The Trace Elements of Human Bone*

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The existence of trace amounts of many elements in human tissue is receiving increased attention. The works of Tipton and associates [25,26] Schroeder [18,19], and Koch and associates [10] have added considerably to our knowledge of the concentrations of elements in the tissues of the body, as well as of their variations with age, environment, and state of health. However, we have been able to locate only two general analytical studies limited to human bone, and these dealt solely with rib, vertebrae, and skull [13,27].

Considerable interest has developed within the past several years in the electrical properties of bone matrix [2,3,4,5,6,7,8,22,23,24]. It is well known that trace amounts of certain elements can profoundly affect these properties in organic polymers [28] and in inorganic semiconducting crystalline materials [15]. Collagen has been described as a macromolecular assembly with possible solid state properties (that is, semiconduction) [16], and obviously bone contains, in addition, an inorganic crystalline phase—presumably apatite. It, therefore, appears possible that trace elements may in fact be associated with either the organic or inorganic component and may influence the electrical or physical properties of bone. Since the physical properties of bone certainly are of functional and pathological significance and since the electrical properties also may be, knowledge of the trace elements in bone is of more than academic interest. The present paper reports an analysis of trace elements in human bones of recent origin from Syracuse, New York, in comparison with specimens of ancient human bone from burial sites in Peru and Pennsylvania. It was hoped that such a geographical and temporal comparison would differentiate those elements consistently associated with the matrix, and possibly of structural significance, from those resulting from dietary and environmental variations and probably not functionally essential to the matrix.

The Peruvian samples were particularly well suited to this study, being in an excellent state of preservation. These were excavated in the vicinity of Chancay, Peru, from burial grounds dating back to the Chancay civilization. This area has had an almost moisture-free atmosphere since at least 200 B. C. The bodies, untreated with preservatives, had been wrapped in cotton and buried at depths of from six to ten feet in the sand. This combination of circumstances resulted in the preservation of much of the collagenous soft structures by simple desiccation (Fig. 1). The absence of exposure of bones to water prevented the leaching of elements from the surrounding soil into the bone or, conversely, loss of elements from the bone by a similar process. The Pennsylvanian specimen was obtained from the Indian burial ground, Mayview site, Allegheny County. There was no preservation of soft parts, and the bone was noticeably lighter than the other samples, both modern and Peruvian.

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Since this state of preservation is more usual, we thought it desirable to analyze this specimen for comparison.

Methods

1. *Bone Samples*

The tibia or femur from five different individuals was used in both the modern and the Peruvian groups. The one sample from the Pennsylvania burial ground, a femur, was known to be approximately 500 years old. The Peruvian samples were estimated to range from 500 to 600 years in age. Modern samples were obtained from amputation specimens, the surgery having been occasioned in all cases by mechanical orthopaedic conditions unaccompanied by osseous pathological lesions.

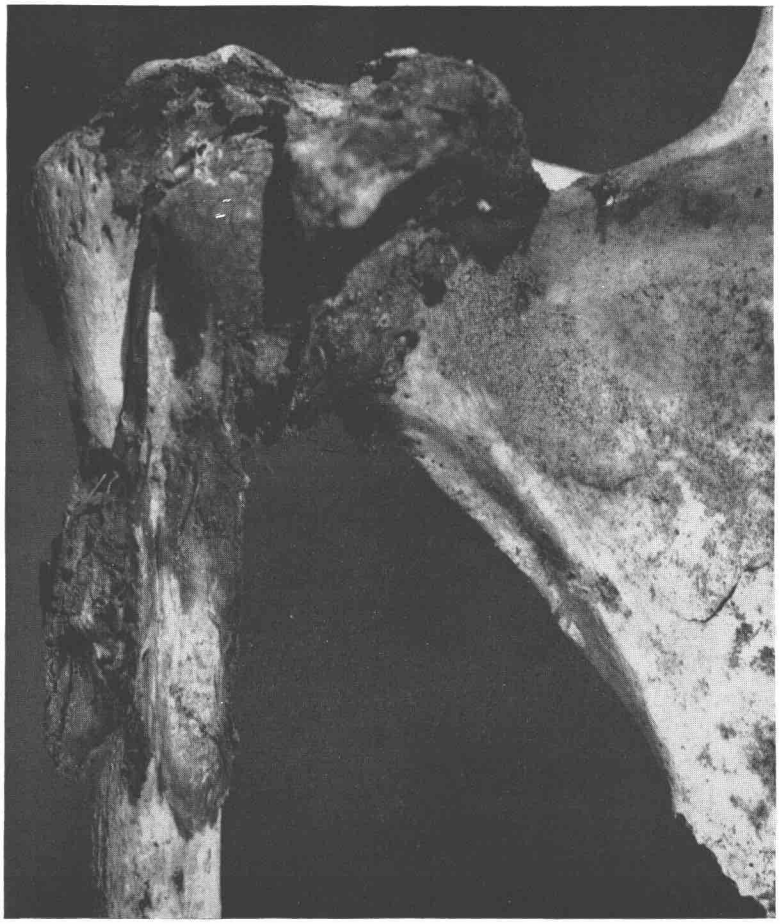

FIG. 1

Intact shoulder joint from Peruvian specimen. The excellent state of preservation is demonstrated in contrast to the usual loss of soft tissues in such specimens. The joint capsule is intact, and the normal scapulohumeral articulation is shown. The biceps tendon also is intact, occupying its normal position in the bicipital groove and retaining its attachment to the superior rim of the glenoid. A remnant of the biceps muscle fascia is also visible.

Carefully isolated interior sections of the cortex of untreated air-dried bone were ashed for twenty-four hours in a muffle furnace at 450 degrees centigrade in Vycor. The brittle ashed bone in small plastic vials was then ground into a fine powder on a ball mill. The powdered ash was mixed with an equal amount of a buffer of one part lithium carbonate and one part graphite (S.P.K.)—both spectrographic grade. The lithium carbonate was used to increase sensitivity to many elements [1].

The drying and ashing behavior was determined by cutting duplicate samples from the stock material and weighing them immediately. Their weight loss was monitored first after equilibration for eighteen hours under room conditions; then during drying at 110 degrees centigrade for twenty-four hours; during and after ashing at 450 degrees centigrade for forty-eight hours; and again after equilibration to room conditions.

2. *Standards*

A general method of semiquantitative spectrographic analysis with sensitive direct current arc excitation was employed for analysis and found very satisfactory for bone, where low sensitivity and high ash content are inherent. Semiquantitative standards were made, using tribasic calcium phosphate of reagent grade, the composition of which is much like bone ash, as well as lithium carbonate and specially prepared standard mixtures of known amounts of forty-five elements (Jarrell-Ash Co.). The calcium phosphate was mixed with equal parts of the lithium carbonate—graphite mixture as previously—with enough of the standard powders substituting for the graphite to make standards of 5, 25, 50, 125, and 250 parts per million. Internal standardization was deemed unnecessary for the desired precision.

The method of trace analysis chosen for this work was that of arc emission spectroscopy, chiefly because of its fairly high sensitivity (one part per million or less for many elements) and its easy accessibility to a wide range of elements (sixty or more in one arcing). Furthermore, since the sample preparation for this technique is minimum, involving only ashing, powdering, mixing, and loading into electrodes, the possibility of contamination is reduced.

Other techniques of trace analysis finding wide application to biological materials are atomic absorption spectroscopy and neutron activation analysis. Atomic absorption has a very high sensitivity (10→100 parts per billion commonly), but each element must be measured individually. A general analysis by this technique would involve considerable time and expense. Neutron activation analysis is extremely sensitive (a few parts per billion is easily detected) and requires little sample preparation when interferences are absent. The high calcium and phosphorus content in bone, however, gives troublesome interferences in the gamma-ray spectrum, making involved separation and concentration procedures necessary to extend the range of available elements. Moreover, neutron activation analysis requires access to a nuclear reactor (or other high neutron flux source) and gamma-ray spectrometric equipment, not available to us. Future studies in particular trace elements in bone may find these powerful techniques quite valuable, but, for the present general analysis, emission spectroscopy is far more suitable.

3. *Analysis*

The buffered samples and standards were packed into 3/16-inch graphite preformed electrodes (SPK-L-4206), arced, and photographed under the following conditions: electrodes SPK-L-4206; anode cup, ten-milligram charge; direct current arc, 9.5 amperes; separation, ten millimeters; exposure, fifty seconds; slit, ten μ; neutral density attenuator, wiremesh; spectrograph, Jarrell-Ash 1.5 meter Wadsworth; grating, 15,000 l. p. i., second order, l. r. d.-5.4 Angström units per millimeter; film, Kodak spectrum analysis #1; developer, D-19—three minutes.

The lines were measured on a microphotometer-comparator (Jarrell-Ash) and the results obtained from analytical curves of transmission versus log concentration. The results are semiquantitative in that they are precise to within ± 25 per cent of their value. The variations from sample to sample were far greater than this for all but copper, making further precision unnecessary for the purposes of this experi-

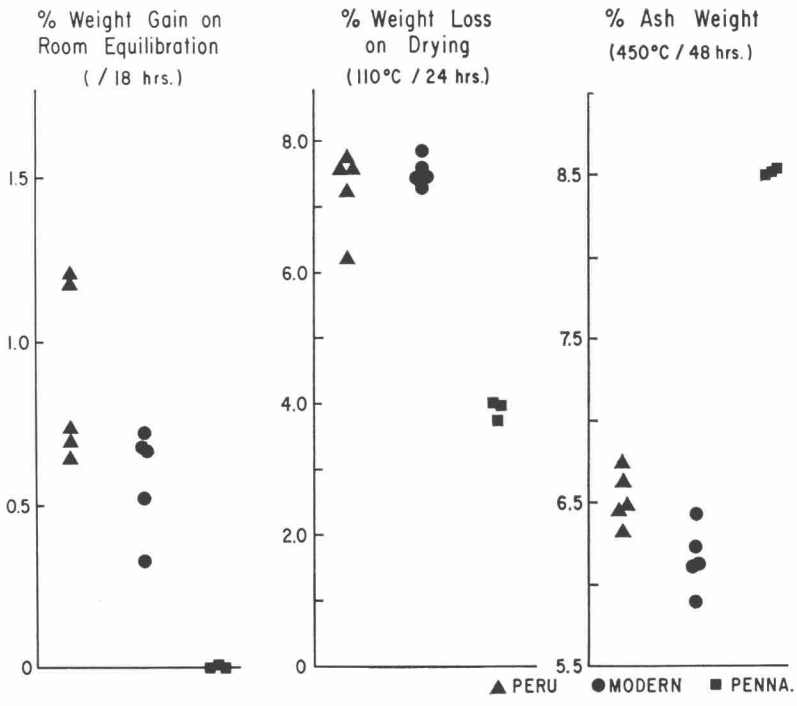

CHART I

Drying and ashing weight determinations of all specimens. The percentile weights are recorded with respect to the initial room-equilibrated weight for each sample. Column 1 indicates freshly sectioned sample weights before initial-room equilibration (eighteen hours). Column 2 indicates the weights after drying at 110 degrees centigrade for twenty-four hours, and Column 3 shows the weights after ashing at 450 degrees centigrade for forty-eight hours. The similarities between Peruvian and modern samples are clearly seen. In contrast, the abnormally low moisture content and high ash weight of the triplicates of the Pennsylvania samples are evident.

ment. A result of < five parts per million should be interpreted as being between one part per million and five parts per million. No direct check for accuracy was made, but the results agree well with those of other investigators [10,14,27].

For zinc, Eastman S.W.R. film was used enabling the recording of the sensitive

TABLE I*

CONCENTRATION OF ELEMENTS DETECTED IN ALL BONE SAMPLES EXPRESSED IN PARTS PER MILLION

Element Detection Limit	No. of Specimen	Al <5	B <5	Cu <5	Fe <5	Pb <5	Mn <<5	Si 5	Ag <5	Sr ?	Sn <5	V <5	Zn 10
	1	5	ND	<5	4	110	<<5		<5	130	<5	25	60
	2	5	ND	5	60	75	<<5		5	130	8	5	85
Modern	2	<5	ND	5	<5	5	ND	<5	ND		<5	5	67
(Ca. 1963)	4	5	ND	5	5	45	ND	50	5	62	<5	10	82
	5	<5	ND	5	<5	16	ND	25		120	12	10	74
	1	5	<5	<5	75	<5	<5		<5	130	9	<5	25
	2	11	5	<3	12	<5	5	60	ND	62	ND	<5	57
Peruvian	3	30	9	<3	55	<5	5	145		110	25	<5	67
(Ca. 1300 A.D.)	4	4	33	<3	11	<5	60	36	ND	210	ND	<5	98
	5	21	5	<3	100	<5	5	155	<5	130	ND	<5	69
Pennsylvania Indian (Ca. 1400 A.D.)	1	74	ND	5	44	ND	<5	110	ND	375	ND	<5	105

* For clarification of detection limits and precision, see "Methods" section of text. ND, not detected.

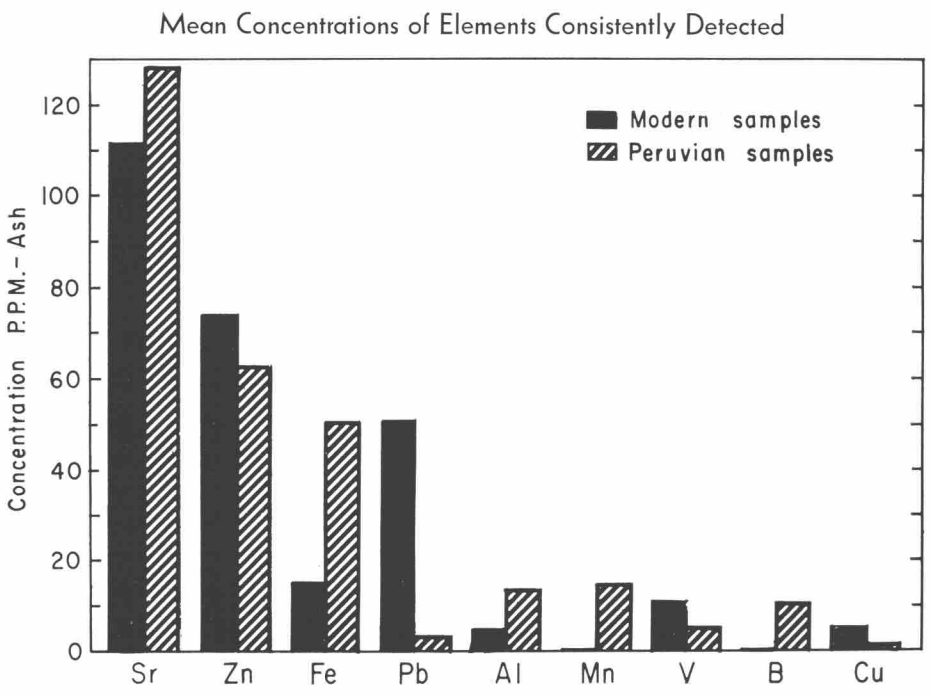

CHART II

The elements routinely detected in all specimens of ancient Peruvian and modern human bone. The elements are arranged in descending order of abundance, and the mean concentration in parts per million is depicted. The rather close similarity in concentrations of copper and zinc in both the ancient and modern samples is evident. In addition, the remarkable increase in lead (and to a lesser extent in vanadium) in modern bone is also quite evident. For a detailed analysis of each specimen, see Table I.

Zn 2138 line; for vanadium, the transmission values of V 3185.396 were converted to intensities and corrected for the interference of Ca 3185. 38 [1]. The precision was the same as above for these elements.

An analysis was made of the sand (Table III) in which the Peruvian skeletons were found in order to ascertain the possible leaching of ions. The sand was ground, mixed 1:1 and 1:100 with graphite, and arced as above. The standards were merely the standard mixtures without dilution. Attention was concentrated on the more plentiful elements, and the results are semiquantitative in nature.

TABLE II

ELEMENTS (GROUP 2) NOT DETECTED—LIMITS ESTIMATED (P.P.M./ASH).

Antimony	50	Mercury	25
Arsenic	>250	Molybdenum	25
Barium	>250	Nickel	5
Beryllium	>5	Rubidium*	25
Bismuth	5	Tantalum	>250
Cadmium	25	Tellurium	>250
Cerium	>250	Thallium	25
Cobalt	5	Thorium	>250
Chromium	25	Titanium	10
Gallium	5	Tungsten	>250
Germanium	5	Uranium	>250
Hafnium	>250	Zirconium	125
Indium	5		

* Rubidium detected (≈ 50 p.p.m.) in Peruvian sample No. 4.

Results

The results of the dry-weight and ash-weight determinations are shown in Chart I in which all percentile weights are taken with respect to the initial-room-equilibrated weight for each sample. It is seen that the modern and Peruvian samples have very similar drying characteristics (assuming water is the volatile component) and differ by only 3.7 per cent in mean ash content. The Pennsylvania Indian sample, on the other hand, held about one-half as much moisture at room temperature as did the others and had a 23.6 per cent higher ash content. This bone was also considerably less dense and far more fragile than either the modern or Peruvian specimens.

The elements sought were divided into three groups. Group 1 consists of twelve elements detected in most samples. Their concentrations were measured and are summarized in Table I and Chart II. Group 2 contains twenty-five elements not detected in any of the samples. Their limits of detection are listed (Table II). Group 3 has twenty-four elements (dysprosium, erbium, europium, gadolinium, gold, holmium, iridium, lanthanum, lutetium, neodymium, niobium, osmium, palladium, platinum, praseodymium, rhenium, rhodium, ruthenium, samarium, scandium, terbium, thulium, ytterbium, yttrium) which also were not detected, but detection limits for these were not estimated. The results for Groups 1 and 2 are semiquantitative, and individual variations were large. Estimation of the concentrations of the major elements in bone—calcium, phosphorus, magnesium, potassium, and sodium —was postponed. Their determination presents some difficult spectrographic problems, such as mutual suppression in the arc [17], variation in volatization, and self-absorption, to name a few.

Significant differences and similarities are noted in Group 1. Among these is the distinctly higher concentration of lead (ten-fold) in modern samples. The remarkably small amounts detected in Peruvian and Pennsylvania Indian bone, as well as the large variations in modern samples, suggest that lead is not a required component although it appears in the skeleton in significantly higher quantities than in any other tissue of the body [14]. The apparent ability of bone to concentrate lead and its appearance in all samples examined suggest, nevertheless, the possibility of a definite structural relationship. Vanadium and tin similarly showed a higher concentration in modern samples compared with the Peruvian, but the difference was less than for lead. Because of its detection in all samples, the presence of vanadium in bone may be significant.

The copper and zinc values appeared remarkably constant in all samples. Their constancy seems unchallenged by the modern environment and suggests a functional role.

Boron was notably undetected in any modern sample analyzed but appeared in every Peruvian sample, possibly because of the 200 parts per million concentration of boron in the sand surrounding the Peruvian specimens (Table III) or because of a high dietary intake. Bone, like all human tissue, does not seem to structure boron despite its ubiquity in the earth's crust and in sea water.

Aluminum, iron, manganese, silicon, and strontium were present in higher concentrations in Peruvian and Pennsylvanian samples than in modern bones. This could easily be explained as the result of contamination since the Peruvian sand held these elements in high concentrations. Iron is usually reported at higher concentrations than those found in this study [14,27]. However, these reports are based on whole bone analyses of either rib or vertebrae, thus including spongiosa and marrow with associated hematopoietic elements. Manganese was sporadically detected at low concentrations in modern samples.

Titanium was not detected (>5 parts per million) in this experiment despite its

high concentration in Peruvian sand. Rubidium was found in only one sample of Peruvian origin despite its biological ubiquity and chemical similarity to potassium.

The involvement of elements in trace concentrations (100 parts per million or less) in biological processes is becoming evident. (See, for example, Schroeder's excellent general review of the subject [19].) Metalloenzymes depending on manganese, cobalt, copper, zinc, and molybdenum have been shown to be active in human metabolism [18]. Many other metals are found to activate or inhibit reactions *in vivo* and *in vitro*. Vanadium and strontium, for example, cause a marked stimulation of mineralization in bone [18]. Inversely, calcium decreases the uptake of most trace element cations in higher animals by decreasing membrane permeability, by forming insoluble salts, or by some other mechanism [19].

In bone, as mentioned at the outset, the possible existence of semiconduction as well as enzymatic processes suggests even more strongly than for soft tissues that a knowledge of its functional, normal, and contaminant trace elements is of utmost importance.

Discussion

The elements detected in the various types of specimens appear to fall into three groups. The first comprises copper, zinc, strontium, iron, silicon, vanadium, and aluminum, which are detected rather consistently in all specimens. In this group, the constant range of concentration of copper and zinc, despite such factors as chronological specimen age, occurrence of leaching, and the like, is remarkable. Lithwick and associates [11] have reported a higher concentration of these elements in young osteons, and a previous publication from this laboratory [2] has speculated on the role of copper in a possible energy transport mechanism between apatite and collagen. It appears likely, therefore, that some of these elements may be structured in the matrix in a functionally important fashion.

The second group consists of elements sporadically detected, such as manganese, silver, tin, and rubidium. The likelihood of these elements being essential to bone is

TABLE III

ANALYSIS OF SAND AROUND PERUVIAN BONES

Major Components	Per cent	Minor Components	Parts per Million
Al	5.0	Ba	165
Fe	5.6	Cu	65
Mn	0.12	Pb	55
Si	∞ 20.0	Ga	10
Sr	0.35	Ni	10
Ca	4.4	Tl	90
Mg	1.3	P	∞ 100
Na	∞ 1.0	V	150
Ti	0.35	B	200

small, but their occurrence may be attributable to local environmental or occupational factors.

The third group contains two elements, lead and vanadium, detected in all but one sample (lead not detected in the Pennsylvania Indian sample) but invariably present in very small concentrations (< 5 parts per million) in ancient bone and in much higher concentrations (Pb, 50 parts per million, V, 11 parts per million) in modern bone.

Increasing contamination of the modern Western environment with lead [20,27], derived particularly from automobile fumes, and the wide use of vanadium in metallic alloys may well be contributing factors to the increased concentration of

these elements in modern bone. Although the normal modern dietary intake of lead is considerably higher than the respiratory intake, only about ten per cent of ingested lead is absorbed. Thus, the quantity of lead inhaled (most of which is absorbed) is a significant source of contamination in man [9]. If we assume a dietary intake for the Peruvian Indians roughly similar to that of twentieth-century Americans, the ten-fold increase in the modern bone examined is a striking reflection of contemporary air pollution. To account for such high lead levels by ingestion alone, the modern dietary intake would have to be one hundred times that of the Peruvian Indians.

While the details of lead absorption in bone are still unclear, a few facts are known. First, lead can and probably does exchange for calcium in bone mineral [12] although it is not, most likely, metabolized with calcium [20]. Secondly, lead concentrations in bone increase with age, showing a saturation in later years [13,20]. This suggests, as do our results, that lead is not important for growth. The effects of lead on the physical properties of bone are yet undetermined.

Elements not detected in this study may be present in bone and also may be of functional and structural importance. Foremost among these is tellurium. The sensitivity of emission spectroscopy for detection of tellurium is very low, but atomic absorption and neutron activation analysis indicate that it is the fourth most abundant trace element in the body and is concentrated by 90 per cent in bone [21]. Tellurium is only the seventy-fifth most abundant element in the earth's crust, and, therefore, its occurrence in bone in such amounts indicates active concentration in the bone matrix. This element is also one of the naturally occurring semiconductors. Its presence in bone certainly warrants further investigation.

From the present study, we conclude that there are certain trace elements that are possibly functional components of the matrix and others that are introduced in abnormally high concentration by pollution of the present environment. It is a distinct possibility that alterations in concentration of some of these trace elements may be associated with various disease states [18].

As data are reported here for only whole bone samples, no attempt is made to associate any of the elements detected with either the mineral or the organic phase of bone. More detailed analyses will be required to distinguish the elements associated with each phase and those characteristic only of the composite bone system.

It should be noted, in conclusion, that from the anatomical appearance and the drying and ashing measurements, the Chancay tissues enjoyed a remarkable physical and chemical preservation unlike that of the Pennsylvania Indian bone. This lack of preservation applies to most available archeological specimens, where leaching, decomposition, contamination, and petrification are common. In order to clarify the state of preservation of bone samples used in future experiments, it would be well that they be compared with modern samples not only in their appearance, density, and so forth, but in their hydration characteristics and ash content as well as their content of trace elements.

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