Analysis of Lead in Circumpulpal Dentin of Deciduous Teeth*

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ABSTRACT

Absorbed lead continuously adds to the lead deposited in the vascularized circumpulpal dentin of the teeth. Thus, this dental tissue is expected to contain a lead concentration which reflects the integrated lead exposure during the time from completion of tooth formation to tooth extraction or shedding. A method has been developed to assess the lead level in the dentin surrounding the pulp chamber in deciduous teeth. Variation within the tooth is minimal, but upper medial incisors show a slightly lower lead level than do other incisors, as assessed in 714 teeth from first-grade Danish school children. This tendency was not confirmed, however, in a small number of paired teeth from the same children. The new method appears advantageous for epidemiological studies of lead neurotoxicity in children.

Introduction

Determination of lead concentrations in shed deciduous teeth has become a useful measure of past lead exposures in children. This metal accumulates in calcified tissues owing to the formation of sparingly soluble lead phosphate. The development of deciduous teeth starts already in prenatal life, and the average lead level in the shed teeth has been used as an integrated measure of the total exposure during early life. Lead levels may vary, however, between different tooth tissues and between different tooth types. Thus, the highest lead concentrations are found in the inner and outer surfaces of the tooth, i.e., the outer layer of the enamel and the circumpulpal dentin.1,6 The circumpulpal dentin, which is formed after the tooth formation has been completed, continues to accumulate lead until the tooth is shed.6,11 Primary dentin contains much lower lead concentrations. In addition to variations within individual teeth, the lead concentration in whole teeth (or crowns) tends to decrease from the medial incisors to the premolars.2,8 A more extensive report

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recently concluded that the largest difference between incisor lead levels was found when comparing upper medial incisors to lower lateral incisors. However, such variations have not been found in less extensive studies of circumpulpal dentin levels. The possibility exists that the variation related to tooth type could be partly due to a differential proportion of dental tissues in the different tooth types.

Three different methods have been used previously: (1) whole tooth or crown, (2) a 300 μm fragment of dentin from a 600 μm thick section of the tooth, and (3) circumpulpal dentin obtained from the root canal with a reamer. Each of these methods may suffer from drawbacks with regard to speed of analysis, inter-tooth variability or biological interpretation. Circumpulpal dentin attracts particular interest, because only this tissue has a blood supply and, in principle, accumulates lead shortly after the time of tooth eruption and until the tooth is shed (or extracted). Owing to root resorption prior to the shedding of deciduous teeth, little circumpulpal dentin remains apart from the tissue lining the pulp chamber. A method has therefore been developed to prepare secondary dentin from the pulp chamber of shed deciduous teeth.

Materials and Methods

Deciduous teeth are currently being collected from children in first-grade classes of schools in four municipalities in Denmark. The 714 teeth used in the present study all originated from six to seven-year-old children from the city of Aarhus. This epidemiological study is coordinated by ONH; all teeth were examined and classified by KL, and analyses were performed at Odense University, Denmark. Only teeth without fillings were used. Some teeth could be split into two parts of similar size by use of a pointed or sharp instrument (preferably a mandrel). Otherwise, a small groove is cut vertically in the midline of the anterior and posterior surfaces by means of a thin diamond grinder. The tooth is then placed in a special vise which splits the tooth into two halves (figure 1). Soft tissue residues are then removed from the pulp chamber by means of a dental explorer, followed by a one-minute immersion in about one ml of 10 percent hydrogen peroxide. Remaining surface contamination is removed by subsequent washing of the tooth fragment for 20 seconds in an ultrasonic bath with concentrated ethanol followed by drying. Clean laboratory techniques are used throughout. The dentin bordering the pulp chamber is removed by a small wofram carbide rosette burr. About one percent of the tooth weight, i.e., an average of about 0.65 mg, is sampled in this way. The powdered dentin is weighed directly into a sampling cup, and 100 μl of concentrated nitric acid are added for overnight dissolution. The following day, 400 μl of redistilled water are added, and the solution is analyzed in two independent runs by electrothermal atomic absorption spectrometry. Small amounts of dentin cause no discernible matrix effect; nitric acid is added to all standard solutions. The temperature program for a 20 μl sample is: drying at 100°C for 25 seconds,

* Borer rund 008, Hager & Meisinger GMBH, D-4000 Düsseldorf 1, Federal Republic of Germany.
† Perkin-Elmer model 305A with an HGA-76 graphite atomizer, autosampler AS-1, deuterium background corrector and recorder.
ashing at an increasing (rate 2) temperature up to 700°C, atomization for three seconds with gas stop at 2100°C followed by three seconds at maximum temperature. By the technique described, an average of about 20 teeth can be analyzed per day.

Three different solid "reference materials" were used. A sample of powdered tooth was kindly provided; this sample consisted mainly of particles of 0.1 to 0.5 mm diameter. The IAEA standard bone material,§ which has a certified lead concentration for aliquots of at least 100 mg, was also used. Inhomogeneity of these materials could cause some analytical variability when using aliquots of about 1 mg. Finally, commercial hydroxyapatite was used as a low-lead-level, dentin-like reference material.

Results

Although day-to-day variations in lead levels of standard solutions were minimal, larger variations occurred in the solid materials (table I). For the bone and tooth materials, some of the variation may be due to inhomogeneity of the samples.

The reproducibility of the method was assessed by determining the lead level of circumpulpal dentin prepared independently from both halves of each of twelve deciduous teeth. The average lead concentrations varied between 0.54 and 16.6 μg per g, and individual values for half-teeth differed by an average of 1.2 μg per g from the tooth mean, i.e., an average of 10.7 percent, only one value being above 20 percent. Additional studies concerned five teeth where three subsequent samplings of about one mg from each tooth were made in order to penetrate into deeper dentin layers. The data in table II indicate that lead concentrations decrease with subsequent samplings into the deeper dentin tissue. Thus, a homogeneous sample of circumpulpal dentin must be as small as allowed by the sensitivity of the detection method. A sample of 0.5 mg seems sufficient, because it will result in 0.02 ng of lead in the 20 μl of solution injected into the graphite tube, if the dentin lead level is 1 μg per g.

The total of 714 shed deciduous teeth represents roughly one-half of the teeth collected in Aarhus. A comparison of lead levels in different incisors is shown in table III. Although the lead levels in each of the tooth types varied widely (average coefficient of variation, 56 percent), each group of incisors showed almost the same average lead level. However, upper medial incisors showed lead levels which were lower than those of

| TABLE I
| Day-to Day Variation of Micro Lead Analysis (μg/g) of "Reference Materials"
<table>
<thead>
<tr>
<th>Material</th>
<th>Number of Analyses</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA bone*</td>
<td>41</td>
<td>3.09 ± 0.41</td>
</tr>
<tr>
<td>Tooth powder†</td>
<td>5</td>
<td>9.08 ± 2.87</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>47</td>
<td>0.41 ± 0.11</td>
</tr>
</tbody>
</table>

*Certified lead level, 3.1 ± 0.28 μg/g; variation of "accepted" levels in interlaboratory comparison, 3.0 ± 1.08 μg/g
†Mean of 13 laboratories, 8.1 μg/g; range, 4.3 – 15.5 μg/g (reference 10)

| TABLE II
| Lead Levels (μg/g) in Subsequent Layers of Circumpulpal Dentin of Five Deciduous Teeth
<table>
<thead>
<tr>
<th>First</th>
<th>Second</th>
<th>Third</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>4.3</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>5.2</td>
<td>3.1</td>
<td>2.9</td>
</tr>
<tr>
<td>6.3</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>9.6</td>
<td>7.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

x = 5.7 x = 3.2 x = 2.5

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Dr. M. Stack, MRC Dental Unit, Bristol, U.K.
§ Reference Material H-5, IAEA, P.O. Box 100, 1400 Vienna, Austria.
| Bio-Rad Laboratories, Richmond, CA 94804.
upper lateral incisors ($t = 4.44; p < 0.001$) and those of lower medial incisors ($t = 3.24; p < 0.005$). Within the same tooth types, right and left side teeth showed no statistically significant differences.

The differences related to tooth type were further studied in several pairs of teeth which originated from the same children. Unfortunately, most of these pairs are right-left pairs of the same tooth type, because these teeth tend to be shed at about the same time. The results from 26 such pairs are available for examination at this time. One pair of lateral incisors showed 29.1 $\mu$g per g in one tooth and 9.2 $\mu$g per g in the other. Otherwise, the mean difference from the pair average was 14.1 percent of the pair average for 11 medial incisors and 11.7 percent for 14 lateral incisors (14.5 percent when the aberrant pair is included). These variations are only slightly larger than the variations found within individual teeth. In addition to the right-left pairs, six pairs of upper medial-lateral incisors were available. In two of these pairs, the medial incisor was higher in lead than was the lateral incisor from the same child, and the numerical difference from the pair mean averaged 14.3 percent of the pair means.

**Discussion**

Much emphasis has been placed on the use of lead deposition in teeth as an indicator of integrated lead exposure during the early years of life when the nervous system is particularly susceptible to the toxic effects of lead. Unfortunately, no uniform approach to tooth analysis has appeared. Several authors have recommended the analysis of whole tooth for lead:2,3,5,9 the procedure is simple, contamination during analysis is unlikely, and sufficient material is available to obtain results of good precision. However, although most shed deciduous teeth only represent the crown, some may occasionally contain root parts, especially if the tooth has been extracted or if it has been shed at an angle. Also, interlaboratory variation is quite large.10 Moreover, most of the deciduous tooth tissue is formed during a relatively short period in early life, and the average lead level in the tooth may not in any accurate way reflect the lead exposure of the body during the life-time of the child. Based on these considerations, circumpulpal dentin would appear to be a superior tissue to analyze, if the problems of small aliquots and risk of contamination can be overcome.

Our method is quite similar to the "reaming" method described by Shapiro et al ten years ago.6 Although the depth of penetration with the rosette burr is difficult to standardize, the results in table II suggest that little variation will be seen when aliquot sizes are kept below one mg. The reproducibility of the method is further indicated by the small differences found when comparing lead levels in two halves of the same tooth or in two teeth from the same child.

Quality control procedures are particularly difficult when working with solid materials with nonhomogeneous distribution of the element assessed. The results obtained when analyzing the IAEA* powdered bone material show the expected average, but the coefficient of variation of 13 percent for our microanalysis is higher than the certified nine percent for aliquots of at least 100 mg.

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**TABLE III**

<table>
<thead>
<tr>
<th>Tooth Type</th>
<th>Number</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial, upper right</td>
<td>100</td>
<td>8.6</td>
</tr>
<tr>
<td>left</td>
<td>97</td>
<td>9.5</td>
</tr>
<tr>
<td>lower right</td>
<td>55</td>
<td>12.5</td>
</tr>
<tr>
<td>left</td>
<td>59</td>
<td>10.6</td>
</tr>
<tr>
<td>Lateral, upper right</td>
<td>113</td>
<td>11.2</td>
</tr>
<tr>
<td>left</td>
<td>102</td>
<td>12.5</td>
</tr>
<tr>
<td>lower right</td>
<td>89</td>
<td>11.7</td>
</tr>
<tr>
<td>left</td>
<td>99</td>
<td>11.8</td>
</tr>
</tbody>
</table>

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*IAEA: International Atomic Energy Agency*
However, it is much below the coefficient of variation of 36 percent found in the IAEA interlaboratory comparison study. Similarly, only a few grains of the relatively coarse tooth powder used in a comparison study could be analyzed by our new method, and a considerable variation was found by us, although the average was as expected. The best material for the purpose of internal quality control is probably commercial hydroxyapatite, usually employed for column chromatography, especially if an additional product with a higher lead concentration can be obtained.

The question regarding lead levels related to tooth type may not be completely solved when using circumpulpal dentin for analysis. Thus, the data in table III suggest that upper medial incisors contain less lead than do other incisors. However, this tendency remains to be assessed in paired teeth from the same individuals. A previous study of one whole set of teeth from an adult individual failed to indicate any tooth-type relationship in circumpulpal dentin.

On the contrary, recent studies of whole teeth (crowns) have suggested that upper medial incisors contain more lead than do other incisors. Thus, additional information on the possible variations in circumpulpal dentin would be needed to allow a more definite judgment.

The diagnostic validity of results obtained by the present method is difficult to assess. However, increased lead levels in circumpulpal dentin are no doubt related to increased lead concentrations in the circulating blood. We have had the occasion to analyze an incisor from a seven-year-old boy who has been exposed to high lead levels. The blood lead concentration has been about 50 μg per 100 ml on four determinations during the previous two years but had decreased to 31 μg per 100 ml at the time when the incisor was shed. The circumpulpal dentin showed a lead level of 314 μg per g, about 30-fold above the average of other children and much higher than any result ever seen in circumpulpal dentin in our laboratory. Although the detailed exposure history of this child is unknown, this case may indicate that lead analysis of circumpulpal dentin as described is indeed a sensitive indicator of past lead exposures.

Acknowledgments

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References

