed with the Perkin-Elmer model 403 atomic absorption spectrometer with HGA-70 graphite tube atomizer. The sample of methylisobutyl ketone is 50 μl. Drying is performed at 100° C. for 30 seconds, ashing is performed at 1100° C. for 60 seconds, and atomization is performed at 2600° C. for 25 seconds. A typical recorder tracing for nickel analyses in standard and serum samples by nonflame atomic absorption spectrometry is shown in Figure 11. Samples 1a and 1b are reagent blanks. Samples 2, 3, and 4 are duplicate standard samples containing, respectively, 5, 10, and 15 μg. of nickel per liter. Sample 5 is a normal serum and sample 6 is the same serum with addition of 10 μg. of nickel per liter. The recovery of added nickel averages 100 per cent; the within-run precision of replicate analyses is approximately 5 per cent (coefficient of variation).

Normal values for concentrations of nickel in serum or plasma and for the excretion of nickel in urine are listed in Table 16.

ADDITIONAL USES OF ATOMIC ABSORPTION SPECTROMETRY

In Table 17 are listed additional applications of atomic absorption spectrometry to measurements of trace metals in body fluids and tissues. This compilation of methods is intended to serve as a ready source of reference and to illustrate the multitude of situations in which atomic absorption spectrometry can contribute to clinical diagnosis. The methods that are

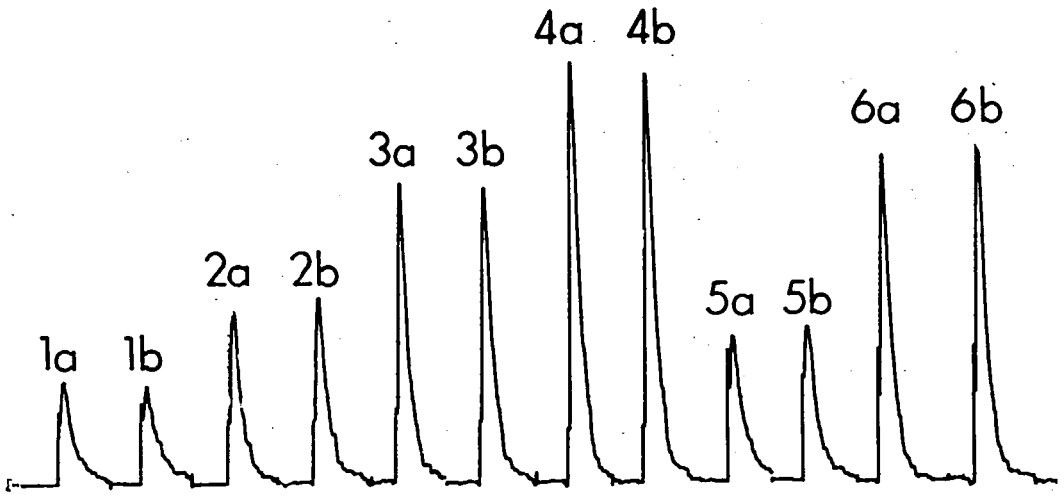


Figure 11. Typical recorder tracing for measurements of serum nickel by means of the graphite tube furnace (see text).

marked with an asterisk in Table 17 have been evaluated in the author's laboratory and have served satisfactorily for the stated clinical application.

Analyses of samples of hair are being used increasingly in order to estimate the body burdens of trace metals. A tabulation of applications of atomic absorption spectrometry to measurements of trace metals in human hair and nails is presented in Table 18. Studies by Klevay,[243–245] Hambidge,[176–177] and Nechay and Sunderman[190] have indicated that it is necessary to obtain segments of hair that are clipped within 2 to 5 cm. from the scalp in order to achieve consistent analytical results. Particular attention must be focused upon the method of washing the hair prior to atomic absorption spectrometry. If adequate precautions are exercised in sampling and washing, hair can be a valuable tissue for measurements of trace metals. The author

TABLE 16. NORMAL VALUES FOR NICKEL IN SERUM (S), PLASMA (P), AND URINE (U) BY AAS

Authors	*Date*	*Sample*	*Preparation*	*No. of Healthy Subjects*	*Normal Values* *Mean ± S.D.*	*Range*
					μg./dl.	
Schaller et al.[197]	1968	P	TCA deproteinization, APDC-MIBK extraction	26	2.1 ± 0.9	0.6–3.7
Nomoto and Sunderman[4]	1970	S	TCA deproteinization, APDC-MIBK extraction	40	0.26 ± 0.08	0.11–0.46
Nomoto[191a]	1971	S	TCA deproteinization, APDC-MIBK extraction	23	0.21 ± 0.11	
Pekarek and Hauer[184]	1972	S	Direct analysis (50 μl.), graphite furnace	20	1.5 ± 0.5	
McNeely et al.[189]	1972	S	TCA deproteinization, APDC-MIBK extraction	26	0.26 ± 0.10	0.08–0.52
					μg./day	
Sunderman[3]	1965	U	Acid digestion, DMG-$CHCl_3$ extraction	17	20 ± 10	7.2–38
Lehnert et al.[198]	1970	U	TCA treatment, APDC-MIBK extraction	15	9.3 ± 3.5	5.7–12.7
Nomoto and Sunderman[4]	1970	U	Acid digestion, APDC-MIBK extraction	26	2.4 ± 1.1	1.0–5.6
McNeely et al.[189]	1972	U	Acid digestion, APDC-MIBK extraction	20	2.5 ± 1.4	0.5–6.0

TABLE 17. ANALYSIS OF VARIOUS TRACE METALS IN BIOLOGICAL MATERIALS BY AAS

Metal	*Wavelength (nm.)*	*Authors*	*Date*	*Sample*	*Preparation*	*Application*
Ag	328.0	Buneaux and Fabiani[199]	1970	U	DDC-MIBK extraction	Ag poisoning
Al	396.1	Krishnan et al.[200]	1972	SF,T	Acid digestion	Exper. toxicology
As	313.3	Devoto[201]	1968	U	Arsenomolybdic acid extraction in cyclohexanone; Mo analysis by AAS	As poisoning
Au	242.8	Frajola and Mitchell[202]	1967	S,U,F	APDC-MIBK extraction	Au therapy of rheumatoid arthritis
		Lorber et al.[203]	1968	S, U	Dilution (1:1) with SDS solution, internal standardization	Same
		Dietz and Rubenstein[204]	1969	S	H_2O dilution (1:1)	Same
		Dunckley[205]	1971	S	H_2O dilution (1:4)	Same
		Balazs et al.[206]	1972	S,U	$KMnO_4$ oxidation, $AuCl_3$-MIBK extraction	Same
		*Harth et al.[207]	1973	B,S,U	APDC-MIBK extraction	Same
Be	234.9	Bokowski[208]	1968	U	Acid digestion, acetylacetone-MIBK extraction	Industrial monitoring
Bi	223.1	Devoto[209]	1968	U	Dry ashing, APDC-MIBK extraction	Bi poisoning
Bo	249.7	Bader and Brandenberger[210]	1968	B,U,T	Acid digestion	Boric acid poisoning
Cd	228.8	Berman[211]	1967	B	TCA deproteinization, DDC-MIBK extraction	Cd poisoning
		*Lehnert et al.[212, 213]	1968, 1969	S,U	Acid digestion, APDC-MIBK extraction	Industrial monitoring
		Curry and Knott[214]	1970	T	Acid digestion	Environmental exposure
		Hauser et al.[215]	1972	B	Low-temp. ashing, tantalum sampling boat	Industrial monitoring
Co	240.7	Sullivan et al.[216]	1968	T	Acid digestion	Beer drinker's cardiomyopathy
Hg	253.7	Berman[211]	1963	B	TCA deproteinization, APDC-MIBK extraction	Hg poisoning
		Toribara and Shields[217]	1968	T	Cold digestion (HCl and $NaNO_3$), ion exchange chromatog., CdS adsorption	Same
		*Mesman et al.[218]	1970	U	APDC-MIBK extraction	Same
		Hermann et al.[219]	1970	B,U	Pyrolyzing furnace for Hg vaporization	Same
		Krauser et al.[220]	1971	B,U	Acidification, $SnCl_2$ reduction, volatilization	Same
		Schaller et al.[221]	1971	U	Electrolysis on copper wire; nonflame atomization	Same
		Kubasik et al.[222]	1972	P,U	Cold digestion (H_2SO_4-$KMnO_4$), $SnCl_2$ reduction, volatilization	Same

Table 17 *continued on opposite page.*

shares Klevay's view[243–245] that analyses of hair as a biopsy material may become an established method in clinical pathology within the next few years.

Atomic absorption spectrometry of trace metals can also be employed in clinical pathology laboratories for indirect measurements of various nonmetallic constituents of body fluids. Nine examples may be cited:

1. *Serum chloride* can be precipitated as silver chloride by adding an excess of silver nitrate, and the silver that remains in solution is measured by atomic absorption.[250]

2. *Urine bromide* can be extracted as gold bromide and the gold measured by atomic absorption.[251]

3. *Inorganic phosphorus* can be measured by reaction with ammonium molybdate solution. The phosphomolybdate complex is extracted into octanol or isobutyl acetate, and the molybdenum is measured by atomic absorption.[252, 253]

4. *Urine sulfate* can be precipitated as barium sulfate. The precipitate is dissolved in EDTA solution, and barium is determined by atomic absorption.[254]

5. *α-Amino acids* in plasma and urine can be reacted with copper to form stable complexes and the copper content of the complexes measured by atomic absorption.[255–257]

6. *Nonesterified fatty acids* in serum can be extracted into an organic solvent and reacted with copper to form copper com-

TABLE 17. ANALYSIS OF VARIOUS TRACE METALS IN BIOLOGICAL MATERIALS BY AAS

Metal	*Wavelength (nm.)*	*Authors*	*Date*	*Sample*	*Preparation*	*Application*
Li	670.8	Blijenberg and Leijnse[223]	1968	S	Ethanol deproteinization	Li therapy of manic psychosis
		Lehmann[224]	1968	S	HCl dilution (1:10)	Same
		Tompsett[225]	1968	S	TCA deproteinization	Same
		Little et al.[226]	1968	S,U	H_2O dilution (1:5-1:20)	Same
		Zettner et al.[227]	1968	S,U	H_2O dilution (1:2-1:100)	Same
		*Pybus and Bowers[228]	1970	S	H_2O dilution (1:10)	Same
		Levy and Katz[229]	1970	S	H_2O dilution (1:10)	Same
		Frazer et al.[230]	1972	P,B,E	H_2O dilution (1:50)	Same
Mn	279.8	Mahoney et al.[231]	1969	S	H_2O dilution (1:2)	Normal subjects
		Ajemian and Whitman[231a]	1969	U	Dry ashing; 8-quinolinol extraction into MIBK-$CHCl_3$	Industrial workers
		Bek et al.[232]	1972	S	Dry ashing, nonflame atomization	Normal subjects
Mo	313.3	Pierce and Cholak[128]	1966	B,U	Dry ashing, APDC-MIBK extrac.	Normal subjects
Rb	780.0	Sutter et al.[233]	1970	S,B,U	H_2O dilution (1:5)	Rb therapy of psychosis
Sr	460.7	Descube et al.[234]	1967	U	Dilution (1:10) with HCl-$LaCl_3$	Sr therapy of osteoporosis
		Curnow et al.[235]	1968	S,U	Coprecipitation with Ca-oxalate, La addition	Same
		Tompsett[225]	1968	U	Dry ashing, ion-exchange	Same
		Montford and Cribbs[236]	1969	U	La-coprecipitation	Industrial monitoring
		*Warren and Spencer[237]	1972	U,F	Dry ashing, La-addition	Sr therapy
		Bek et al.[232]	1972	S	Dry ashing, nonflame atomization	Normal subjects
Tl	276.8	Berman[211]	1967	B,U	TCA deproteinization, DDC-MIBK extraction	Tl poisoning
		*Savory et al.[5]	1968	S,U	Acid digestion, HBr-ether extraction	Same

*Recommended method.

B = Blood
E = Erythrocytes
F = Feces
P = Plasma
S = Serum
SF = Spinal fluid
T = Tissues
U = Urine

TABLE 18. ATOMIC ABSORPTION SPECTROMETRY OF TRACE METALS IN HAIR (H) AND NAILS (N)

Authors	*Date*	*Sample*	*Washing*	*Preparation*	*Metals Analyzed*
Kopito et al.[238]	1967	H	H_2O boiling	Acid digestion	Pb
Schroeder and Nason[239]	1969	H	CCl_4	Dry ashing	Mg,Cu,Zn,Cr,Cd,Co,Pb,Ni
Bradfield et al.[240]	1969	H	Detergent, ether, ethanol	Dry ashing	Zn
Backer[241]	1969	H	Triton X-100, H_2O	Chloric acid digestion	Fe,Cu,Zn
Harrison et al.[242]	1969	H	"7X-O-Matic" detergent, H_2O	Acid digestion	Mg,Fe,Cu,Zn
Klevay[243-245]	1970,1973	H	Acetone, ether, Na lauryl-SO_4, H_2O	Acid digestion	Cu,Zn,Pb
Petering et al.[246]	1971	H	Acetone, ether, Na lauryl-SO_4, H_2O	Acid digestion	Cu,Zn
Harrison and Tyree[247]	1971	N	"7X-O-Matic" detergent, H_2O	Acid digestion	Ca,Mg,Fe,Cu,Zn
Hammer et al.[248]	1971	H	Detergent, ethanol, EDTA (boiling)	Acid digestion	Cu,Au,Cd,Pb
Barnett and Kahn[89]	1972	N	None	Graphite furnace	Cu
Renshaw et al.[157]	1972	H	Ether (reflux)	Graphite furnace	Pb
Briggs et al.[249]	1972	H	Detergent or ether	Dry ashing	Fe,Cu,Zn
Nechay and Sunderman[190]	1973	H	"7X-O-Matic" detergent, H_2O	Acid digestion	Ni

plexes, which are determined by atomic absorption.[258]

7. *Hemoglobin analyses* can be standardized by measurements of hemoglobin iron, using atomic absorption.[259, 260]

8. *Uroporphyrins, coproporphyrins, and protoporphyrins* in urine and blood can be isolated by ion exchange chromatography or by extraction into organic solvents. The porphyrins are then complexed with copper, and the copper complexes are determined by atomic absorption.[261]

9. *Cardiac glycosides* can be estimated in biological fluids, on the basis of their inhibition of rubidium uptake by erythrocytes. Rubidium analyses of washed red cells are performed by atomic absorption.[262]

For additional references to applications of atomic absorption spectrometry to measurements of trace metals in animal and plant materials, the reader may consult the recent review by Christian.[263]

CONCLUSIONS

In conclusion, it may be appropriate to speculate regarding the future role of atomic absorption spectrometry in clinical pathology. By the mid 1980's, the author predicts that atomic absorption spectrometry will be one of six automated instrumental methods that will constitute the basic analytical techniques for use in clinical chemistry laboratories. The other five instruments will probably include a rapid kinetic spectrophotometer for enzymatic analyses; a gas chromatograph-mass spectrometer for analyses of steroid hormones, drugs, and volatile metabolites; a radioactivity counter for radioimmunoassays of protein and polypeptide hormones, antibiotics, and various other drugs; a multichannel electrometer for rapid measurements of electrolytes by means of ion-specific electrodes; and a multichannel nephelometer for immunological assays of specific proteins in body fluids. Sample identification and accumulation of analytical results from the six basic instruments will undoubtedly be accomplished by means of a centralized laboratory computer. As one of the major instrumental methods in clinical chemistry laboratories, atomic absorption spectrometry of trace metals will provide the means for recognition of diverse new diseases, as well as providing data of value in the diagnosis and prognosis of diseases of trace metal metabolism that are already recognized in clinical medicine.

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Department of Laboratory Medicine
University of Connecticut School of Medicine
P.O. Box G
Farmington, Connecticut 06032