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Influence of Calcitonin on the Initial Uptake of Lead and Mercury by Bone (40498)

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It has been known for some time that after entrance into the body most heavy metals find their way to bone. Based on studies utilizing radionuclides, these have been divided into metals that "seek bone surfaces" and those that "seek bone volumes" (1). However, the first contact of the metal must be with the bone surface. The fact that lead reacts with hard tissues in the body was first utilized by Okada and Mimura (2) in a vital staining technique for the study of tooth development. It has since been used by several investigators as a method for evaluating the growth of calcifying tissues (3–7).

Recently, it has been proposed that the hormone calcitonin (CT) has as one of its primary target sites cells lining bone surfaces (8). Not only do these cells respond rapidly to this hormone, but changes in the fluid between these cells and bone surfaces and around osteocytes can be identified (9).

The study to follow was carried out to determine whether pretreatment of rats with CT would influence the rapid uptake of lead by bone. For comparison, mercury, another heavy metal, was also studied.

Materials and methods. These experiments utilized male Sprague-Dawley rats, 185–225 g in body wt. They were maintained on stock laboratory rat chow. Routinely, the rats were fasted overnight before use in the experiments. CT (salmon CT generously supplied by Armour Pharmaceutical Company) was administered as a single injection (0.1 mU/g body wt), or the same amount was given hourly for several hours. Lead was injected as the acetate (20–40 mg Pb acetate/kg body wt) into a jugular vein (IV). Controls were similarly injected with sodium acetate. Mercury was injected as the chloride (3 mg $HgCl_2$/kg body wt). Controls for mercury experiments received saline. The dosages employed for both metals are in the toxic, but not acutely lethal, range. The dose for lead was chosen for its ability to produce a hypercalcemia (10). The dose for mercury was 10% of the acutely lethal dose. Rats were sacrificed 5 min to 1 day after injection of the heavy metal. Rat tibiae were fixed in 1% glutaraldehyde with 0.1 *M* phosphate buffer at pH 7.2 for 2–3 hr, followed by a 2-hr rinse in 0.25 *M* sucrose. Tissues were decalcified in 0.2 *N* HCl and saturated with H_2S gas for 3–4 days (2, 5). Transverse sections of the diaphysis and longitudinal sections of the epiphyseal–metaphyseal area were incubated in 0.1% gold chloride solution at 37° for 30 min, then dipped in 5% sodium thiosulfate solution for 5–10 min. Sections were mounted with glycerin. Using this procedure, lead is identified by colors varying from red to almost black; mercury appears as a lavendar-to-purple precipitate.

Results. Histological preparations were made from tibiae of rats sacrificed sequentially after injection of the heavy metals. The presence of both metals can be identified in bone within 5 min after their injection. There are some differences in the distribution of the two metals, due primarily to the characteristic of the specific metal. Lead reacts strongly at the surfaces of bone with calcium and phosphate, becoming permanently attached at the first point of contact. It is removed primarily by bone resorptive processes. Mercury does not react strongly with calcium and phosphate. If it attaches at all, it is easily displaced. Its distribution appears to be that of a volume seeker (1). This metal probably follows fluid flow through bone and becomes more diffusely distributed than lead. By 24 hr after injection, the presence of lead can be seen as a line deposit on bone surfaces. Mercury, with its diffuse distribution, gradually disperses.

1. *The effect of CT on metaphyseal uptake of lead and mercury.* For the study of the

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metaphysis, the time period, 30 min after metal injections, was chosen for presentation (Fig. 1). The effect of CT, given in one or more injections, on the uptake of lead and mercury could be easily visualized. The heaviest precipitate resulting from the fixation reaction with either of the two metals was in this trabecular bone, probably due to the source or amount of blood supplied. Minor differences in localization were noted. The heaviest concentration of lead was in the primary spongiosa, in the area where only a layer of bone coats the underlying cartilage. For mercury, the heaviest concentration was further down the trabeculae, in that area usually termed as secondary spongiosa.

The important point for our study is that pretreatment with CT inhibited the uptake of both metals in all areas of metaphyseal bone reached by the particular metal. This can be seen by comparing Panels a and c of Fig. 1 (controls) with Panels b and d (CT-pretreated). Since these metals, particularly lead, reach bone even in CT-treated rats, it can be assumed that the presence of this hormone produces a condition at the surfaces of bone which slows the movement of these metals and their contact with bone surfaces.

2. *Demonstration of CT-induced inhibition of the uptake of lead or mercury by compact bone.* Lead and mercury can be identified on compact bone surfaces of the tibia within five minutes after injection, but the concentrations are much lower than in metaphyseal areas. At this time, lead reaction precipitate appears as a thin line on both periosteal and endosteal surfaces and in the walls of vascular channels (Fig. 2a). A rough estimation of lead uptake could be made by measuring the percentage of bone surfaces (both periosteal and endosteal) on which the red precipitate could be identified in thick (50 μm) cross-sections of the shaft (Table I). By 5 min after iv injection, essentially all periosteal surfaces showed traces of lead, while about 50% of the endosteal surfaces had accumulated sufficient lead for the precipitate to be recognizable. There was the suggestion of a transient decrease at 15 min. However, increasing amounts could be seen from 30 min through 2 hr. At 24 hr the lead line was thick and covered all surfaces (Fig. 2b).

Examination of the histological preparations demonstrated that pretreatment with CT decreased, at least temporarily, the amount of lead on bone surfaces in the shaft. In Table I are given the percentages of bone surfaces containing identifiable precipitate as measured with an ocular grid under light microscopy. These measurements are subject to visual recognition of the reactive material, rendering them somewhat subjective in nature. Under these conditions, minor differences would be questionable. However, because CT reduced the surface measurement of identifiable precipitate by as much as 50%, it can be concluded that preadministration of CT reduces the rate of lead uptake by surfaces of compact bone. These differences are not maintained. Not shown are data from measurements taken at 12 and 24 hr after lead injection. At these times, no statistical difference could be seen between the two groups by the methodology used.

The effect of calcitonin on the uptake of mercury in the shaft of the tibia was not clearly demonstrable by this methodology. Mercury accumulation in the shaft is diffuse, appearing to penetrate into osteocyte lacunae. Vascular channels were usually filled with the reaction precipitate. The peak uptake of mercury was around 15 min after its injection. While visual examination suggested that calcitonin decreased the uptake of mercury in the shaft, this was impossible to quantitate.

Discussion. The primary purpose of these experiments was to determine from histological preparations whether calcitonin changed the pattern of uptake of either lead or mercury at bone surfaces. The doses used were in the toxic range, as comparison was to be made between the amounts of these elements accumulating on bone surfaces in control and calcitonin-treated rats. They should not be compared with the doses of lead used by Okada and Mimura (2), who were using lead as a vital stain to study changes in bone histology.

Rosen and Wexler (11) have reported that the addition of CT to bone in organ culture inhibited the release of lead from bone into the medium. This was attributed to the hormone's ability to inhibit bone resorption, a well-documented action. In contrast, in our study the reduction in the rate in which these metals reached bone following pretreatment

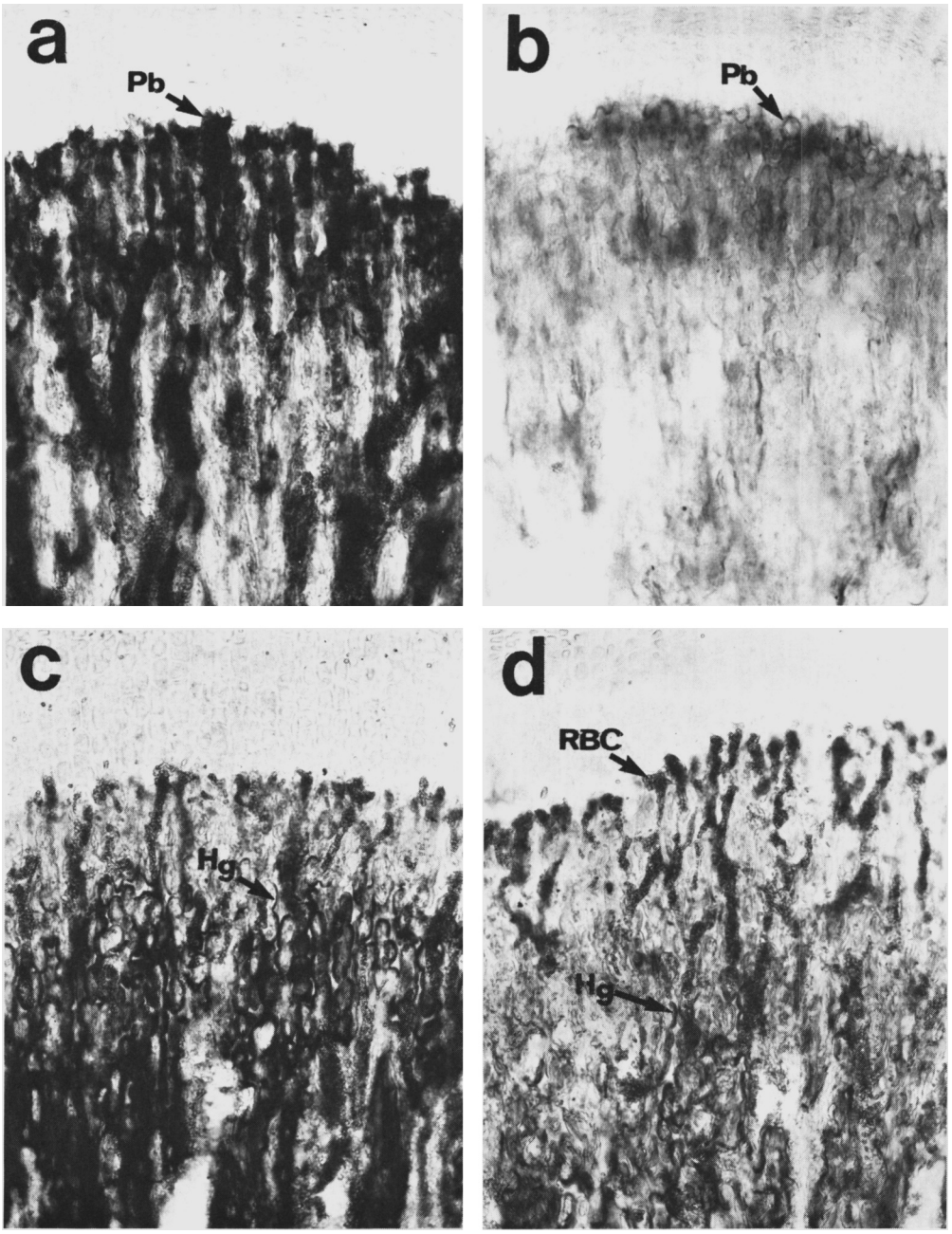

FIG. 1. Effect of pretreatment of rats with calcitonin on the distribution of lead and mercury in the metaphysis of the tibia. Rats sacrificed 30 mins after injection of metal. Longitudinal Sections × 100. (a) Injection of lead acetate (30 mg/kg) into control rats; (b) Injection of lead acetate into rats pretreated for 4 hrs with calcitonin (0.1 mU/g body w/hr); (c) injection of mercuric chloride (3 mg/kg) into control rats; (d) injection of mercuric chloride into rats pretreated with CT; Pb = PbS precipitate localization; Hg = HgS. RBC = localization of red blood cells.

with CT was a surface phenomenon, occurring at the interfaces of bone with extracellular fluid.

Lead is a "surface seeker," in that it attaches almost irreversibly to the first bone surface the ions contact. This may be due to

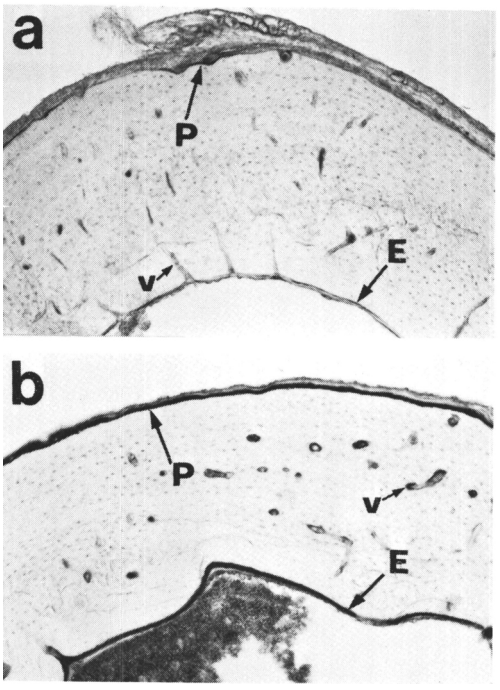

FIG. 2. Cross-section of the shaft of the tibia of normal rats after injection with lead acetate (× 100). (a) Rat killed 5 min after lead injection, P = periosteum; E = endosteum; V = vascular channel. (b) Rat sacrificed 24 hr after lead injection.

the affinity of lead for phosphate. Mercury is a "volume seeker," in that it attaches little, if at all, to bone surfaces.

This report has been limited to establishing that calcitonin reduces or slows the uptake of both lead and mercury by trabecular bone. Inhibition of lead uptake also occurs in compact bone. No specific information is provided to explain the method by which the hormone accomplishes this task. However, the conclusion appears valid that calcitonin effects must occur on all bone surfaces and that its action is not limited to specific processes in bone, such as those involved in bone formation or resorption. We are suggesting, therefore, that calcitonin, by its action on cells covering bone surfaces, must produce changes in bone fluid and/or on bone surfaces, which results in the slowing of the progress of these heavy metals onto the surface or through bone.

Summary. Lead acetate (20 to 40 mg/kg body weight) or mercuric chloride (3 mg/kg body weight) was injected intravenously into normal rats and those pretreated with calcitonin (0.1 mU/g body wt/hr for 1–4 hr). Rats were sacrificed at intervals from 5 min to 24 hr after heavy metal injection. Tibias were prepared histologically, using Okada and Mimura's vital staining technique. The metaphyses and diaphyses were examined under the light microscope to study the effect of calcitonin on the uptake and distribution of these two metals in bone. Calcitonin dramatically reduced the initial uptake of lead and mercury by bone. This reduction, at least for lead, occurred in both trabecular and compact bone. The mechanism(s) by which calcitonin influences the distribution of lead and mercury in bone has not yet been determined.

TABLE I. PERCENT SURFACE AREA OF TIBIA SHAFT COATED WITH LEAD.[a]

Group	Control group	CT pretreated	*P* values
A. 5 Min postlead-injection			
Periosteal surfaces	97.3 ± 1.1	28.5 ± 7.6	<.001
Endosteal surfaces	53.1 ± 3.4	22.0 ± 7.8	<.005
B. 15 Min postlead-injection			
Periosteal surfaces	89.6 ± 3.4	28.1 ± 5.4	<.001
Endosteal surfaces	46.3 ± 3.9	6.3 ± 3.6	<.001
C. 30 Min postlead-injection			
Periosteal surfaces	96.1 ± 2.2	61.4 ± 7.2	<.001
Endosteal surfaces	97.5 ± 1.9	53.9 ± 6.8	<.001

[a] Values are means ± SE of the percent of each surface on which identifiable precipitate could be visualized, obtained by measurement with an ocular micrometer of four cross-sections of the tibia shaft for each of two rats.

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