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The Multielemental Analysis of Bone

A Review

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ABBREVIATIONS

AAS	atomic absorption spectroscopy
AES	atomic emission spectroscopy
ASV	anodic stripping voltammetry
CCP	capacitively coupled plasma
CMP	capacitive microwave plasma
CT	computer tomography
DPASV	difference pulse ASV
EDAX	energy disperse analysis of X-rays
EPMA	electron probe microanalysis
ES	emission spectroscopy
ESR	electron spin resonance
ETA-AAS	electro-thermal atomization AAS
EXAFS	extended X-ray absorption fine structure
FAAS	flame AAS
FAES	flame AES
GF-AAS	graphite furnace AAS
HCES	hollow cathode discharge emission spectroscopy
IAEA	International Atomic Energy Agency
ICAP	inductively coupled argon plasma
ICP	inductively coupled plasma
INAA	instrumental neutron activation analysis
IR	infrared spectroscopy

ISE	ion selective electrodes
ISFET	ion selective field effect transistors
LAMMA	laser microprobe mass analysis
MEA	multielemental analysis
MIP	microwave induced plasma
MS	mass spectrometry
NAA	neutron activation analysis
OAES	optical atomic emission spectroscopy
PAA	photon activation analysis
PAS	photo acoustic spectroscopy
PIGE	proton induced prompt gamma ray emission
PIXE	particle/ or proton induced X-ray emission
RBS	Rutherford back scattering
RNAA	radiochemical NAA
SEM	scanning electron microscope
SSMS	spark source MS
TDPAC	time differential PAC
UV	ultra violet spectroscopy
XRF	X-ray fluorescence analysis
XMA	X-ray microanalysis
XRD	X-ray diffraction

ABSTRACT

Numerous elements, especially metals, possess biological activity. But until now, they were of interest mainly as ingredients of foods.

This caused the grouping in essential and nonessential elements, and characteristic changes of their concentrations in tissues and body fluids of man are often accompanied by pathologic alterations. The growing interest in environmental influences on human, animal, and plant health has focused our view on the toxicity of certain elements. Often it depends on concentration (*1*) or chemical form (e.g., Cr^{3+} is essential whereas Cr^{6+} is venomous) if an element is vital or poisonous.

Index Entries: Bones, metals in; elements, essential and nonessential; Co; Cu; Fe; Ni; Pb; F; Mg; Na.

INTRODUCTION

Today very little is known about the accumulation (long term effect) of the trace elements, because their "normal levels" in different tissues and fluids are hardly known and are difficult to define. In this respect bone is not an exceptional case. Mermet and Hubert (*2*) start from the

following list of essential and toxic elements (T. Nishima (*3*) lists also B but does not mention As and Ni): essential elements, Na, K, Mg, Ca, C, N, O, P, S, Cl, H; essential trace elements, F, I, Se, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Si, Sn, As; and toxic elements, Li, Be, Ba, F, Cl, Br, As, Sb, Bi, Pb, Sn, Tl, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg.

Analysis of these elements can be performed by different techniques. However, routine analyses for single elements may be erroneous and may lead to questionable clinical diagnosis and therapeutic recommendations.

Simultaneous multielement analysis (MEA) is preferable especially if complemented by multivariate statistic approaches (*4*).

MEA can be accomplished by the well established flame atomic absorption and emission spectroscopy (FAAS and FAES) and AAS with electrothermal atomization (ETA-AAS) in the form of sequential analysis of separate fractions of a large sample. Sample mass can be much reduced by discrete (pulsed) nebulization (*5*). In addition, ac arc emission spectroscopy (ES) and in particular ES with dc arc can be used. More recently laser emission methods have been employed. Papers referring to the above-mentioned methods are cited by Delves (*6*).

A simultaneous MEA of single samples over a wide range of analyte concentrations is possible by ES with the aid of direct current plasma, high frequency plasmas (inductively coupled plasma, ICP, or capacitively coupled plasma, CCP), and microwave plasmas (capacitive microwave plasma, CMP, or microwave induced plasma, MIP). A review of applications of plasma-AES is given by Mermet and Hubert (*2*). Concerning the efficiency of ICP for the determination of trace elements, *see* Schramel, Klose, and Hasse (*7*).

MEA of bone samples are often carried out via activation analyses; their stage of development has been depicted extensively by Krivan (*8*).

The application of nuclear activation techniques in the life sciences was reviewed by Takeuchi (*9*) and he summarized the proceedings of the IAEA symposium held in 1978. The results of qualitative analytical bone investigations were compiled in a bibliography of measurements using radiation absorption and scattering (Preuss and Bolin (*10*)).

In some cases, electroanalytical procedures render possible MEA (*11*). However, spectrophotometric methods in the UV- and visible regions of the spectrum are seldom useful for MEA. A versatile and sensitive method to detect metals is the spark source mass spectrometry (SSMS) in its various techniques (*12*). (As photo plates are mainly used for registration, the results are often obtained with much expense and errors with up to 15% occurring.) Measurements with ion selective electrodes (ISE) are now replacing some methods of FAES and FAAS, e.g., for analysis of Na^+, K^+, and progressively Ca^{2+}. Well established is the determination of F^- by ISE (*13*). The percentage of emergence of ion-selective field-effect transistors (ISFET) promises the applicability for MEA even *in vivo*.

To what extent future improvements of computer assisted tomography (CT) allow determination of concentration in the sense of MEA has to be awaited. First attempts are made to measure concentrations of single elements (e.g., Ca in (*185*)). In future photo acoustic spectroscopy (PAS, optoacoustic spectroscopy) applications could be found in the analysis of solid samples. Though this method yields results similar to those of molecular spectroscopic methods (UV, IR), elemental analyses can be done too (*see 14*). The foundations of PAS and applications to condensed phases and solid materials were recently reviewed (*14–16*). Applications of laser microprobe mass analysis (LAMMA) also could be suitable as reported by Gabriel et al. (*17*) for special cases of fluorine in dental hard tissues.

THE BONE

Kidd "points out that: Bone macroscopically consists of two different types of structures: cortical or compact bone which is found in the shafts of long bones and surfaces of flat bones; and trabecular cancellous bone which is found in the metaphyseal region of long bones and within the cortical coverings of flat and short bones.

Trabecular bone is made up of a network of hundreds of fine interlacing bony spicules called trabeculae, whereas cortical bone is much more dense.

Bone is an active, living tissue formed from a mineralized protein complex, that is 30% organic and 70% inorganic. Here we are interested in the inorganic part which is made up mainly of the crystal structure of hydroxyapatite ($Ca_{10}(PO_4)_6$ $(OH)_2$). The ratio of calcium to phosphorous in the pure hydroxyapatite crystal is 3.13, but in bone and some synthetic calcium phosphates this ratio may be as low as 1.66.

The main constituents of bone salts are calcium (Ca^{2+}), phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), hydroxyl ions (OH^-), and water (H_3O^+), with smaller amounts of magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), and chlorine (Cl^-) ions. As the bone matures, there are also increasing traces of other ions, notably of fluorine (F^-)" et al. (*18*). As cortical and cancellous bones differ structurally, it is believed that these bones may also differ biochemically. However, there is no scientific evidence to support this view. Susheela and Iha (*19*), starting from this point of view evaluated some biochemical characteristics of both types of bone from rabbits. In the parameters, they investigated significant differences ($p > 0.05$) were found in the cases of hydroxyproline, hexosamines, N, Ca, and Mg. "It is therefore unlikely that the responses of cortical and cancellous bone to various pathological conditions will be the same," the authors conclude.

SAMPLING AND STORAGE

Prior to discussion of this paper, it should be necessary to refer to some presampling factors that may affect the elemental composition of our specimens.

In his report on this subject, Iyengar (*20*) defines the presampling factors "as events associated with a biological specimen *in situ* and before its arrival at the laboratory for analysis. Irrespective of the mode of collection, the samples are subject to influences from *biological variations,* postmortem changes, and intrinsic errors." The biological variations are divided into genetic factors, long-term (e.g., age, sex, and pregnancy) and short-term physiological influences (circadian rhythm, foods, and stress as examples) (*21*) and seasonal changes (grouped under physiologic and climatic categories) (*22*). Postmortem changes can be caused by autolysis, inhibition, and cell swelling. The intrinsic errors involve medication, hemolysis, subclinical conditions, and medical restrictions. Finally, differences between specific organ segments have to be taken into consideration (*see* below).

To underline the importance of presampling factors, we remember the opinion of Thiers (*23*): "Unless the complete history of any sample is known with certainty, the analyst is well advised not to spend his time in analysing it."

The sampling and storage of hard tissue seem not to present any special problem. "Samples should be stored at −20°C until ready for analysis and 'formalin' preparations avoided unless shown to be trace element free. Subsections for analysis should be cut with quartz knives to avoid contamination with trace metals" (*6*).

However, in the case of trace element analysis, where extremely small amounts of elements to be analyzed are extracted from very low sample masses (for biopsies of bone: less than 10 mg), even the slightest impurities can lead to considerable errors. These errors can be attributed to two main causes: contamination or element losses because of mechanical abrasion from the sampling tools, adsorption on or desorption from the surfaces of vessels (Plasticware should be washed in 2*N* HNO_3 (and EDTA, 6 g/L) and rinsed with deionized water before use). Other error sources are changes in tissue composition. This case can lead to errors, if an element is distributed nonhomogeneously in a tissue and if the analysis is performed spotwise. On the other hand, if the total content is required, a homogenization step has to be included. A survey of all possible errors is given by Behne (*24*), including the special case of human bone biopsies.

An error traced back to insufficient splitting of compact and spongious bone is seen in the analysis for Cd as reported by Simon and Liese (*11*). The spongy bone contains elevated average Cd amounts because of

adhesive biological matter such as marrow and blood. Against it, the average Pb content is lower. Furthermore, it is essential to know that in cases of acute or subacute lead poisoning, Pb is predominantly enriched in the compact regions of bone, whereas "normal" bone shows no difference (*25,26*).

Susheela and Iha (*19*) have investigated the biochemical characteristics of cortical and cancellous rabbit bone. They found fundamental differences, e.g., cortical bone had significantly more Mg^{2+} and Ca^{2+} than did cancellous bone, but Mg/Ca being similar in both bone types.

According to Sansoni and Iyengar (*27*), freezing or freeze-drying of samples are good methods for long storage. But freeze drying is not suitable for Hg or I determinations, since these elements partially volatilize during the drying process.

SAMPLE TREATMENT

To look at the "philosophy" of trace element analysis and to understand the analytical problems that often lie in the systematic errors inherent in the methods employed the papers of Tölg should be consulted (*28,29*).

Sample treatment culminating in the detection step (the measuring of signals) strongly influences accuracy and precision of the results obtained. Often the causes leading to random errors affecting the precision of a determination can be found and the errors minimized. The systematic errors, however, remain and may lead to questionable accuracy. Interlaboratory comparisons of the observed values obtained by different techniques reveal that excellent precision isn't a guarantee for accuracy, and even with the same technique, identical samples can give quite different results depending on the pretreatment prior to injection. Thus, direct atomization of nitric acid-dissolved bone resulted in aluminum values that were systematically higher than those from a neutron activation analysis procedure. Garmestani et al. (*30*) showed this to be a result of calcium and phosphate interference on the aluminum signal. (Phosphate ions tend to enhance the aluminum response more than the calcium tends to suppress it.)

Digestion methods in trace analysis have been recently surveyed by Dorner (*31*). Vigler et al. (*32*) reviewed sample preparation techniques for AAS and ICP. Initially, samples often have to be defatted. This can be achieved with a variety of solvents, e.g., acetone (*18*), methanol:diethyl ether (3:1) (*33*), or ether:acetone (1:1, v/v) (*19*).

Drying Procedures

Most analytical results are expressed on a dry weight basis. Therefore drying of tissue samples is an indispensable step in the analytical procedure. ("When the dry weight concentrations [of bone samples] are

compared with ash weights the results have been multiplied by 1.4." (*34*). Our own experience resulting from series of rat bones is a factor of (mean) 1.3 to compare fresh and dried samples. This is in agreement with other authors' relation 10 g fresh = 6 g dry = 3 g ash.)

Drying is commonly carried out in a thermal oven. The drying time required ranges up to 72 h. Hence, the drying step is often the rate-limiting step in the analytical procedure and attempts are made to reduce this time. One possibility is the use of microwaves.

The frequency of this type of electromagnetic waves ranges 10^2–10^6 MHz, they generate heat rapidly by energizing dipolar molecules. Koh (*35*) used a commercial microwave oven to dry 10 g portions of biological tissue for trace element determination without significant ($p > 0.95$) loss of elements compared with thermal ovens. The influence of various drying temperatures was investigated by Iyengar et al. (*36*). Oven drying at 120°C resulted in losses of Sn (10–15%) and of Mn, Se, and Ce (up to 5%). The results are also organ dependent.

These observations show the need for precautions before dry ashing, e.g., acid extractive pretreatment.

Extraction of Elements

Delves et al. (*37*) described the determination of 11 metals in a 1 mL solution of an oxidized blood sample. "The metals Fe, Cu, Bi, Zn, Ca, Pb, Co, Ni, Mn, Sr, and Li are selectively extracted into small (0.30 to 0.50 mL) volumes of methyl isobutyl ketone (MIBK) as their chelates or ion-association complexes, and are determined in the organic phases by AAS. The enhancement effect of the organic solvent combined with the extraction and concentration of the metals results in average sensitivity increases of seven times that obtained by a direct determination of the aqueous solutions." The recovery of added metals was quantitative and (without Pb and Bi) a precision of 8% was achieved at the 0.1 ppm level (*37*).

A similar strategy was followed by Agemian and coworkers (*38*). They reported a method specially set up for high-fat fish tissue up to 5 g using an aluminum hot-block. After digestion with nitric acid and sulfuric acid, the acid extracts of the sample were analyzed by direct FAAS for Cu, Zn, and Cr, whereas Cd, Ni, and Pb were concentrated by chelation with ammonium tetramethylene dithiocarbamate (APDC, ammonium pyrrolidine dithiocarbamate) followed by solvent extraction with MIBK and determined by FAAS. (Drasch et al. (*39,40*) recently published a quantitative gas-chromatographic determination of Pb and Cd in biological materials preceded by liquid-liquid separation of their diethyldithiocarbamates.)

Neutron irradiated samples of dental enamel are treated with APDC after dissolving, and the chelates are extracted with ethyl acetate. The activity of the extracted complexes then was measured by Deschamps et al. (*41*).

After removal of lipid and acid digestion of bone samples Kamata et al. (*42*) separated Cd from the matrix by two extractions with dithizone in CCl_4. The first extraction removes Cd; the second extraction at pH 8.5 in the presence of 2-hydroxyethylethylene diaminetriacetic acid is needed to prevent suppression of the Cd signal by Zn. Cd is finally measured after back extraction into 0.24*M* HCl.

Nakashima et al. (*43*) studied a separation procedure of trace elements in perchloric acid medium of bone digest using mixed reagent containing 1% of APDC and diethylammonium diethyldithiocarbamate. Optimum pH and acid concentrations were presented for the metals (Co, Cu, Ni, and Pb) in the presence of calcium phosphate or sodium pyrophosphate. The organic layer was back extracted by shaking with concentrated nitric acid.

The success of a chelating agent can be method dependent. The often used 8-hydroxyquinoline, e.g., is unsuitable in electron impact ionization mass spectrometry of the ratio of stable Cu isotopes, $^{65}Cu/^{63}Cu$ in biological materials (blood plasma, liver, and feces). In this special case, TPP (tetraphenylporphine) was used by Buckley et al. (*44*).

The separation of elements can also be achieved by methods of coprecipitation, as reported by Hölgye (*45*), who separated Pu from bones with bismuth phosphate.

Dissolution of Organic Substances

Organic matter present in the sample is a potential source of interference with the analytical procedure because of nonspecific matrix effects.

Jackson et al. (*46*) investigated the use of Soluene-100 (contains a 2% w/v solution APDC), a quaternary ammonium hydroxide tissue solubilizer. Employing this product, one can prepare tissue samples for atomic absorption quickly and with minimal handling, thus reducing sample loss or possible contamination from excessive sample preparation. Another major advantage of this procedure is that the organic-based solubilizer enhances the sensitivity for the metals investigated (Zn, Cu, and Fe). The deproteination using hydrazine also was employed prior to the investigation of bone mineral as reported by Doi et al. (*47*). In this connection they refer to Termine et al. (*48*) and note that the procedure induces only minor chemical changes and no structure alterations in minerals.

Complete Oxidation

Despite the many variations, almost all methods fall into one of two main classes, i.e., dry ashing and wet digestion. In the former, oxidation is accomplished by heating the sample to a relatively high temperature, usually between 400°–700°C. Chemical compounds may sometimes be added to aid the process; when a bomb technique is used, oxygen under pressure is used in place of air. (Dry ashing is generally recommended

for trace element analysis (*23*). However, dry ashing elements from the container and the atmosphere may be introduced into the sample. Certain classes of biological material tend to froth and char. Others, high in nitrogen, may ignite upon heating. Many substances are converted into refractory, difficultly soluble compounds. Serious losses are caused by diffusion and volatilization depending on oxidation state and composition of the substrate; therefore no accurate predictions are possible.)

In wet digestions, the temperatures are much lower, liquid conditions are maintained throughout, and oxidations are carried out by oxidizing agents in solution. Wet ashing, used extensively with mixtures of hot mineral acids, is generally tedious and potentially hazardous. The anions that are introduced tend to interfere with subsequent analyses. A significant problem is also produced by impurities in reagents.

The recovery for analysis of trace elements using the mentioned strategies was surveyed by Gorsuch (*49*) (radiochemical investigations). Generally, wet digestion provides better recovery for easily volatized elements and dry ashing requires less manpower and attention.

Some problems in complete oxidation of bone with respect to the applied physical detection method (e.g., DPASV and AAS) were discussed in the dissertation of Liese (*50*). It is generally not possible to choose the same digestion procedure for all the different methods of determination.

A comparison of wet and dry ashing for the analysis of biological materials by AAS was presented by Watling and Wardale (*51*) (liver, heart, and brain). Blanusa and Breski (*52*) performed a comparison of dry and wet ashing procedures for Cd and Fe determinations, e.g., in carcasses of rats using ^{115m}Cd and ^{59}Fe. They found no loss of Cd or Fe by volatilization during dry ashing at 600° and 450°C, respectively. During ashing at 450°C significant quantities of Fe are bound to porcelain or glass, but no adsorption was observed if acidified solutions (10% HNO_3) of the ashed samples are stored.

The decomposition methods for the determination of trace elements in biological materials were recently reviewed by Knapp (*53*). Sources of potential systematic errors and ways to eliminate them are discussed as well as suitable decomposition procedures and their characteristics.

Dry Ashing

A dry ashing procedure under low temperature conditions was proposed by Carter and Yeoman (*54*). After drying 10 μL aliquots of whole blood (added to 50 μL of deionized, distilled water) on a hot plate at 110°C for 2 min, a vacuum of 1 mm Hg or less had been produced and a gas mixture of oxygen and carbon tetrafluoride was allowed to bleed into the system at a rate of 30 mL min^{-1}. Then a plasma was produced by radio frequency. After ashing, the samples were analyzed for Cd concentration by AAS. With an apparatus similar to that used in high temperature dry ashing, Gleit and Holland (*55*) performed the decomposition of organic substances. They used a stream of oxygen excited by a radio fre-

quency discharge, whereby temperatures of less than 100°C can be maintained. Radioactive tracer studies demonstrate that 17 representative elements can be quantitatively recovered after complete oxidation of the organic substrate.

Animal tissues containing bone are dry ashed in a more conventional way by Menden et al. (*56*). (The described method has also been found to be useful for the preparation of microsamples of tissue for anodic stripping voltammetry.) For "samples with more bone in them" they recommend a method consisting of successive additions and evaporations of 5-mL vol of aqua regia. The elements Zn, Co, Cd, Pb, Fe, Mn, Mg, and Ca are determined by AAS. Before analyzing lead in bone of field hares using AAS, Klingler et al. (*57*) applied a rapid new method of dry ashing, which gives precise results and is therefore recommended for routine examinations.

The incineration was carried out in oxygen-flushed flasks. The bone samples were wrapped in paraffin foil (to aid combustion) and a filter strip (to aid ignition). The samples were ignited by means of an electrode in a platinum container. (The only disadvantage—weakness of solder points in the electrode.)

Witkowski et al. (*58*) used a digestion technique with ashing of samples in a muffle furnace at 400°C for 12 h as a first step. Spiked samples yielded a mean of 90.4% lead recovery in the whole procedure. But this step was reported to produce poor reproducible losses of lead because of the formation of volatile compounds (this occurs at 300°C, according to Klingler (*57*)). The low temperature ashing with three different exited gases (O_2, Ar, and CO_2) was found by Tochon-Danguy et al. (*59*) to cause important alterations, both of paramagnetism and crystallographic structure of the mineral substance of human bone and of tooth enamel. This should be considered in studying bone metabolism.

A low temperature oxygen plasma ashing unit was also applied by Parkinson et al. (*60*) to analyze Al in human bones using flameless AAS. Recently, Williams (*61*) reduced ashing time considerably by introducing fluorine into the oxygen plasma prior to the determination of Sn, Fe, Pb, and Cr in biological materials by AAS. The novel and efficient method of achieving this is based on the use of poly-tetrafluoroethylene (PTFE) as both the sample container (crucibles or dishes) and the source of fluorine.

Wet Ashing

A wet ashing of biological samples (liver) in a microwave oven is reported by Abu-Samra et al. (*62*). Some disadvantages of wet ashing (close and constant operator attention, need for special hoods to handle perchloric acid fumes safely, and danger of explosion) are minimized in a specially adapted commercial microwave oven. Acid mixtures (4:1 of reagent grade nitric and perchloric acid) are heated internally by the

oscillating electromagnetic field, resulting in very rapid, safe, and efficient ashing.

This type of digestion system was applied with some modifications by Barrett and coworkers (*63*) to the analysis of hard tissue (human teeth) for Hg by ASV. They placed the dried section of a tooth weighing 5–15 mg into an ASV cell (Pyrex) and added 300 μL of an acid mix (79.5% nitric, 20% perchloric, and 0.5% (v/v) sulfuric acids). The time for completing the dryness typically was 10 min.

A simple technique is reported by Johnson (*64*) for postmortem liver, which gives good recoveries and appears applicable to other tissues. 30 g samples were oven-dried at 100 ± 5°C overnight, weighed into a digestion flask and 25 mL of a (1 + 1) mixture of 16*M* nitric acid and 12*M* hydrochloric acid was added. The samples were digested on an asbestos-covered hot plate at 100 ± 20°C until only the lipid fraction remained undigested (clear oily layer above the hot digestion). This fraction sank on cooling. After centrifuging, the supernatant liquid was removed, and the remaining lipid was extracted with hot distilled water, cooled, and recentrifuged. The supernatant portions were combined, evaporated at 100 ± 20°C to 25 mL, and then diluted to 50 mL with distilled water with a clear yellow solution being obtained. (The samples were kept in polypropylene containers under refrigeration before being analyzed by AAS.) Digestion of tissue samples (liver) for analysis by ICP-OAES is described by McQuaker et al. (*65*) (HNO_3, HAO_4).

A wet ashing procedure using a 1:1 mixture of HNO_3 and $HClO_4$ was used by Liese (*50*) before determining Pb and Cd in human rib bones. Working very well in the case of DPASV, the procedure could not be adopted for AAS investigations.

Drasch (*66*) recently reported a simple (but time consuming) way of digestion. He added 500 μL nitric acid to about 100 mg of bone sample and allowed the mixture to stand covered for about 10 d at 20°C. "After this period the samples were totally dissolved." Similar in its simplicity is the digestion used by LEC (*67*): 1 g dried bone is dissolved in 25 mL 2*M* HCl. Nakashima et al. (*43*) found no difference between acid digestion and dry ashing (<500°C) in the case of Co, Cu, Fe, Ni, and Pb in bone.

The influence of ashing techniques on the analysis of trace elements in animal tissues was investigated by Clegg and coworkers (*68*). They found nitric acid digestion of liver samples to be superior to other acids, acid combinations, or bases. Mean concentrations for Fe, Cu, and Zn differed from NBS certified values by less than 1.5%, whereas those for Mn differed by 4%. (The determination of Mn was for that reason elaborated in detail, *see* Clegg et al. (*69*.)

Animal bone reference material was mineralized with a saturated $Mg(NO_3)_2$ solution by Siripone et al. (*70*). The method was designed for larger quantities (several grams), which were treated with 5 mL aliquots, heated until dryness, and baked at ~ 450°C. The residue was dissolved

in mineral acid and diluted to ~ 150 mL (pH 2). "The isolation of trace elements with active carbon is based on sequential scavenging of the chelated elements at five different pH values, 0.2, 1.5, 2.5, 5, and 7.5."

Wet ashing with HNO_3 + $HClO_4$ mixtures leads to the formation of 56 insoluble compounds, according to Bajo and Suter (*71*). The problem was overcome by adding H_2SO_4 to the solution. (Unfortunately, the authors used no bony material.)

Pressure Digestion

A pressure decomposition device for a rapid treatment of large amounts of biological materials was developed and described by Scheubeck et al. (*72,73*).

Drying and burning in oxygen under elevated pressure and the absorption of reaction products are performed in the same vessel, thus allowing a high recovery rate of heavy metal traces. "Pressurized decomposition, mainly using about 65% HNO_3 as the oxidant has two main disadvantages: the probability of unexpected explosions which can cause hazards of staff, if no security precautions are taken; and the fact, that, in most cases, a clear (more or less yellow colored) analyte solution is obtained, does not mean that the treated material is completely mineralized."

It has been shown (*74*) that the carbon balance, determined by means of gas-chromatography indicated in no case (of a pressure pattern in closed teflon crucibles followed after addition of nitric acid and heating) a complete ashing and a very strong dependence of the decomposition rate on the kind of investigated material. Another method of decomposition of biological materials (but not of hard tissues) is reported by Han et al. from the Tölg group (*75*). Cd, Tl, Pb, and Bi recoveries are analyzed after a dynamic combustion process using pure oxygen.

Liese (*50*) has evaluated a pressure digestion of human rib bones using only HNO_3, for at the presence of $HClO_4$, large interferences arose in case of AAS determination of Pb and Cd (Cd was not found). A new bomb construction to achieve fast and safe wet digestion, low blanks, good precision, and accuracy was presented by Uhrberg (*76*).

Preconcentration Techniques

Such techniques mainly have to be applied in the case of trace element analysis. Recently, Werner (*77*) has reviewed the methods of enrichment before trace analysis, and an extensive review of multielement concentration techniques for trace elements was given by Bächmann (*78*).

Analyte Applications

The application of the analyte containing medium depends on the chosen analytical method. Only very few techniques allow the application of an undestructed solid bone. INAA and PIXE investigations are examples. Most of all the samples have to be in liquid form, which can be

achieved by dry ashing followed by extraction step(s), wet ashing, or other digestion procedures.

It also may be necessary to ash the bone first and then to match a solid sample, e.g., as reported by Webb and Wordingham (*79*) for the spectrographic determination of barium. In this work the bone ash was ground with a spectrographic buffer composed of equal parts of anhydrous copper sulfate and graphite powder. This procedure was the same as applied in the determination of total strontium (*80*) in bone samples.

SOME METHODS APPLIED TO BONE

AAS for Bone Analyses

In his review, *Direct Analysis of Solids by Atomic-absorption Spectrophotometry*, Langmyhr (*81*) only cites one paper dealing with bone. The analysis therein is done for Ag and Zn. At the same time, the author published the analysis of animal bone for Cd, Pb, and Mn (*82*). Phosphorus was determined by Langmyhr and Dahl using three AAS techniques (*83*), but unfortunately, bone samples were not included.

The interference influences (inorganic depression effects and specific and unspecific absorption) and their elimination when using a graphite cuvette in the determination of Pb and Cd are discussed by Oelschläger and Lautenschläger (*84*). Tissues are not analyzed.

Pb and Cd in human bones were determined by Simon and Liese (*11*) and the results were compared with those evaluated by DPASV. The results agreed well. More detailed information can be obtained from the work of Liese (*50*). It only should be noted that it was necessary to apply different ways of wet digestion for both analytical methods, especially that Cd could not be detected by AAS, although the digestion procedure worked very well in the case of DPASV.

A method of dry ashing of animal tissues (bodies of 1-d-old rat pups) for AAS determination of Zn, Co, Cd, Pb, Fe, Mn, Mg, and Ca was applied by Menden et al. (*56*) and proposed for "samples with more bone in them." Garmestani et al. (*30*) compared AAS results for Al in bone with NAA obtained values and found a Ca and phosphate interference of the Al signal. They offer an optimum procedure to overcome the difficulties.

Ba was separated from calcium in animal and human bone and AAS analyzed by Kawamura and coworkers (*85*). The origin of human bone is quoted as "fetus, child, adult."

An interesting approach to direct solid tissue analysis was presented by Fry and Mohamed (*86,87*). The procedure involves a 2 min tissue homogenization followed by direct atomization of the homogenate with a special version of a clogfree nebulizer. The results are in excellent agreement with conventional dry/wet ashing methods for the determination of Cu, Mn, and Zn, but unfortunately not in bone.

In the determination of Ca, it is of interest to note, that its absorbance becomes independent of the phosphate concentration if a sufficient amount of metavanadate (metavanadate and molybdate, respectively) is present in the solution or sprayed into the air–acetylene flame. This was shown by Sarudi (*88*) in the analysis of calcium in feedstuffs. (This permits the use of the colored solution containing phosphovanado-molybdate, prepared for the determination of phosphates and the calcium content.)

Eleven elements (Ca, Mg, Cd, Co, Cu, Mn, Ni, Li, Pb, Sr, and Zn) are determined by Lappalainen et al. (*34*) in human cancellous bone. They performed a linear multiple regression analysis and found, e.g., that age and sex did not significantly affect the concentration of Zn and Cu. Knuutila et al. (*89*) have also reported a statistically significant inverse relation of Ni to Li and a positive correlation between Sr and Ni. The strong interrelation between Li, Sr, and Ni suggests that these elements should be analyzed together in studying the role of these elements in mineralization.

Brätter and coworkers (*90*) analyzed lead distributions in human skeletons by flameless AAS. Their main work was concerned with the distribution of many other trace elements evaluated with NAA (*see* chapter 5).

Flameless AAS was used to determine Cu and Cd after wet digestion (HNO_3, 60% $HClO_4$) of tibiae (epi and metaphysis) and diaphysis and bone extracts from rats, but flame atomic absorption spectrophotometry was employed for determination of Zn to evaluate the effect of these metals on the bone collagen metabolism (*91*).

Lead analyses of 105 femur bones of hares were made by the aid of AAS (flame) using a new method of incineration by Klingler et al. (*57*). (For description of this new dry ashing variant, *see* dry ashing.) Lead analyses in bone biopsies were also performed by Kijewski and Lowitz (*25*) using the hydride technique in flameless AAS. (For other papers concerning lead determination in human bones, *see* (*92*.))

The lead burden in prehistorical, historical, and modern human bones was carefully evaluated by Drasch (*66*) in a total of approximately 650 specimens with graphite furnace AAS. The dependence of the lead concentrations found were compared with the age, sex, type of bone, conditions of preservation, and storage.

Deer mandibles were analyzed for lead by Witkowski et al. (*58*). Their study indicated little influence of age, sex, and county on lead levels in teeth too. The authors proposed additional studies to establish the reliability of using deer teeth and/or mandibles as suitable bioindicators.

A method for the routine determination of Al in bone by flameless AAS was proposed by Parkinson et al. (*60*). After 2 extractions with dithizone and back extraction into 0.24*M* HCl, Kamata et al. (*42*) determined ng g^{-1} levels of Cd in bone by ETA-AAS. 10 ng g^{-1} Cd in fresh bone can be determined within a relative SD of 10%. (For sample treat-

ment details, *see* Extraction of Elements.) Other extraction conditions were studied by the same group to determine Co, Cu, Ni, and Pb in bone using GF-AAS (*43*).

The direct lead content analysis of milligram quantities of bone ash was performed by Wittmers et al. (*93*) by GF-AAS. Flameless AAS results of Pb in human leg bones are compared with previously determined concentrations using XRF by Wielopolski et al. (*94*).

Bone Examined by ICP

The very recent survey of Mermet and Hubert (*2*) contains only one hint at an investigation of bone using ICP. But the aim of the work of Kluckner and Brown (*96*) was the analysis of teeth and the analyzed calcined animal bone sample obtained from IAEA was to check the accuracy of the determinations. IAEA bone samples were also analyzed by Abercrombie et al. (*97*) and the results are compared with Barringer Research Samples.

In another feature article written by Dahlquist and Knoll (*95*), the analysis of ancient bone tissues for Al and Ti is touched upon. (For more technical reviews, *see* (*225,226*).) Schramel et al. (*7*) critically reviewed the efficiency of ICP emission spectroscopy for the determination of trace elements in biomedical samples. (Results for bone are not given.) Interelement effects (e.g., from Na, K, Mg, Ca, and Fe) in trace element analysis (for Cd, Cu, Mn, Al, and Zn) were evaluated by Schramel and Xu-Li-Giang (*98*). (Hard tissues were not analyzed.)

Comparative analyses for lead (Pb) in several acid-digested (HNO_3/$HClO_4$), bone-meal food supplements are enclosed in the contribution of Jones and Boyer (*99*). The ICP values are obtained on wavelength-modulated, background-corrected systems against matrix matched standards (10,000 μg Ca/mL; 5000 μg P/mL; and 200 μg Mg/mL) and are compared with ASV measurements. Bone meal samples were also analyzed by Capar and Gould (*100*) and levels for Al, Cr, Cu, Fe, Mn, Mo, Ni, Ti, and Zn were reported. Lee (*67*) evaluated the spectral interferences in bone matrix dependent on various concentrations of Ca at a molar quotient Ca/P = 1.5. He found signal depressions up to 12% for Cu, Zn, B, and Se and deviations up to 24% for Fe, Mn, Sn, Ni, Pb, Co, Cr, and Mo.

Twenty elements in human bones were determined by Mahanti and Barnes (*101*). The authors used hydrid and elemental generation for As, Se, Hg, electrothermal vaporization for Cu, and a 30-fold preconcentration achieved by poly-dithiocarbamate chelating resins for Cd, Cu, Co, Mo, Ni, Pb, Ti, and V.

Related Methods of OAES

Bone ash was analyzed for Sr using the dc arc (*86*). The same principle was applied by Webb and Wordingham (*79*) to determine total barium content of bone ash. Recently, Kinsey and Comtois (*102*) reported on

Table 1
Some AAS and ICP Results of Human Bones

Source/Type	Method	Elem.	Concentrations in ppm	Ref. rem.
rib, compacta	AAS	Pb	7.4 (15)	*11*
		Cd	24.4 (30)	
iliac crest	AAS	Pb	4 – 18 (26 – 410)	*25*[a]
Cancell.	AAS	Zn	113.9 ± 40.7	*34*[b]
		Cu	1.3 ± .5	
femur	AAS	Pb	5.13	*66*
pelvis			2.54	
cranium			4.08	
sternum			1.95	
cancell.	AAS	Li	.23	*89*
iliac crest		Sr	65.5	
		Ni	1.29	
human	GF-AAS, AAS	Pb	14.08 ± 1.74 and 60.85 ± 5.24	*93* ash
tibia cortex	AAS (XRF)	Pb	15 – 35	*94* wet wt.
human	AAS	Zn	33 – 71 (fresh bone) 189 (ashed)	*200*
rib	AAS	Mn	5.7 ± 2.2 2.3 ± .6 2.8 ± 2.1	*208*
human	AAS	Ba	4.7 (fetus) 10.2 (child) 13.4 (ad.)	*210* ash
limb. alv.	AAS	Pb	4.9 ± 1.5 (1.8 – 7.7, n = 20)	*213*
skull (os temp.)	AAS	Pb	2	*214*
human	ICP	Ca	271 000 ± 3 000	*101*
		P	138 000 ± 2 000	
		Mg	2 260 ± 40	
		Na	10 660 ± 2 00	
		Sr	149 ± 2	
		Fe	29.4 ± .6	
		Zn	102.0 ± 1	
		Ba	21.0 ± .4	
		Al	42.9 ± .8	
		Mn	1.56 ± .08	
		Pb	25.0 ± 2	
		As	.011 ± .0005	
		Se	.101 ± .002	
		Hg	.012 ± .0003	
		Cu	1.0 ± .03	
		Pb	22.4 ± .4	
		Ni	2.1 ± .02	
		Co	.4 ± .01	
		Ti	.5 ± .01	
		V	1.1 ± .03	

Table 1
Some AAS and ICP Results of Human Bones (*Con't*)

Source/Type	Method	Elem.	Concentrations in ppm	Ref. rem.
skull	ICAP	Ca	294 000 (means, n = 3)	*211*
		P	125 000	
		Mg	3 100	
		Mn	6.2	

[a]Dried substance; 5 control specimens; 13 patients with nephropathy given in parenthesis
[b]Means of 51 ♀ and 87 ♂ samples
[c]Normals / renal insufficients / ren. ins. with Al therapy

the determination of "critical trace elements" in macaque monkey bones by dc plasma.

Activation Analyses of Bone Samples

In recent years, activation analysis has contributed significantly to accumulate an immense amount of bone data, especially referred to fossil bones. But there are also techniques applicable *in vivo* to the whole body of living subjects. (But the *in vivo* neutron activation technique is not suitable for measurement of the distribution of the major elements since variations along the bone could not be obtained with sufficient precision (*18*:*7*). *See*, also (*104*). One of the well established techniques is activation with thermal or fast neutrons (NAA).

This technique was applied to small biopsy samples by Batra and Bewley (*103*) to analyze bone for Na, Cl, K, and P. The samples (dried, fat-extracted, and weighed) were enclosed in polyethylene tubes and irradiated with neutrons of mean energy 7.5 MeV that stemmed from a cyclotron and the chosen nuclear reactions were the following: $^{23}Na(n,\gamma)$ ^{24}Na, $^{37}Cl(n,\gamma)$ ^{38}Cl, $^{48}Ca(n,\gamma)$ ^{49}Ca, $^{41}K(n, p)$ ^{41}Ar, and $^{31}P(n, \alpha)$ ^{28}Al.

As bone is a target organ for fluorine, this element has been determined very often. A paper also discussing the error possibilities in detail is that of Brätter and coworkers (*106*). An interesting point they mentioned is the dependence of error on the mass of bone samples.

Fluorine was determined by Woittiez and Das (*107*) with the reaction $^{19}F(n, 2n)$ ^{18}F during irradiation with fast neutrons, measuring the annihilation radiation of the radionuclide formed. The two other reactions $^{19}F(n, p)$ ^{19}O, $^{19}F(n, \alpha)$ ^{16}N suffer from serious oxygen interference. Furthermore, the authors determined calcium via $^{44}Ca(n, p)$ ^{44}K, and phosphorus by $^{31}P(n, \alpha)$ ^{28}Al, because the possible interfering element Si (via $^{28}Si(n, p)$ ^{28}Al) is very low in content (17 μg/g). After Batra and Bewley, the paper of Gatschke et al. (*108*) was the second concerned with the determination of P in bone by NAA. The level of P and Al was measured via the short lived radionuclide ^{28}Al after the samples had been irradiated twice, once with a cadmium shield, and thus separating the contributions of both elements.

The determination of phosphorus in sheep bone by *in vivo* NAA was reported by Whineray and colleagues (*104*). The possible source of interference from ^{38}Cl-γ-rays was shown to be unimportant as far as it concerns the blood-transported chlorine.

A very broad investigation of elemental concentrations in fossil bones as a function of depth below the outer surface was recently presented by Badone and Farquhar (*109*). The work shows that, e.g., some of the bones have been exposed to more than one set of environmental conditions. The authors used three separate irradiations with slow neutrons to activate fluorine first, short half-life isotopes of U, Ba, Sr, Na, V, Al, Mn, La, Eu, Sm, and Ca, second and production of longer lived activities in Fe, Co, Cr, Sc, and Th last. (*See* also *110–112*).

The work of Geidel (*113*) in trace element studies for Mississippian skeletal remains showed that dietary differences did exist between status groups for V, Mn, Zn, Cu, and Sr. 14-MeV NAA is employed by Chindhade (*114*) to the estimation of fluorine and phosphorus in bone.

The analysis of human skeletons by the Brätter group (*90*) showed that trace elements are distributed in varying degrees within a bone, and throughout the skeleton their variations seem to be related to functional and structural conditions. The element content was observed to be higher at epiphyseal areas of long bones than in the shaft and higher in trabecular than in cortical bones. Furthermore, it was found that the element content of a single bone sample depends largely on the mass ratio spongiosa/compacta at the sampling site. The elements F, Pb, Sr, and Zn could be useful in the study of health problems involving bone tissue. For 23 elements, the authors give the distribution between mineral and collagen of bones. (F, Sr, and Pb are not detected in collagen, the Ca and Na content is lower than 1%. Zn is 1.0%, Ba and Mn 5.2 and 6%, respectively.)

Sontag (*115*) investigated the effect of the bone turnover rate on the distribution of radionuclides and found in 100-d-old rats a greater radioactivity in the femur endosteum than in the periosteum. If 50-d-old animals were injected with ^{239}Pu citrate, only a little difference between endosteum and periosteum was found.

The relative amounts of cortical to trabecular bone for nine sections across the bone are measured as are the variations in concentrations of the elements Ca, P, O, Mg, Na, Cl, Zn, and Br (*18*). Kidd et al. analyzed a human tibia using NAA and CT-scanning as well as electron-microprobe analysis. (For results from three modes of neutron activation, *see* Table 7.2.) To evaluate the suitability of the iliac crest biopsy in the analysis of bone and marrow Gawlik et al. (*105*) determined the matrix elements Ca, P, Na, and nine trace elements (*105,108*).

The authors suggest that rubidium is a suitable "scout" element for the determination of the remaining blood in the bone samples because of its large blood/bone content ratio. (It is assumed that 1% of blood relates

to 90 μg Rb/kg bone.) *In vivo* activation analysis and its problems were also discussed.

A comparison of trace aluminium determination in bone by destructive NAA with pre-injection wet chemistry atomic absorption (AA) showed that the AA procedure results in data with lower relative SD values (*30*).

Irradiated samples of dental enamel were treated in a way that could also be used to investigate bones. Samples (about 50 mg) were dissolved in 10 mL of 6*N* HNO_3 in the presence of 50 μg Cu as a carrier (pH adjusted to 4), 1 mL of aqueous 4% APDC and, after 2 min at 60°C, the complexes were extracted into ethyl acetate. After purification, the activity was measured (*41*). Levels of Zn, Cu, Mn, Co, Fe, Se, Hg, P, Ca, Na, and Cl were reported.

It may be useful to mention the potential interferences inherent in reactor NAA of trace elements as recently reviewed by Cornelis et al. (*116*). The authors discuss effects, e.g., of the irradiation process on biological samples (in this respect the method should "never be described as a non-destructive" one), causing blank values by the irradiation process, nuclear interferences, and systematic errors resulting from measurement of the radiation (counting process and interpretation of the γ-ray spectra). The possibilities of detection systems are extensively evaluated and presented by Görner (*117*).

INAA of bone using cyclotron neutrons of 5.5 MeV (flux $10^8 n$ $cm^{-2}s^{-1}$, exposition time 600 s) was performed by Eichhorn et al. (*118*). Because of hardware conditions, the authors reported only analytical values for Ca and P in spinal bone.

Radiochemical NAA of IAEA H-5 "animal bone" after a special mineralizing procedure (*see* wet ashing) was performed by Siripone et al. (*70*) to determine 10 trace elements. Retief et al. (*119*) determined and compared 16 elements in human enamel and dentin. Other MEA of enamel and dentin can be found (*120*).

In vivo analysis of Ca of the hand was performed by Maziere et al. (*121*). Using ^{252}Cf as a neutron source, they measured ^{49}Ca (8.8 min, 3.10 MeV). Most *in vivo* determinations are based on ^{252}Cf radiation but are concerned mainly with Ca measurement. One of the papers reporting multielement analyses in this field is that of Seiler (*122*). The error of *in vivo* analysis (hand) was $\geq 3\%$ and $\geq 6\%$ in the case of Ca and P respectively. Behne et al. (*123*) found that F in spongiosa is higher by a factor of 3 compared to compacta. Detailed values are not only given for femur. Human mandibular compact bone was analyzed by Söremark and Bergman (*124*). They determined Na, Ca, Mn, Cu, Zn, Sr, Rb, Cd, W, and Au in postmortem material (five males, five females, 56–79 y) by γ-spectrometry preceded by a chemical group separation. Another method to perform activation analysis is based on the particle induced emission of γ-rays. Proton induced prompt gamma-ray emission (PIGE)

is a method especially suited for determining light elements. For this method only small low energy accelerators are sufficient. To compare with NAA: If the proton beam is high enough in energy, nuclear reactions such as (p, $p'\gamma$), (p, γ), and (p, $\alpha\gamma$) will take place and similar to NAA, the energy of γ-rays is typical for the product nucleus.

The first application of PIGE in bone studies is reported by Hyvönen-Dabek (*125*). For 15 subjects, the concentrations of Li, B, O, F, Na, Mg, Al, P, and Ca are given. The measurement of bone decalcification is achieved by Wilson and Adelstein (*126*) *in vivo* using the reaction

$$^{40}Ca + p \rightarrow {}^{38}K + 2p + n, \text{ and } {}^{38}K \rightarrow {}^{38}Ar + \beta^+ + \gamma \text{ (2.17 MeV)}.$$

PIXE, XRF, and Related Methods

PIXE, XRF

A microprobe analysis of lead in human femur by PIXE was published by Lindh and colleagues (*127*). Skeleton remains were analyzed for major, minor, and trace element content by means of PIXE (and INAA and RBS) in the work of Maenhaut et al. (*111*).

Results are given for Ca, V, Mn, Fe, Cu, Zn, Sr, and Pb for different spots on bone samples. (It can be concluded that PIXE results generally produce greater measuring errors than INAA.) Hyvönen-Dabek et al. (*128*) tabulated results for Cr, Mn, Fe, Ni, Cu, Zn, Pb, Br, and Sr and investigated the same samples used in PIGE analyses.

XRF analyses are often used to achieve *in vivo*/*in situ* measurements of single elements (*129*). Ahlgren et al. (*129*) determined Pb in forefingers irradiating the second phalanx with two collimated ^{57}Co sources of a total activity of 0.8 GBq. (The mean absorbed dose is 2.5 mGy, compared to an ordinary X-ray picture of a hand: 0.65 mGy.)

Snyder and Secord (*130*) performed the *in situ* measurement of Sr after rabbits were injected with $SrCl_2$. Fluorescent radiation was obtained from a ^{109}Cd source (37 MBq) which produces Ag K_α (22.1 keV) and K_β (25.1 KeV) X-rays and about 4% 87.7 keV γ-rays via ^{108}Cd (n,γ) ^{109}Cd. Measurements were also made of the skull and medial surface of the tibial crest of an adult dog. The technique may serve to determine Sr of surface near bone.

The characteristic L X-rays of Pb induced by ^{125}I were used to test a non-invasive analysis in the legs of six adults (postmortem). Wielopolski et al. (*94*) subsequently compared their results with flameless AAS and found a linear correlation coefficient of $R = 0.90$. Just recently, Gonsior and Roth (*132*) reviewed the trace element analysis by particle and photon-induced X-ray emission spectroscopy. They compiled only two works on bone, which are already included in this text.

Table 2
Some Results of Activation Analyses

Source/Type	Method	Elem.	Concentrations in ppm								Ref. rem.
tibia	INAA	Ca	244 800 ± 18 900								*18*[a]
		P	77 600 ± 4 200								
		Mg	2 680 ± 390								
		O	160 000 ± 11 200								
		Na	6 550 ± 530								
		Cl	501 ± 165								
		Zn	84 ± 1								
		Br	.56 ± .27								
see[b]	INAA	Br	6.8	3.5	3.1	2.4	1.2	3.3	3.4	1.7	*90*[b]
		Ca%	22.0	21.4	22.1	21.4	19.9	21.7	19.7	19.3	
		Co	.6	.28	.52	.86	.06	.4	.31	.05	
		Eu ppb	40	24	69	22	4	23	27	4	
		F	416		289	—430—			—400—		
		Fe	843	333	554	253	29	235	341	34	
		Hg	4.7	2.1	2.3	1.2	.2	2.2	2.1	.25	
		La	.4	.54	.92	.32	.06	.56	.54	.13	
		Na%	.4	.41	.33	.41	.44	.42	.45	.45	
		Zn	2.5	3.62	3.24		1.22	4.1	2.95	1.37	
		Sc ppb	217	117	304	102	9	188	140	10	
		Sr	270	252	192	217	216	215	218	198	
	AAS	Pb	221	114	138	95	73	104	114	81	
post mortem	INAA	Ca	238	500							*103*[c]
		P	103	444							
iliac crests	INAA	Ca	213 000 ± 1 100					87 000 ± 5 000			*105*[d]
		P	98 000 ± 6 000					54 000 ± 3 000			
		Na	4 900 ± 900					500 ± 300			
		F	626 ± 573					243 ± 106			
		Fe	183 ± 78					2080 ± 700			
		Zn	151 ± 22					280 ± 20			
		Sr	79 ± 23					180 ± 30			
		Al	19.5 ± 6.1								

Table 2
Some Results of Activation Analyses (*Con't*)

Source/Type	Method	Elem.	Concentrations in ppm	Ref. rem.
		Se	.13 ± .4 .50 ± .06	
		Co	.046 ± .037 .139 ± .012	
		Rb	<.04 Not detect.	
		Sc	.0014 ± .0007	
humerus,	INAA	F	121 – 794	*106*
femur	PAA	F	325 – 1000	
left radius	PIXE	Ca	200 000 ± 32 000	*111*[r]
	INAA	Fe	5 100 ± 600	
		Zn	760 ± 120	
		Pb	236 ± 72	
		V	.84 ± .45	
		Mn	.23 ± .06	
		Cu	.15 ± .03	
		Sr	.29 ± .45	
human?,	INAA	Ca	344 000 ± 21 000/289 000 ± 115 000	*118*[t]
spinal cord		P	156 000 ± 25 000/104 000 ± 41 000	
femur	INAA	Ca	258 000 252 000, 254 000	*123*[s]
		F	358 1 020, 1 070	
mandib.	NAA	Ca	250 000 ± 23 900	
		Na	14 100 ± 9 800	
		Zn	117.3 ± 58.2	
		Sr	42.6 ± 14.0	
		Rb	5.11 (2 cases)	
		Cd	1.28 ± .50	
		Cu	1.00 ± 1.73	
		Au	.016 ± .014	
		W	.000254 ± .000195	
rib	NAA	Na	8050 and 2 500 (dry and wet)	*205*
		Mg	1 00 310	
		K	4 190 1 300	
skull	INAA	Ca	245 100 255 300 259 480	*206*[h]
See[h]		Na	14 010 13 090 14 760	

		Mg	3 690	2 600	3 090	
		Cl	262	356	228	
		Sr	48.8	71.7	48.2	
		Mn	7.6	3.0	3.5	
see[i]	INAA	Ca	64 000 – 287 000			*4, 217*
		Na	3 500 – 7 400			
		Mg	1 600 – 5 500			
		Cl	458 – 1 197			
		Zn	59 – 244			
		Fe	50 – 345			
		Sr	40 – 112			
		Ba	17 – 314			
		Rb	2 – 62			
		Co	.48 – 3.84			
		Mn	.32 – 1.36			
		Sb	.004 – .081			
femur shaft	PIGE	Ca	184 000 – 231 000			*125*
		P	84 200 – 105 000			
		O	304 000 – 381 000			
		N	114 000 – 130 000			
		Na	5 230 – 6 720			
		Mg	1 580 – 2 550			
		F	178 – 1 630			
		Al	<22 – 54			
		B	<2 – 14			
		Li	.3 – 1.1			

[a]Mean values over all tibia sections
[f]The columns' meaning: lumbar vertebra/ clavicle and rib/ pelvis: iliac crest/ humerus/ ulna radius/ patella foot/ femur/ tibia fibula; standard deviations are given in the original paper
[i]Dried and fat extracted
[d]Second column: bone marrow of the iliac crest (dry, defatted)
[r]To compare the range: values given are only for one part of one spot; see original paper for full information
[l]Cyclotron neutrons; first column; bone meal, second: spine
[s]First column: compacta, second: spongiosa; numerous values for other skeleton parts are given in the original paper
[t]The columns are due to os parietale, processus mastoides, and os frontale
[r]Range between upper jaw, lower jaw and iliac crest

EDAX, XMA

The distributions of Ca and P and of Na, K, S, and Cl were studied in mineralized matrices in a SEM by the method of energy dispersive X-ray analysis (*133*).

Ca and P in bone and teeth were determined by X-ray microanalysis by Nicholson and Dempster (*134*). They performed quantitative microprobe analysis on intracellular mineral deposits, osteoids, and mineralized matrix in trabecular bone from rachitic, vitamin D-treated, and normal rats.

A microregion analysis on implant (biomaterials) and dental materials was performed by Meyer and Schuler (*135*). They analyzed the layer structure of the transition from the implant to the original bone for O, Na, Mg, Si, P, S, Cl, K, and Ca. Burger (*136*) used EDAX to evaluate the intracellular distribution of calcium in mineralizing tissues.

Effects of fluoride on the chemical composition of inorganic bone substance (in mice; Ca, P, and F) were studied by Bang (*137*). After bone sections had been microradiographed, they were investigated by EPMA. Using the same method, Wollast and Burny (*131*) studied the mineralization at the microscopic level in diseased persons and compared total bone to Schaltlamelle and osteons.

Freeze dried, unembedded cryostat sections of shock frozen tissues are measured utilizing mixtures of salts as standards (EPMA). Krefting et al. (*138*) measured profiles of the elements S, P, and Ca in the epiphyseal growth plate of the ulna of pigs. Mineralized regions are not included. It is demonstrated that P content is intracellularly higher than extracellularly (4/0.2%) contrary to S (1.5/4.5%), and Ca (0.4/0.6%).

The EPMA has been applied to investigate another mineralizing system: turkey tibia tendon (*139*). Zn content of cancellous bone from the iliac crest was determined by XRF in an autopsy series (28 women, 66 men) by Alhava et al. (*140*). Osteofluorosis in rabbits was analyzed using EPMA by West and Malcolm (*141*). They found that Ca:P ratio appeared to show a variation related to the F concentration.

Scattering Methods

RBS was applied to skeleton remains by Maenhaut et al. (*111*). Kerr et al. (*142*) reported on a method based entirely on the detection of coherently scattered photons (103.2 keV, ^{153}Sm) which is very sensitive to changes in mineral composition. The mineral concentration in trabecular bone is assessed.

Mössbauer Spectrometry

One study devoted to the *in vitro* uptake of alkaline earths and rare earths in the mineral bone matrix was that of Marshall (*143*) who obtained a Mössbauer spectrum of a ^{133}Ba source deposited on bone powder after incubation in a radioactive barium chloride solution.

Table 3
Some Results Obtained by PIXE and Other Methods

Source/type	Method	Elem.	Concentrations in ppm	Ref. rem.
See[a]	PIXE	Zn	144 ± 27	*128*
		Sr	47.7 ± 14.3	
		Br	12.4 ± 5.5	
		Pb	12.2 ± 2.5	
		Fe	7.58 ± 1.55	
		Cu	3.58 ± 2.16	
		Ni	<2.4	
		Mn	<2.3	
		Cr	<2.0	
forefing.	XRF	Pb	20 – 140 ± 15	*129*[b]
femur	EPMA	Ca	399 000 ± 3 000	*131*[c]
		P	178 000 ± 4 000	
compacta	MS	Tl	.00218 – .00288	*204*[d]
See[d]		Tl	.00144 – .00219	
iliac crest	ISE	F	77, 104 and 4 320 (fluorosis)	*207*
iliac crest	ISE	F	1 036 ± 627 (controls) 5 617 ± 2 143 (fluorosis)	*202*
medulla	pulse	Au	<.5 and 3.7, 5 – 10	*171*[e]
cortex	polaro-graphy	Au	<.5 and 3, 2 – 3	
rib		F	610 (dry, defatt.) 1 100 (ash)	*176*
iliac		F	530 960	
rib	DPASV	Pb	7.7 (15)	*50*
compacta		Cd	24.7 (30)	
femur	ASV	Pb	8 – 32.1 (means.) 18.8	*26*[f]
tibia		Pb	12.2 – 28.1 18.9	
rib		Pb	13.6 – 35.2 23.0	
vertebra		Pb	14.4 – 53.1 26.6	
human	ASV	Au	<.5	*215*[g]

[a]Shaft and neck of femur, iliac crest

[b]*In vivo* determination; 140 persons being occupationally exposed to lead over 1–30 years

[c]Persons over 60 years old; The authors also provide concentrations for osteoporosis and morbus Paget exostosis.

[d]Wet weight; range over sex and age; first line: compacta, second line: calvaria, ribs, and thigh compacta

[e]Fresh bone; first: mean value of 10 controls, second: value and range for two patients

[f]Ash referred; range covers sex and age (newborn–71 years)

[g]Fresh bone; values for rheumatoid arthritis patients who had undergone Au therapy 9 years before are reported to be 5–20-fold greater

This experimental method was used by Kellershohn et al. (*144*). They observed a Mössbauer effect for ^{161}Tb uptake in bone crystals and in mouse bones (*145*). The time differential perturbed angular correlation (TDPAC) was applied to overcome some difficulties of the above mentioned works. Rimbert et al. (*146*) used TDPAC for studies of the ^{133}Ba ion uptake in bone crystals obtained from cortical ox bone.

Emission Mössbauer spectra and PAC measurement led Rimbert et al. (*147*) to the conclusion that the uptake modes of rare and alkaline-earth ions on the bone matrix are different. Rare earth ions may be adsorbed on the surface bone in an environment of hydroxyl groups. The alkaline-earth uptake process may be described by two steps: surface adsorption on the hydroxylapatite in a hydroxide environment, and cationic exchange with the calcium phosphate groups into bone crystals.

TRACE ELEMENTS IN BONE

In a recent review, Zumkley and Bertram (*148*) start with the statement that trace elements in medicine, seldom noticed in the past because of methodological reasons, are growing in significance. Deviations in their turnover are observed as cause or as consequence of numerous diseases.

Dividing the bone seeking elements into volume seekers (e.g., Ca, Sr, and Ba) and surface seekers (Am, Ce, Th, Cf, and Y) (*18*), trace elements are mainly placed into the last group. Only few results from the literature may be added.

Fluorine

Rabbits given NaF intragastrically showed significant changes both in inorganic and organic constituents of cortical and cancellous bone (*188*).

The study of endemic skeletal fluorosis in India revealed F in bone only half as high as in controls (*189*).

The mobilization of Pb storage in the organism to dentin and bone is accelerated by ip injections of sodium citrate or of FCH_2CO_2H (*186*).

Experimental fluorosis caused gross disturbances in the distribution of essential trace elements (nutrients) such as Zn, Cu, and Fe (*187*).

The administration of NaF to rats caused proliferation of periosteal cells in iliac bone and fibrosis. The activity of acid phosphatase in liver is also increased (*165*).

"The reciprocal action and effect of Mg and fluoride on the bones" were studied by Anders et al. (*169*).

Suzuki (*176*) found more F in males than in females. Ca, P, and Mg did not correlate.

Selenium

The uptake of ^{35}S by bone and cartilage and the incorporation of ^{35}S into chondroitin sulfate were studied in rats fed diets (*153*) and it was found that the uptake/incorporation was high in control and Se-enriched fed animals and lowest in the Se-deficient group.

Vanadium

The inhibition of human alkaline phosphatases by vanadate was reported by Seargeant and Stinson (*152*).

Hansen et al. (*158*) reported on the Vanadium distribution in the rat body. V^{5+} as $Na^{48}VO_3$ and V^{4+} as $^{48}VOSO_4$ were given to male Wistar rats at a dose of 5 μmol/kg. Two days after injection kidney had 28 mmol/g wet wt followed by spleen, liver, bone, blood plasma, testis, lung, erythrocytes, and brain. In the case of V^{4+} spleen and bone seemed to bind V to a higher degree than the other organs.

Chromium

From ESR studies of enamel, dentin, and bone Doi et al. (*47*) concluded that Cr in calcified tissues is mainly associated with the organic constituents. In hydrazine-deproteinated bone, the concentration of chromium is decreased to about 1/100th.

Manganese

As found by De Oliveira et al. (*161*) Mn did not affect the proportional fixation of F in the femur of rats (60-d experiment).

Nickel

Recently Kirchgessner and Schnegg investigated the iron metabolism in nickel deficiency. In rats, Ni deficiency (15 ppb in feed) decreased bone Ca (19%) and P (15%) and increased Mg contents of bone (20%) (*157*).

Copper

An investigation of the role of copper in bone metabolism was undertaken by Wilson et al. (*151*). Active bone resorption was found to be inhibited significantly in dependence of copper concentrations. The influence of $ZnSO_4$, Au(I), and Au(III) salts was tested as well.

The deposition of Ca and Mg in the bone of hens was unaffected by various levels of dietary Cu (0.446–1.521 mg/100 g feed) given over two generations (*166*).

Milachowski et al. (*172*) investigated the influence of Cu and Zn on bone healing in rabbits.

Zinc

Piglets (26 d) fed 110 mg Zn/kg for 80 d show increasing bone contents, whereas no significant changes were found in blood indices (*190*).

Another observation is zinc depletion following experimental fluorosis in mice (*196*).

On the mobilization of Zn from bone during a developing zinc deficiency is reported by Brown et al. (*155*).

Murray and Messer (*162*) investigated the "turnover of Zn during normal and accelerated bone loss in rats". Murray et al. (*163*) also investigated the mandibular bone turnover during calcium deficiency in the rat.

Tissue zinc levels are an index of body zinc status (*167*).

Yamaguchi et al. (*156*) also reported on the "stimulatory effect of Zn on bone growth in weanling rats".

Concentrations of Zn, P, and F did not differ significantly in cortical and cancellous bone of rabbits, whereas Mg and Ca did (hydroxyproline, nitrogen, and hexosamines did so as well) (*19*).

Effects of Zn and Ca deficiencies on the rate of bone resorption in rats were studied by Murray et al. (*183*). There was no effect of Zn deficiency in either normally growing or Ca deficient animals. But a partial substitution of Zn for Ca in bone mineral was suggested as Zn concentrations were almost double those of Ca-supplemented animals.

Hsieh and Navia (*184*) reported that a Zn-diet fed group (1 ± 0.2 ppm) of guinea pigs (175–195 g) had two–four times less Zn in femur (and in bone containing nylon implants) after two wk than control animals.

Tin

Sn decreases femoral calcium independently of calcium homeostasis in rats (*149*).

As found by Yamaguchi et al. (*156*), $SnCl_2$ oral doses administered to weanling male rats (1.0 mg Sn/kg at 12-h intervals for 28 d) apparently causes the inhibition of collagen synthesis prior to the suppression of DNA synthesis in the femoral epiphysis of the animals.

Lithium

Birch (*89*) studied the lithium accumulation in bone following its oral administration in rats and in humans. Li decreases bone Ca in rats and suppresses bone mineralization and organic matrix synthesis in growing rats. Li treatment in humans can induce hypercalcemia.

Lead

From the investigation of ^{210}Pb metabolism in isolated bone cell populations, it is known that osteoclasts appear to be the major cell type involved (*191*).

In biopsy specimens from the iliac crests of 14 patients and five control specimens, Kijewski and Lowitz (*25*) determined the lead contents.

Some actions of Pb in interaction with other trace elements in bone are discussed by Rosen et al. (*177*). They investigated the effects of $CaNa_2EDTA$ on lead and trace metal metabolism in bone organ culture. Decreases of Pb, Zn, Cr, Mn, and Fe were found in a dose-related manner as increasing concentrations of $CaNa_2EDTA$ (0.001–0.05 m*M*) were added to the culture medium.

Pb treated and control dogs showed no statistical differences between morphometric parameters (cortical thickness and width), but Pb levels in blood and bone were higher in experimental animals. This was revealed by Delaquerriere-Richardson et al. (*179*) via radiographic studies. Growing rats were fed with 5 or 20% casein and with or without $Pb(OAc)_2$, 1 mmol/kg diet. Kizuki (*181*) reported that Pb deposition in bone was higher in the rats fed with 20% protein.

Cadmium

The inhibition of hydroxyproline syntheses by Cd^{2+} is observed in cultured embryonic chick bone (*192*).

Also found is the inhibitory effect of Cd on the calcification of embryonic chick femurs in tissue culture (*193*).

Oral administration of $Cd(OAc)_2$ decreased the Ca content in rat bones and hastened the disappearance of $Ca_3(PO_4)_2$ resulting in Ca:P ratios characterizing apatites (*194*). The collagen content is slightly decreased.

The same effect (lowering of P and Ca levels) is produced by injections of $CdCl_2$ (*195*).

There is *in vitro/in vivo* evidence that bone lysyl oxidase activity was largely inhibited by Cd. Zn ($5 \cdot 10^{-4}$ mol l^{-1}) in vitro inhibited activity by 69%; Fe, Mg, and Cu did not; and Mn and Hg slightly enhanced activity (*154*). (The authors state that this is the first case demonstrating the striking inhibitory effect of Cd on bone collagen metabolism both in vitro and in vivo.

It was demonstrated that Cd (predominantly accumulated in the epi- and metaphyses rather than diaphyses of rat) inhibited bone lysyl oxidase both in vitro and in vivo and subsequently increased the solubility of bone collagen (*91*). The influence of Zn and Cu (Hg) was also investigated.

Kamata et al. (*42*) determined Cd (and Zn) in bone after dithizone extractions of swine humerus and compared the results with some literature values from swine mandibles, rat femurs and human bones.

Fauran-Clavel et al. (*180*) found in radioisotopic studies that chronic oral Cd administration in rats: inhibited the absorption of Ca by active transport (intestine); acted on the bone crystallization process (decrease of Ca in the deep bone compartment by Cd^{2+}).

Gold

Preliminary results published by Hutin et al. (*171*) show that Au accumulated in the bony tissue of patients undergoing chrysotherapy; the concentrations were 5–20 times higher than those found in controls.

Alkaline Earth Metals

The ^{89}Sr, ^{85}Sr rat femur retention is inversely proportional to the concentration of dietary Ca (*150*).

Mg depletion in rats results in narrowing of the epiphyseal zone and metaphysis and a pronounced radiolucent zone in the metaphysis of radius and ulna. Combined Ca and Mg deficiency also developed osteomalacia and secondary hyperparathyroidism but histological changes were lesser marked than in the Ca deficiency group (*182*).

The distribution of Sr in the skeleton of Japanese men is given by Tanaka et al. (*173*).

Paleodietary information can be obtained from the Sr:Ca ratio as demonstrated by Sillen (*174*). Sr is also used for dietary reconstruction by Schoeninger (*175*) (high Sr content suggests that the diet contained less meat than other diets).

Several Elements

Trace element levels in various organs (not in bones) of normal rats have been determined by Sato and Kato (*178*). "The results seemed to be closely related to the dietary intake in rats." (INAA: Cu, Mn, Fe, Zn, Rb, Sb, Se, Cr, Co, Cs, and Sc) Can it therefore be concluded that also the bone levels may be influenced by diets?

The first measurement of trace element content of bone marrow was carried out by Gawlik et al. (*105*) using NAA. Co and Sr are enriched very highly compared to blood and bone. Enriched with respect to bone are also: Se, Zn, Fe (Al and Sc not determined). Concentrations of Zn and Pb in hippocampus and spinal cord are presented by Frederickson et al. (*159*).

Dresner et al. (*160*) examined the effect of trace elements (Cu, Au, Cd, Zn, and Pb) on heme synthetic and degradatory enzymes in rat bone marrow suspensions. And Parker and Toots (*164*) reported about "trace elements in bones as paleobiological indicators".

Typical batch stock diet for experiments on laboratory animals was analyzed for trace elements by Sabbioni et al. (*170*).

Investigations with bone-seeking radionuclides (^{47}Ca, ^{99m}Tc-Pyrophosphate, and ^{113}Sn) performed by Kutzner et al. (*168*) in rats of different age demonstrated a true accumulation in teeth as compared to bone. Büll et al. cited in *168* found higher enrichments of ^{99m}Tc-PP in spongious regions than in compact regions of bone.

The action of trace elements in the mineralization of hard tissues (including bone) has been reviewed by Weatherell and Robinson (*223*).

Tables

Some numerical results from human bone analyses are compiled for comparisons in Tables 1, 2, and 3, respectively. Animal bone values may be found, e.g., in references (*198, 199, 201, 203, 209, 212, 216*).

Especially in trace analysis there is a lack of conveniently designed reference material in particular with regard to the chemical speciation of elements. In this case it is often necessary to use different analytical methods to assure true values, *see* Table 4.

In addition to quantitative results, Table 5 lists some few qualitative investigations.

Table 4
Intercomparisons of Analytical Methods

Methods compared	Elements	Reference
AAS, NAA	Al	*30*
AAS, DPASV	Pb, Cd	*11, 50*
AAS, NAA	Sr	*175*
AAS, XRF	Pb	*94*
AAS, INAA, PIXE	Sb, As, Cd, Cu, Pb, Hg, Se, Ag, Sn, Zn	*218*
AAS, HCES, arc AES	Al	*219*
AAS, NAA	Pb	*227*
INAA, PIXE	Ca, V, Mn, Fe, Cu, Zn, Sr, Pb	*111*
INAA, PAA	F	*106*
INAA, ICAP	Ca, Mg, Mn	*217*
INAA, XRF	N, F, Na, Mg, Al, Si, P, K, Ca, Mn, Fe, Zn, Sr, Ba	*220*
ICP, ASV	Pb	*99*

Table 5
Some Qualitative Investigations

Method	Material	Features	Reference
XRD	human cortical femur	paramagnetic and cryst. effects	*59*
ESR	bone, tooth enamel		
EDAX (SEM)	white mice; sections (enamel, dentin, . . .)	Na, Mg, P, S, Cl, K, Ca	*133*
EDAX	bone sections	Ca	*136*
XMA	pig ulna; cryostat sections	P, S, Ca (epiphyseal growth)	*138*
NAA	bones from autopsy or biopsy	Ca:P ratio in relation to histological data	*221*
AES	fixed and unfixed	*in situ* correlation of	*222*
EPMA	specimens	structural and chemical information	
EXAFS	rat tibia mineral	Ca (AAS), P (wet chemic.)	*224*
AAS			

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