Ultrasound and Lysosomal Histochemistry of ia Rat Osteoclasts

B. H. Schofield
Division of Orthopaedic Surgery, Johns Hopkins University, School of Medicine

L. Stefan Levin
Division of Medical Genetics, Johns Hopkins University, School of Medicine

S. B. Doty
Division of Orthopaedic Surgery, Johns Hopkins University, School of Medicine

Received May 25, accepted October 16, 1973

Histochemical techniques for light and electron microscopy showed that metaphyseal osteoclasts in "incisors absent" rats contained greater than normal amounts of lysosomal acid phosphatase, aryl sulfatase and acid trimetaphosphatase. Lysosomal phosphatase activity at neutral pH was also elevated in the metaphyseal osteoclasts except in those cells immediately beneath the growth plate, where this enzyme was absent. The failure of any discernable resorption of organic matrix appeared to correlate with the absence of a ruffled border and a concomitant absence of extracellular lysosomal enzyme. Despite this failure, electron microscopic evidence of inorganic crystal removal was noted, suggesting that mineral dissolution represents a separate process from the enzymatic breakdown of organic matrix.

Key words: Lysosomes -- Bone -- Resorption -- Osteoclast.

Introduction

The ia strain of rats is characterized by a defect in the bone resorptive process inherited as an autosomal recessive trait (Greep, 1941). It has been postulated that...
this phenomenon is due to a failure of normal osteoclastic activity (Schour et al., 1949). Bhaskar et al. (1950) have reported that failure of resorption is a transient phenomenon, beginning shortly before birth. They have shown radiographic evidence of resorption in the tibia beginning at 30 days of age; by 283 days, the tibia appears normal. These affected animals therefore, provide a model for the correlation of the structure and enzymatic activity of osteoclasts with the mechanisms of calcified tissue resorption.

Material and Methods

Thirty male homozygous \( ia \) rats between the ages of 17 and 24 days of age were starved for 24 h prior to killing by ether. The proximal portions of the tibiae and the distal portions of the femora were immediately removed, split lengthwise, and fixed in 6% cacodylate-buffered glutaraldehyde for 3 h at 4°C. Samples of metaphyseal bone were postfixed in 1% osmium tetroxide and embedded in Epon 812 for electron microscopic examination. The remaining tissue was decalcified in EDTA, and 36 \( \mu \) thick frozen sections were stained for the demonstration of lysosomal acid phosphatase (Gomori, 1950); "neutral" phosphatase at pH 7.4 using ATP and \( p \)-nitrophenylphosphate as substrates (Doty and Schofield, 1972); acid trimetaphosphatase (Berg, 1960); and aryl sulfatase (Hopsu-Havu et al., 1967). In addition serum calcium was measured at the time of sacrifice.

Sprague-Dawley rats of comparable age and sex were used as controls.

Results

Osteoclasts in \( ia \) rat metaphysis were increased in number and exhibited greater than normal acid phosphatase activity as has been previously shown (Handelman et al., 1967; Marks, 1973). In addition, these cells were more reactive for acid trimetaphosphatase and aryl sulfatase than normal controls (Figs. 1 and 2). "Neutral" phosphatase activity was also greater than normal with the exception of those osteoclasts immediately under the growth plate. In those cells, lysosomal staining was strikingly absent (Fig. 3) when compared with normal osteoclasts (Fig. 4).

Electron microscopic examination revealed marked differences in osteoclast morphology between normal and \( ia \) rats. Active osteoclasts in metaphyseal regions of control animals exhibited characteristic ruffled border cell surface specializations (Fig. 5). Immediately adjacent to the ruffled border, normal osteoclasts contained large number of dense bodies and vacuoles which previously have been shown to be histochemically reactive for the various lysosomal enzymes. Reaction product was also present on bone matrix in contact with the normal ruffled border

Fig. 1. Femoral metaphysis from a 23-day-old normal rat stained for aryl sulphatase. Incubated for 20 min at 37°C. \( GP \) growth plate cartilage, \( \times \) 70

Fig. 2. Femoral metaphysis from a 23-day old \( ia \) rat stained for aryl sulphatase. Incubated for 20 min at 37°C. \( GP \) growth plate cartilage, \( \times \) 70

Fig. 3. Femoral metaphysis from a 23-day-old \( ia \) rat metaphysis. Neutral phosphatase staining is not present in osteoclasts immediately beneath growth plate. Incubated 20 min at 37°C. \( GP \) growth plate; \( \times \) 120

Fig. 4. Femoral metaphysis from a 23-day old normal rat. Neutral phosphatase activity is intense in osteoclasts immediately beneath growth plate. Incubated for 20 min 37°C. \( GP \) growth plate, \( \times \) 120

Figs. 1–4 are photomicrographs