conditions of oxygenation and of anoxia are given for the different sample positions and pion doses. This ratio between the damage observed after the same dose under 2 conditions is not a value of OER but in the absence of complete dose-response curves provides a realistic approximation[16]. In the 1.0 Gy peak and plateau positions results are inconclusive because of the limited number of cells which can be analyzed. However for the 3.0 Gy samples the plateau value of 3.7 for the oxygenated:anoxic ratio is significantly greater than the peak ratio of 1.9. This peak ratio of damage agrees very well with a value of 1.7–1.9 obtained for the ratio of chromatid aberrations in *Vicia faba* root meristem cells irradiated with pions under similar conditions[3].

Positions in the phantom were chosen such that plateau and post-peak doses were approximately equal. The average peak:plateau ionization ratio was 1.55 and the average peak:post peak ionization ratio was 1.59. At the dose levels achieved in the plateau (1.94 Gy and 0.65 Gy) OER values for 250 KVp X-rays of 3.2 and 4.1 would be expected[11]. Comparison of these expected values with the observed damage ratios in table 2 (3.7 and 4.0) leads to the conclusion that the plateau radiation is of comparable OER to 250 KVp X-rays implying a similar LET to X-rays. The ratios given in table 2 show that damage in the post-peak however is significantly less affected by the presence of oxygen; suggesting that the radiation in this part of the beam is of high LET as observed with *Vicia faba* at CERN[13] and, on the pion beam from NIMROD, with HeLa cells[14,15].

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Normal osteoclast number and function in rat pups lacking parathyroid hormone[1]

M. Krukowski and A.J. Kahn

Department of Biology, Washington University, St. Louis (MO 63130), and Division of Bone and Mineral Metabolism, School of Dental Medicine, Washington University, St. Louis (MO 63110, USA), 25 October 1979

Summary. In parathyroidectomized suckling rats, bone modeling and the number and activity of cells in the osteoclast population are normal. These findings are at variance with observations in older animals and suggest that factors other than parathyroid hormone influence osteoclast formation and function in the neonate.

Levels of circulating parathyroid hormone are low before birth[2–5] yet fetal bone contains substantial numbers of osteoclasts which appear active in the modeling process in vivo, and are responsive to parathyroid hormone in vitro[6]. By contrast, hypoparathyroidism in young and adult animals results in a pronounced decrease in osteoclast number and function[7–10]. Precisely when during development the osteoclast population becomes dependent upon parathyroid hormone has not been established. The present study was undertaken to determine whether the transition to hormone dependency occurs during the suckling period.

Materials and methods. Parathyroid glands were removed by dissection from 1-day-old rat pups under cold anesthesia. Sham-operated littermates served as controls. Following surgery, the pups were returned to their mothers and nursed until sacrificed at 14 or 19 days of age. At sacrifice, blood was collected for analysis of calcium[11] and phosphorus[12], and femurs and trachea-thyroid complexes were removed for histological evaluation. Tissues were fixed in 10% neutral formalin, and stored in 5% neutral formalin until embedded. For electron microscopy, bones were demineralized in 7.5% EDTA in 0.1 M phosphate buffer overnight, placed in 3% gluteraldehyde in phosphate buffer for 1 h, and postfixed in OsO_4 in 0.1 M cacodylate buffer for an additional h. They were then washed in buffer, dehydrated in alcohols, and embedded in Araldite 502. Thin sections were cut on an LKB ultramicrotome and stained with uranyl acetate and lead citrate. For light microscopy, femurs were demineralized in 7.5% EDTA in 5% formalin, and embedded in paraffin. Trachea-thyroid complexes were similarly embedded and serially sectioned to ascertain completeness of parathyroidectomy. The 5 µm sections were stained with hematoxylin and eosin. Osteoclast quantitation was done using a Merz-Schenk grid at a magnification $\times 450$. The metaphyseal region of each bone was sequentially scanned and the number of osteoclasts and the amount of bone per field was determined. The results were expressed as osteoclasts/mm^2 bone. The number of nuclei/osteoclast was also established.

Results. Successfully parathyroidectomized (Ptx) animals showed no evidence of residual parathyroid tissue in trachea-thyroid sections, and were characteristically hypocalcemic and hyperphosphatemic. Mean blood calcium for Ptx pups was 7.1 mg/dl (range 6.4–8.4) as compared to 10.9 mg/dl (range 9.8–12.6) for sham-operated littermates; mean Ptx blood phosphorus was 17.9 mg/dl (range 15.6–21.0) vs 10.4 mg/dl (range 8.8–11.6) for controls. Despite the absence of parathyroid hormone, histomorphometric analysis of bone sections revealed that osteoclast number and size (i.e., No. nuclei/cell) were comparable in normal

and Ptx pups. The average number of osteoclasts in Ptx animals was 7.54 cells/mm^2 bone (range 6.41–9.09) as compared to 7.37 osteoclasts/mm^2 bone (range 5.10–10.18) in controls. Similarly, the average number of nuclei/osteoclast was essentially the same for both treated and control animals being 2.99 (range 2.55–3.20) for normal pups vs 2.80 (range 2.48–3.18) for Ptx animals. The osteoclasts in bones of Ptx pups were typically juxtaposed to bone surfaces and displayed pronounced cytoplasmic vacuolation that was apparent even at the light microscope level (figure 1). Moreover, at the electron microscope level, these cells showed the clear zone and ruffled membrane characteristic of osteoclasts actively engaged in resorption[13] (figure 2). That the osteoclasts of the Ptx pups were indeed active in bone matrix degradation was borne out by the normal trabecular morphology and volume of bone in the treated animals.

Discussion. A considerable body of evidence has contributed to the conclusion that the osteoclast population, both in size and level of activity, is dependent upon parathyroid hormone. For example, parathyroidectomy in young and adult rodents and in humans produces a marked reduction in the osteoclast population and a diminution in resorptive function[7-10]. Moreover, administration of exogenous parathyroid hormone to aparathyroid animals restores both osteoclast number and activity[6,9]. Similarly, hyperparathyroidism is characterized by among other things, large numbers of highly active osteoclasts[14]. In the present study, we have shown that removal of the parathyroid glands from suckling rats evokes no apparent change in either the size or function of the osteoclast population. That the glands normally produce biologically active hormone during this period is demonstrated by the marked and sustained changes in blood calcium and phosphorus levels resulting from parathyroidectomy. That the osteoclasts of young (prenatal) animals are capable of responding to parathyroid hormone has been repeatedly demonstrated in vitro[15,16]. What then, if not parathyroid hormone, influences osteoclast formation and function in fetal and neonatal animals? Bone matrix and bone matrix constituents have been shown to be chemotactic for monocytes[17,18], the putative osteoclast precursor[19,20], and appear to be essential for osteoclast differentiation[21]. Moreover, both lymphocytes and monocytes (macrophages) synthesize and release agents capable of stimulating osteoclast function: Lymphocytes produce osteoclast activating factor[22] (OAF) and monocytes prostaglandins of the E series[23,24]. We suggest, therefore, that in the fetus and neonate, the osteoclast population is being influenced by factors which are intraosseous in origin, and arise from the extracellular calcified matrix (bone or cartilage) and/or from marrow lymphocytes and monocytes.

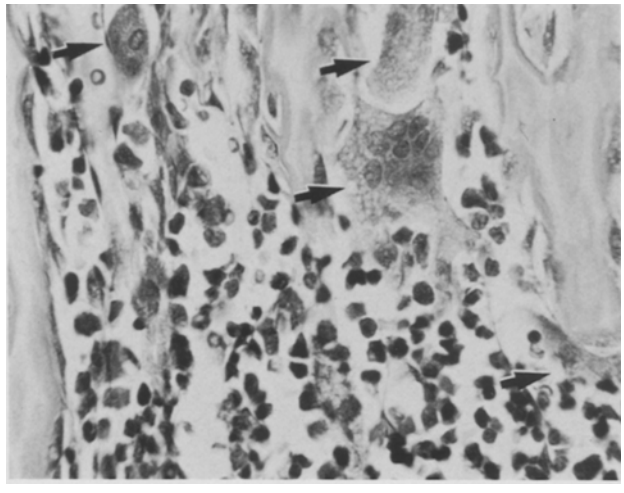

Fig. 1. Typical osteoclasts (arrows) as they appear in bone of 19-day-old parathyroidectomized rat pup. Paraffin embedded section stained with hematoxylin-eosin. × 440.

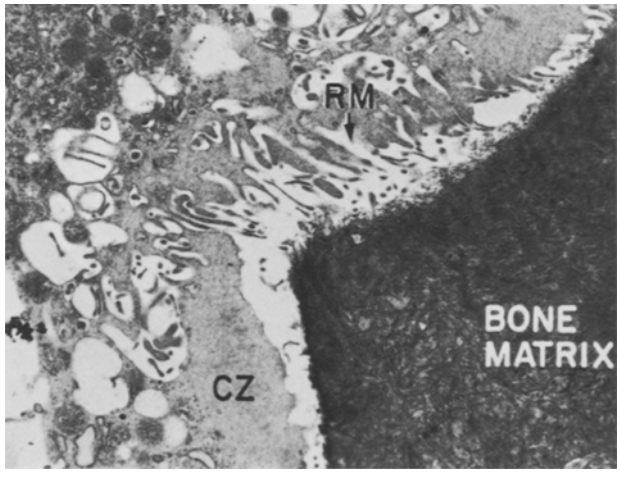

Fig. 2. Active osteoclast with clear zone (CZ) and ruffled membrane (RM). 14-day-old parathyroidectomized rat pup. Plastic embedded section stained with uranyl acetate and lead citrate. × 8800.

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