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Stable Lead Isotopes in Teeth as Indicators of Past Domicile—A Potential New Tool in Forensic Science?

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ABSTRACT: A pilot study using stable lead isotope analyses has shown that permanent and deciduous teeth from Eastern and Southern European subjects have completely different lead isotopic compositions to those of Australian subjects. There are statistically significant differences between groups of teeth from subjects from the former Soviet Union (CIS), the former Yugoslavia, United Kingdom, and Lebanon. The isotopic analyses confirm the stability of lead in enamel but suggest that there is exchange of European lead with Australian lead in dentine amounting to about 1% per year. The isotopic differences in, and exchange of, European lead and Australian lead offer an exciting and powerful tool for forensic identification.

KEYWORDS: forensic science, human identification, teeth, lead isotope ratios, missing persons, country of origin

In the state of New South Wales (NSW) Australia, over 200 unidentified bodies per year are recorded by authorities. Dental records are a prime source of information for identification of information in forensic science but with the ease of travel nowadays and extensive migration during the past century, access to dental records may not always be possible or useful.

The lead isotope "fingerprinting" technique using teeth offers a potential new approach to identification of remains of missing persons because of the isotopic differences between Australian subjects and those from other countries (1).

Minimal use has been made of the lead isotope technique in the past in forensic science (2–5), probably because its main use has been in the earth and environmental sciences and more recently in the health field. A summary of past uses in forensic science is described in Andrasko et al. (5).

The lead isotope technique uses the four isotopes of lead. Three are the stable end products of radioactive decay of uranium and thorium: ^{238}U to ^{206}Pb, ^{235}U to ^{207}Pb, and ^{232}Th to ^{208}Pb. The abundance of fourth, ^{204}Pb has been essentially constant since the Earth began and this isotope is commonly used as a reference isotope. Because three isotopes of lead are produced by radioactive decay, the amounts (abundances) have changed over geological time and this is reflected in the geological source of the lead. The abundances are usually expressed as ratios so that lead from the geologically old (~1700 million years old) lead-zinc-silver deposits of Broken Hill in NSW has an abundance ratio of the ^{206}Pb isotope to the ^{204}Pb isotope ($^{206}Pb/^{204}Pb$) of 16.00 whereas the ratio is 18.1–18.3 for geologically young deposits (500–400 million years old) on the same continent or from the northern hemisphere. It is these isotopic differences which are used to evaluate the source of lead in the environment, humans, and animals. Interpretations of lead isotopic data may not be straightforward because lead in the environment or animals may be a mixture of lead from different sources (mines). Hence, lead that is introduced to the body from air/food/water is largely dependent on the source of lead in the environment, which in turn is dependent on the age, and hence isotopic composition of the rocks/ores from which the lead in the environment is derived.

Gulson and Wilson (6) and Gulson (7) demonstrated the use of the lead isotope technique, combined with the well-established histology of teeth, in evaluating *in utero* and early childhood lead exposure from slices of deciduous teeth. In this approach, analysis of the enamel provides evidence of *in utero* exposure. Analysis of dentine provides evidence of exposure during the early childhood years, when hand-to-mouth activity is usually an important contributor to lead body burden, and potentially up to the time of tooth exfoliation. In children exposed to lead sources such as from mining, paint, or petrol in communities such as the Broken Hill lead mining community, Gulson and Wilson (6) and Gulson (7) have shown from isotopic composition and lead concentration that the source of lead from the incisal and cervical sections of deciduous teeth is different, reflecting the change in lead from *in utero* exposure to early childhood.

The aims of this pilot study were: (i) to establish if any isotopic differences in teeth exist between Australian and European subjects, and (ii) to determine if there were isotopic differences within the European subjects. A companion paper reports on the isotopic changes occurring when the subjects migrate to Australia reflecting an exchange of lead sequestered in teeth with lead in the Australian environment (8).

Materials and Methods

Samples and Sample Preparation

Permanent teeth of Australian migrants were obtained from dental practitioners throughout Sydney. The subjects were mainly from Eastern and Southern Europe. The teeth studied were also assessed for their suitability in a related project: "Biokinetics of Lead in Human Pregnancy". Information regarding country and city of origin, age of subject, and residence time in Australia up until tooth extraction was requested, but not always obtained.

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Deciduous teeth were generally those from children whose mothers were enrolled in a related project and were analyzed to provide evidence for the skeletal lead isotopic signature of the mother. In one subject it was possible to analyze trabecular bone attached to the root of a permanent tooth.

Deciduous and permanent teeth were analyzed. The deciduous teeth were crowns only as the roots had been resorbed. Wherever possible, central and/or lateral incisors were analyzed to allow comparison with other studies. For upper incisors, the crowns were cut transversely into 1–2 mm thick from the incisal and cervical areas using a diamond-impregnated stainless steel disc. The incisal section consisted of enamel and varying amounts of coronal dentine.[4] The locations from which the incisal, cervical, and root sections were cut for the analyses in this paper are shown in Fig. 1 of Gulson (7). The lower incisors were also cut into 1–2 mm transverse slices. As enamel and coronal dentine are formed prior to the crawling stage of most children (10,11), they provide an indicator for *in utero* and earliest childhood lead exposure. For determinations on enamel samples, as much attached dentine as possible was removed, and vice versa. The lower central portion of the incisal section was reamed to ensure removal of any later-formed circumpulpal dentine. As much as possible of the thin veneer of enamel was cut from the cervical section using a dental bur, leaving mainly dentine. The pulpal canal in the cervical section was usually resorbed to varying degrees but to ensure minimal contribution from secondary (circumpulpal) dentine, approximately 2 mm of the pulpal canal and dentine was reamed out. The dentine in the cervical section provides an integrated exposure to lead from the time of eruption of the tooth until exfoliation (12–13).

For permanent teeth, 1–2 mm sections of the outer crown and the root sample was taken from an area approximately 1–2 mm from the root tip (Fig. 1 in Gulson (7)). In some cases, the circumpulpal dentine was reamed out to evaluate the effect of the circumpulpal dentine on lead isotopic composition and concentration.

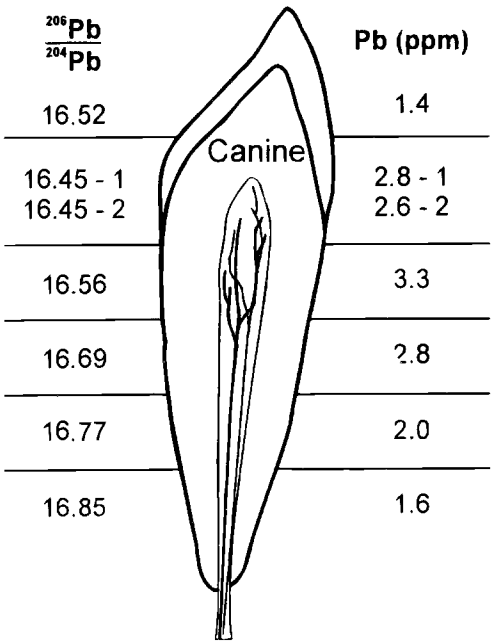

FIG. 1—*Lead isotopic and concentration results for various sections through a permanent canine from a 20 year-old Australian male subject. The two sets of results (−1, −2) in the section at the enamel-cementum junction are for two different canines from the same subject.*

[4]In this paper, the nomenclature of Shapiro et al. (9) is followed; i.e., enamel, dentine, secondary dentine (the dentine zone around the pulpal canal, also called circumpulpal dentine) and pulp.

Isotopic and Lead Concentration Analysis

Tooth slices weighing 2–60 mg were decontaminated and analyzed as described by Gulson (7). The isotope dilution method was used to analyze the lead isotope abundances and lead concentrations in the sample. This involves adding to the test sample, prior to digestion, a known amount of a 46% ^{202}Pb solution of known isotopic abundance (composition). The lead is separated from interfering elements by anion exchange chromatography.

Processing "blank" levels were <150 picograms Pb; no corrections for this "blank" have been made to the data as it is insignificant compared with the amount of lead in the analyzed sample. High precision isotope ratios were measured on a VG Isomass 54E thermal ionization mass spectrometer in fully automatic mode. The external precision of the $^{207}Pb/^{206}Pb$ isotope ratios is ±0.06% (2 sigma), based on over 1700 analyses in the CSIRO laboratories of the international NBS Lead Standards SRM 981 and 982 and natural samples. The precision for the $^{206}Pb/^{204}Pb$ ratio is ±0.1%. An estimate of the precision can also be obtained from: (i) a comparison of analyses of different teeth from the same subject (e.g., 806 in Table 1), (ii) analyses of equivalent sections of the same tooth (e.g., 2015, RUCi-1, -2), and (iii) teeth from siblings (1024 in Table 1). An analysis of SRM 981 was performed with each batch of samples. Accuracy of the measured isotope ratios in the teeth samples is by way of normalization of the ratios to those given by NBS.

Results and Discussion

Australian Teeth

The isotopic signature for Australian subjects is based mainly on deciduous teeth analyses, especially teeth for "non-exposed" children from the Broken Hill mining community. For "non-exposed" children from Broken Hill and several other adults and children from varying locations, the mean $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{206}Pb$ ratios are 16.56 and 0.9318, respectively (Table 2). These data are consistent with that obtained for the isotopic composition of lead in blood from over 200 Australian subjects (1).

From comparisons of permanent and deciduous teeth, the isotopic composition has remained remarkably uniform over more than a 30-year period, indicated by the relatively small standard deviation for the data.

Data for various slices through a single permanent canine exhibit some variation in isotopic composition and lead concentration (Fig. 1) but this is negligible compared with the differences observed between Australian and immigrant subjects.

Immigrant Teeth

Results for both deciduous and permanent teeth from immigrant subjects to Australia are listed in Table 1 and shown in Fig. 2. Only one permanent tooth from the former CIS has been analyzed so far but the data for deciduous teeth are generally quite uniform in isotopic composition and have surprisingly low lead concentrations given the purported environmental pollution in this country.

At this stage, there appears to be overall similarities in the isotopic composition of teeth from subjects from the Eastern Block countries (CIS, Bulgaria, Czechoslovakia, Poland, and former

TABLE 1—*Lead isotope and lead concentrations for teeth.*

Identifier	Tooth Type	Tooth Section	207Pb/ 206Pb	206Pb/ 204Pb	Pb (ppm)
Former CIS					
805	Uci	C	0.8746	17.77	1.3
806- 1*	Uci	I	0.8644	17.99	0.8
		C	0.8637	18.01	1.5
806- 2*	Lci	I	0.8646	18.01	0.9
		C	0.8640	18.00	1.5
816	Uci	Mi	0.8654	17.99	0.9
		I	0.8731	17.89	0.4
2015	LUci	I-1	0.8653	18.01	0.7
	RUci-1	I-2	0.8650	17.99	0.5
	RUci-2	I-2	0.8651	17.99	0.5
		C-1	0.8653	17.99	0.9
		C-2	0.8647	18.02	0.7
2023	LOCa	I	0.8647	18.01	0.5
		C	0.8651	18.01	0.8
2046	LLi	I	0.8667	18.06	0.2
		C	0.8654	18.00	0.6
803	Li	I	0.8732	17.89	0.2
		C	0.8672	18.01	0.6
1072	UCi	I	0.8637	18.00	0.3
		C-1	0.8647	17.91	0.3
		C-2	0.8633	18.03	0.3
1077	M	CR	0.8674	17.98	0.3
		RT	0.8633	18.04	2.1
1054	L1stM	CR	0.8618	18.11	0.3
		RT	0.8656	18.01	3.2
1024- 1o	LLLi	I	0.8671	17.95	0.2
		C	0.8643	18.02	0.5
1024- 20	ULMo	I	0.8698	17.93	0.6
		C	0.8615	18.11	0.8
2051	Mo	I	0.8621	18.10	0.2
		C	0.8630	18.07	0.9
Romania					
H907	M	R	0.8528	18.30	7.3
Bulgaria					
Bulgaria 1	LI	CR	0.8544	18.27	16.2
		RT	0.8594	18.13	25.4
Bulgaria 2	LI	CR	0.8555	18.20	16.9
		RT	0.8624	18.08	24.1
H916	LI	RT	0.8594	18.14	3.0
1042	LOLCa	I	0.8570	18.21	0.9
		C	0.8485	18.41	1.4
Former Czechoslovakia					
Czech 1	na	CR	0.8588	18.17	4.6
		RT	0.8691	17.92	20.0
Czech 2	na	CR	0.8670	17.94	4.1
		RT	0.8690	17.92	14.5
Poland					
Poland 1	PM	CR	0.8720	17.84	2.5
		RT	0.9091	17.02	11.3
Poland 2	CA	CR	0.8733	17.79	3.4
		RT	0.9252	16.70	63.0
Poland 3	UCi	C	0.8531	18.26	3.3
		MID	0.8535	18.27	1.2
Poland 4	UCI	I	0.8599	18.11	0.7
		C	0.8672	17.97	0.3
1043	URM	CR	0.8544	18.22	0.8
			0.8571	18.20	2.2
2052	I		0.8569	18.19	0.3
	C		0.8633	18.08	0.3
Former Yugoslavia					
Croatia 1	LOM	CR	0.8494	18.39	6.5
		RT	0.8544	18.25	17.2
Croatia 2	URM	CR	0.8494	18.45	6.5
		RT	0.8612	18.10	7.6
Croatia 3/1	UM/1	CR	0.8516	18.33	1.7
		RT	0.8546	18.26	4.1
Croatia 3/2#	UM/2	CR	0.8508	18.34	2.2
		RT	0.8539	18.28	10.7
Croatia 3/2-2		BONE	0.9014	17.19	1.8
Croatia 4	LOM	I	0.8614	18.08	1.0
		C	0.8573	18.20	2.4
Yugoslav 4/1	M/1	CR	0.8524	18.36	0.4
		RT	0.8691	17.93	5.9
Yugoslav 4/2#	M/2	CR	0.8530	18.34	0.4
		RT	0.8700	17.90	3.0
1496	LOLM	CR	0.8614	18.08	1.0
		RT	0.8573	18.20	2.4
H919		RT	0.8663	18.00	10.5
I827	PM	RT	0.8640	18.08	23.8
K312	PM	CR	0.8624	18.11	0.5
		RT	0.8527	18.36	7.0
K316	LOM	CR	0.8529	18.32	3.3
		RT	0.8677	17.97	4.5
2031-1	Li	I	0.8620	18.11	0.7
		C	0.8618	18.13	0.8
2031-2	Li	I	0.8679	18.00	0.9
		C	0.8599	18.14	0.5
Romania					
H907	M	R	0.8528	18.30	7.3
Spain					
Spain	URI	CR	0.8732	17.82	4.4
		RT	0.8736	17.82	28.4
Spain#	ULM	CR	0.8749	17.78	5.1
		RT	0.8705	17.91	18.8
Greece	UR 3rdM	CR	0.8602	18.17	5.0
		RT	0.8567	18.23	24.3
Lebanon					
Lebanon 1	PM	CR	0.8794	17.64	2.8
		RT	0.8932	17.35	8.1
Lebanon 2	LOM	CR	0.8748	17.77	1.0
		RT	0.8763	17.73	5.4
Lebanon 3	LOM	CR	0.8798	17.66	1.4
		RT	0.8837	17.59	6.1
Lebanon 4	PM	CR	0.8774	17.70	1.8
		RT	0.8882	17.45	12.6
Lebanon	LOL 2nd M	CR	0.8666	18.00	4.9
		RT	0.9215	16.81	29.0
Lebanon	UR CI	CR	0.8868	17.54	1.8
		RT	0.8771	17.72	15.1
Lebanon	UR 1st M	CR	0.8823	17.63	2.2
		RT	0.8949	17.33	7.7
Lebanon	LOL CA	CR	0.9130	16.98	1.0
		RT	0.8897	17.47	11.2
Syria/1	UL CA	CR	0.8949	17.33	2.3
		RT	0.8808	17.65	21.9
Syria/2#	UR CI	CR	0.8900	17.45	2.4
		RT	0.8840	17.57	8.2
Egypt	2nd M	CR	0.8910	17.43	2.3
		RT	0.8818	17.63	6.3
Turkey	UR 1st M	CR	0.8796	17.68	3.1
		RT	0.8673	17.99	20.9
Turkey	LO 1st M	CR	0.8647	17.99	1.0
		RT	0.8792	17.69	5.3
Uruguay	URI	CR	0.8640	18.03	6.9
		RT	0.8779	17.78	22.2
U.K.	ULI	CR	0.9067	17.12	1.7
		RT	0.9237	16.72	5.9
Italy 1	LOLI	CR	0.8591	18.21	4.2
		RT	0.8797	17.78	28.5
Italy 2	URM	CR	0.8670	18.00	1.6
		RT	0.8771	17.76	19.8

*Different teeth from same subject; o—Siblings.
Permanent teeth: CR—Crown, RT—Root, LO—Lower, U—Upper
ULI—Upper Left Incisor, URI—Upper Right Incisor.
Deciduous teeth: I—Incisal section, C—Cervical section,
M—Molar, PM—Premolar, LOM—Lower Molar,
MI—Middle section, UM—Upper Molar,
Uci—Upper central incisor, Ca—Canine, Mo—Molar,
Lli—Left lateral incisor.
NA: Information not available.

TABLE 2—*Mean and standard deviations for selected groups of immigrant teeth (enamel).*

	Australia (n = 29)	CIS (n = 14)	Yugoslavia (n = 13)	Lebanon (n = 8)	Poland (n = 6)
Mean 206Pb/204Pb	16.56	17.98	18.23	17.62	18.07
SD	0.17	0.06	0.15	0.29	0.20
Mean 207Pb/206Pb	0.9318	0.8664	0.8566	0.8825	0.8617
SD	0.0088	0.0033	0.0063	0.0136	0.0088
Mean Pb (ppm)	2.0	0.5	2.0	2.1	1.5
SD	1.4	0.3	2.2	1.3	1.2

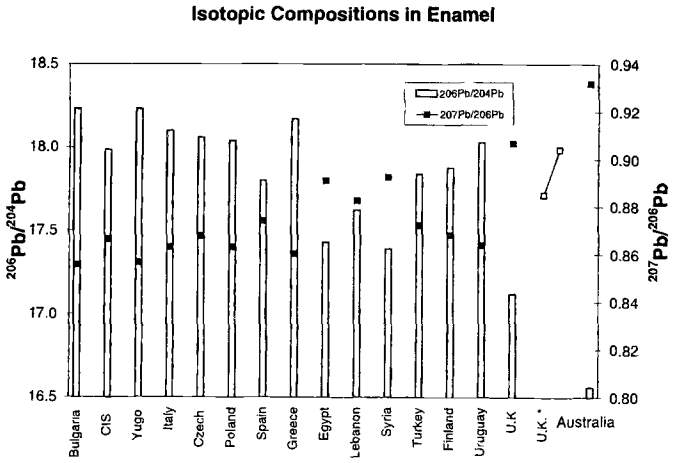

FIG. 2—*Diagrams illustrating variation in $^{207}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ ratios for enamel from deciduous teeth (D) and permanent teeth for subjects from several European countries. The data for Finland are 7 bone samples measured by Keinonen ((15)1989). These data contrast with the range measured for Australian teeth and blood lead values. The $^{207}Pb/^{206}Pb$ data for the U.K.* are for 237 deciduous teeth measured by ICP-MS (Alexander et al. (14)1993).*

Yugoslavia). Nevertheless, there are statistically significant differences in data for the former Yugoslavia and CIS (Table 3). There may be similarities for teeth of subjects from the Mediterranean countries of Egypt, Lebanon, and Syria but which, as a group, are significantly different from teeth from the Eastern Block countries. There are statistically significant differences in data for the former Yugoslavia and Lebanon (Table 3). The single analyses from Greece and Turkey (and Chile) are different from the other Mediterranean countries. The data for the U.K. are totally different from those of the other European countries but with closer affinities to the Australian teeth. The similarity of Australian and U.K. teeth is probably because Australian lead is the dominant component in the manufacture of alkyl lead in the U.K.

Some of the variability for teeth within a given country appears related to the age of the subject. For example, the permanent teeth for two Polish subjects have a lower $^{206}Pb/^{204}Pb$ than that for deciduous teeth. The significance of this is that there has been a change in the source of environmental lead from one generation to the next, a not unexpected phenomenon given internationalization of trade and hence potential changes in sources of lead.

TABLE 3—*Test of variance for selected groups of immigrant teeth.*

Group	DF	p-value 207Pb/206Pb	p-value 206Pb/204Pb	p-value Pb (ppm)
Yugoslavia/CIS	42	7 × e-6	4 × e-7	0.006
Yugoslavia/Lebanon	19	1 × e-5	4 × e-6	0.85

Other Data

Limited isotopic data are available from other studies. Alexander et al. (14) measured the lead isotopic compositions in 237 whole deciduous teeth from the U.K. by the Inductively Coupled Plasma Mass Spectrometer Method. This method has an order of magnitude lower accuracy and precision than the method used in our paper and only data for the $^{207}Pb/^{206}Pb$ ratio are available. The range in the U.K. teeth are plotted in Fig. 2. Other data which allow expansion of the data base are high precision isotopic measurements on bones from Finland (15); these data are also plotted in Fig. 2.

Lead Exchange and Residence Time in Australia

The differences in isotopic composition, and lower lead concentrations in enamel compared with dentine, are consistent with the hypothesis that no systemic absorption occurs in enamel after ameloblast degeneration. The small isotopic differences observed in incisal versus cervical sections of some deciduous teeth probably reflect changes in the source of environmental lead compared with maternal lead. Nevertheless, the uniformity of, for example, CIS incisal (enamel) data, which is a reflection of the source of maternal lead, provides a good indication of the environmental lead prevailing at about the time of pregnancy. As the children are of different ages and from different parts of the CIS, the teeth isotopic data provide evidence for the uniformity of the source of environmental lead, potentially over decades. A similar uniformity in isotopic composition over decades is observed in Australian teeth.

In a companion paper, it was shown that there was a positive correlation between the change in isotopic composition between enamel and dentine and residence time in Australia providing evidence for ongoing exchange of lead in dentine and that in blood, probably via the dentinal tubules. The rate of exchange between European and Australian lead in dentine was approximately 1% per year, similar to the suggested rate of turnover of lead in cortical bone (16).

In contrast there was almost complete isotopic exchange in socket bone (trabecular bone) lead with Australian lead for one subject over a 15-year period, consistent with data for turnover of lead in trabecular bone estimated from measurements on tibia/calcaneus/finger by the K-XRF method and blood lead analyses on occupationally-exposed subjects (17–21) and stable lead isotope analyses (22).

Limitations of the Pilot Study

There are several limitations to the present pilot study which include: (i) The lack of data for all countries and/or regions. (ii) The potential dilution of the statistical significance of the isotope ratios over time due to the exchange of lead in dentine as discussed above. This is not a problem, however, if enamel is analyzed. and (iii) A comparison with regional anthropological data (race differences).

Conclusions

The results of this pilot investigation of teeth support blood lead data showing that subjects from some European countries have

sources of lead that are totally different from long-term Australian residents. There are statistically significant differences between some countries such as the Eastern Block countries and for example, Lebanon and the U.K. A systematic study should determine whether it is possible to discriminate teeth from other countries. Nevertheless, even at this stage, the lead isotope data could provide evidence of a non-Australian origin for unidentified persons.

No teeth data have been measured for subjects of Asiatic origin but blood (unpublished data) and air lead analyses (23) from the south east Asian region show significant isotopic differences between, for example, subjects from China and Hong Kong and subjects from the Philippines and Thailand. Nevertheless, there is a significant isotopic difference between these countries and Australian subjects.

The difference in rate of exchange of lead in enamel, dentine, and jawbone (and also blood), if confirmed from other analyses, could permit not only identification of the country of origin but also an estimate of the residence time of the subject in Australia.

The results of this investigation confirm earlier investigations in these laboratories which demonstrated the importance of analyzing various sections of teeth rather than whole teeth. The latter procedure may camouflage important exposure information.

Acknowledgments

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