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# Effect of Cadmium on Bone Resorption in Cultured Fetal Bones

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Itai-itai disease which occurred in Toyama Prefecture, Japan, was thought to be due, at least partly, to chronic cadmium poisoning(FRIBERG et al 1971). Patients suffered severe pain in the waist, back and joints as well as kyphosis spinal column. In addition, x-ray film of these patients revealed abnormalities in the humerus and ribs. These bone lesions have been considered to be caused secondarily by dysfunction of other tissues, especially that of the kidneys, but there are some reports that the bone lesions appear before the occurrence of pathological changes in the kidneys of Cd-administered rat(MATSUE et al. 1970, YOSHIKI et al. 1975). It is currently unclear whether bone lesions by Cd are due to the direct action on the bone or indirect action which is caused by dysfunction of the kidney or intestine.

To clarify the direct action of Cd on the bone, we studied the effect of Cd on the ossification of chick-embryo cultured bones biochemically and histologically(SAKAI et al. 1975, MIYAHARA et al. 1978). The results showed that Cd inhibited the bone matrix formation and brought about a malfunction in the ossification process. In the present work the effect of Cd on demineralization was studied using $^{45}Ca$-prelabeled bone in tissue culture and low levels of Cd were found to stimulate $^{45}Ca$ release from the bone.

## METHODS

Nineteen-day-old rat fetuses were obtained from mothers which had been injected subcutaneously with 200 µCi of $^{45}Ca$ as $CaCl_2$ solution 40 h prior to sacrifice(RAISZ & NIEMAN 1969). The radius and ulna were dissected free of muscle and placed on squares of Millipore filter resting on a glass ring in a Petri dish. To test the effect of Cd, single radius and ulna were compared with those of opposite limbs from the same fetus. The culture dish contained 0.5 mL of a chemically defined medium(NAGATA et al. 1977) and was equilibrated with 5 % $CO_2$ and 95 % air at 37°C in a $CO_2$ incubator. The final concentration of Ca was 1.4 mM

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and Pi 1.0 mM. A 24-h preculture was used before the addition of Cd to remove most of the exchangeable $^{45}Ca$ from the bone and thereafter the medium was changed every two days. After cultivation, the bones were extracted with cold 0.1 M acetate buffer containing 0.01 M EDTA(pH 5.0) for two days. The extracts and media were analyzed for $^{45}Ca$ by liquid scintillation counting. The amount of $^{45}Ca$ released from the bones during cultures was expressed as percentage of the total radioactivity initially present, calculated from the total dpm in media and bone. The significance in $^{45}Ca$ release after culture was determined by Student's t-test. "Heat-inactivated" bones were prepared by heating the bones to 75°C in the culture medium for 5 min prior to explantation. The medium was also measured for lactic acid enzymatically(LUNDHOLM et al. 1963). To see whether or not our experimental conditions were suitable, we tested the effect of bovine parathyroid hormone(PTH) on $^{45}Ca$ release in the presence of various levels of bovine serum albumin (fraction V) which were added to prevent the PTH from adhering to the glass wall of the culture vessels. As shown in Table 1, PTH produced a significant increase in $^{45}Ca$ release in levels of bovine serum albumin above 0.7 %. In the following experiments 1 % bovine serum albumin was added to the culture medium.

TABLE 1. Effect of Albumin Concentration on Release of Previously Incorporated $^{45}Ca$ from Fetal Rat Bones Cultured for Three Days in a Chemically Defined Medium with and without Parathyroid Hormone(5 U/mL)

| Albumin concentration (%) | $^{45}Ca$ release (% total radioactivity) Control | PTH |
|---|---|---|
| 0 | 14.8 ± 0.7 | 13.6 ± 0.6 |
| 0.1 | 14.1 ± 0.4 | 15.2 ± 0.4 |
| 0.4 | 15.2 ± 0.1 | 16.8 ± 1.0 |
| 0.7 | 16.1 ± 0.4 | 19.6 ± 0.9* |
| 1.0 | 16.6 ± 0.2 | 19.7 ± 1.4* |

Values are the means ± SE of 4 bones.
*Significantly different from paired control cultures, $p<0.05$

## RESULTS

Fetal rat bones prelabeled with $^{45}Ca$ were cultured with 0.03 to 30 ppm Cd for 6 days. As shown in Table 2, $^{45}Ca$ release from the bones was significantly stimulated by 0.1 to 1 ppm Cd, although Cd did not affect $^{45}Ca$ release at 0.03 ppm and at levels exceed-

TABLE 2. Effect of Cd Concentration on Release of Previously Incorporated $^{45}Ca$ from the Fetal Rat Bones Cultured for Six Days with and without Cd

| Cd concentration (ppm) | $^{45}Ca$ release (% total radioactivity) Control | Cd |
|---|---|---|
| 0.03 | 24.3 ± 0.4 | 24.2 ± 0.4 |
| 0.1 | 24.4 ± 0.6 | 25.7 ± 0.8* |
| 0.3 | 23.3 ± 0.2 | 24.1 ± 0.1* |
| 1.0 | 24.0 ± 0.2 | 26.8 ± 0.1* |
| 3.0 | 26.4 ± 1.1 | 25.9 ± 0.3 |
| 10.0 | 24.1 ± 0.3 | 24.5 ± 0.2 |
| 30.0 | 23.4 ± 0.5 | 23.8 ± 0.3 |

Values are the means ± SE of 4 bones.
*Significantly different from paired control cultures, $p<0.05$

ing 3 ppm. To see whether the enhancement of $^{45}Ca$ release by Cd is due to an active resorption or passive dissolution, $^{45}Ca$ release from living bones in the presence of 1 ppm Cd was compared with that from "heat-inactivated" ones. Stimulative effects of Cd on $^{45}Ca$ release in living bones were not observed in heat-inactivated ones. As a stimulation of $^{45}Ca$ release by PTH has been recognized to be ascribed to an increase in the release of lactic acid(VAES 1968), it was investigated whether lactate release was stimulated by Cd. At Cd concentrations of 0 and 1 ppm, the lactate release was 45 and 71 μg, respectively, under aerobic conditions and 78 and 76 μg under anaerobic conditions, respectively ; each value is the mean of two samples. A 1 ppm Cd caused an increase in the release of lactic acid under an aerobic condition, although a lactate release from bones cultured anaerobically was not stimulated by Cd.

## DISCUSSION

Bone resorption, characterized by the solubilization of both the mineral and organic components of the osseous matrix, can be observed in tissue culture. Considering the similarity of the bone resorption in vivo and in the tissue culture system, the investigation of bone resorption in the tissue culture system might be useful to clarify the mechanism of bone demineralization in Itai-itai disease. This report shows that mineral loss is stimulated by 0.1 to 1.0 ppm Cd. A stimulation of demineralization by a low level of Cd suggests the possibility that Cd has a direct effect on mineral loss.

As the stimulative effect of Cd on the demineralization was not recognized in heat-inactivated bone, it was demonstrated that the active resorption played an important role in the stimulation of mineral loss by Cd. VAES(1968) has studied the action of PTH on bone resorption and showed a following hypothesis responsible for bone resorption : the lysosomal acid hydrolase is responsible for the resorption of organic matrix of bone and acid, originating possibly from the stimulation of glycolysis, cares for concominant solubilization of bone mineral and also favors the hydrolytic action of the lysosomal enzymes.

Cd was found to stimulate lactate production at a concentration similar to those which enhanced demineralization. This result suggests that an enhancement of mineral loss by Cd is ascribed, at least partly, to an increased lactate formation. The mechanism responsible for a stimulative effect of Cd on the formation of lactic acid is unknown. As the stimulation of lactate production by Cd was not observed under an anaerobic condition, Cd might stimulate an aerobic glycolysis. Embryonic chick cultured bone showed results similar to those in this report(MIYAHARA et al. 1980).

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