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Studies of lead transport in bone organ culture

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The skeleton is the reservoir for the majority of the body burden of lead [1]. Factors that control the metabolism of lead (Pb) in bone are particularly critical: (1) bone is the major source of Pb mobilized during chelation therapy [2], and (2) "subtoxic" amounts of Pb may be mobilized during skeletal remodeling. Yet, little is known concerning the metabolism of the rapidly mobile Pb compartment loosely bound to bone [3], compared to tightly and deeply bound Pb in bone [3]. Recent studies have suggested that it is the former component of bone Pb that is metabolically active and readily miscible with other soft tissue compartments of Pb [3].

The methods of bone organ culture have provided, in many cases, the link between observations *in vivo* and those made at the molecular level [4]. Moreover, it is evident that ionic changes in the extracellular fluid control, in part, the osteolytic process and that hormones act through such changes within a specific ionic environment [5]. The present studies in bone organ culture indicate that one skeletal Pb compartment, comprising up to 20 per cent of total ^{210}Pb incorporated into fetal rat bones, is controlled by cardinal agents regulating normal skeletal growth and remodeling, namely, parathyroid hormone (PTH), calcitonin (CT), calcium (Ca), inorganic phosphate (P_i) and magnesium (Mg).

Pregnant rats were intoxicated with stable Pb throughout gestation by providing a drinking solution of deionized water that contained Pb at a concentration of 5 mg/ml. According to methods described previously [6], on day 18 of gestation, 50 μCi ^{210}Pb were injected into the tail vein, and 24 hr later, paired fetal radii and ulnae, dissected free of cartilage, were planted on grids in 5 ml of modified BGJ medium that contained no Pb. After a continuous culture period of 48 hr, at 37° under 5% CO_2 in air, the total amount of ^{210}Pb released into the experimental medium (EM) from treated bones (eight to ten bones/culture dish) was measured by liquid scintillation counting and compared to that released from paired control bones into the appropriate control medium (CM). The effects of adding one hormone (PTH or CT) to the EM of all treated bones and changing the concentration of one ion (Ca, P_i or Mg) in the EM and CM, unless the ionic concentrations in the "Standard Medium" were used (Tables 1–3), were expressed as cpm EM/CM ratios. The concentration of other ions in the EM and CM remained constant, if the level of one ion was changed in both media. The EM/CM ratios of five to twelve paired cultures were calculated as the mean ± S. E., and the significance of each EM/CM ratio, compared to 1.00, was evaluated by Student's t-test. In contrast to 48-hr continuous culture periods, timed experiments were also carried out: paired bones (eight/culture dish) were treated with and without PTH for varying periods of time, four to eight treated and control dishes/time period. Both the EM and CM contained the same concentration of ions as those in the standard medium. At the end of the specific time period, medium aliquots were removed from each petri dish (EM and CM), and the total amount of ^{210}Pb released was measured in the EM and CM as described above. Treated and control bones for timed and continuous cultures were ashed and analyzed for Ca and Pb content by flameless and flame atomic absorption spectroscopy respectively [7, 8]. These

Table 1. Effects of PTH on the efflux of ^{210}Pb in various media in continuous cultures*

Group	Ion conc (mM) Ca	P_i	Mg	48-Hr release of ^{210}Pb (EM/CM)	(μg/100 Pb mg bone ash) Treated	Control
Ca	0.70	1.0	0.8	1.06 ± 0.07†	7.23 ± 0.16	7.04 ± 0.10
	1.40	1.0	0.8	2.30 ± 0.14‡	7.18 ± 0.19	8.98 ± 0.17§
	2.80	1.0	0.8	1.46 ± 0.16†,‡	8.25 ± 0.17	9.15 ± 0.17§
P_i	1.40	0.5	0.8	3.45 ± 0.09‡	7.16 ± 0.10	8.43 ± 0.16§
	1.40	1.0	0.8	3.30 ± 0.14‡	7.00 ± 0.16	8.52 ± 0.14§
	1.40	2.0	0.8	1.78 ± 0.10†,‡	8.69 ± 0.13	8.64 ± 0.17
	1.40	4.0	0.8	1.12 ± 0.17†	8.55 ± 0.15	8.25 ± 0.14
Mg	1.40	1.0	0.4	1.59 ± 0.19†,‡	5.00 ± 0.10	5.65 ± 0.17
	1.40	1.0	0.8	2.05 ± 0.10‡	5.68 ± 0.10	6.61 ± 0.19§
	1.40	1.0	1.6	2.22 ± 0.10‡	5.52 ± 0.11	6.49 ± 0.12§

* Underlined ion concentrations are those in the standard medium. Values are expressed as means ± S. E. Each culture dish contained ten bones. The concentrations of PTH used per ml of the EM were: Ca: 250 ng (3885 I.U./mg); P_i: 1.0 μg (1000 I.U./mg); and Mg: 1.5 μg (1500 I.U./mg).

† EM/CM ratio different from that found in standard medium, $P < 0.01$.

‡ Different from 1.00, $P < 0.01$.

§ Different Pb levels in control compared to treated bones within each group, $P < 0.01$.

‖ Different Pb levels in treated compared to other treated bones, at different ionic concentrations, within each group, $P < 0.01$.

Table 2. Effects of PTH on the efflux of ^{210}Pb at various times*

Time of PTH exposure	Release of ^{210}Pb (EM/CM)	Pb (μg/100 mg bone ash)	Ca (mg/100 mg bone ash)
0	1.00	9.90 ± 0.16	11.20 ± 0.64
10 min	0.82 ± 0.05	9.93 ± 0.14	11.32 ± 0.57
2 hr	1.12 ± 0.04	9.85 ± 0.10	8.96 ± 0.52†
6 hr	1.59 ± 0.08‡	9.00 ± 0.14†	8.15 ± 0.48†
24 hr	3.69 ± 0.15‡	8.10 ± 0.15†	8.00 ± 0.59†
48 hr	3.75 ± 0.09‡	8.07 ± 0.06†	8.10 ± 0.53†

* Ion concentrations were the same in the EM and CM as those in the standard medium (Ca: 1.40 mM; P_i: 1.0 mM; and Mg: 0.8 mM). The values represent means ± S. E. Each culture dish contained eight bones. The concentration of PTH (3885 I.U./mg) used in all treated cultures was 375 ng/ml of medium.

† Different concentrations of Pb and Ca in ashed bones, $P < 0.01$, compared to 0- and 10-min times for Ca and 0-, 10-min and 2-hr times for Pb respectively.

‡ Different from 1.00, $P < 0.01$.

values were similary evaluated by Student's *t*-test. Bovine PTH (1000–3855 I.U./mg) at concentrations of 250 ng to 1.50 μg/ml of medium was used. Therefore, since PTH of a different potency was used within each experimental group (Tables 1 and 3), only comparisons within each group have statistical meaning.

At a low medium concentration of Ca, 0.7 mM, a high rate of bone resorption (cell-mediated solubilization of bone mineral and matrix) occurred in both control and treated cultures, and there was no further increase in ^{210}Pb release by adding PTH to medium of low Ca concentration (Table 1). Hence, the EM/CM ratio of 1.06 is not different from 1.00. Moreover, the Pb content of control bones cultured in low Ca medium was comparable to that of PTH-treated bones at both low and standard medium levels of Ca (Table 1).

In low Ca medium, this high rate of active resorption was sustained and dose-related (see legend of Table 1), and this suggests that active resorption was increased. This suggestion was supported by comparing the efflux of ^{210}Pb in living vs heat-killed bones—the efflux of ^{210}Pb was far greater and consistent in living than in killed bones, in which the efflux of ^{210}Pb was negligible (EM/CM: $\leqq$0.10). Thus, net removal of label had occurred, and this was not simply due to physiocochemical exchange (Table 1). The consistent, dose-related and highly reproducible EM/CM ratios in living bones, noted in these experiments and the others reported herein, indicated that cell-mediated stimulation or inhibition of ^{210}Pb release was present, compared to the very minor effects observed in heat-killed bones. On the other hand, at a high medium level of Ca, 2.80 mM, PTH action was inhibited (Table 1). These effects of a high medium level of Ca were reflected by a significant increase in the stable Pb content of ashed bones (Table 1).

After bones were exposed to relatively high concentrations of PTH in timed experiments (Table 2), a significant stimulation of ^{210}Pb and stable Pb release was observed as early as 6 hr. Moreover, by 24 hr, there was no apparent difference in either EM/CM ratios or stable Pb concentrations, compared to those observed in 48-hr continuous cultures. The release of ^{210}Pb was accompanied by a proportional loss of stable Pb (Table 2), indicating that there was net removal of lead rather than only an increase in exchange of ^{210}Pb. The release of Ca and Pb from PTH-treated bones followed, in general, a comparable time course, though the net release of Ca occurred somewhat earlier than that observed for the efflux of Pb. The larger net release of Ca, measured in ashed bones, was most likely a reflection of its larger concentration and/or greater mobility in this and other systems [5].

By increasing the P_i concentration to 2 mM from the standard level of 1 mM, inhibition of PTH action on ^{210}Pb efflux was found in both treated and untreated cultures (Table 1). Nevertheless, a significant EM/CM ratio was noted. P_i (4 mM) inhibited PTH action further and, since the efflux of ^{210}Pb was similarly inhibited in control cultures, the EM/CM ratio was not significantly different from 1.00. In this instance, and at a medium P_i level of 2 mM, significant rises in the stable Pb content of ashed bones were noted. In these continuous 48-hr experiments with Ca and P_i, and the following ones, this mobile Pb compartment comprises up to 20 per cent of total ^{210}Pb previously incorporated.

At a low medium concentration of Mg, 0.4 mM, compared to the standard level of 0.8 mM, the efflux of ^{210}Pb was inhibited in PTH-treated and control cultures (Table 1). Nevertheless, a significant EM/CM ratio and a significantly lower net loss of stable Pb were demonstrated. At a high concentration of Mg, 1.60 mM, no effect was found. Salmon CT, when added to the standard medium containing 1 mM P_i, inhibited the efflux of ^{210}Pb. The combined effects of CT and rising medium levels of P_i appeared progressively additive, as EM/CM ratios decreased while the stable Pb content of ashed bones increased (Table 3).

These data indicate: (1) that PTH enhances cell-mediated and rapid net release of ^{210}Pb from bone; this release is accompanied by a proportional loss of stable Pb, which generally parallels in time concurrent removal of Ca from treated bones. In continuous cultures, this effect on ^{210}Pb efflux can be inhibited by P_i; (2) that CT inhibits ^{210}Pb release. CT and P_i produce responses in the same direction; and (3) that low medium levels of Ca increase both bone resorption and ^{210}Pb release, while high levels produce the opposite effects. Thus, the metabolism of Pb in this compartment is qualitatively similar to that of bone mineral, studied similarly *in vitro* [4, 9], though its size is smaller than that of Ca as suggested by the larger decrease of stable Ca noted in the timed experiments. It is not surprising that the size (20 per cent) of this prelabeled and mobile compartment of ^{210}Pb is smaller than that of preincorporated ^{45}Ca (30–70 per cent) [4, 9–11], studied under similar conditions, since protein inclusions are present in bone [12], and it is reasonable to suggest that nondiffusible Pb protein complexes [13] predominate over soluble-exchangeable ones. However, the net release of previously incorporated ^{210}Pb increases 2- to 3-fold when one adds EDTA to the medium or when the culture period extends to 72 and 120 hr (J. F. Rosen and E. E. Wexler, unpublished observations).

Though model(s) to explain the interaction of CT, PTH, Ca, Mg and P_i have been proposed at the cellular level [5, 14] these models have yet to be extensively tested. Nevertheless, the observations *in vitro* reported herein may possibly yield information to explain previous studies *in*

Table 3. Effects of CT on the efflux of ^{210}Pb in various media in continuous cultures*

	Ion concn (mM) Ca	P_i	Mg	48-Hr release of ^{210}Pb (EM/CM)	(μg/100 mg bone ash) Treated	Control
CT	1.40	1.0	0.8	0.78 ± 0.14†	11.90 ± 0.16	10.81 ± 0.11‡
	1.40	2.0	0.8	0.35 ± 0.11†,§	12.00 ± 0.10	10.20 ± 0.15‡
	1.40	4.0	0.8	0.20 ± 0.09†,§	12.80 ± 0.17‖	10.10 ± 0.16‡

* Thirty mU/ml of salmon CT (1500 M.R.C.U./mg) was added to each culture dish. Underlined ion concentrations are those in the standard medium. Values are expressed as means ± S. E. Each culture dish contained ten bones.

† Different from 1.00, $P < 0.01$.

‡ Different Pb levels in control compared to treated bones within each group, $P < 0.01$.

§ EM/CM ratio different from that found in standard medium, $P < 0.01$.

‖ Different Pb levels in treated compared to other treated bones, at different ionic concentrations, within each group, $P < 0.01$.

vivo of Pb metabolism. The present evidence indicates that the release of Pb from bone increases as medium levels of Ca decrease. Hence, low Ca levels in the extracellular fluid may lead to increased release of Pb from bone and subsequent redistribution to soft tissues and blood. Indeed, it has been demonstrated that low dietary Ca enhances Pb toxicity in rats [15], in part, by increasing bone turnover of Pb with sequestration in soft tissues [15]. Moreover, low dietary intakes of Ca and low serum levels of Ca have been found in a large group of children with Pb intoxication (blood Pb $\geq 60\,\mu$g/dl) [16]. Though experimental studies *in vivo* on the effects of Pb metabolism produced by various dietary P_i intakes have not been reported, the data in this report, as in others [5, 9, 10, 14], indicate that high medium (extra-cellular) levels of P_i inhibit bone resorption and hence the efflux of Pb from bone.

This culture system may well enable further study and definition of hormonal, ionic and other factors that control a rapidly mobile compartment of bone Pb regulated like bone mineral.

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