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In Vivo Bone Lead Measurements: A Rapid Monitoring Method for Cumulative Lead Exposure

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Lead concentrations (μg/g wet weight) in human bone (tibia) were measured noninvasively in vivo employing an X-ray fluorescence technique. Forty-five workers who had been subjected to chronic industrial exposure were found to have a mean bone lead content of 52.9 μg/g wet weight (0 to 198 μg/g). In addition to bone lead content, blood lead, body burden of lead as assessed by urinary lead excretion after EDTA chelation, zinc protoporphyrin, and unstimulated urinary lead excretion were evaluated. The results suggest that the in vivo measurement of tibia lead content may serve as an acceptable indicator of body lead burden and provide a practical technique for lead screening purposes. The correlation coefficient between X-ray fluorescence findings and lead excretion following Ca-EDTA administration is 0.69; $p < 0.001$.

Key words: lead, in vivo, X-ray fluorescence, bone

INTRODUCTION

Short- and long-term toxic effects of exposure to lead have been observed for many years [Waldron and Stofen, 1974; Goyer and Rhine, 1973]. Although the group at major risk are occupationally exposed workers, the general population, to a lesser degree, is also exposed to lead. Children constitute the most susceptible exposure group in the general population since neurotoxic effects of lead may bring about permanent damage to the developing central nervous system [Waldron and Stofen, 1974; Lin-Fu, 1982; Needleman and Landrigan, 1981].

Almost all criteria for the detection and diagnosis of lead exposure rely heavily, if not exclusively, on a determination of the lead in blood [Joselow and Carnow, 1976]. Such blood measurements, together with unstimulated urine lead measurements, reflect recent lead absorption, and are known to be unreliable as indicators of long term exposure [Vitale et al, 1975]. Recently, zinc protoporphyrin (ZPP) has been introduced as an indicator of chronic lead intoxication [Eisinger et al, 1978], but

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a definitive measurement of the so-called "body burden" of lead can be established only with a lead chelator, ethylenediaminetetraacetic acid (EDTA). This provocative test was introduced in the early 1960s and involves measurement of urinary lead excretion 24 to 72 h after intravenous infusion of calcium-EDTA [Emmerson and Thiele, 1960; Emmerson, 1973]. This test is more sensitive than standard blood tests and reflects to some degree the lead accumulation in the body. However, as it requires hospitalization for up to three days and administration of a drug with potentially adverse side effects [Meltzer et al, 1961], it is cumbersome and impractical as a screening test and is usually reserved for subjects already identified as having elevated blood lead levels.

It has been estimated that about 90% of total body lead in adults accumulates in the skeleton [Schroeder and Tipton, 1968]. Furthermore, the turnover time of the lead in the skeleton is approximately 20 years [Rabinowitz et al, 1976]. It is desirable, therefore, in estimating the total body burden of lead, to measure lead directly in the largest and, more important, the longest lived compartment, namely, the skeleton. Skeletal lead levels have been studied in autopsy materials [Schroeder and Tipton, 1968; Grandjean and Holma, 1973; Barry, 1975; Lindh et al, 1978] and in biopsy samples [Westerman et al, 1965]. Noninvasive measurements of lead in teeth and in bone in vivo have been performed employing the X-ray fluorescence method [Needleman et al, 1974; Ahlgren et al, 1976, 1980; Christoffersson et al, 1984; Wielopolski et al, 1981, 1983a, 1983b].

METHOD AND SUBJECTS

The X-ray fluorescence (XRF) technique is brief and noninvasive, and carries low risk (localized skin dose of ~ 1 rad to an area of 1 cm^2). It is based on the specific atomic property of lead to emit characteristic X-rays upon stimulation induced by external irradiation. The stimulated radiation is monitored externally by a solid state detector and can be expressed in terms of the lead concentration in the bone. In previous studies [Ahlgren et al, 1976], a ^{57}Co source was used to induce K X-rays from lead. At these energies (74.957 and 84.922 keV), the lead is sampled throughout the bone volume. In the present work, a ^{109}Cd source was used to induce L X-rays [Wielopolski et al, 1981, 1983]. At these energies (10.549 and 12.611 keV), only lead in the surface layer of the bone can be sampled. One mean free path for 12.611 keV X-rays in the bone is about 0.5 mm, where, for K X-rays, it is about 25 mm. Since the emitted radiation is considerably attenuated by the overlying tissue, the tibial shaft has been chosen as the measurement site because of its thin and fairly constant (3–6 mm) overlying skin thickness as determined using a 7-MHz ultrasound unit. The measurements are corrected for the overlying tissue by normalizing the results to a constant 3mm tissue thickness. Calibration of the system was determined by correlating X-ray intensities obtained from cadaver's leg and subsequent atomic absorption analysis of a bone specimen from the same site (Wielopolski et al, 1983). In the present work the minimum detection limit, defined as three times the square root of the background, is about 20 $\mu g/g$ wet bone.

The XRF technique was used to measure the lead content in the bone of 45 male workers. These workers were engaged in either the production of a leaded glass product which is then converted to porcelain (Coating Division), or the manufacture of paint pigments (Color Division). A NIOSH evaluation of the plant [Landrigan et

al, 1982] showed increased air exposure to lead in the Coating Division and to cadmium in the Color Division. Complete clinical evaluations of 37 of the workers were carried out on an in-patient basis over a 3-day period. These evaluations (performed 2–9 months before the XRF measurements) included work histories, physical examinations, standard blood and urine chemistries, blood lead levels and 24-h urine lead excretion before and after the intravenous (35 workers) or intramuscular (two workers) administration of 1 g of Ca-EDTA. The results of these studies will be described in detail elsewhere. At the time of the XRF measurements, blood samples were also obtained and analyzed for lead and ZPP. Urine samples were also examined and analyzed for lead.

RESULTS

Routine clinical chemistry tests did not indicate any significant abnormalities in the population tested. The range of values for the various lead exposure indices is summarized in Table I.

A correlation coefficient derived from a regression line, $r = 0.69$ ($p < 0.001$), exists between bone lead concentrations and the cumulative total urine lead output for 48 h following the administration of Ca-EDTA. The data are presented in Figure 1. The upper limit of normal for lead excretion, 600 μg/48 h, after Ca-EDTA is marked as a dashed line. The lead level in bone, 30 μg/g marked in Figure 1, has arbitrarily been set 50% above the detection limit of the instrument in order to increase the confidence level in the lead measurements by XRF. The straight line is a standard deviation line rather than a regression line (see discussion). Consequently, the bone lead level which corresponds to the upper level of lead excretion in normal population (600 μg/48 h) is 70 μg/g wet bone.

The regression lines of bone lead measurements versus Pb-blood (measured at the time of XRF measurements), ZPP, and Pb-urine had correlation coefficients and p values $r = 0.44$, $p = 0.004$; $r = 0.39$, $p = 0.015$; and $r = 0.40$, $p = 0.01$, respectively.

TABLE I. Lead Exposure Indices and Fraction of the Workers Above Currently Accepted Limits

Parameter	Number of workers	Mean value (units)	Range	Standard deviation	Upper limit[a] of normal (ULN)	Fraction above ULN (%)
Age	45	49.4 (y)	35–77	10.3	—	—
Exposure[b]	45	20.9 (y)	13–38	7.4	—	—
Pb-Urine[c]	41	20.3 (μg/l)	1–56	16.2	80	—
Pb-Blood[c]	41	23.1 (μg/dl)	5–58	11.6	40	9.8
ZPP[c]	41	27.4 (μg/dl)	7–87	19.4	50	17.0
Pb-Blood[d]	34	31.2 (μg/dl)	9–60	—	40	28.6
Ca-EDTA[d]	36	663.0 (μg/48 h)	111–2134	465.0	600	38.9
BUN[d]	38	15.7 (mg/dl)	9–25	3.4	20	5.2
Creatinine[d]	38	1.0 (mg/dl)	0.5–1.45	0.2	1.5	0.0
Pb-Bone	43	52.9 (μg/g wet)	0–198	47.4	30[e]	61.4

[a]Upper limit value for general population.
[b]Years in the plant.
[c]Blood or urine samples taken at the time of XRF measurements.
[d]Determinations performed 2–9 months before XRF measurements.
[e]This is not derived from normal population studies.

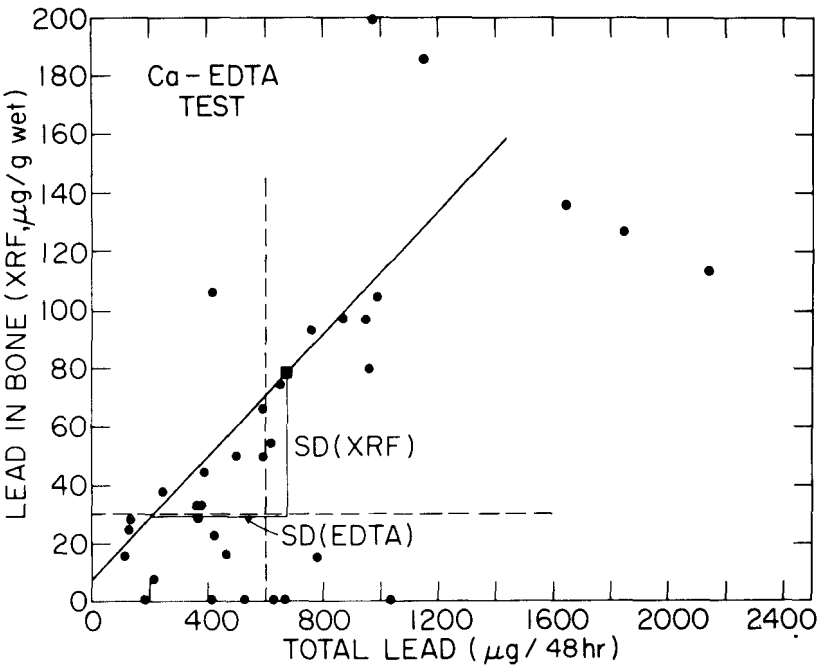

Fig. 1. Lead in bone determined by XRF versus 48-h lead excretion following Ca-EDTA test. The sloping line represents standard deviation line. The horizontal dashed line is set arbitrarily at 50% above the detection limit of the XRF system; the vertical dashed line represents the upper level of Ca-EDTA lead excretion in the general population.

DISCUSSION

This study demonstrates the applicability of the X-ray method for lead determination in bone. In the present work, since low energy lead L X-rays are used, the lead is measured in the surface of the cortical bone only. Consequently, the difficulties in defining the geometry encountered in calibration when high energy K X-rays are used [Ahlgren et al, 1976; Ahlgren and Mattsson, 1979] do not exist. Selection of tibial shaft as the measurement site was dictated by relatively constant and thin, 3- to 6-mm overlying tissue, to minimize attenuation of the signal. Tibia has also been suggested as a more sensitive site for lead measurement than finger bone [Craswell et al, 1984].

A standard deviation line used in Figure 1 was chosen over regression line because a) it treats the variables independently of each other, b) values below the detection limit are discarded from the analysis, and c) it is less sensitive to fluctuations in the data points. The line goes through the mean value of each population with a slope being the ratio of the standard deviation of each population. It can be shown that this slope equals that of the regression line divided by the correlation coefficient r. The bone lead level of 70 μg/g, obtained from the chelatable lead upper limit of normal population requires further clarification and confirmation; however, it appears to be five to seven times higher than that reported in the general population [Waldron and Stofen, 1974]. It should be pointed out that the reported values refer to the bulk bone, while the present measurements reflect the surface bone. Nevertheless, one possible reason for that discrepancy may be that since the source of the chelatable

lead pool and its relationship to the cortical or surface bone lead has not been established definitely, lower levels of bone lead in general population may be indicative of past lead exposures, while the chelatable lead is still within accepted normal levels (below 600 μg/48 h). Thus, the bone lead level of 70 μg/g is unrealistically high to represent the upper limit for the general population. It can be seen in Figure 1 that four out of 34 patients have low lead levels in bone, while the stimulated lead in urine is elevated, eight have high bone lead while low lead in urine, and 22 overlap. These results together with the results in Table I suggest that this method might be used for screening purposes, once an upper limit for lead in bone in general population is established.

Furthermore, only three patients had elevated blood lead, six had elevated ZPP values, and none had elevated unstimulated urine lead. These results support the claim that the Ca-EDTA provocative test is more indicative of cumulative lead exposure than the standard biochemical tests. In addition, the correlation of these results with bone lead were worse than that of the Ca-EDTA test. Ahlgren et al [1982] reported that the lead concentration in blood appears to correlate better with bone lead at blood lead levels below 40 μg/dl. These results were not confirmed in the present work.

With further refinement of the instrument and improved standardization procedures, it should be possible to use the XRF measurement of bone lead for screening purposes. It is more reliable than blood lead as an index of cumulative lead exposure and is also significantly easier to perform than the CA-EDTA test. XRF holds promise for evaluating the pathophysiology of such chronic effects of exposure to lead as lead nephropathy. Standard tests offer little insight into the development of such long-term effects. Furthermore, the XRF technique may be helpful in identifying those bone compartments which are affected following lead chelation therapy. Current improvements in the present instrument will lower the detection limit to a level which may allow its application in the screening of children with potential lead exposure.

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