TECHNICAL NOTE

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Reassembling Scattered and Mixed Human Bones by Trace Element Ratios

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ABSTRACT: Trace metal ratios in human bones were examined to determine if there were ratios that were sufficiently consistent within an individual yet varying sufficiently from the bones of another individual so that bones in a mixed grave could be reassembled. The concentrations of 21 elements sampled at 54 places on 30 human bones in each of 5 skeletons indicated that the magnesium/zinc ratio was the most reliable and that the zinc/sodium, magnesium/sodium, and chromium/sodium ratios could be used as supplements to help reassemble human bones belonging to the same individual after all standard techniques had been used.

KEYWORDS: physical anthropology, musculoskeletal system, trace elements, bones, chromium, magnesium, sodium, zinc

This problem of reassembling scattered and mixed human bones originated with a helicopter crash in Ethiopia in which the bones from three bodies had been stripped clean by wild life and scattered over a large area. The problem was to reassemble the bones corresponding to the proper people. Two bodies were the same height and within two years of age of each other and definite assignments of all of the bones could not be made. An additional method is therefore required for use in the future to supplement existing techniques. It was decided to examine the trace elements present in the bones to determine if sufficient individuality existed to be useful.

It is known that many elements at the trace level reside in bone material [*1-6*]. Lambert [*7-10*] has demonstrated the applicability of bone elemental analysis to the study of diets of ancient peoples. His studies have shown substantial concentrations of magnesium (Mg), zinc (Zn), strontium (Sr), iron (Fe), aluminum (Al), potassium (K), and calcium (Ca). He has also documented the distribution of these elements in bone material. Electron microprobe

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experiments [*11*] were performed to determine how the elements are distributed in the cross section of a bone. The effects of soil on the equilibrium concentrations of several elements in buried bones have been examined [*7*], and it was found that Ca leached from the bones to the soil, Fe, Al, and K leached from soil into the bones, while Zn, Mg, and Sr did not leach. The primary bones used in his studies were the femur and the rib. Strontium currently is "state of the art" as a dietary indicator [*12–18*] to determine diet, age, and race of ancient peoples.

The two hypotheses to be tested and reported on here are based on the assumptions that each human is not only genetically different, but each eats a different diet, facts which might be reflected in the trace metals deposited in human bones. Therefore it was desired to determine if (1) the actual values or ratios of pairs of elements will be similar from bone to bone within one body, but (2) measurably different from another body.

Experimental Procedure

Apparatus

The sampling tool was a 6.25-mm diameter tungsten carbide twist drill. The digestion apparatus was a six port micro Kjeldahl apparatus, Precision Scientific Model No. 85372, equipped with 100-mL flasks. The analytical balance was a Mettler single pan Model H-5. The Jarrell-Ash Model No. 950 Atomcomp inductively coupled plasma atomic emission spectrophotometer was controlled by a DEC PDP-8A computer. The Triga Mark II reactor was used for neutron activation analysis. The gamma ray counter was a Canberra 7000 series Ge(Li) detector. The multichannel analyzer was a Canberra Series 80. The computer was an ADAC LSI-11.

The vials were 7 dram (12 g), 2.8 by 5.5 cm plastic prescription type. These were soaked in 50% perchloric acid $(HClO)_4$ for 48 h, rinsed with distilled-deionized water, air-dried, the tops replaced, and then stored in a covered box until used to store samples. A blank for each element to be measured was run on the leachate and on water allowed to be in the vials for seven days. No detectable amounts were found.

Chemicals

The chemicals used were concentrated nitric acid 3/1 with $HClO_4$ and perchloric acid 70% (G. F. Smith Chemical Co.).

The standards were commercially prepared stock solutions:

1000 ppb: silver (Ag), titanium (Ti), molybdenum (Mo), vanadium (V), cobalt (Co), Al, arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), Fe, manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), and Zn;

100 ppm: Ca, sodium (Na), and K; and

50 ppm: Mg.

Procedure

A sample is obtained by drilling into the bone at the desired place. A 0.1- to 0.4-g sample is placed in a micro Kjeldahl flask, 10 mL of mixed nitric/perchloric acid $(HNO_3\text{-}HClO_4)$ (3:1) and the sample digested. The digested sample is cooled, quantitatively transferred with small portions of water to a 25-mL volumetric flask, and diluted to volume with distilled-deionized water. Aliquots of the acid digest mixture were treated in the same manner as the samples to serve as blanks in the digestion process of the experiment. Standards were run every five samples to check instrument drift. Once the raw data for the samples were obtained, they were blank corrected by subtracting the values for each element present in the acid digest blank from the corresponding element value for each sample.

Results and Discussion

Table 1 lists the characteristics of the skeletons examined. They include three males, two females, white and Mongoloid, and ages ranging from approximately 40 to 65+ years.

Table 2 lists the places on these skeletons where samples, both left and right side, were obtained. The samples were obtained by one person who coded the samples. The analyses were run by another person as a single blind.

Table 3 lists the elements determined and the detection limit for each element based upon the analysis procedure. These were the elements the EPA instrument was set up to measure, most of which would have been selected for this study anyway.

The skeletons were numbered by the anthropologist taking the samples as 1, 3, 4, 5, and 6 for the first set of analyses and 7, 8, 9, 10, and 11 for the second set.

A duplicate set of samples were analyzed for Skeleton 7 (also as a blind) to determine the precision to be expected for the instrumental portion. This set duplicated all parameters except for redrilling.

Based on a "pool" standard deviation and average mean for these duplicate determinations of all of the 54 sites, the calculated precision is given to be slightly less than 3% relative standard deviation (RSD). This is therefore a measure of what can be expected of this procedure based on instrument variables, standards preparation, and general technique.

TABLE 1—*Skeletal information.*

Skeleton	Age	Race	Stature	Origin	Comments
1 (9)	40-50	Caucasoid	male	South Asia	chemically processed biological supply
3 (8)	40	Caucasoid	male	Iowa	cadaver material
4 (7)	60+	Caucasoid	female	Iowa	cadaver material
5 (10)	60+	Mongoloid	male	Kansas[a]	buried 800 to 1000 yrs
6 (11)	45-55	Mongoloid	female	Kansas[a]	buried 800 to 1000 yrs

[a]Calovich Indian Mounds, Wyandotte Co. Site 14WY7.

TABLE 2—*Sampling positions (left and right side) on each skeleton.*

Bone	Positions Sampled
Humerus	end, center, end
Radius	end, center, end
Ulna	end, center, end
Femur	end, center, end
Tibia	end, center, end
Fibula	end, center, end
Ilium	end
Talus	end
Calcaneus	end
Metatarsal	end
Carpal	end
Proximal (T.)	end
Large rib	end
Manubrium	end
1st Thoracic	end
1st Lumbar	end
Frontal	center

TABLE 3—*Elements determined and their detection limits.*

Element	Detection limit, ppm[a]
Silver[b]	0.005
Aluminum	0.02
Arsenic	0.05
Barium[b]	0.002
Beryllium	0.002
Cadmium[b]	0.002
Cobalt	0.005
Chromium[b]	0.005
Copper[b]	0.01
Iron[b]	0.05
Manganese	0.002
Molybdenum	0.005
Nickel[b]	0.01
Lead[b]	0.05
Antimony	0.05
Selenium[b]	0.5
Titanium	0.005
Vanadium	0.005
Zinc[b]	0.02
Magnesium[b]	2.0
Sodium[b]	2.0

[a]Determined by EPA.
[b]Elements determined in Skeleton 1.

An entire duplicate set of samples (blind) on all five skeletons were obtained by redrilling next to the original drilling site. Ten of these fifty-four sites were examined by a different instrument, the results in general averaging the same as the initial two experiments.

In the initial study, 5184 individual determinations were made and examined as single elements. The values for the individual elements within a skeleton were averaged and a standard deviation obtained. The values from one bone to another were then compared, as were those from one skeleton to another. Only arsenic appeared to be of value in distinguishing the bones of one skeleton from another.

The large spread in values was believed to be in part due to the nature of the bone. Some bones did not fully digest even though a HNO_3-$HClO_4$ mixture was used. Traces of what was believed to be sand were sometimes present. To filter those samples, dry and weigh the residue, and make an appropriate correction was deemed too time-consuming to be practical. By using the ratios of elements, sample size cancels out of the calculation as does the occasional loss of sample by bumping during the digestion. The ratios of 2 elements, of which 420 were possible, were then examined. It was found that the variation was too great to be of any real value except for Zn/Mg, Mg/Zn, Zn/Na, Mg/Na, and Cr/Na. The 5 skeletons were then resampled (blind sampling technique) and the entire process, including standards, was repeated. By using the above ratios and arsenic as a single element, it was possible to match each of the original 5 skeletons (1, 3, 4, 5, 6) with the second sample set (9, 8, 7, 10, 11). These data are shown in Tables 4 and 5.

The Cr/Na ratio was initially questioned since the samples were obtained by drilling with a tungsten carbide tipped steel drill and it was suspected that the source of the chromium was the bit. A sample of the chuck end of the bit was melted off, so as not to contaminate the sample with cutting tool particles, and the elements determined by neutron activation analysis. The bit was then irradiated, allowed to decay to a safe handling level, and used to obtain a bone sample. The sample was examined for traces of radioactive chromium and none was found. Therefore the source of the chromium was the bones and the Cr/Na ratio is valid.

TABLE 4—*Skeleton matching summary based on the Mg/Na and Zn/Na ratios.*

Skeleton	Mg/Na	Zn/Na
1	0.337 ± 0.019	0.012 2 ± 0.001 1
9	0.280 ± 0.011	0.009 5 ± 0.000 7
3	0.787 ± 0.046	0.188 ± 0.041
8	0.450 ± 0.021	0.099 8 ± 0.042 0
4	0.614 ± 0.026	0.049 2 ± 0.006 7
7	0.436 ± 0.043	0.003 37 ± 0.009 29
5	0.934 ± 0.166	0.045 6 ± 0.004 1
10	1.22 ± 0.43	0.030 8 ± 0.003 4
6	1.47 ± 0.93	0.030 4 ± 0.007 3
11	1.26 ± 0.54	0.020 7 ± 0.003 2

TABLE 5—*Skeleton matching summary based on the Cr/Na ratio and arsenic.*

Skeleton	Cr/Na	As, ppm
1	$5.46 \times 10^{-4} \pm 6.70 \times 10^{-5}$	NA[a]
9	$3.54 \times 10^{-4} \pm 3.60 \times 10^{-5}$	BDL[b]
3	$1.69 \times 10^{-3} \pm 9.00 \times 10^{-5}$	1890 ± 161
8	$6.53 \times 10^{-4} \pm 3.44 \times 10^{-5}$	1130 ± 105
4	$1.19 \times 10^{-3} \pm 1.00 \times 10^{-4}$	216 ± 46
7	$6.52 \times 10^{-4} \pm 6.63 \times 10^{-5}$	128 ± 24
5	$3.03 \times 10^{-3} \pm 4.10 \times 10^{-4}$	1.37 ± 1.01
10	$2.55 \times 10^{-3} \pm 7.33 \times 10^{-4}$	8.03 ± 2.99
6	$2.77 \times 10^{-3} \pm 1.30 \times 10^{-3}$	2.83 ± 3.40
11	$1.73 \times 10^{-3} \pm 4.12 \times 10^{-3}$	9.16 ± 4.03

[a]NA = not applicable, as not available at that time.
[b]BDL = below detection limit.

For the initial screening, 54 sample sites were chosen in 30 bones. Once these data were obtained it was intended to determine how many of those sampling points could be eliminated for future work and if the elimination would improve the relative standard deviation sufficiently to be of value. If only the middle sample position of the long bones were used, then the relative standard deviation decreased significantly and either the Zn/Mg or Mg/Zn ratio (2 elements that do not leach in or out) became more reliable. Table 6 shows the data for all of the sites and Table 7 for the extremity bones only.

Two hypotheses were tested statistically: (1) the values of the Zn/Mg and Mg/Zn are uniform within one skeleton, and (2) the five skeletons are all equal based on the Zn/Mg and Mg/Zn ratios.

The model used was a two-way mixed model—*bone* (fixed, same for each skeleton and *body* (random). To test these hypotheses, statistical p values were calculated using a Statistical Analysis System (SAS) statistics program.

The general test is: if α value $>$ p value, then reject the hypothesis. But if α value $<$ p value, then accept the hypothesis (95% confidence $\alpha = 0.05$, 97.5% $\alpha = 0.025$, and 99% $\alpha = 0.001$). The results are summarized in Table 8.

The conclusions that can be drawn from Tables 6, 7, and 8 are that of the two ratios, the Mg/Zn is somewhat more uniform than the Zn/Mg if all of the bone data is used. If only the extremity bones are considered, then there is no practical significant difference between the

TABLE 6—*Skeleton matching summary based on the Zn/Mg and Mg/Zn ratios.*

Skeleton	Zn/MG	Mg/Zn
1	0.0358 ± 0.0022	29.2 ± 1.6
9	0.0343 ± 0.0018	30.2 ± 1.6
3	0.215 ± 0.036	6.52 ± 1.01
8	0.206 ± 0.078	8.74 ± 1.37
4	0.0797 ± 0.0097	14.7 ± 1.43
7	0.0754 ± 0.0160	16.8 ± 1.64
5	0.0412 ± 0.0122	42.0 ± 12.5
10	0.0416 ± 0.0083	38.2 ± 8.83
6	0.0225 ± 0.0057	65.7 ± 17.3
11	0.0249 ± 0.0045	52.1 ± 13.1

TABLE 7—*Skeleton matching summary based on the Zn/Mg and Mg/Zn ratios at the extremities of the long bones.*

Skeleton	Zn/Mg	Mg/Zn
1	0.0300 ± 0.0022	33.7 ± 2.6
9	0.0313 ± 0.0029	32.7 ± 3.2
3	0.115 ± 0.023	9.71 ± 2.22
8	0.0804 ± 0.020	14.0 ± 2.86
4	0.0485 ± 0.0054	21.1 ± 1.8
7	0.0412 ± 0.0029	24.5 ± 1.6
5	0.0394 ± 0.0130	40.1 ± 22.9
10	0.0447 ± 0.0177	34.4 ± 20.0
6	0.0201 ± 0.0115	79.2 ± 53.6
11	0.0224 ± 0.0087	53.5 ± 23.8

TABLE 8—*The* p *value significance test for the Zn/Mg, Mg/Zn ratios in human bones.*

Ratio	Hypothesis	*p* Value	Comparison	Conclusion
Zn/Mg	1	0.2996	0.05 < 0.2996	accept 1
All bones	2	0.0001	0.05 > 0.0001	reject 2
Mg/Zn	1	0.4238	0.05 < 0.4238	accept 1
All bones	2	0.0001	0.05 > 0.0001	reject 2
Zn/Mg	1	0.4872	0.05 < 0.4872	accept 1
Extremities	2	0.0008	0.05 > 0.0008	reject 2
Mg/Zn	1	0.4183	0.05 < 0.4183	accept 1
Extremities	2	0.0001	0.05 > 0.0001	reject 2

ratios. In addition, the values for the ratios Zn/Mg and Mg/Zn are uniform at the 95% confidence level within the bones of each of the five skeletons and different between skeletons.

This most likely will not be the case if a larger number of skeletons are sampled, but it does indicate the range of usefulness for the Zn/Mg ratio.

Conclusion

A total of 11 958 individual element determinations were obtained to make the statistical evaluations. Based on that data the following conclusions were made.

Because of the normal variation in measuring techniques and the amounts of trace elements in human bones, there is not sufficient individuality to reassemble a number of skeletons bone for bone from a mass grave using only individual elements in a trace element technique. However, if combined with current standard anthropological techniques, then either the Zn/Mg or Mg/Zn ratio, supplemented by the Zn/Na, Mg/Na, and Cr/Na ratios could be of value to decide questionable bone assignments. Occasionally, the presence of a specific trace element, such as As, might be of assistance.

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