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Blood Lead—Tooth Lead Relationship Among Boston Children

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The amount of lead in deciduous teeth has been used extensively as a marker for infant lead exposure and body burden (Needlman et al. 1972). Elevated tooth lead levels have been seen in children who had lead poisoning (Brudervold et al. 1977). Also, on a population wide basis tooth lead levels appear to vary according to housing status and presumably lead exposure (Ewers et al. 1982 and Gilberg et al. 1982). This exposure index has been applied using varying techniques in Denmark (Grandjean et al. 1984), South Africa (Grobler et al. 1985), and the United Kingdom (Mackie et al. 1977).

Because of the neurotoxicity of lead, the tooth lead levels of retarded and normal children have been compared (Pinchin et al. 1978). Most recently, in research of lead and child development, tooth lead levels have been used as markers of past lead exposure (Needleman et al. 1979). Despite the widespread use of tooth lead values, very little is known about the exact time course of lead deposition in tooth from blood. This report compares blood lead levels at different ages to tooth lead levels in a group of Boston children.

MATERIALS AND METHODS

The base population consisted of 11,837 consecutive births at the Boston Hospital for Women between April 1979 and April 1981. Details of the population and blood sampling have been published (Rabinowitz et al. 1984). Briefly, babies were eligible for inclusion in this follow up study if their umbilical cord blood lead levels were in the highest, middle or lowest decile. The participating mothers had a mean age of 29 years and were well educated (e.g. 15 years mean schooling). In general the 249 enrolled children represent healthy products of uneventful pregnancies and live in areas that placed them at relatively low risk for lead poisoning.

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The annual attrition rate was less than 10%; a total of 202 children completed the 2 year follow-up. Both blood and tooth were available from 102 children. In addition to umbilical cord blood, capillary blood was sampled at 6,12,18, and 24 months and venous blood at 57 months of age. These specimens were analyzed in duplicate or triplicate with a model 3010A anodic stripping voltammeter (ESA, Bedford, MA). The average difference between the duplicates was 1 ug/dl and exceeded 3.5 in only 10% of the pairs.

Shed primary teeth of the study children were requested from the parents by mail. Teeth were returned by mail, wrapped in plastic padding, inside a cardboard box in a reinforced envelope. Parents supplied information about the tooth's location in the mouth and the date the tooth was shed by indications on a reply card supplied to them. After receipt, the teeth's outer surfaces were usually brushed by hand with pumice powder that had been pre-cleaned with warm EDTA. This was done to enable better visual detection of enamel defects and was unrelated to the dentin lead assay.

All subsequent steps were completed in a room with controlled air flow which was continually recirculated by two HEPA air filters (model 100 Plus, Environmental Air Control, Albuquerque NM). This yielded laboratory air containing 1 ng per cubic M of lead, versus Woods Hole outdoor air of 4 and Boston laboratory air of 10 to 50 at that time. Inside the sample digestion box which was fitted with an additional HEPA filter, the air lead level were 0.03 ng per cu M. The entrance to the room was fitted with a sticky adhesive mat for dust control.

The tooth lead was determined in two portions of dentin taken from the zone presumably representative of post-natal deposition. Cross-sectional slabs of tooth material (FIGURE 1) were obtained by sagittally slicing the tooth with a low-speed Buehler saw fitted with two diamond impregnated blades separated with a thin spacer. Water was the only cutting fluid. Because of the low relative humidity in the laboratory no special efforts were made to dry the samples. This slab was covered with a sheet of Parafilm and placed on an anvil. A chisel and mallet were used to isolate different portions of the slab for chemical analysis; the sampled sites within each tooth were located visually. A cut was made from just below the cementum-enamel junction, which was visible in the silhouette of the tooth, to a point midway between the top of the pulp cavity and the crown. For incisors this cut approximates the neonatal line (Schour 1936). A midline cut was also made. For deciduous teeth with the normal amount of root resorption, this yielded two specimens of 10-15 mg each from the post-natal dentin.

These portions were digested in a Pyrex tube with 250 uL of re-distilled nitric acid (GF Smith). Blank tubes and tubes with a calcium-based standard accompanied each batch. These were heated in the HEPA filtered air chamber. After refluxing and dissolution, they were heated further to reduce the volume to near dryness (less than 30 uL). Just prior to analysis, the samples were

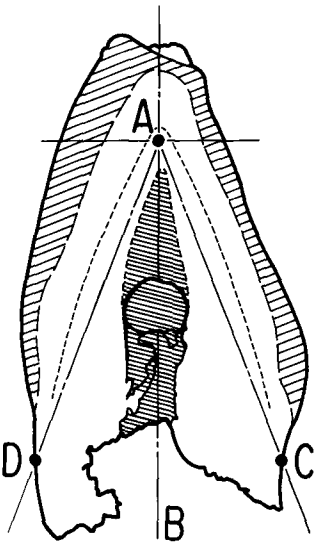

Figure 1: Tabular sagittal cross section of typical deciduous incisor (about 10 X magnification). Note crown wear and partially resorbed root. Hatched areas indicate enamel and the pulp chamber; dashed line is approximate location of the neonatal line. Samples were obtained from the postnatal zones between DAB and BAC.

redissolved in place with 2.5 mL of 1.0 M sodium acetate, 0.2 M chloride and perchloric acid buffer, pH to 5.8. This buffer was made nearly lead-free by batch electroplating for as many as 50 hours at 1.2 volts using a model 3010A anodic stripping voltameter (ESA, Bedford MA) with a mercury coated graphite electrode. Initial plating was at 1.25 volts for 5 minutes, the sweep rate was 15.00 mv per step, and final potential was 220 mv. Although a strip charter recorder was used to monitor the machine performance, the actual lead evaluation was done with the peak integrator. These methods have several advantages.

First, using a fast stripping rate and peak integrator provides large and sharp current surge whose measurement is unaffected by slight lead peak asymmetries, which would cause inaccuracies if peak height were used instead. Second, our choice of buffer allows for the unavoidable variations in the residual amounts of acid left in the test tubes, which would affect the location of the lead peak. Third, any copper peak is further from the lead peak in this matrix, thus minimizing interferences expected with a chromium chloride based matrix.

The amount of lead represented by each peak was calculated from the best-fit straight line fit to at least 5 aqueous lead standards run among the samples on the same day. The average r-squared associated with this line was 0.990 (std dev = .011). Procedural lead blanks averaged 3.0 ng (std dev = 2.3) per sample and represent about 6% of the lead present. An internal standard of lead-enriched calcium chloride was prepared to monitor the long-term behavior of the system. The concentration of lead in this solution and the aqueous lead standards was determined by Professor William Manton of the University of Texas using isotope dilution mass spectrometry. The observed mean value of lead in this standard, which accompanied each of the 215 batches of teeth, which included those reported here, was 5.48 (std dev = 0.38) ug/g , with a range of 4.6 to 6.3. The 95 % confidence interval was 5.44 to 5.54 ug/g. The value obtained by isotope dilution, an absolute reference method, was 5.52 (std dev =0.04). The standard did not exhibit any time trend. The correlation of the observed values with time was not significant, r=-.05, p=.48.

We obtained two lead concentrations for each tooth. These values were averaged if they differed by 2.5 ug/g or less. Otherwise, two additional portions of the tooth were prepared, and the three closest values for the tooth averaged. This was necessary for fewer than 15 % of the teeth. When multiple teeth were submitted for a child, we considered only the first tooth received for most of the analyses reported here. 95 of the 102 teeth received were primary incisors; for 5 the location was uncertain.

RESULTS AND DISCUSSION

The mean lead level in these 85 primary incisors was 2.8 ug/g (std dev = 1.8, range 0.3 to 12.8). The average lead level of central incisors did not differ from that of lateral incisors (2.8, std dev = 1.8 vs 2.6, std dev = 1.4). Similarly, maxillary incisors did not differ from mandibular incisors (3.2, std dev = 2.3 vs 2.5, std dev = 1.1). For the smaller number of children who supplied multiple teeth, paired analysis also showed no difference between types of incisor.

Figure 2 consists of six scatter plots of the dentin lead and blood lead values for various ages between birth and 57 months. As displayed in Table 1, the association between tooth lead and blood lead increases with age, first achieving statistical significance at age 18 months. By 57 months the correlation coefficient is 0.56. This is similar to the value of 0.47 found between current blood lead and incisors among 302 German children (Ewers et al. 1982).

Although calcification never totally stops, in the sampled part of the deciduous incisor calcification is complete by the end of the first year of age and of the entire tooth before the end of the second year (Schour and Massler 1941). These findings imply that this dentin continues to accumulate the lead present in blood at older ages. Although about 80 percent of the dentin specimen assayed was calcified prior to age 18 months, less than 10 percent of the explained tooth lead variance is accounted for by blood lead

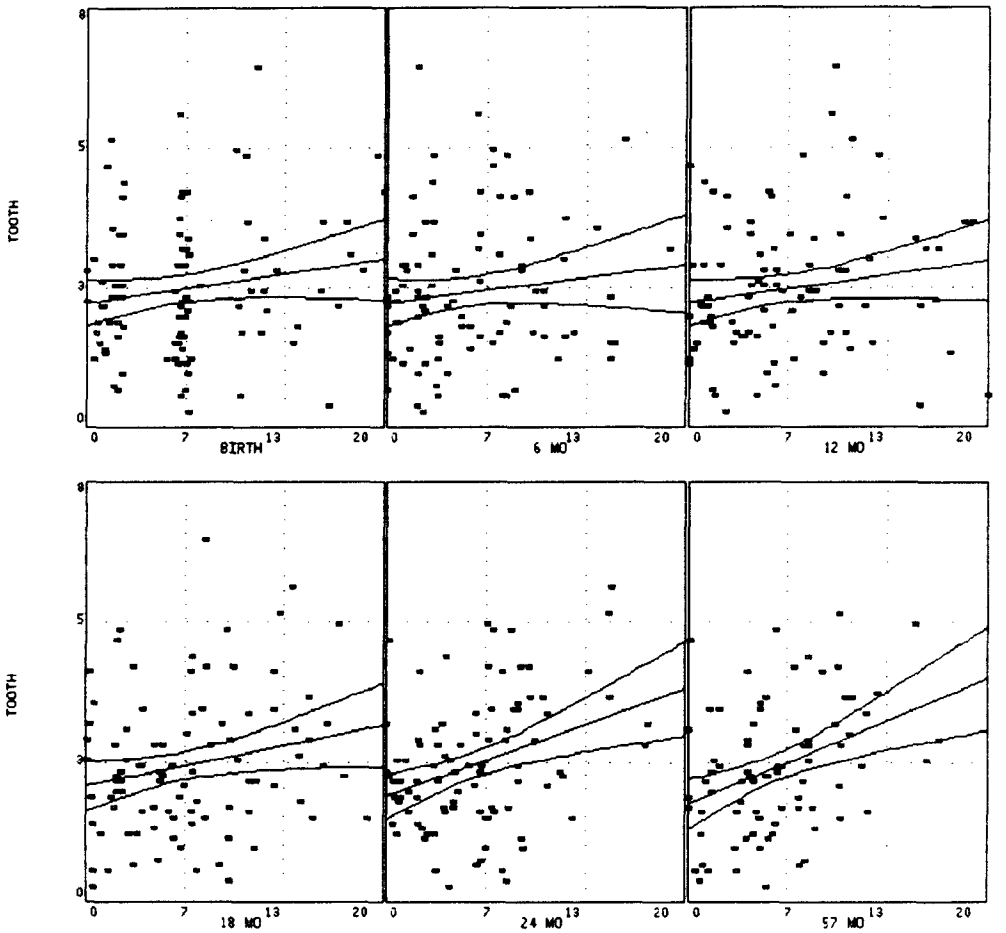

Figure 2: Scatter plots and best linear fits of tooth lead and blood lead at six ages, birth through 57 months. Dentin lead averaged 2.8 ug/g, blood lead about 68 ug/L. Note the increase of slope with age.

measurements at or before 18 months. Any lead deposited earlier can be lost through exchange. If the dentin did accumulate lead uniformly with time, then the regression intercept would increase with age. Because the intercepts do not increase with age, we infer that dentin does not retain deposited lead uniformly over time during the early years of development (Table 1). Additional support for this view is provided by the observation that averages of the blood lead at various combinations of ages did not correlate better with the tooth lead than did the blood at the last age only.

As young dentin matures, the microtubule structure changes and becomes less permeable (Thomas 1985). Even fully mature dentin is somewhat permeable to fluids (Pashley et al. 1984). Also, zinc and strontium diffuse through dentin, but on a sub-millimeter scale (Ashrafi et al. 1983 and Stazen et al. 1977). Thus, it is not surprising that the dentin appears able to lose lead for as much as a year after initial calcification is complete, only later becoming a more sealed repository. In contrast, permanent teeth appear to accumulate lead over many decades (Steehout and Portois 1981).

Table 1: Pearson Correlation Coefficients (r) and Bi-variate Regression Slopes (β) and Intercepts (Int) of Shed Insicors and Blood at Various Ages.

Age	N	r	β	S.E.	p	Int	S.E.
Birth	102	.23	.07	.03	.03	2.3	.3
6 mo	97	.14	.04	.03	.2	2.5	.3
12	99	.11	.04	.03	.2	2.5	.3
18	102	.23	.08	.03	.01	2.2	.3
24	101	.48	.15	.03	.0001	1.8	.2
57	88	.56	.21	.03	.0001	1.5	.3
Average	102	.19	.08	.03	.02	2.1	.2

Table 2. Correlation of Blood Lead at 57 months with Earlier Blood Lead Levels and with the Dentin Lead Measurement.

Sample	r	
Blood Lead		
Birth	.09	
6 mo	.14	
12	.24	
18	.38	*
24	.42	**
Dentin Lead	.56	**

* p <0.01 ** p <0.0001

Although individual infant blood lead levels fluctuate over time in this population, variability seems to decrease with increasing age (Table 2) (Rabinowitz et al. 1984). Much of the variance in 24 month blood lead is shared with the 57 month value. Thus, the apparent correlation of tooth lead with the 24 month blood value may be a result of the intercorrelations among these blood lead measures.

In this population mean blood lead levels did not change substantially with age. However, in populations where children receive the preponderance of their total lead exposure during a narrow time interval, the patterns of correlation between blood lead levels at particular ages and tooth lead levels might be different.

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