Alteration of Bone Metabolism in Tissue Culture in Response to Parathyroid Extract.* (28420)

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(Introduced by I. Zipkin)
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Since citric acid is able to solubilize bone mineral(1,2) and is found in large quantities in bone(3), it has been suggested that this anion is responsible for the demineralization phase of bone resorption(4). Although this hypothesis received support from *in vivo* studies on parathyroid hormone induced resorption, a number of *in vitro* studies on bone failed to confirm these results (see Ref. 5 for review). *In vitro* evidence compatible with this concept was obtained by analyzing the supernatant media of resorbing bone in tissue culture. Consistent increases in citric acid, calcium and phosphorus were found which paralleled the bone resorption seen morphologically(6). In the non-resorbing, control cultures lactic acid accumulated. However, this pattern of acid accumulation might have been coincidental, related more to the aerobic metabolism of the experimental cultures (high oxygen in gas phase) and the anaerobic metabolism of the control cultures (low oxygen in gas phase) than to active bone resorption *per se*.

The recent development of proceduces for obtaining marked bone resorption in tissue culture in response to parathyroid extract (PTE) (both control and experimental tubes being gassed with 50% O_2)(7,8), has led us to reinvestigate the question of citrate *vs.* lactate production by resorbing calvaria in this new system.

Methods. Whole calvaria were removed aseptically from 5- to 6-day-old Swiss albino mice of the Webster strain and placed in a Petri dish containing nutrient medium. After removal of the occipital bone the remainder of the calvarium was centered on a small rectangular coverslip and embedded in a plasma clot composed of 2 drops of heparinized chicken plasma plus 1 drop of chick embryo extract. The coverslip cultures were inserted into Leighton tubes, and to each culture was added 2 ml of supernatant medium made up of Gey's balanced salt solution, 100 units of penicillin and streptomycin in Gey's balanced salt solution and heated horse serum in a ratio of 1:1:8. Lilly parathyroid extract was added to the media of the experimental cultures in place of some of the balanced salt solution to give a final concentration of 0.5 unit/ml. All tubes were gassed briefly with a mixture of 50% O_2 + 50% N_2, stoppered and placed horizontally in a rotating drum at 37° C. The cultures were refed and gassed every 2 days.

Tubes were removed at various times and the calvaria separated from the media. The media were analyzed for calcium(9), phosphate(10), citrate(11), lactate(12), glucose (13), and hydroxyproline(14). Calvaria were weighed and placed in 1 N HCl prior to calcium, phosphorus and citrate analysis. At least 3 separate samples were analyzed at each time period.

Results. Marked differences in the composition of media obtained from control and PTE calvaria were noted (Fig. 1). By the fourth day calcium, phosphorus, citrate and lactate were significantly elevated above the control in the PTE cultures. The composition† of control media in mg % at this time was calcium 10.0 ± 0.66, phosphorus 1.32 ± 0.22, citrate 2.6 ± 0.3, lactate 97 ± 5, and glucose 50.1 ± 2.36. The media from PTE cultures contained in mg % 14.7 ± 1.0 calcium, 2.0 ± 0.18 phosphorus, 7.9 ± 0.08 citrate, 115 ± 7 lactate and 36.5 ± 3.16 glucose. At this time, PTE treated calvaria utilized more glucose (0.57 mg) than controls (0.46 mg). At later times, the differences between the two groups decreased. By

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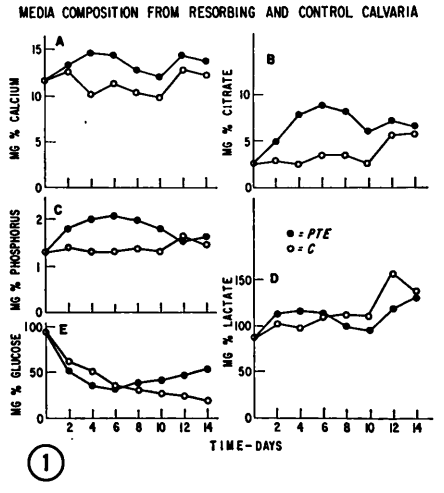
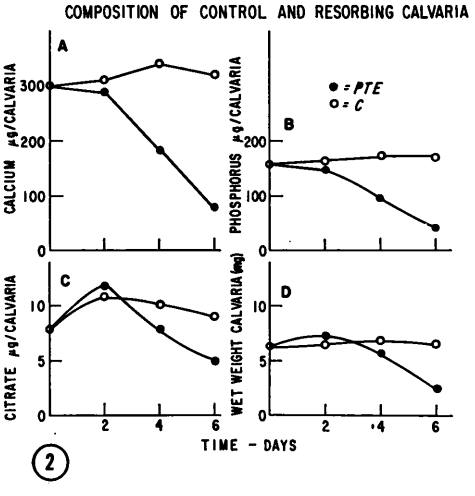
† Values given represent the mean of at least 3 analyses followed by standard error.





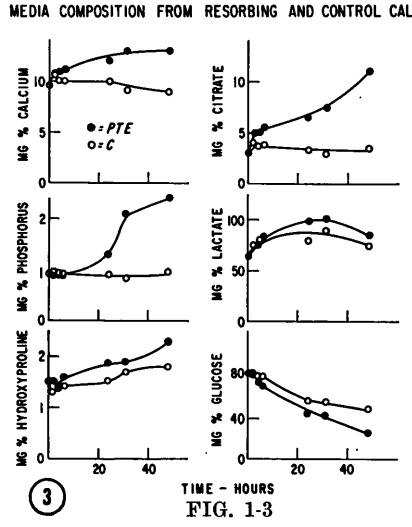


FIG. 1-3

the eighth day the control calvaria utilized more glucose and produced more lactate than did the PTE treated samples. Citrate levels were greatly increased in the media from PTE treated cultures and closely paralleled calcium and phosphate alterations. Moreover the composition of the calvaria reflected these changes in media composition (Fig 2.) Calcium, phosphorus, citrate and the weight of the calvaria decreased rapidly during the peak period of resorption (2-6 days).

To obtain data concerning the chemical changes taking place within a 48 hour culture period, media were analyzed at various intervals during the second to fourth day of culture, when calcium levels usually reached their maximum. During this period PTE treated calvaria utilized glucose and produced lactate at a greater rate than did control calvaria. After a lag period, calcium, phosphorus and citrate increased in parallel fashion in the media of PTE treated cultures. Hydroxyproline was also elevated in the media from PTE treated calvaria (Fig. 3).

Discussion. Calvaria, cultured *in vitro* in the manner described, are markedly responsive to PTE. A small dose of PTE induces rapid resorption within the calvaria. The elevation in media calcium in the resorbing cultures is similar to that observed when PTE is administered *in vivo*(2). The action of PTE on carbohydrate metabolism in these cultures is also similar to that previously observed *in vivo*(16,17). In both cases citrate and lactate levels are increased in the environmental fluids by PTE. In addition, utilization of glucose by bone is also increased in both cases by parathyroid hormone. It is of particular interest that the effects of PTE on carbohydrate metabolism appeared to precede changes in calcium and phosphate.

The lag phase preceding mineral mobilization during the first 6 hours after refeeding the cultures is possibly related to the period needed for "conditioning" of the medium (15). The difference between this study and previous *in vitro* studies which have been largely limited to short incubation times could be related in part to this fact.

The increase in media citrate in the cul-

tures receiving PTE are due to the synthesis and subsequent release of citrate from the bone cells rather than release of citrate from bone mineral, since more than 40 times the original content of citrate in the calvaria appeared in the media. These results are consistent with the theory that the mobilization of mineral during bone resorption is brought about by the increased accumulation of metabolic acids(16). Both of the acids examined here (citrate and lactate) increased in the media in parallel to calcium and phosphate in the PTE cultures. Of the two, it seems likely that citrate plays a greater role than lactate since lactate also increased markedly in control cultures where calcium levels remained constant. Similarly, Kenny(18) has found that epinephrine increases lactate production by bone *in vitro* without changing mineral metabolism. However, it is possible that acid production (as indicated by citrate and lactate levels) may be additive to the solubilizing action of the citrate ion, since citrate solubilizes hydroxyapatite (and bone) by a variety of mechanisms including chelation of calcium and the displacement of phosphate from crystal surfaces. The increases in media hydroxyproline in the experimental cultures, provide chemical evidence that the organic matrix is destroyed during the process of resorption in our system. However, from these data it is not possible to state with certainty that removal of matrix coincides with dissolution of mineral.

Summary. The metabolism of mouse calvaria grown in tissue culture is markedly altered by parathyroid hormone. Accompanying the resorption of bone in the parathyroid hormone treated culture is an increased production of citrate. An enhanced production of lactate occurred during the first 4 days of the experiment which appeared to reflect an increased utilization of glucose during this period. Increased amounts of hydroxyproline were also found in the media indicating that the organic matrix was being destroyed. These results are consistent with the theory that the mobilization of mineral during bone resorption is brought about by the increased accumulation of metabolic acids. Of the 2 acids examined here, citrate would appear to play a greater role since it more closely paralleled mineral dissolution.

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