Bone Analysis: Silent Testimony of Lead Exposures in the Past

PHILLIPPE GRANDJEAN

Grandjean, P. 1989. Bone Analysis: Silent Testimony of Lead Exposures in the Past. Meddr Grønland, Man & Soc. 12: 156–160. Copenhagen 1990–01–26.

Modern-day environmental pollution by lead results in considerable adverse health effects. Increased lead exposures are a result of modern technology, and pre-industrial exposures were presumably very low. This possibility may be examined by analysis of mummified human tissues from the past. Bone samples are particularly well suited for this purpose, because the skeleton contains most of the body burden of lead, and because well preserved bone tissue is unlikely to be contaminated post mortem. Trabecular bone from a lumbar vertebral body was taken from each of the mummified bodies from Qilakitsoq, except for the youngest child. The lead concentrations were determined by atomic absorption spectrometry with routine procedures against laboratory contamination. The lead levels varied between 0.12 and 0.30 µg/g dry weight with a median of 0.21 µg/g. Although slightly lower levels may be obtained by using more rigorous clean-laboratory techniques, the results found are similar to minimal levels measured in bone tissue from Nubians of Northern Sudan and Precolumbian Peruvians. Thus, the Qilakitsoq Eskimos lived in a pristine environment low in lead. This result offers an independent indication that modern-day lead exposures are considerably in excess of base-line levels to which humans have originally adapted.

Philippe Grandjean, Department of Environmental Medicine, Odense University, J. B. Winsløws Vej 19, DK-5000 Odense C.

Chemical information contained within preserved skeletal tissues may be used to provide historical and prehistorical insights into the activities and environmental conditions of past populations. The analytical data derived from skeletal remains can be considered a testimony of previous events. In particular, testimonies provided by these long dead "silent witnesses" may expand our knowledge concerning human lead exposure in different geographical areas over a range of time periods.

Weighty testimonies by such "silent witnesses" especially relate to arguments concerning the relative magnitude of modern-day lead exposure. Patterson (1965) estimated that environmental pollution had increased the human body burden of lead by 100-fold above the "natural" or "unpolluted" level. Publication of this proposal with its significance for low-level lead toxicity initiated a major controversy which has not yet been fully resolved.

Analysis of the skeletal system provides most of our reliable information about past lead exposures. Bone and tooth are frequently the only remaining tissues from ancient burials, and lead is, fortunately, preferentially stored in calcified tissues. However, as these tissues are complex and subject to contamination by exogenous material, caution should be exercised in their use and in data analysis. In addition, lead analysis of calcified tissue is associated with many problems and pitfalls. Based on detailed evaluation, the weight of the evidence provided by these "silent witnesses" should be carefully considered. A few analyses of human calcified tissues from premetallurgical eras have suggested that large increases in lead exposure have occurered since then (Ericsson *et al.* 1979; Grandjean *et al.* 1979) and this chemical testimony therefore supports Patterson's views. In this way chemical information extracted from well-preserved mineralized tissues may be used to answer a contemporary question regarding environmental pollution.

The Qilakitsoq mummies provide an exceptional opportunity to examine Patterson's (1965) hypothesis. The burials took place about 500 years ago, when metallurgy and lead technology was unknown among the Eskimos. The bodies were mummified by the dry and very cold conditions in the cave, and the bone tissues were well protected against any post mortem contamination in the grave. In addition, long-range transport of lead aerosols from European smelting of lead was minimal, as indicated by the very low lead concentrations found in Medieval snow layers from Greenland inland ice (Murozumi *et al.* 1969).

Lead accumulation in the skeleton

Following absorption, lead ions in the blood are efficiently retained in the calcified tissues. Indeed, the skeleton can be likened to a huge ion exchance column with the bone mineral phase serving as the exchange medium. The effectiveness of this scavenging system is such that over 90% of the body burden of lead is located in the skeleton (Barry 1978).

A major factor influencing the bone retention of lead is the metabolic activity of the tissue. Local lead uptake varies due to differences in vascularity of the tissue. Indeed, compared with Haversian bone, active periosteal and sub-epiphyseal bone have very high affinities for lead. However, the lead content of a bone is a function of both the rate of ion uptake and the rate of its release. Compartmental analyses suggest that the average biological half-life for skeletal lead is about ten years, but it varies, of course, with bone type and composition. Thus, the average rate of bone remodelling in adults is about 3.5% per year, but this rate varies from a high of 8% in vertebrae to a low of 1.5% in femurs (ICRP 1975). In agreement with this observation is the finding that cortical bone contains more lead than trabecular bone. Our own studies have shown that temporal bone contained 50% more lead than the vertebral body (Grandjean 1975), and similar results were found by Drasch (1982). Further, because of the very long half-lives of lead in bones, the accumulation of lead may continue throughout the entire life of a human being. When considering bone lead levels, the biological age should therefore be taken into account.

Selection of materials

The main problem in the selection (and preparation) of archaeological samples is the avoidance of post-mortem changes in lead concentration. In our experience, the best specimens are those which have been well preserved in extremely dry environments. Although lead is firmly bound in the calcified tissues, some solid-solution migration and re-crystallization may take place over extended time periods. Migration may occur because the apatite retains its ion exchange characteristics; this property facilitates both lead uptake from the external environment and lead release from the bone. Of course, if exogenous water is unavailable, loss of bone lead would be minimal. In humid conditions, particularly when the soil pH is low, some lead leaching and migration could occur. Unfortunately, the direction and extent of lead transport and migration are difficult to evaluate. Cases of severe lead contamination of bones from a lead coffin have been described by Waldron (1981).

An additional problem which gives rise to abnormally high lead levels is post-mortem contamination of the specimen with dust particles. Exogenous lead-containing particles can adhere to tissue samples and microparticles can become incorporated into the apatite structure. Such particles may arise from the soil and the burial vessel. More recently, anthropogenic lead aerosols have become an atmospheric source of exogenous lead.

Decontamination methods may not completely solve these problems. When sparingly soluble lead salts from soil water become incorporated in bone mineral, the lead ion enters the apatite lattice and undergoes heteroionic exchance with Ca^{2+} or even Mg^{2+}. Thus, the importance of post-mortem contamination of archaeological samples should not be overlooked. Clearly, as environmental lead pollution is ubiquitous, this type of contamination represents a major research obstacle.

When comparing mummified bones to bone samples from present-day autopsies, the possible difference in contents of organic materials should be taken into account. On a dry weight basis, fresh samples contain relatively less lead than do samples without the organic matrix. For femoral bone, Drasch (1982) found a difference of 28%.

Occasionally, tissues other than bone or teeth may be available for chemical analysis. For example, we have examined mummified brain tissue from Sudanese Nubia (Grandjean *et al.* 1977). Unfortunately, postmortem contamination is difficult to remove or exclude, because the tissue may have been permeated by exogenous lead. In addition, lead concentrations cannot be given with reference to dry weight because organic components of the mummified brain have been degraded. For this reason, we chose to express the lead concentration as a function of brain copper. Copper has a long biological half-life and, in contrast to lead, is found in relatively constant amounts in brain. Although the Nubian samples exhibited very low bone lead levels, the lead/copper ratios varied widely but were not significantly different from present-day values. However, a child who exhibited excessive lead concentrations in teeth and bones also had the highest lead/copper ratio found in the Nubian material (Shapiro *et al.* 1980). These results suggest that while the soft tissues may reflect the level of lead exposure, the actual analytical data derived from mummified tissues may be difficult to interpret.

Finally, some comments about lead analysis of hair may be appropriate. While this complex structure is frequently used for toxicological studies, in only a few instances have hair samples from historical and prehistorical times been utilized for lead analyses. The use of this tissue entails some problems similar to those of bone. The most important one is exogenous contamination; indeed, to remove this type of contamination without changing the endogenous lead level is not an easy undertaking (Grandjean 1978). Further, Lockeretz (1973) suggested that the significance of such conta-

mination may increase with time and make interpretation of lead analyses virtually impossible, particularly if the hair was exposed to moisture. Thus, although hair analysis may be utilized as a screening procedure for the measurement of current lead exposure, the use of hair for archaeological sample evaluation has some severe drawbacks.

Calcified tissues are therefore useful for lead analysis for three reasons: 1) most of the body lead burden is retained within these tissues; 2) bones and teeth may be preserved for millenia under dry conditions without major changes in their mineral contents; and 3) surface contamination may be easily removed.

Analytical Procedures

Three critical steps are notable in the measurement of lead in calcified tissues: (1) specimen decontamination, (2) specimen preparation, and (3) sample analysis.

1. Specimen decontamination

A number of specimen cleaning procedures have been described in the litterature. No one method has received universal acceptance. As we have obtained favourable results using an ultrasonic bath to clean hair of surface contamination, we have used this technique to wash bone as previously described (Grandjean *et al.* 1979). More thorough washing techniques have been based on exposure of the tissue to dilute acid (Ericsson *et al.* 1979), but this approach is much more drastic, and extreme care must be exercised in its use. All handling should take place under clean laboratory conditions.

2. Specimen preparation

Frequently, the research worker does not have the luxury of deciding which piece of bone or tooth should be utilized for lead analysis. However, in this study where specimen size was not limiting, the following guidelines were observed. Comparable morphological fragments were selected from the trabecular part of each vertebral body. As in a Japanese study (Ishinishi 1978), interior sections of bone were used to avoid surface contamination. Care was taken to use lead-free surgical scalpel.

3. Sample analysis

Calcified tissues may be dissolved for analysis after ashing the sample in an oven or by wet digestion with acid. Dry ashing may pose a problem because lead is lost from the sample at temperatures above 500°C. Low temperature ashing with excited oxygen is inefficient. Acid digestion is currently the preferred method, and both nitric and perchloric acid may be used. Fresh samples are more easily demineralized with perchloric acid than by nitric acid. However the presence of perchloric acid can produce matrix effects which interfere with flameless atomic absorption detection techniques. In the present study, which was carried out in 1983, concentrated, ultrapure nitric acid was used as previously described (Grandjean *et al.* 1979).

A number of different, sensitive detection methods are available. We have used, with favourable results, atomic absorption spectroscopy (Grandjean *et al.* 1979). The results obtained by this method are reproducible and correlate well with those obtained by anodic stripping voltammetry. All specimens were separated in two subsamples which were both analysed in duplicate on a Perkin-Elmer atomic absorption spectrometer with deuterium background correction and a graphite furnace HGA-76. The coefficient of variation of duplicate results averaged 21%. The IAEA reference material of animal bone was analysed repeatedly in the laboratory with excellent results (Grandjean *et al.* 1984). However, the lead level in this material is about 15-fold above that in the Qilakitsoq mummies and may not be relevant for the analytical quality assurance. On the other hand, repeated assessment of lead concentrations in a commercial hydroxyapatite preparation has shown a coefficient of variation of 27% at an average level of 0.41 µg/g which is closer to the mummy lead levels. However, these determinations were made on 1-mg aliquots of the material. Thus, analytical and biological variations may be relatively large at such low lead concentrations.

The Qilakitsoq testimony

The lead concentrations found are given in Table 1. All results are averages of duplicate analyses of two samples from each mummy. Due to the possibility of minor laboratory contamination, these results should be re-

Table 1. Lead concentrations (µg/g dry weight) in vertebral bodies from the Qilakitsoq mummies.

Identification number	Sex and age (years)	Lead concentration
I/2	♂,3½–4	0.23
I/3	♀,c.25	0.30
I/4	♀,c.30	0.19
I/5	♀,c.45	0.26
II/6	♀,c.50	0.12
II/7	♀,18–22	0.14
II/8	♀,c.50	0.22

Table 2. Lead concentrations (µg/g dry weight) in well preserved bone tissue of adults from preindustrial and contemporary civilizations

Time period	Burial site	Number examined	Bone tissue	Average lead concentration	Source
3300–2900 BC	Sudanese Nubia	9	Temporal	0.6	Grandjean *et al.* 1979
Before 600 AD	Peru	2	Mandible	0.14	Ericsson *et al.* 1979
About 1475 AD	Qilakitsoq	6	Vertebral body	0.21	This study
1977	Copenhagen	17	Temporal	5.5	Grandjean *et al.* 1979

garded as upper limits. The true, *in vivo* lead levels in the Qilakitsoq Eskimos 500 years ago may have been somewhat lower than the results given in Table 1. Additional analyses of bone and tooth samples are now underway in a more sophisticated laboratory with improved contamination control.

From the results obtained so far, no age relationship is apparent. This finding may be surprising, because lead is known to accumulate with age, but the small number of specimens available from Qilakitsoq limits the validity of this apparent lack of correlation.

Other testimonies of past lead exposures

"Silent witnessess" have been examined for lead exposures in several laboratories. Each study of skeletal remains has been carried out with a different method which may make comparison difficult. The types of calcified tissues selected, the age groups examined, the sample cleaning and preparation, and the analytical method are important parameters which may influence the results.

The oldest Nubian and Peruvian samples originate from premetallurgical eras, and the lead levels reflect the magnitude of "natural" or prepollution exposures (Table 2). When compared to present-day levels the analyses show bone ratios of about 0.1 and tooth ratios of 0.01 and below. Both studies (Grandjean *et al.* 1979; Ericsson *et al.* 1979) reveal information which indicates that the bones, despite excellent preservation in a dry environment, were somewhat contaminated, and that tooth lead concentrations may be better indicators of the original exposure levels. Both in Nubia and in Peru the natural tooth lead levels were 1%, or less, of current lead levels in North America.

Bone samples from similar time periods were available from other sources (Grandjean 1975; Drasch *et al.* 1979; Waldron & Wells 1979; Ishinishi *et al.* 1978), but the results are difficult to interpret in detail because the samples were exposed to soil moisture and most likely were somewhat contaminated. However, an increasing lead retention with time as lead technology was introduced was found both in Nubia, Denmark, England and Japan. Medieval samples contained even more lead than present-day controls. A similar pattern was found in Jaworowski's original study (1968) from Poland where excessive lead doses were identified for the 17th-19th centuries. High bone lead levels were prevalent even in the remote Faroe Islands during past centuries (Nielsen *et al.* 1982). Such retention levels were related to the use of pewter, glazed earthenware, lead water pipes, lead-containing drugs and other sources which were common in the past. From the information available, such sources of lead never reached the Qilakitsoq group studied.

In concert, the studies of lead levels in archaeological skeletal samples justify two conclusions: 1) prepollution exposures were lower than 1–10% of present-day exposures in North America and Europe; and 2) with increasing use of lead, exposures increased and have, during some periods, averaged considerably more than today.

Significance of Silent Witnesses

The magnitude of lead exposure levels has been examined by an expert committee under the auspices of the US National Academy of Sciences (1980). On the basis of complex estimates from ecological models and data provided by "silent witnesses", the committee concluded that present-day exposures were 10-to-1,000-fold above those in which Homo sapiens originally adapted. The evidence provided by the chemical testimony of these human ancestors was considered important and in agreement with independent estimates from other sources.

When compared to natural levels, the current lead exposure levels of most industrialized nations are rather excessive. Despite the fact that lead exposures of many contemporary control groups and industrialized populations may well be 100-fold greater than the background level, very little is known of the possible health effects of lead in humans with "habitual" environmental exposures. Environmental lead pollution is not restricted to industrialized countries; airborne lead and other sources of lead intake have probably permeated even the remotest areas of the world. Thus, present-day blood lead levels in Greenland are similar to levels

found in Denmark and other countries (Hansen *et al.* 1984). Nevertheless, some population groups have been identified with comparatively low blood lead concentrations. Perhaps by using such populations as controls, we might learn more about the possible effects of lead at low exposure levels.

Governmental monitoring and control of several important uses of lead has resulted in a decrease in the general lead exposure levels. On the other hand, about 10% of the annual lead production is still used for the production of alkyl lead additives for gasoline. The atmospheric lead pollution that results from the lead halides of automobile exhaust also contaminates the food chains. This type of contamination is in sharp contrast with historical times, when excess exposure was caused by a limited number of single sources. Today, environmental lead pollution reaches most people through complex interacting pathways. As might be expected, with the sophistication of modern societies, prevention of lead exposure has not become simpler.

In conclusion, chemical testimonies of lead exposures in the past have been successfully obtained in several laboratories. The examination of "silent witnesses" of past times have contributed significantly to our knowledge of previous lead exposures, and current exposure levels have been put in a new perspective. The Qilakitsoq mummies provide a relatively recent testimony that an almost lead-free environment persisted in Greenland about 500 years ago before the influence of Western civilization.

Acknowledgements

The author is grateful to Irving M. Shapiro and Ole Vagn Nielsen for stimulating discussions, to J. P. Hart Hansen for carefully providing the samples and to Ranja Bjerring for technical assistance.

References

Barry, P. S. I. 1978. Distribution and storage of lead in human tissues. – In: Nriagu, J. O. (ed.), The biogeochemistry of lead in the Environment, Part B: 97–150. Elsevier/North-Holland Biomedical Press, Amsterdam.

Drasch, G., Meyer, L. v. and Kreglinger, R. 1979. Untersuchungen zum Bleigehalt menschlicher Knochen. – Arbeitsmed. Sozialmed. Präventivmed. 14: 31–34.

Drasch, G. A. 1982. Lead burden in prehistorical, historical and modern human bones. – Sci. Total Environ. 24: 199–231.

Ericsson, J. E., Shirahata, H. and Patterson, C. C. 1979. Skeletal concentrations of lead in ancient Peruvians. – N. Engl. J. Med. 300: 946–951.

Grandjean, P. 1978. Lead concentrations in single hairs as a monitor of occupational lead exposure. – Intern. Arch. Occup. Environ. Health 42: 69–81.

Grandjean, P. 1975. Lead in Danes, historical and toxicological studies. – In: Griffin, T. B. and Knelson, J. H. (eds.), Environmental Quality and Safety Suppl. Vol. 2, Lead: 6–75. Academic Press, New York.

Grandjean, P., Fjerdingstad, E. and Nielsen, O. V. 1977. Lead concentration in mummified Nubian brains. – In: Proceedings of the International Conference of Heavy Metals in the Environment. Toronto, Canada, October 27–31, 1975, Vol. III: 171–179.

Grandjean, P., Nielsen, O. V. and Shapiro, I. M. 1979. Lead retention in ancient Nubian and contemporary populations. – J. Environ. Pathol. Toxicol. 2: 781–787.

Grandjean, P., Hansen, O. N. and Lyngbye, K. 1984. Analysis of lead in circumpulpal dentin of deciduous teeth. – Ann. Clin. Lab. Sci. 14: 270–275.

Hansen, J. C., Christensen, R. B., Allermand, H., Albøge, K. and Rasmussen, R. 1984. Concentrations of mercury, selenium and lead in blood samples from mothers and their newborn babies in four Greenlandic hunting districts. – Meddr Grønland, Man & Soc. 5: 1–19.

International Commission on Radiological Protection (ICRP) 1975. Report of the Task Group on Reference Man. – Pergamon, Oxford.

Ishinishi, N., Eguchi, Y. and Hisanaga, A. 1978. Comparison of lead concentration in the rib bone cortex of ancients with moderns. – Paper presented at the XIX International Congress on Occupational Health, Dubrovnik, Yugoslavia, September 25–30, 1978, Abstracts volume: 77–78.

Jaworowski, Z. 1968. Stable lead in fossil ice and bones. – Nature (Lond.) 217: 152–153.

Locheretz, W. 1973. On the lead content of human hair (1871–1971). – Science 180: 1080.

Murozumi, M., Chow, T. J. and Patterson, C. 1969. Chemical concentrations of pollutant lead aerosols, terrestrial dusts and sea salts in Greenland and Antarctic snow strata. – Geochim. Cosmochim. Acta 33: 1247–1294.

National Academy of Sciences (NAS) 1980. Lead in the human Environment. – National Academy of Sciences, Washington, D. C.

Nielsen, O. V., Grandjean, P. and Bennike, P. 1982. Chemical analyses of archaeological bone samples, evidence for high lead exposure on the Faroe Islands. – Danish Journal of Archaeology 1: 145–148.

Patterson, C. C. 1965. Contaminated and natural lead environments of man. – Arch. environ. Health 11: 344–360.

Shapiro, I. M., Grandjean, P. and Nielsen, O. V. 1980. Lead levels in bones and teeth of children in ancient Nubia, evidence of both minimal lead exposure and lead poisoning. – In: Needleman, H. L. (ed.), Low Level Lead Exposure: The Clinical Implications of Current Research: 35–41. Raven Press, New York.

Waldron, H. A. 1981. Postmortem absorption of lead by the skeleton. – Am. J. Phys. Anthrop. 55: 395–398.

Waldron, H. A. and Wells, C. 1979. Exposure to lead in ancient populations. – Trans. Stud. Coll. Physicians Phila. 1: 102–115.