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SUBCOMMITTEE ON ENVIRONMENTAL AND OCCUPATIONAL TOXICOLOGY OF NICKEL*

# ANALYTICAL BIOCHEMISTRY OF NICKEL

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# ANALYTICAL BIOCHEMISTRY OF NICKEL*

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Abstract - Measurements of nickel in biological materials are reviewed, with emphasis on (a) preliminary steps for oxidation or removal of organic matter; (b) pre-concentration and separation procedures; (c) instrumental techniques for quantitation of nickel; and (d) reference values for nickel concentrations in human body fluids, tissues, and excreta. Electrothermal atomic absorption spectrometry is currently the most sensitive, convenient, and reliable technique for determination of nickel in biological materials, but it is rivalled in sensitivity by three other techniques (differential pulse polarography, particle-induced x-ray emission spectrometry, and gas chromatography). Isotope dilution gas chromatography-mass spectrometry may eventually become the definitive method for analysis of nickel in biological materials. Until such a definitive method has been developed, the IUPAC provisional reference method (electrothermal atomic absorption spectrometry) will serve for comparative evaluations of other procedures for clinical measurements of nickel concentrations.

## INTRODUCTION

Within the past decade, clinical chemistry and toxicology laboratories throughout the world have begun to measure nickel concentrations in human body fluids, tissues, and excreta in order to monitor exposures to nickel compounds which may result in acute or chronic toxicity. The IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel has conducted two interlaboratory surveys in which urine specimens were distributed to laboratories in seven countries for measurements of nickel concentrations by atomic absorption spectrometry. Both surveys disclosed wide discrepancies in the nickel concentrations that were obtained by participating laboratories. Adams et al (1) reported these findings and pointed out the serious need for improved accuracy of nickel determinations in body fluids. The interlaboratory comparisons showed that atomic absorption procedures with preliminary oxidation and extraction steps were generally superior to direct electrothermal atomization techniques in (a) analytical sensitivity; (b) recovery of added nickel; (c) interlaboratory precision; and (d) concordance of ranking of urine samples in order of increasing nickel concentrations. Adams et al (1) suggested that a reference procedure for analysis of nickel in biological materials was needed to harmonize the discordant results of nickel determinations.

The IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel sponsored an International Conference on Nickel Toxicology which was held in Kristiansand, Norway in May 1978 (2). The Working Group on Nickel Analysis at the Kristiansand Conference agreed upon a provisional reference method for analysis of nickel in serum and urine. This method is currently being evaluated in laboratories in the United States, Canada, England, West Germany, Finland, and Japan. Contingent upon the outcome of these trials, the provisional reference method will be published for critique by scientists throughout the world. Supporting documents for the provisional reference method include a report by Ader and Stoeppler (3) on radiochemical measurements of the recovery and analysis of nickel in urine and the present review on the analytical biochemistry of nickel. This article is intended to fill a void in the scientific literature, since methods for analysis of nickel in biological materials have not previously been reviewed. In contrast, the metabolism, clinical biochemistry, radiochemistry, toxicology, and carcinogenicity of nickel have been summarized in several recent articles and monographs (4-9).

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## OXIDATION OR REMOVAL OF ORGANIC MATTER

Methods for nickel determinations in biological materials usually involve preliminary steps for oxidation or removal of organic constituents. Seven procedures that have been employed for this purpose are listed in Table 1, with comments about their advantages and disadvantages. The cited references include descriptions of the basic techniques as well as their applications to analyses of nickel and other trace metals. Zachariasen et al (18) compared wet digestion using a combination of oxidizing acids versus dry ashing in a muffle furnace at 560°C as preliminary steps for analysis of nickel in whole blood, plasma, and urine. They concluded that dry ashing was superior because it required less attention and minimized use of chemical reagents which are sources of nickel contamination. Torjussen, Andersen, and Zachariasen (14) reached the opposite conclusion in a subsequent paper on analysis of nickel in tissues. They found that wet digestion was preferable to dry ashing because it saved time and because the sample could remain in the same vessel throughout the analysis.

Mikac-Dević et al (13) evaluated four techniques for destruction of organic constituents of serum and urine, including dry ashing in a muffle furnace and three different procedures for wet digestion. They obtained the best results by heating the sample together with mixed ultrapure acids ($HNO_3$, $H_2SO_4$, and $HClO_4$) in a Pyrex digestion tube inside an electric block heater. This procedure had the advantages that (a) the digestion, chelation, and extraction steps were performed in a single tube without necessity of quantitative transfer, (b) a constant volume of acid digestion mixture was used for blank, standard, and unknown samples, (c) the samples did not require constant attention since bumping and foaming were avoided by careful regulation of the heating block temperature, and (d) the samples did not evaporate to dryness since $H_2SO_4$ refluxed in the tubes when the digestion was completed. Most importantly, acid digestion was not attended by sporadic losses of nickel such as occurred from adsorption of nickel onto quartz crucibles during dry ashing in a muffle furnace.

Ader and Stoeppler (2) used $^{63}Ni$ as a tracer in order to compare losses during acid digestion and dry ashing of urine samples. They found that acid digestion in quartz tubes yielded quantitative recovery of $^{63}Ni$. In contrast, variable amounts of $^{63}Ni$ were lost during dry ashing in quartz crucibles in a muffle furnace, owing to formation of insoluble nickel silicates. Watling and Wardale (16) evaluated five techniques for oxidation of tissue samples, including two dry ashing procedures, two wet digestion procedures, and a low temperature ashing technique using the Tracerlab LTA 600 apparatus. One of the dry ashing procedures proved to be unsatisfactory for nickel analysis, owing to nickel contamination of manganese nitrate which was added as an ashing aid. The other four procedures yielded practically equivalent results for nickel analyses. After considering the pros and cons, Watling and Wardale (16) concluded that their personal preference was for wet digestion with $HNO_3$ and $HClO_4$.

TABLE 1. Digestion, Ashing or Protein-Precipitation Techniques for Analyses of Nickel in Biological Materials

| Technique and References | Reagents | Equipment | Comments |
|---|---|---|---|
| Wet digestion (10-16) | $HNO_3$, $H_2SO_4$, $HClO_4$, or $H_2O_2$ | Kjeldahl burners or electric block heater | Care to prevent foaming and bumping |
| Dry ashing (16-19) | HCl | Quartz or Pt crucibles, muffle furnace | Variable adsorption of Ni to quartz |
| Low temp. ashing (20-22) | $O_2$ plasma | Quartz boats, low temp. asher to excite $O_2$ | Few samples per run; slow, awkward transfer |
| Pressurized digestion (23-26) | $HNO_3$ | Teflon vessels in combustion bombs | Ni contamination problem; explosion hazard |
| Microwave digestion (27) | $HNO_3$, $HClO_4$ | Microwave oven with sample chamber | Few samples per run; microwave hazard |
| TMAH digestion (28-31) | Tetramethyl-ammonium hydroxide | Water bath | Small sample capacity; slow |
| Precipitation (12,32-34) | Trichloroacetic acid | Centrifuge | Ni is not released from urease, a Ni-metalloenzyme |

Quarternary ammonium compounds that were originally developed for solubilization of tissues for liquid scintillation counting have been used for analyses of trace metals in tissues (28-31). For example, Kaplan et al (29) analyzed nickel and other trace metals in rat lung samples (0.3 g, wet weight) following solubilization by incubation at 60°C for 24 h in 6 ml of a toluene solution of tetramethylammonium hydroxide. These procedures are labor-saving and do not require any special equipment, but they are slow and accommodate relatively small amounts of water and protein in the sample.

Several investigators (12,32-34) have reported that oxidation of organic matter can be circumvented by precipitation of serum or plasma proteins with trichloroacetic acid and HCl. At low pH, Ni[II] is liberated from binding to serum albumin and amino acids, and Ni[II] can be chelated and extracted from the protein-free supernatant fluid. The simplicity and convenience of trichloroacetic acid precipitation make this procedure attractive for routine use in measuring nickel concentrations in serum or plasma specimens from nickel-exposed workers. The present author has reservations about use of this procedure for analyses of nickel concentrations in pathological sera, since he has found that trichloroacetic acid does not quantitatively release nickel from jackbean urease, a nickel metalloprotein. Artefactitiously low values for serum nickel concentrations would be obtained if similar nickel metalloproteins exist in tissues and are released into serum under pathological conditions. Trichloroacetic acid treatment has been suggested for analysis of nickel concentrations in urine (34), but the validity of this approach has not been thoroughly documented.

The present author has evaluated each technique in Table 1 for use in analyses of nickel in urine, serum, and/or tissue specimens by electrothermal atomic absorption spectrometry. Quantitative recovery of nickel was achieved by dry ashing, provided that platinum crucibles were used instead of quartz crucibles and that the temperature of the muffle furnace did not exceed 525°C. Particular care was necessary to increase the temperature gradually from 100°C to 525°C and to avoid cross-contamination from dissemination of fluffy ash by air drafts when the door of the muffle furnace was opened. Trials of pressure digestion with $HNO_3$ in Teflon vessels inside Parr combustion bombs were disappointing, since leakage of $HNO_3$ fumes into the stainless steel casing resulted in sporadic nickel contamination. Oxidation with excited $O_2$ by means of the Tracerlab LTA 600 low temperature ashing apparatus was cumbersome because of (a) limited sample capacity, (b) few samples per run, (c) formation of protein crusts that were refractory to oxidation, and (d) difficulty in dissolving the inorganic residues in HCl and transferring the residues quantitatively from the shallow quartz combustion boats into test tubes. Acid digestion by use of a microwave oven was convenient for use with a few samples but was impractical for analysis of a routine batch of 36 to 42 samples.

The Working Party on Nickel Analysis at the Kristiansand Conference on Nickel Toxicology selected the wet digestion technique of Mikac-Dević et al (13) for the IUPAC provisional reference method for nickel analysis in serum or urine. This method can also serve for analyses of nickel in samples of tissue (<0.5 g, wet weight), provided that larger volumes of mixed acid are used. The present author recommends the wet digestion procedures of Nomoto and Sunderman (12) or Elakhovskaya et al (15) when larger samples of tissue (1-5 g, wet weight) are analyzed. The wet digestion of tissues is conveniently performed in 125 ml Erlenmeyer flasks on a large electric hot plate that furnishes an easily regulated and uniform heating surface.

## PRE-CONCENTRATION AND SEPARATION PROCEDURES

Nickel concentrations in human body fluids, tissues, and excreta are low in comparison to the sensitivities of most of the available analytical techniques. Therefore, clinical methods for nickel analyses customarily include preliminary steps to concentrate the nickel prior to quantitation. These steps also may help to separate nickel from substances that interfere with quantitation. Preconcentration and separation techniques that have been used for nickel determinations in body fluids are listed in Table 2. Several other techniques have been used for preconcentration of nickel from aqueous solutions, including (a) precipitation with benzildioxime (42), polyvinylhydroxyquinoline (43), or tris-(pyrrolidinedithiocarbamato)-cobalt (44); (b) adsorption on polyamine-polymer resin (45), dimethylglyoxime-impregnated polyurethane foam (46), or ion-exchange membrane filters (47); and (c) solvent extraction following complexation with thiothenoyltrifluoroacetate (48) or with a mixture of dithizone, quinolinol and acetylacetone (49).

Resin adsorption techniques for preconcentration of nickel from urine have been reported by two groups of investigators. Following acid digestion of urine, Janik and Jankowski (35) neutralized the digestion mixture and filtered it through a column of MK-2 ion exchange resin at pH 9.5. The adsorbed nickel was quantitatively eluted by addition of dilute HCl. Barnes and Genna (37) passed filtered samples of acidified urine (250 ml) directly through a miniature column of polydithiocarbamate resin. The sequestered nickel was quantitatively recovered by total acid digestion of the resin. The studies of Janik and Jankowski (35) and Barnes and Genna (36) were both preliminary investigations, and neither of them included

TABLE 2. Pre-concentration and Separation Techniques for Analyses of Nickel in Biological Materials

| Technique | Chelation Reagent | pH | Extraction Reagent | Authors |
|---|---|---|---|---|
| Resin adsorption | Ion-exchange resin (MK-2) | 9.5 | Dilute HCl | Janik & Jankowski (35) |
| | Polydithiocarbamate resin | 1-2 | $HNO_3$, $H_2SO_4$ | Barnes & Genna (36) |
| Chelation-extraction | Diethyldithiocarbamate | 8.5 | Isoamyl alcohol | Sunderman (37) |
| | Dimethylglyoxime | 8.5 | MIBK or $CCl_4$ | Zachariasen et al (18,19); Sunderman (37); Kincaid et al (38); Morgan (39) |
| | Pyrrolidinedithiocarbamate | 2.5<br>7.0 | MIBK | Nomoto (12,40,41)<br>Zachariasen et al (14,34) |
| | α-Furildioxime | 9.0 | MIBK | Mikac-Dević et al (13) |

comparisons with results of nickel analyses by other procedures or reference values for nickel concentrations in urine from non-exposed, healthy subjects.

Mikac-Dević et al (13) tested four chelating agents [ammonium pyrrolidinedithiocarbamate (APDC), dimethylglyoxime (DMG), α-furildioxime (FD), and benzildioxime (BD)] for extraction of Ni[II] from digests of urine or serum into methylisobutylketone (MIBK) and n-butylacetate. The optimal analytical sensitivities by electrothermal atomic absorption spectrometry were achieved with FD or APDC as the chelating agents and MIBK as the extraction solvent. Ader and Stoeppler (2) used $^{63}Ni$ as a tracer to compare the efficiencies for extraction of nickel by three chelating agents (APDC, DMG, and FD). Under the same conditions that were employed by Mikac-Dević et al (13), Ader and Stoeppler (2) found that the recovery of $^{63}Ni$ from urine digests into MIBK averaged 99% for APDC, 89% for FD, and 88% for DMG. Ader and Stoeppler (2) showed that the enhanced analytical sensitivity that was observed by Mikac-Dević et al (13) with FD as the complexing agent may have been caused by co-extraction of excess FD into MIBK. Zachariasen, Andersen, and their coworkers (14,18,19,34) evaluated DMG and APDC for analyses of nickel in biological materials and concluded (34) that APDC is the best of these chelating agents since it can be used for analyses of other trace metals as well as nickel. There is general agreement regarding the superiority of MIBK as the organic solvent for extraction of nickel chelates of APDC, DMG, or FD (2,13,34). Based upon these results, the Working Party on Nickel Analysis at the Kristiansand Conference on Nickel Toxicology selected APDC as the chelating agent and MIBK as the extraction solvent for use in the IUPAC provisional reference method for nickel analysis in serum and urine.

Based upon a study of the APDC-MIBK chelation-extraction procedure, Blanton et al (50) reported that the efficiency of extraction of $^{63}Ni$ from aqueous solutions was constant from pH 1 to pH 8 and was independent of the initial nickel concentration in the sample solution up to a concentration of 100 μg Ni/litre. Nomoto and Sunderman (12) found that pH 2.5 was optimal for extraction of nickel pyrrolidinedithiocarbamate (Ni-PDC) into MIBK. On the other hand, Andersen et al (34) employed pH 9.0 for the extraction of Ni-PDC into MIBK. The present author has recently found that pH 7.0-7.5 is superior to pH 2.5-3.0 or pH 5.0-5.5 for the extraction of Ni-PDC into MIBK. The critical factor appears to be gradual decomposition of the Ni-PDC complex. At pH 7.0-7.5 the Ni-PDC solution in MIBK remains stable for at least 3 h at room temperature and 24 h at 0°C. In contrast, at pH 2.5-3.0 and 5.0-5.5, variable diminutions of nickel concentrations in the MIBK extracts are observed upon storage. The decomposition of Ni-PDC in MIBK is more pronounced in extracts of urine or serum than in extracts of nickel standard solutions. Quantitative recovery of nickel added to urine is consistently obtained when the extraction is performed at pH 7.0-7.5, whereas low recovery values are frequently encountered when the extractions are performed at pH 2.5-3.0 or pH 5.0-5.5. Time-dependent decomposition of Ni-PDC in MIBK at pH 2.6 has also been noted by Ader and Stoeppler (2), who advised that nickel quantitation be performed within 1 h after the APDC-MIBK extraction. On the other hand, Jenne and Ball (51) found that Ni-PDC extracted from distilled water or tap water at pH >4 was stable in MIBK for at least 15 h at room temperature and up to one week at 4°C. In the light of these various observations, pH 7.0-7.5 has been selected for extraction of Ni-PDC into MIBK in the IUPAC provisional reference method.

## SPECTROPHOTOMETRIC AND FLUOROMETRIC METHODS

The molar lineic absorbances of various color reagents which are employed for spectrophotometric measurements of nickel are listed in Table 3. α-Furildioxime has been selected by the U.S. National Bureau of Standards as the preferred reagent for spectrophotometric determinations of nickel in biological materials (61). The extraction of nickel furildioximate into chloroform can be made very selective, since interference by iron and aluminium can be prevented by addition of citrate or tartrate to the reaction mixture, and interference by cobalt and copper can be eliminated by backwashing the chloroform extract with ammonia. The NBS method for analysis of nickel in biological materials (e.g., orchard leaves and bovine liver) involves the following steps: (a) wet ashing with $HNO_3$ and $HClO_4$; (b) addition of ammonium citrate and α-furildioxime solutions; (c) extraction of nickel furildioximate into chloroform and backwashing with dilute $NH_4OH$; and (d) spectrophotometry of the chloroform extract at 435 nm. The NBS spectrophtometric method is insufficiently sensitive for most clinical applications, since it requires that the samples contain 0.5 to 4 μg of nickel (61).

Zephiramine (1,2-naphthoquinonedioxime-4-sulfonic acid) and diethyldithiocarbamate are the most sensitive of the spectrophotometric reagents that are listed in Table 3, but they both lack specificity for nickel. Zephiramine has not been used for analyses of nickel in biological materials. Sunderman (37) employed diethyldithiocarbamate as the spectrophotometric reagent in a nickel assay that involved the following steps: (a) wet ashing of biological materials with $HNO_3$, $H_2SO_4$, and $H_2O_2$; (b) separation of nickel from interfering elements by chloroform extraction of nickel dimethylglyoximate in citrate buffer at pH 8.5; (c) back-extraction of nickel with HCl; and (d) conversion of nickel into the diethyldithiocarbamate complex and extraction into isoamyl alcohol at pH 8.5. The absorbance of nickel bisdiethyldithiocarbamate was measured at 325 nm. This method was suitable when the concentrations of nickel in serum or urine exceed 10 μg/litre, but it was insufficiently sensitive to measure the lower concentrations of nickel which are now recognized to occur in serum or urine of healthy subjects who have no occupational exposures to nickel compounds.

A fluorometric technique for nickel analysis has been described by Schenk et al (62) based upon quenching by nickel of the fluorescence of aluminium-1-(2-pyridylazo)-2-naphthol. This procedure is sensitive to nickel in concentrations as low as 1 μg/litre, but it is subject

TABLE 3. Spectrophotometric Reagents for Nickel Analysis

| Reagent | Solvent | Abs. Max. (nm) | Molar Lineic Absorbance ($x10^6 A \cdot m^{-1} \cdot mol^{-1}$) | Authors |
|---|---|---|---|---|
| Dimethylglyoxime | Chloroform | 335 | 0.5 | Kuse et al (52) |
| Benzildioxime | Chloroform | 406 | 1.1 | Banks & Barnum (53) |
| KCN & $NH_4OH$ | Water | 267 | 1.2 | Scoggins (54) |
| Thiothenoyl-trifluoroacetone | Carbon tetra-chloride | 480 | 1.2 | Muyle & Khopkar (55) |
| Cyclohexane-1,2-dionedioxime | Water | 460 | 1.4 | Perez et al (56) |
| α-Furildioxime | Chloroform | 435 | 1.6 | Bodart (57) |
| 6-Nitroquinoxaline-2,3-dithiol | MIBK | 710 | 2.1 | Bhaskare & Jagadale (58) |
| 3-Nitroso-4-hydroxy-5,6-benzocoumarin | Acetone | 395 | 2.5 | Kohli & Singh (59) |
| Thiotrifluoro-acetylacetone | Chloroform | 256 | 3.4 | Barratt et al (60) |
| Diethyldithio-carbamate | Isoamyl alcohol | 325 | 3.7 | Sunderman (37) |
| Zephiramine | Chloroform | 307 | 5.1 | Kuse et al (52) |

to interference by other metals and by chloride, sulfate, and phosphate ions. Therefore, it hardly appears to be suitable for nickel analysis of biological materials.

## REACTION RATE METHODS

Mealor and Townshend (63) developed a kinetic method for microdetermination of nickel based upon its catalytic effect on the decomposition of permanganate in alkaline solution in the presence of acetodiphosphoric acid. The rate of the reaction is proportional to the square of the nickel concentration. This reaction has been employed by Hadjiioannou et al (64) for an automated spectrophotometric reaction-rate system. Amounts of nickel in the range of 0.3-2.1 µg/sample can be determined with coefficients of variation of 2.5% within measurement times of 10 to 50 seconds (64). Kurzawa and Kubaszewski (65) described a kinetic method for nickel analysis based on a reaction of sodium azide with iodine that is catalyzed by sodium diethyldithiocarbamate. Nickel diethyldithiocarbamate does not catalyze the iodine-azide reaction. Therefore, the concentration of nickel in the reaction mixture is inversely related to the velocity of the iodine-azide reaction. Kurzawa and Kubaszewski (65) applied the reaction system to analyses of samples containing 0.14-14 µg of nickel, including measurements of nickel concentrations in margarine and drugs.

## DIFFERENTIAL PULSE POLAROGRAPHY

Polarographic techniques for nickel analysis have been described by several workers (49,66-68), but these techniques have lacked sufficient sensitivity to be employed for nickel determinations in biological materials. Recently Flora and Nieboer (69) found that addition of dimethylglyoxime to ammoniacal tartrate or citrate buffers enhances by a factor of 15 the sensitivity of derivative polarography of nickel at a dropping mercury electrode. This enhancement phenomenon has also been noted by Vinogradova and Prokhorova (70) and Astafeva et al (71). By means of dimethylglyoxime-sensitized differential pulse polarography, Flora and Nieboer (69) detected nickel concentrations as low as 2-3 µg/litre in buffered reaction mixtures. In a preliminary study, Nieboer et al (72) applied their procedure to measurements of nickel in human urine and blood following oxidation of organic constituents by dry ashing. Good agreement was observed between nickel analyses in body fluids by pulse polarography and by electrothermal atomic absorption spectrometry. Nieboer (personal communication) is currently refining the dimethylglyoxime-sensitized polarographic technique for analysis of nickel in biological materials, and he anticipates that the technique can serve as a means for independent assessment of the IUPAC provisional reference method.

## X-RAY FLUORESCENCE SPECTROMETRY

Concentrations of nickel that are found in human body fluids, tissues, and excreta are too low to permit direct measurements of nickel by standard techniques of x-ray fluorescence spectrometry. Forssen (17) ashed human tissues in a muffle furnace and compressed 30 mg aliquots of the ash into a wafer (17 mm in diameter) by means of a hydraulic press. The wafer served as the target of the incident x-ray beam. The spectral emission lines of 13 elements including nickel were scanned with a lithium fluoride detector. Forssen (13) reported that the detection limit for nickel was approximately 10 µg/gram of ash. She was able to measure nickel in only 20 of 665 tissue samples. Kessler and Mitchell (73) substantially increased the sensitivity of x-ray fluorescence spectrometry by preliminary co-precipitation of trace metals by addition of titanium in the presence of diethyldithiocarbamate. The precipitate was confined to a microdot (1.3 mm in diameter) on a filter disc which served as the x-ray target. Nickel was detected in aqueous solution in amounts as small as 0.6 ng/sample, equivalent to a nickel concentration of approximately 2 µg/litre. Kessler and Mitchell (73) did not employ their procedure for measurements of nickel in biological materials.

## PARTICLE-INDUCED X-RAY EMISSION SPECTROMETRY

Particle-induced x-ray emission ("PIXE") spectrometry is more sensitive than x-ray fluorescence spectrometry, and it has recently been used for detection and quantitation of various trace metals in tissues and body fluids (73-76). In practice, a 2-4 MeV proton beam from a Van de Graff generator is focused magnetically upon a dried sample inside a vacuum chamber. The sample is deposited as a spot (10-20 mm in diameter) on a target that is composed of a thin organic film (e.g., "Mylar," "Nucleopore," or carbon-impregnated polycarbonate). The proton beam dislodges inner shell electrons from atoms in the sample, and the inner shell vacancies are immediately filled by outer shell electrons. This process induces release of x-rays with energies that are characteristic of the element from which they were derived. The intensity of x-ray emission at each specific energy level is detected by a silicon detector and is quantified with a multi-channel analyzer. The intensity of x-ray emission at a specific energy level is proportional to the concentration of the corresponding element in the sample. Quantitation of PIXE analyses is accomplished either by use of internal standards such as strontium, or by spiking the sample with known amounts of the element(s) to be analyzed (74-77).

Three groups of investigators (78-80) have attempted to perform direct measurements of nickel concentrations in dried human blood serum by the PIXE technique, but the analytical sensitivities were barely sufficient to detect the presence of nickel. Campbell (77) reviewed these data and concluded that ashing and pre-concentration will be required for quantitation of nickel in human serum by the PIXE technique. Chen et al (81) successfully used PIXE for measurements of nickel concentrations in tissue specimens obtained at autopsy from control patients and from patients who died of Legionnaires' disease. Samples (0.1-0.5 g, wet weight) were digested in $HNO_3$ and 10 µl aliquots were evaporated to dryness and analyzed by PIXE with a 2 MeV proton beam. The x-ray intensity at the 7.472 KeV energy level that is characteristic of nickel atoms was used to estimate nickel concentrations by the standard additions method. The correlation coefficient for nickel concentrations obtained by Chen et al (81) using the PIXE method and those obtained by the present author's laboratory using the IUPAC provisional reference method was 0.936, based upon paired measurements of 5 samples of lung tissue. These limited data suggest that PIXE is a promising approach to analysis of nickel in tissues for laboratories that possess the requisite instrumentation.

## NEUTRON AND CHARGED PARTICLE ACTIVATION ANALYSIS

The practical usefulness of neutron activation analysis of nickel in biological materials is limited by the relative insensitivity of this technique for nickel. Lux and Zeisler (82) employed activation analysis using reactor irradiation and γ-spectrometry with a Ge(Li) well-type detector for measurements of trace metals in human connective tissue samples (0.1-0.2 g, wet weight). The detection limit for nickel was 0.5 µg/g (wet weight). Lux and Zeisler (82) did not detect nickel in normal connective tissue, but they did demonstrate the presence of nickel in connective tissue samples taken near nickel-containing metal implants. Swanson and Truesdale (83) used neutron activation for analysis of nickel and other metals in human lenses which had been lyophilized after quenching in liquid nitrogen. Swanson and Truesdale (83) speculated that nickel accumulation might be involved in the pathogenesis of senile cataracts, since nickel was inconstantly detected in lenses from young patients, but it was present in readily measured concentrations in cataractous lenses from senile patients. Swindle and Schweikert (84) described a procedure for analysis of nickel by charged particle activation analysis using an 88-inch cyclotron, based upon the reaction $^{58}Ni(p,pn)^{57}Ni$ ($t_{½}$ = 36 h). Post-irradiation chemical separation of $^{57}Ni$ resulted in a detection limit for nickel of approximately 1 µg/g in inorganic reference materials. Versieck et al (85) measured $^{58}Co$ produced by the reaction $^{58}Ni(p,n)^{58}Co$ ($t_{½}$ = 71 da) to study the influence of contamination from needles and scalpels upon the nickel concentration in human liver.

## ISOTOPE DILUTION MASS SPECTROMETRY

Paulsen et al (86) and Moore et al (87) described techniques for nickel analysis by stable isotope dilution and spark source mass spectrometry. In the procedure of Moore et al (87) one aliquot of a dissolved sample was spiked with stable $^{62}Ni$, and nickel was extracted as the dimethylglyoxime complex from an ammoniacal solution into chloroform and back extracted from the chloroform with dilute $HNO_3$. Nickel was separated by cation exchange chromatography, and the ratios of $^{58}Ni/^{62}Ni$ and $^{60}Ni/^{62}Ni$ were determined by mass spectrometry with a thermal ionization technique at 2060°C. A rhenium ribbon filament was used to reduce nickel background. The concentration of nickel was calculated from the relative abundances of $^{58}Ni$, $^{60}Ni$, and $^{62}Ni$ in the spiked and natural samples. Moore et al (87) applied this procedure to measurements of nickel in fuel oil, coal, and fly ash. To date, isotope dilution mass spectrometry has not been used for measurements of nickel in biological materials, but such measurements should be feasible in view of the recent success of Marino (88) and Veillon et al (89) in application of stable isotope dilution to determination of chromium in biological materials. Veillon et al (89) ashed lyophilized urine samples in an $O_2$ plasma discharge and spiked the samples with $^{50}Cr$. A volatile, thermally stable trifluoroacetylacetone chelate of chromium was isolated and the isotope ratio of $^{50}Cr/^{52}Cr$ was measured by combined gas chromatography-mass spectrometry using a Finnigan quadrupole mass spectrometer. The concentration of chromium in pooled urine from healthy adults averaged 0.32±0.02 µg/litre, which was about an order of magnitude lower than previously believed (89). The investigations of Veillon et al (89) and Marino (88) have shown that isotope dilution mass spectrometry with gas chromatographic separation is an extremely powerful analytical method for trace metals in biological materials. Attempts to adapt the method of Veillon et al (89) for analysis of nickel in body fluids are currently in progress in the present author's laboratory.

## RADIODISPLACEMENT ANALYSIS

German et al (90) developed a radioactive tracer displacement technique for determination of small quantities of nickel. Nickel is first isolated by dimethylglyoxime extraction and then determined by the displacement reaction between Ni[II] and $^{65}ZnEDTA$. The $^{65}Zn$ which is displaced from $^{65}ZnEDTA$ is extracted into a dithizone-$CCl_4$ solution, and $^{65}Zn$ is measured by γ-spectrometry. German et al (90) demonstrated that samples containing as little as 0.5 µg

of nickel could be analyzed by this relatively simple technique. This method has not been used for analyses of nickel in biological materials.

GAS CHROMATOGRAPHY

Gas chromatography of nickel complexes, particularly with ligands of the β-diketone type, has been a topic of intensive investigation during the past decade (91-99). The β-diketones possess thermal stability and volatility which are favorable for gas chromatography and their solubility in polar organic soluents is an advantage for preliminary solvent extraction. Substitution with fluoro or thiol groups increases the sensitivity of these compounds for electron capture detection. The gas chromatographic reagents that are listed in Table 4 are all β-diketone derivatives, excepting dipropyldithiocarbamate, which is also an attractive reagent for it yields excellent separations of nickel from copper and zinc (99).

Barratt et al (94) and Uden et al (95) employed gas chromatography for measurements of nickel in biological materials. For analysis of nickel and copper in mouse liver, lung, and kidney, Uden et al (95) ashed the samples in a muffle furnace and dissolved the residue in acid (0.3 g, wet weight, of tissue/ml of acid). Aliquots (100 µl) of the dissolved ash were alkalinized with gaseous $NH_3$ and 1 ml of an ethanolic solution of the ligand [$H_2$(enTF$A_2$)] was added. After addition of 20 ml of $H_2O$, Ni(enTF$A_2$) and Cu(enTF$A_2$) were extracted into 1 ml of benzene. Gas chromatography was performed under conditions indicated in Table 4. The detection limit was 20 pg/sample (1 µl) injected onto the column, with a $^{63}$Ni electron capture detector and 4 pg/sample with a scandium tritide electron capture detector. The nickel detection limit achieved with the scandium tritide detector was equivalent to approximately 15 µg/kg (wet weight) of tissue. Nickel concentrations in the mouse tissues were not specified, but the authors noted that the results agreed with values obtained by atomic absorption spectrometry. Barratt et al (94) used a similar procedure to measure nickel in samples of instant tea and hydrogenated triglycerides which contained from 4-13 µg/g. Thus, it appears that gas chromatography can serve as a practical technique for measurements of nickel in biological samples. To date, no thorough evaluations have been performed of gas chromatography of nickel in body fluids or excreta.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Uden and Walthers (100) reported that the nickel complex with N,N'-ethylenebis(salicylaldimine) has a molar lineic absorbance of $5.0 \times 10^6 A \cdot m^{-1} \cdot mol^{-1}$ at 254 mm, and they showed that the Ni(enSa$l_2$) complex can be separated from the copper complex, Cu(enSa$l_2$), by high performance liquid chromatography on microparticulate silica with a solvent system consisting of 20% acetonitrile in methylene chloride. By use of an ultraviolet detector at 254 mm and a flow cell with a volume of 8 µl, Uden and Walthers (100) achieved a nickel detection limit

TABLE 4. Chelation Reagents for Nickel Analyses by Gas Chromatography with Electron Capture Detection

| Reagent | Abbreviation | Extraction Solvent | Column Packing | Column Temp.(°C) | Authors |
|---|---|---|---|---|---|
| Trifluoroacetylacetone | TFA | Benzene | 2% Silicone SE-30 on Chromosorb WHP | 165-230 | Tamura et al (93) |
| Monothiotrifluoroacetylacetone | T-TFA | n-Hexane | 5% Silicone E-350 on Universal B | 140-170 | Barratt et al (94) |
| Bis(trifluoroacetylacetone)-ethylenediimine | $H_2$(enTF$A_2$) | Benzene | 1.5% OV-101 on Chromosorb W | 225 | Uden et al (95) |
| | | n-Hexane | 3% Silicone QF-1 on Varaport 30 | 150 | Belcher et al (96) |
| N,N'-propylenebistrifluoroacetylacetoneimine | $H_2$(pnTF$A_2$) | Benzene | 1.5% Dexsil 200 on Chromosorb W | 260 | Uden et al (97) |
| Bis(acetylpivalylmethane)-ethylenediimine | $H_2$(en(APM)$_2$) | Cyclohexane | 5% Silicone E-350 on Universal B | 285 | Belcher et al (98) |
| Dipropyldithiocarbamate | DPDTC | Chloroform | 1% Dexsil 300 on Chromosorb WHP | 245 | Gemmer-Colos & Neeb (99) |

of approximately 5 ng/sample. Linear relationship between absorbance and $Ni(enSal_2)$ concentration was maintained up to the μg level. Liska et al (101-103) investigated the separation of metal complexes of N-substituted dithiocarbamic acids by high performance liquid chromatography. In their latest paper, Liska et al (103) showed that nickel bisdiethyldithiocarbamate can be separated from the corresponding complexes of Zn, Cu, Mn, Pb, Co, Cd, and Fe on microparticulate silica with 10% chloroform in cyclohexane as the solvent. Liska et al (101) noted that the limit of detection of nickel bisdiethydithiocarbamate at 325 mm is $10^{-9}$-$10^{-10}$mol by use of a UV detector attached to the high performance liquid chromatograph. More sensitive detection of nickel can probably be achieved by use of flame or electrothermal atomic absorption detectors, as described by Jones et al (104), Koizumi et al (105), and Vickrey et al (106). The usefulness of high performance liquid chromatography for trace analysis of nickel in biological materials has not yet been demonstrated.

## ATOMIC EMISSION SPECTROMETRY

During the decade from 1955 to 1964, several investigators surveyed the concentrations of trace metals in human blood and autopsy tissues by emission spectrography, and measurements of nickel concentrations were frequently included in the tabulated results of these studies (107-113). Little reliance can be placed upon these measurements of nickel, since the nickel concentrations were either below or barely above the detection limits. When more sensitive atomic absorption procedures were developed during the mid-1960's, measurements of nickel in biological materials by emission spectrography were generally abandoned. Renewed interest in atomic emission techniques for nickel analysis has been evoked by the recent development of inductively coupled plasma-atomic emission spectrometry (36,114). Haas et al (114) have described an instrument for direct multi-element analysis of urine based upon (a) ultrasonic nebulization of the sample; (b) aspiration of the sample vapor by an argon stream into a luminous plasma produced by an induction coil; and (c) simultaneous detection of photoemission at 20 wavelengths by a polychromator. Emission intensities of added amounts of internal reference elements were used to compensate for variations in nebulization efficiency. However, with the simple procedure for sample preparation that was used, the instrument lacked sufficient sensitivity to detect nickel in normal urine. Haas et al (114) stated that the detection limit for nickel was approximately 4-9 μg/litre of urine. Barnes and Genna (36) overcame this limitation by pre-concentration of metals in urine by a factor of 125 by use of a poly(dithiocarbamate) resin. Samples of urine (250 ml) were passed through a resin column, and the sequestered metals were recovered by digestion of the resin to achieve a final sample volume of 2 ml. The sample was then aspirated into the inductively coupled plasma for determination of 10 trace metals including nickel. Barnes and Genna (36) noted that the detection limit for nickel in urine was 0.06 μg/litre. This technique appears to offer advantages for routine analyses of trace metal concentrations in urine specimens.

## ATOMIC FLUORESCENCE SPECTROMETRY

Armentrout (115) pointed out the superior analytical sensitivity of nickel determinations by atomic fluorescence compared to atomic absorption spectrometry. Consistent with this observation, Matousek and Sychra (116) found that the detection limit for nickel analysis at 232.0 nm by flame atomic fluorescence spectrometry was approximately 3 μg/litre compared to 20 μg/ liter by atomic absorption spectrometry with the same spectral source and spectrometer. Use of an organic dye laser as the excitation source for atomic fluorescence spectrometry has enhanced analytical sensitivity and convenience. By use of a tunable dye laser, Weeks et al (117) obtained a detection limit of 2 μg/litre for nickel analysis by flame atomic fluorescence spectrometry at 352.4 mm. However, since lower detection limits for nickel can easily be obtained by electrothermal atomic absorption, there has been little interest in flame atomic fluorescence spectrometry of nickel in biological materials.

## ATOMIC ABSORPTION SPECTROMETRY

In 1960, Allan (118) first reported the use of flame atomic absorption spectrometry for nickel analysis in aqueous solutions. Applications of the technique to measurements of nickel concentrations in human body fluids, tissues, and excreta were soon described by several investigators (12,32,39,40,41,119,120). In the procedure of Nomoto and Sunderman (12) samples of urine (50 ml) were digested with $HNO_3$, $H_2SO_4$, and $HClO_4$, and samples of serum (10 ml) were deproteinized with trichloroacetic acid. Nickel was extracted as nickel bisdiethyldithiocarbamate into MIBK (3 ml), and the concentration of nickel in the MIBK extract was determined by atomic absorption with an acetylene-air flame. This procedure achieved a detection limit for nickel of 0.1 μg/litre of urine or 0.5 μg/litre of serum (12). The coefficients of variation of replicate nickel analyses in urine and serum samples were 10% and 9%, respectively (12). The large sample requirement for serum and the protracted digestion required for analysis of urine made this flame atomic absorption method cumbersome for routine use. In order to achieve greater sensitivity, most laboratories that were engaged in nickel analyses for clinical purposes shifted to electrothermal atomic absorption spectrometry as soon as graphite electrothermal atomizers became commercially available (1,13,18,19,33,34,121,122). In the electrothermal atomic absorption method of

Mikac-Dević et al (13) 1 ml samples of serum or urine are digested with $HNO_3$, $H_2SO_4$, and $HClO_4$, and nickel is extracted as the α-furildioxime complex into MIBK (0.7 ml). Aliquots (50 µl) of the MIBK extract are pipetted into the graphite tube furnace, and the temperature program for drying (up to 120°C), ashing (up to 950°C), and atomization (2600°C) is performed. This procedure achieves a detection limit for nickel in serum or urine of 0.4 µg/litre and coefficients of variation of ±10% and ±7% for analyses of serum and urine, respectively (13).

Recent refinements in instrumentation for electrothermal atomic absorption spectrometry [e.g., (a) automatic sampling devices (123,124); (b) temperature ramping during the drying and charring cycles (125); (c) more sudden heating for atomization (126,127); (d) optical sensors to regulate the atomization temperature (125,128,129); (e) pyrolytic hardening of graphite tubes (123,130-132); (f) improved optical alignment of the $D_2$-background correction system (125,133); and (g) integration circuitry to measure peak areas instead of peak heights (134-136)] have significantly improved the sensitivity and precision of nickel analysis in serum and urine. The IUPAC provisional reference method for nickel analysis, as currently employed in the present author's laboratory, involves: (a) digestion of 2 ml samples of serum or urine as described by Mikac-Dević et al (13); (b) chelation of nickel according to the protocol of Mikac-Dević et al (13) but with substitution of 2% APDC for α-furildioxime and adjustment to pH 7.2 for extraction of the Ni-PDC into 0.7 ml of MIBK; (c) analysis of 20 µl aliquots of MIBK extract by use of a Perkin-Elmer model 5000 atomic absorption spectrometer with model HGA-500 electrothermal atomizer, optical sensing temperature control system, $D_2$-background corrector, and automatic sampling system. The temperature program for the graphite tube furnace will be given below. The nickel concentrations that are present in ultrapure reagents are the factors that determine the detection limit, rather than the sensitivity of the analytical instrument. The detection limit for nickel is approximately 0.3 µg/litre of serum or urine. The coefficient of variation of nickel analysis in urine is 7.8%, based upon 21 analyses on consecutive working days of a single urine specimen from a healthy subject, with a mean nickel concentration of 4.2 µg/litre. The recovery of nickel averages 98% (SD±3.4%), based upon additions of nickel in a concentration of 5 µg/litre to 12 specimens of urine from healthy subjects (mean nickel concentration = 3.9 µg/litre).

Dudas (137) studied the effects of drying parameters upon the sensitivity of electrothermal atomic absorption spectrometry of Ni-PDC in MIBK extracts. He noted that MIBK slowly spread laterally and up the walls of the graphite tube, provided that the drying cycle was delayed for at least 1 min after sample injection. Otherwise, boiling of MIBK caused sputtering of the sample and decreased the analytical sensitivity and reproducibility. The present author has achieved greatest sensitivity and precision for detection of Ni-PDC in 20 µl samples of MIBK by the following drying program for the Perkin-Elmer HGA-500 furnace: 70 sec linear ramp from 25°C to 120°C and 10 sec plateau at 120°C.

Fuller (138) and Findlay et al (139) investigated the loss of nickel during the pre-atomization heating period in electrothermal atomic absorption spectrometry. Fuller (138) found that heating at 750°C for 60 sec was associated with minimal loss of nickel, whereas heating at 1100°C for 30 sec caused 10% to 35% loss of nickel, depending upon the sample matrix. Findlay et al (139) reported that heating at 900°C for 30 sec caused loss of less than 5% of nickel by volatilization. The present author recommends the following program for the Perkin-Elmer HGA-500 furnace: 45 sec linear ramp from 120°C to 1000°C and 15 sec plateau at 1000°C. Argon flow of 300 ml/min is continuous during the drying and ashing cycles in order to sweep the vapors and combustion products out of the graphite tube. Ultrapure argon (99.999%) is used as the purge gas as recommended by Stoeppler et al (123) to achieve maximum reproducibility and prolong the working life of the graphite tube. In the present author's opinion (140) argon is preferable to nitrogen or helium, since the diffusion constants of vaporized metals in argon are lower than in helium (141), and the specific heat and thermal conductivity are lower for argon than for helium or nitrogen (142). Moreover, when nitrogen is used, there is a possibility of forming traces of cyanogen, which has an absorption band in the ultraviolet spectrum (140). On the other hand, Cruz and Van Loon (143) prefer nitrogen as the purge gas for nickel analysis, since the background absorbance at 232.0 nm is slightly lower for nitrogen than for argon. Beaty and Cooksey (125) suggested introducing air into the graphite tube for 10 sec during ashing at 800°C in order to oxidize the organic matrix of serum. They claimed to analyze nickel directly in serum by this technique without preliminary digestion, deproteinization, chelation, or extraction steps. Insufficient experimental details were provided to evaluate their procedure.

The boiling point of nickel is 1453°C (139). Kantor et al (144) studied the vaporization of nickel at temperatures from 1330°C to 2100°C by measuring the atomic absorption at 232.0 nm. Minimal vaporization of nickel occurred at 1330°C, and the plateau of maximum absorption was reached at 2000°C. Kzobik and Matousek (145) found that the plateau of maximum atomic absorption of nickel was reached at 1710°C. The present author has found that maximum sensitivity for nickel in APDC-MIBK extracts and quantitative recovery of nickel added to serum or urine are achieved by the following atomization program for the Perkin-Elmer HGA-500 furnace: 7 sec plateau at 2700°C, with argon flow reduced to 10 ml/min. The advantage of

atomization at the relatively high temperature of 2700°C apparently derives from avoidance of interference by other metals that are present in APDC-MIBK extracts of biological materials. Metal interference in atomic absorption spectrometry of nickel is more troublesome with flame atomization than with electrothermal atomization. Nomoto (40) observed significant interference by Cu (10 mg/litre) and Au, Pt and Cd (2.5 mg/litre) upon atomic absorption of nickel (50 µg/litre) at 232.0 nm in an acetylene-air flame. Sundberg (146) found that Fe, Mn, Cu, and Co (2 g/litre) suppressed atomic absorption of Ni (20 mg/liter) in oxidizing and reducing acetylene-air flames. The interferences were greatly influenced by observation height, and they could be eliminated by careful adjustment of the distance between the optical beam and the burner. Kantor et al (144) and Kzobik and Matousek (145) have both reported that >50 fold excess of copper suppresses atomic absorption of nickel at electrothermal atomization temperatures below 2000° C. Kantor et al (144) did not observe any significant interference by copper on electrothermal atomic absorption of nickel at 2100°C. Mikac-Dević et al (13) found that Fe (30 mg/litre) suppresses the electrothermal atomic absorption of Ni (10 µg/litre) in the furildioxime-MIBK extraction procedure, and they cautioned that Fe might cause interference in measurements of nickel in whole blood or tissues. No interference was noted when Fe was tested at a concentration of 10 mg/litre under the same conditions. Jackson and West (147) observed >15% suppression of electrothermal atomic absorption of nickel by Cr, Be, Sn, Fe, Mg, Mn, Co, Cu, Al, and Ca when these metals were present in a concentration 100 times that of nickel, based upon analyses with carbon filament atomization. Interferences by these metals were reduced to an acceptable level by collimating the optical path with a small retangular slit so that the light beam passed immediately above the carbon filament.

Emara et al (148) found that $HNO_3$, $H_2SO_4$, and $HClO_4$ each caused suppression of nickel measurements at 232.0 nm by flame atomic absorption. Julshamm (149) reported that $HClO_4$ (1 mol/litre) caused 18% suppression of the atomic absorption of nickel (1 mg/litre) at 232.0 nm as determined by electrothermal atomization at 2500°C. This inhibitory effect could be prevented by preliminary evaporation of the $HClO_4$ solution. Sutter and LeRoy (150) found that the effects of Fe upon electrothermal atomic absorption of nickel were strongly influenced by the concentration of $HNO_3$ in the sample. At a low concentration of $HNO_3$ (1.5 mmol/litre), addition of Fe (50 mg/litre) slightly increased the atomic absorption of nickel (40 µg/litre), whereas at a high concentration of $HNO_3$ (1.5 mol/litre), similar addition of Fe strongly suppressed the atomic absorption of nickel (150). The inhibitory effects of acids upon atomic absorption of nickel are avoided in the IUPAC provisional reference method by chelation and extraction of the Ni-PDC complex into MIBK at pH 7.2. However, it is important that $HClO_4$ be completely evaporated during the preliminary digestion step in order to avoid subsequent oxidation of the APDC reagent.

During an investigation of the nickel content of ureases, Grove and Sunderman (151) observed that tris(hydroxymethylamino)methane ("tris") buffer suppressed electrothermal atomic absorption spectrometry of nickel in aqueous standard solutions but did not interfere in analyses of nickel in urease. Thus addition of tris (2 mmol/litre) to an aqueous solution of $NiNO_3$ (0.9 µmol/litre) caused 57% suppression of atomic absorption of nickel at 232 nm under the instrumental conditions described by Mikac-Dević et al (13). Additions of tris (2-50 mmol/litre) to an aqueous solution of jackbean urease (that contained 0.3 µmol/litre of protein-bound nickel) did not affect the atomic absorption of nickel under the same conditions. Grove and Sunderman (151) suggested that the phenomenon of tris inhibition of electrothermal atomic absorption spectrometry of nickel might serve as a rapid and sensitive method to distinguish nickel which is free in solution from nickel which is tightly bound to protein.

## NICKEL CONCENTRATIONS IN HUMAN BODY FLUIDS, TISSUES, AND EXCRETA

Measurements of nickel concentrations in serum or urine specimens from healthy adult inhabitants of several regions of the world are listed in Table 5. The analyses were all performed by atomic absorption spectrometry, and the subjects did not have any occupational exposures to nickel compounds. Excellent agreement was observed between the nickel concentrations in serum or urine from the subjects in West Germany, Japan, Spain, and the United States (2,33,40,152,155). Significant increases were noted in urine and serum nickel concentrations in inhabitants of Sudbury, Canada, which is a site of large nickel deposits and nickel refineries (153). The mean nickel concentration and the range of concentrations that were reported in urine of inhabitants of Kristiansand, Norway (154) appear to be greater than have been observed in inhabitants of West Germany, Japan, and USA. This finding may be related to the presence of a nickel refinery in Kristiansand, Norway. Plasma nickel concentrations in the residents of Kristiansand, Norway were comparable to serum nickel concentrations in residents of Japan, Spain, and USA. In Table 6 are listed reference values for nickel concentrations in body fluids, excreta, and biopsy tissues from living, nonexposed, adult persons, based upon atomic absorption analyses by the present author and his colleagues (12,156-160) and by Torjussen and coworkers (14,154). In Table 7 are listed reference values for nickel concentrations in human, postmortem tissues obtained at autopsy, based upon analyses by Nomoto (41) and Sunderman et al (161).

TABLE 5. Analyses by Atomic Absorption Spectrometry of Nickel Concentrations in Serum and Urine of Healthy Adult Subjects without Occupational Exposures to Nickel Compounds. Each value is the mean ±SD. The numbers of subjects are listed in brackets.

| Locations | Nickel Concentrations (µg/litre) Serum or Plasma | Urine | Authors |
|---|---|---|---|
| Julich, West Germany | | 2.6±1.2 [21] | Ader & Stoeppler (2) |
| Matsumoto, Japan | 2.1±1.1 [24] | 2.7±1.1 [73] | Nomoto (40,152) |
| Santiago, Spain | 2.5±0.5 [5] | | Gonzalez et al (33) |
| Hartford, U.S.A. | 2.6±1.0 [26] | 2.0±0.9 [20] | McNeely et al (153) |
| Sudbury, Canada | 4.6±1.4 [25] | 7.2±3.9 [19] | McNeely et al (153) |
| Kristiansand, Norway | 1.9±1.4 [57] | 4.9±4.2 [57] | Torjussen & Andersen (154) |

TABLE 6. Analyses by Atomic Absorption Spectrometry of Nickel Concentrations in Specimens from Healthy Adult Subjects without Occupational Exposures to Nickel Compounds(155)

| Specimen | Nickel Concentrations in Specimens Mean±SD | Range | No. | Units | Authors |
|---|---|---|---|---|---|
| Whole blood | 4.8±1.3 | 2.9-7.0 | [17] | µg/litre | Nomoto & Sunderman (12) |
| Serum | 2.6±0.9 | 0.8-5.2 | [80] | µg/litre | Sunderman (156) |
| Urine | 2.2±1.2<br>2.6±1.4 | 0.7-5.2<br>0.5-6.4 | [50] | µg/litre<br>µg/day | Sunderman (156) |
| Feces | 14.2±2.7<br>258±126 | 10.8-18.7<br>80-540 | [10] | µg/g (dry)<br>µg/day | Horak & Sunderman (157) |
| Scalp hair | 220±80 | 130-510 | [20] | µg/kg | Nechay & Sunderman (158) |
| Arm sweat | 52±36 | 7-180 | [33] | µg/litre | Hohnadel et al (159) |
| Parotid saliva | 2.2±1.2 | 0.8-4.5 | [20] | µg/litre | Catalanatto & Sunderman (160) |
| Palatine tonsils | 140±70 | 30-280 | [15] | µg/kg (wet) | Torjussen et al (14) |
| Nasal mucosa | 130±200 | | [57] | µg/kg (wet) | Torjussen & Andersen (154) |

## AVOIDANCE OF NICKEL CONTAMINATION

Contamination during specimen collection is a troublesome problem for measurements of nickel concentrations in body fluids, excreta, and tissues. Sweat from the fingers and palms of the hands is rich in nickel (159) and is a common source of contamination of pipets and specimen containers. Procedures to minimize nickel contamination during collection and analysis of serum and urine have been described in detail by Mikac-Dević et al (13) and Sunderman (140). Plastic cone-tips that are used for micropipetting instruments (e.g., "Eppendorf" or "Oxford" pipettors) are frequently contaminated with metals (162,163) and should invariably be cleansed before use by soaking in ultrapure $HNO_3$ followed by multiple rinses with demineralized water which has been distilled in a quartz still (140). Commercially available evacuated tubes for collection of blood (e.g., "Vacutainer" tubes) are often contaminated with trace metals (164,165) unless the tubes and stoppers have been washed with $HNO_3$. Versieck et al (85) demonstrated that use of Menghini biopsy needles or surgical scalpel blades can cause many-fold increases in the nickel concentrations of small biopsies of normal human liver. Segments of glass tubing and plastic knives that have been washed in ultrapure $HNO_3$ are satisfactory tools for collection of postmortem tissues specimens for nickel analysis. For helpful advice on contamination control in trace metal

TABLE 7. Analyses by Atomic Absorption Spectrometry of Nickel Concentrations in Post-Mortem Tissues from Adult Subjects. Subjects A-D were reported in reference 161 and subjects E-L in reference 41. All analyses were performed by the Nomoto-Sunderman technique (12).

| Subjects | Causes of Death | Nickel Concentrations (μg/kg, wet weight) Bone | Lung | Kidney | Liver | Heart |
|---|---|---|---|---|---|---|
| A ♂ 44 | Stab wounds | | 24 | | 5.2 | 6.2 |
| B ♀ 40 | Barbiturate poisoning | | 22 | | 8.6 | 5.7 |
| C ♂ 18 | Hanging | | 8 | | 7.6 | 4.3 |
| D ♀ 22 | CO poisoning | | 10 | | 13.2 | 8.3 |
| E ♀ 60 | Tuberculous meningitis | 340 | 104 | 11.0 | 8.8 | 7.2 |
| F ♂ 48 | Esophageal cancer | 190 | 81 | 6.9 | 7.1 | 4.4 |
| G ♀ 46 | Uterine cancer | 270 | 48 | 14.8 | 7.3 | 5.8 |
| H ♂ 40 | Amyotrophic lateral sclerosis | 360 | 132 | 9.2 | 5.8 | 6.7 |
| I ♀ 58 | Esophageal cancer | | 121 | 6.8 | 6.1 | 4.9 |
| J ♂ 55 | Hepatoma and cirrhosis | 640 | 134 | 18.2 | 10.9 | 8.6 |
| K ♀ 49 | Hepatoma | 290 | 109 | 9.6 | 8.3 | 5.7 |
| L ♀ 72 | Cholangiocarcinoma | 240 | 221 | 7.7 | 9.6 | 9.3 |
| Nickel Concentrations (mean±SD): | | 333±147 | 85±65 | 10.5±4.1 | 8.2±2.3 | 6.4±1.6 |

analysis, readers may consult recent monographs by Zief and Mitchell (166) and LaFleur (167).

## CONCLUSIONS

Electrothermal atomic absorption spectrometry is currently the most sensitive, convenient, and reliable method for analysis of nickel in biological materials. Three other techniques (i.e., dimethylglyoxime-sensitized pulse polarography, particle-induced x-ray emission spectrometry, and gas chromatography with electron capture detection) rival electrothermal atomic absorption spectrometry in analytical sensitivity. With further refinements, each of these techniques may be suitable for clinical applications. Isotope dilution gas chromatography-mass spectrometry may eventually become the definitive method for analysis of nickel in biological materials. Until such a definitive method has been developed, the IUPAC provisional reference method for nickel analysis by electrothermal atomic absorption spectrometry will serve for comparative evaluations of other procedures for clinical measurements of nickel concentrations.

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