

# ACTIVATION OF PARATHYROID HORMONE BY HEPARIN IN VITRO

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Pieces of the parietal bone of inbred C57BL/Mib mice aged 3 days were grown in culture for 3 days. The following substances were added to the culture medium: 0.01 unit/ml (series I) and 0.1 unit/ml (series II) parathormone, 0.1 unit/ml (series III) and 1 unit/ml (series IV) heparin, and 0.01 unit/ml parathormone + 0.1 unit/ml heparin (series V). Resorption of bone tissue of the explants was observed in the experiments of series II and V, but not in those of series I, III, and IV. Parathormone (0.01 unit/ml), combined with heparin (0.1 unit/ml), stimulated resorption of the bone tissue of the explant (series V), whereas if added separately in the same dose it had no such action.

**KEY WORDS:** bone tissue; resorption; parathormone; heparin.

Since many endogenous factors can influence the skeletal system in the developing organism it may be very difficult to demonstrate the role of each of them separately under these conditions. By organ culture experiments the effect of various substances on developing bone tissue can be demonstrated with reasonable certainty [4-9]. The writers have shown that administration of heparin or parathormone to mice with hereditary osteopetrosis can induce resorption of bone tissue in the medullary cavity of the long bones. During combined administration of heparin and parathormone to these mice, resorption of bone tissue in the diaphysis of the long bones takes place just as in response to administration of heparin alone. This suggests that heparin is one of the components that controls the development and resorption of bone [1, 2].

To test this hypothesis and to explain the role of heparin and parathormone in this process experiments were carried out on organ cultures.

## EXPERIMENTAL METHOD

Pieces of parietal bone of inbred C57BL/Mib mice aged 3 days were grown in culture for 3 days in penicillin flasks. A medium of the following composition was used: Earle's solution 4.5 ml, medium No. 199 5 ml, bovine serum 4.5 ml, 40% glucose 0.6 ml. Each flask contained 1 ml of this medium and one explant. Culture in this medium formed the control. In experimental series I 0.01 unit/ml parathormone was added to the culture medium, 0.1 unit/ml parathormone in series II, 0.1 and 1 unit/ml heparin respectively in series III and IV, and 0.01 unit/ml parathormone and 0.1 unit/ml heparin in experimental series V. Each explant was drawn under the microscope by means of the RA-4 drawing apparatus and its area was determined. The relative change in area of the explants was calculated in per cent. The material was fixed in alcohol-formol and stained with alizarin red, toluidine blue, cresyl fast violet, and Ehrlich's hematoxylin with eosin [3]. Each experimental series consisted of 16 explants of the parietal bone. The significance of differences in growth was determined by the Kolmogorov-Smirnov criterion.

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TABLE 1. Effect of Parathormone and Heparin on Relative Growth of Parietal Bone Explant from C57BL/Mib Mice Aged 3 days after Culture for 3 days

Experimental series	Treatment	Dose (in units/ml)	Change in area of explant (in %)
I	Parathormone	0.01	-3.7
II	The same	.	-25.7
III	Heparin	0.1	-8.6
IV	"	1.0	-5.1
V	Parathormone	0.01	-14.6
	Heparin	0.1	
VI	Control		-2.5

Legend: 1. Each series consisted of 16 explants. 2. Significance of differences determined by Kolmogorov-Smirnov criterion:  $P_{VI-I} > 0.5$ ;  $P_{VI-II} < 0.01$ ;  $P_{VI-III} > 0.05$ ;  $P_{VI-IV} > 0.05$ ;  $P_{VI-V} < 0.01$ ;  $P_{I-II} < 0.01$ ;  $P_{I-V} < 0.01$ ;  $P_{II-V} = 0.05$ .

### EXPERIMENTAL RESULTS

In the control series (VI) no significant changes in bone tissue were found after culture for 3 days. The very slight resorption (decrease in area) of the explant was evidently attributable to absorption of the wound surface (Table 1). Mast cells with a few large metachromatically stained granules were observed in the bone tissue. On the addition of 0.01 unit/ml parathormone to the culture medium (experimental series I) no resorption of bone tissue was observed (Table 1). However, the granules of the mast cells became very small and optically empty and they did not stain metachromatically with toluidine blue. Parathormone in a dose of 0.1 unit/ml (experimental series II) induced the same changes in the granules of the mast cells and, in addition, intensive resorption of the explants was observed both at the borders and in the central part, together with a large increase in the number of osteoclasts.

Heparin, when added in doses of 0.1 and 1 unit/ml (experimental series III and IV respectively) did not induce any significant resorption of the explant compared with the control (Table 1). The mast cells of the explant grown in this culture medium had large metachromatically stained granules which filled all the cytoplasm.

In experimental series V 0.1 unit/ml heparin and 0.01 unit/ml parathormone were added so that their combined action could be studied. This combination of heparin and parathormone induced considerable resorption of bone tissue, practically the same as parathormone itself in a dose of 0.1 unit/ml (Table 1). The explants of this series contained more osteoclasts than those of the control series. The mast cells of these explants contained tiny metachromatically stained granules.

Change in the metachromatic staining of the granules of the mast cells in the presence of parathormone (series I and II) suggests that some components of the granules of these cells interact with parathormone and that the mast cells are an essential component in the resorption of bone tissue. Stimulation of resorption of bone tissue in organ cultures by parathormone has been described previously [8] and the results of experimental series II confirm this once again. The absence of resorption of the explant in experimental series III and IV indicates that heparin does not induce resorption of bone tissue. However, a combination of heparin (0.1 unit/ml) with parathormone (0.01 unit/ml) stimulates resorption of the bone tissue of the explant. Parathormone, if added separately in the same dose (experimental series I), had no such action.

Heparin thus activates parathormone in relation to the resorption of bone tissue but by itself it does not induce its resorption.

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