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Calcified-Tissue Investigations Using Synchrotron X-Ray Microscopy

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Abstract. Synchrotron x-ray microscopy in both emission and absorption modes has been used to examine elemental distributions in specimens of rat tibia, human deciduous teeth, and an orthopedic implant phantom. The work was performed with a spatial resolution of 8 μm for the emission work and 25 μm for the absorption work. The results illustrate the usefulness of SXRM for measurements of different types of calcified tissue.

1. Introduction

Calcified tissues are complex substances which are difficult to characterize completely. Diverse morphological structures exist on a scale that demands the use of instruments with high spatial resolution such as the transmission electron microscope. On the other hand measurements of the distributions of toxic elements require the best possible minimum detection limits as well as superior spatial resolution. In this case electron probe techniques are not suitable. In addition, there are also cases where the ability to make non-destructive evaluations of the density distributions in wet bone specimens without sectioning of the material is necessary. Synchrotron x-ray microscopy (SXRM) can be used to meet these needs. It can be used for measurements on sections with spatial resolutions below 10 micrometers and has minimum detection limits for transition elements and heavy toxic elements (lead, mercury) of about 1

part-per-million (dry weight) for thick specimens using energy-dispersive x-ray detectors. Non-destructive measurements can be made by use of computed-microtomography (CMT) methods.

Three measurements made using the methods of SXRM are described here. They include work based on synchrotron-radiation induced x-ray emission (SRIXE) to study the distribution of gallium in rat tibia and lead in human teeth. CMT was used in a pilot experiment to investigate the interface between an orthopedic implant and bone surface in a test phantom. The results show the efficacy of SXRM in attacking a variety of problems in calcified tissue research.

2. Experimental

The experimental work was carried out with x-ray beams from the National Synchrotron Light source. Low-energy x-ray beams from the X-26 bending magnet beam line were used for the SRIXE work. The CMT work utilized high energy beams produced by a 5-T super-conducting wiggler on the X-17 beam line. Beams of about 8 μm × 8 μm produced by use of a mechanical collimator were employed for the SRIXE experiments. The specimen was moved through the beam using a computer-controlled stepper-motor driven x,y,z stage. Fluorescent x rays produced by the beam were detected with a 30 mm^2 Si(Li) detector placed at 90 degrees to the incident beam 4 cm from the sample.

The CMT experiments on the X-17 beam line were carried out with essentially the same experimental approach. In this case the collimator was chosen to give a beam size of 25 μm × 25 μm. The mean energy of the photon beam after filtering with 250 μm of tantalum was about 60 keV. The CMT used a first generation geometry which involved rotation of the specimen as it was moved through the beam. Linear attenuation coefficients were measured by use of a scintillation detector operated in current mode placed at 0 degrees to the incident beam.

3. Results

3.1 Distribution of Gallium in Rat Tibia

Gallium nitrate is a therapeutic agent used in the treatment of hypercalcemia. In order to better understand the pharmaco-dynamics of the absorption of gallium in growing bone, a study in young rats was carried out.

Thin sections of the tibia (10 μm) were measured and fluorescent x rays from gallium and other elements were detected. The results of a linear scan across the cortex of the diaphysis from periosteum to endosteum is shown in figure 1. The gallium distribution is highly non-uniform and correlates with regions of active bone formation. From looking at the systematics of the

distributions it is hoped that it will be possible to gain information on the biological mechanisms that govern the mechanisms of the gallium deposition.

3.2 Distribution of Lead in Deciduous Teeth

The neurotoxic effects of lead exposure in early life have long been under investigation. Until the last decade or so these studies have been cross-sectional in nature. That is, evaluations of neurobehavorial status and of lead exposure were conducted at one or two points in time. It was widely recognized, however, that the neurobehavorial effects noted were possibly irreversible and, therefore, not necessarily related to the current lead exposure as reflected in the blood lead concentrations but, rather, to some exposure earlier in life. Shed deciduous teeth may be a way to determine cumulative lead exposure from birth since the lead in calcified tissue is relatively non-exchangeable. It is possible that enamel lead could represent blood leads at birth since the enamel formation is complete at a very early age, and that dentine lead is related to recent lead exposure.

In order to apply this concept in practice it is necessary to have a detection method with high detection sensitivity and excellent spatial resolution. The SXRM meets these criteria. In order to demonstrate this, a scan taken across a tooth is shown in figure 2. It can be seen that the lead is sharply peaked in regions at the enamel-dentine and dentine-pulp interfaces and that the lead distribution is non-uniform in the dentine. A systematic investigation of the correlation between the distributions and blood leads is being undertaken.

3.3 Application of CMT to the Study of Orthopedic Implants

The long-term prospect for the success of an orthopedic implant is critically dependent on the material tissue properties of the bone and soft tissue adjacent to the implant. Thus, there is a need for the development of methods to perform such characterizations. Previously, it was not possible to use CMT to study the metal remodelled-bone interface region because of artifacts caused by the so-called partial volume effect. These artifacts appear as streaks extending radially outward from metal objects in tissue. In principle, they can be avoided by using an x-ray beam with a narrow energy bandwidth and a sufficiently high energy in combination with a much finer spatial sampling than is normally used for the morphological structures that are to be studied. The bone implant phantom consisted of a 1 mm s.s steel rod cemented into a rat femur with PMMA cement. The tomographic image of the phantom is shown in figure 3.

The image shows that the beam hardening artifacts were successfully eliminated. It is concluded from the result that detailed non-destructive measurements of the bone-implant interface region can be made to study the growth of bone into porous implant materials.

4. Acknowledgements

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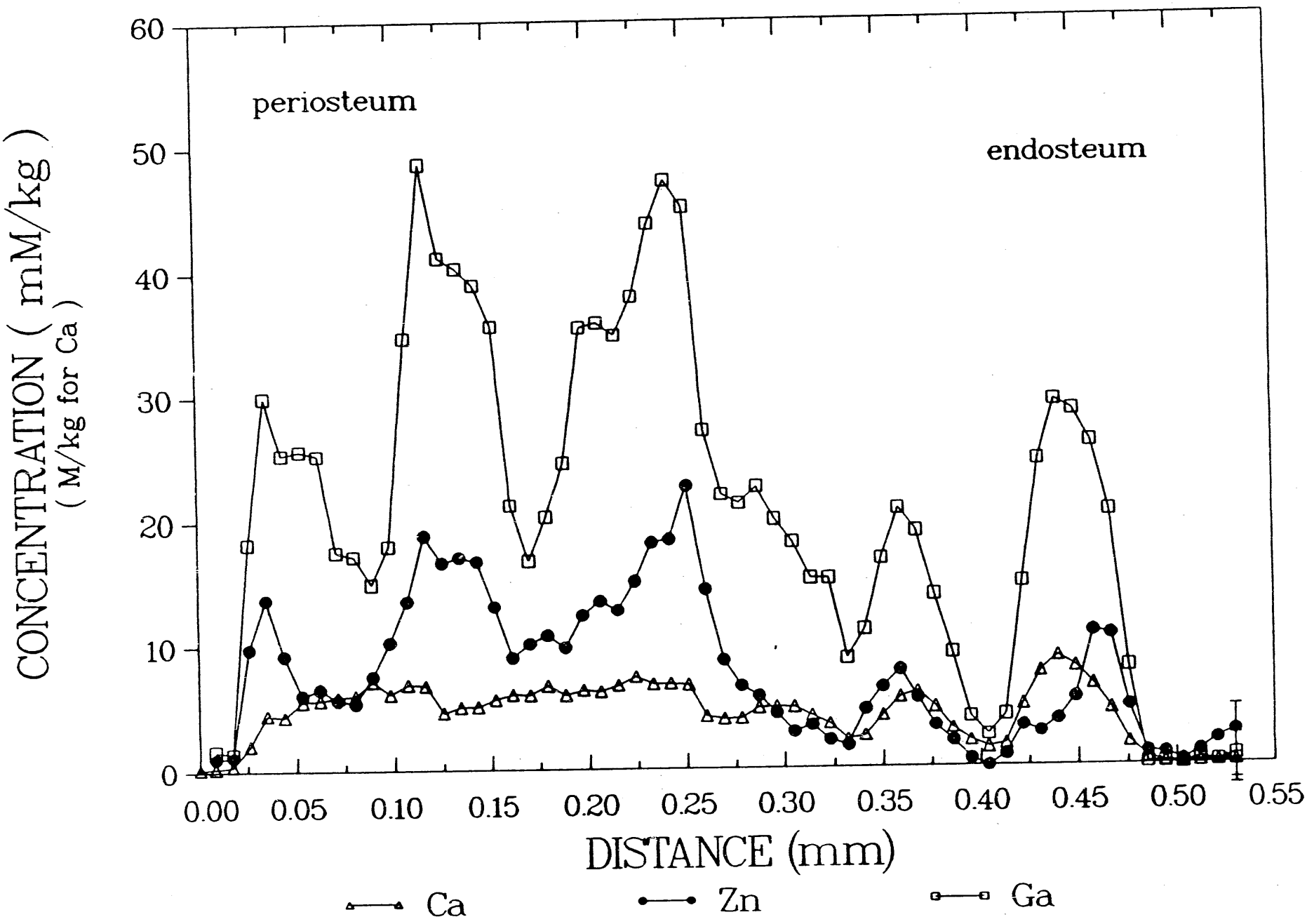
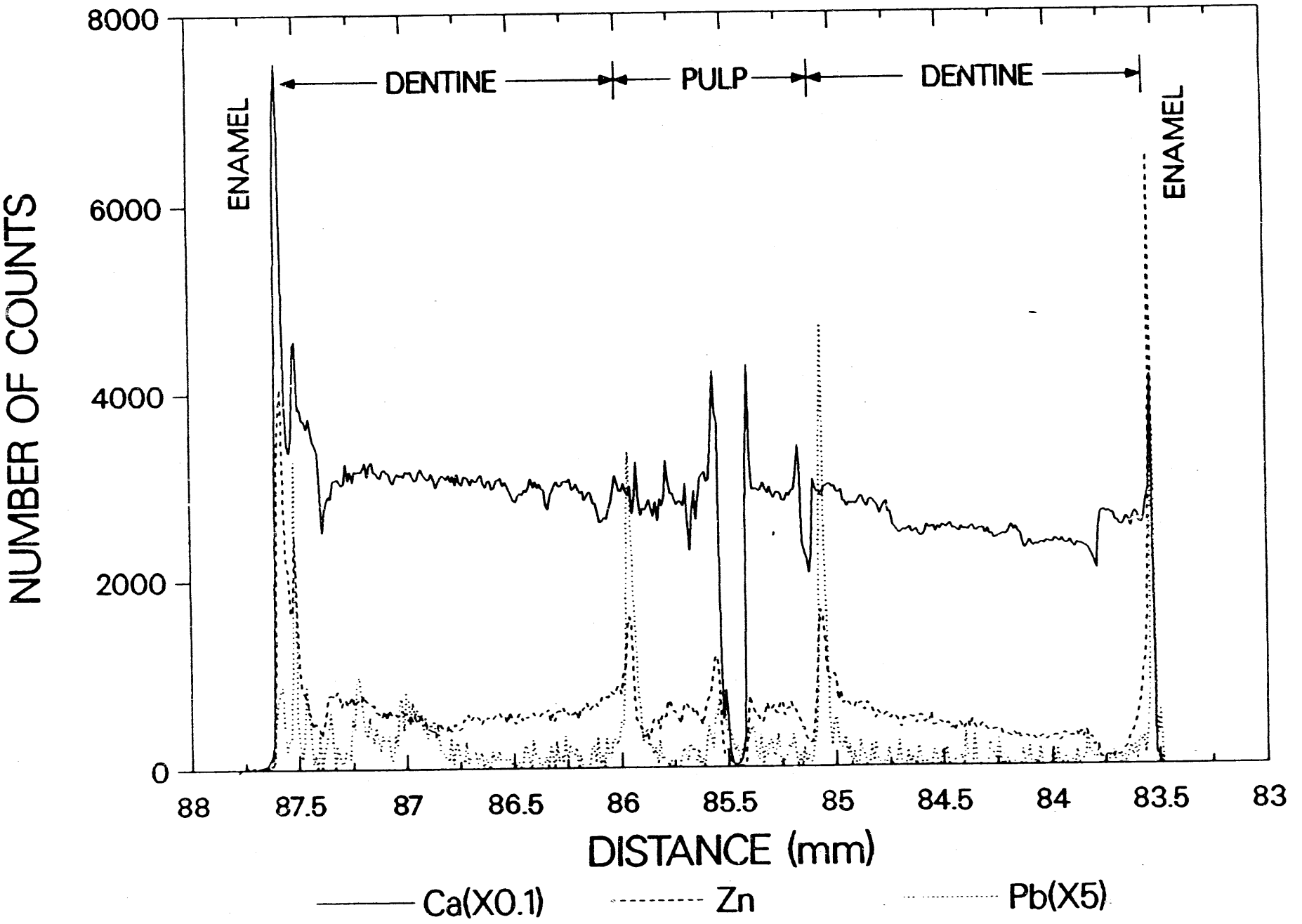
Figure Captions

Figure 1. Linear scan from the periosteal surface to the endosteal surface of cortical bone from the tibia of a rat treated with gallium nitrate. The step size was 9 μm.

Figure 2. Linear scan across section of a shed deciduous tooth. The spatial distribution may be used to understand long-term exposure to lead. The step size was 10 μm.

Figure 3. Tomographic section of an orthopedic implant cemented into a rat femur. CMT is demonstrated to be a viable method for study of the implant-bone interface that does not require sample preparation.


periosteum
endosteum
CONCENTRATION (mM/kg)
(M/kg for Ca)
60
50
40
30
20
10
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
DISTANCE (mm)
Ca
Zn
Ga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4000
2000
0
NUMBER OF COUNTS
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DENTINE
PULP
DENTINE
ENAMEL
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87.5
87
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85
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83.5
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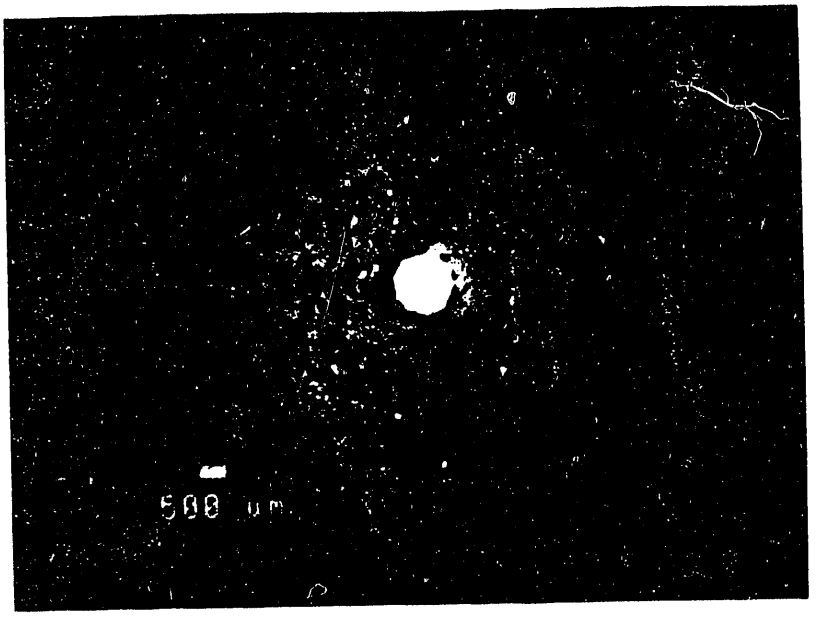
DISTANCE (mm)
Ca(X0.1)
Zn
Pb(X5)



Jones et al. Fig 3.

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