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Morphological and Histochemical Observations on the Lack of Osteoclasis in the "tl" Strain of Rat[1,2] (38396)

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(Introduced by H. J. Keene)

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A spontaneous mutation has been recently described in a colony of Osborne–Mendel rats which may provide a unique model for the study of calcified tissue (1). The animals (designated TLOM/Ndri) are characterized by retarded growth, normal longevity, osteopetrosis, unerupted dentition, extramedullary hemopoiesis and periorbital encrustations. Preliminary evidence indicates that "tl" bone is characterized by a lack of physiological resorption.

The "tl" rat is similar to the "ia" rat (1), except that in the latter there is a transitory lack of bone resorption after which there is a partial eruption of teeth (2). The osteopetrotic bone of the "tl" and "ia" rats appears to be histologically similar, except that the "tl" bone appears devoid of osteoclasts (1), while the "ia" bone has been found to display osteoclasts in normal or increased numbers (3–8). The present study was undertaken in an attempt to identify osteoclasts that may be present within "tl" bone, by the application of histochemical methods.

Alveolar and long bones were obtained from a 275-day-old "tl" rat and from two normal Osborne–Mendel animals of 256 and 286 days of age. The animals were killed by an anesthetic overdose and hard tissue specimens were removed and fixed immediately. All tissues were fixed for 3 hr in cold 4% glyoxal in 0.2 *M* cacodylate buffer and rinsed in chilled Holt's sucrose solution. Specimens were decalcified in buffered EDTA after Fullmer and Link (9). Frozen sections were cut in a cryostat, picked up on clean dry slides and either incubated directly or placed in slide boxes, wrapped in plastic and stored at 0–4°. Some sections were stained with H&E. Acid phosphatase activity was demonstrated by Barka's (10) azo-dye method using either naphthol AS-BI or AS-TR phosphate as substrate and hexazonium pararosaniline as coupler. For *B*-glucuronidase, sections were incubated in media prepared as described by Hayashi *et al.* (11). Tissues from normal animals were processed and incubated simultaneously with "tl" specimens. Control sections were prepared by incubating some sections in media with the substrates omitted.

In normal bone sections, osteoclasts were readily identifiable by their conspicuous display of intense acid phosphatase or *B*-glucuronidase activity (Figs. 1 and 2). The histochemical reaction product appeared as a diffuse cytoplasmic staining for both enzymes. Reactive osteoclasts were observed along periosteal and endosteal surfaces and vascular channels of the maxillary and mandibular bones (Fig. 1). Reactive osteoclasts were observed in normal long bones along the scalloped periosteal surface of proximal metaphyses and to a lesser extent along endosteal surfaces (Fig. 2). Both the acid phosphatase and *B*-glucuronidase reactions appeared to provide a selective method for the visualization and identification of osteoclasts in normal bone sections.

Osteoclasts were not observed in decalcified bone sections from "tl" specimens. Acid phosphatase and *B*-glucuronidase activity was demonstrable in other cells (*e.g.*, neurones, granulocytes) and in certain areas of the bone matrix in "tl" specimens, but osteoclasts were not observed (Fig. 3).

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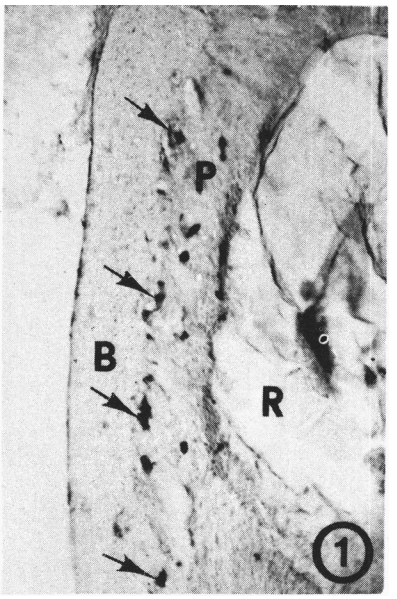

FIG. 1. Section of normal rat maxilla stained for the demonstration of *B*-glucuronidase activity. Reactive osteoclasts (arrows) are conspicuous along the inner surface of the alveolar crypt. B = bone, P = periodontal membrane, R = root portion of a molar tooth (×90).

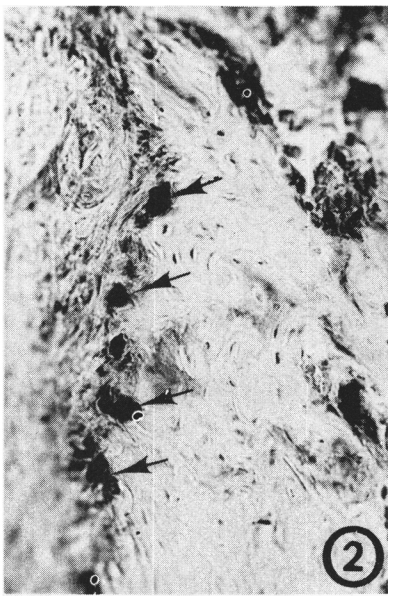

FIG. 2. Metaphyseal region of a normal rat femur. Osteoclasts (arrows) display intense acid phosphatase activity throughout their cytoplasm (×200).

Based upon the inability to identify osteoclasts histochemically, it would appear that a normal osteoclast cell population is not a component of the osteopetrotic tissues in the adult "tl" rat. This tends to support previous observations based on morphologic impression alone. Continued study of additional animals will be required to ascertain whether this mutation offers a unique model for calcified tissue research by virtue of the animal's normal longevity and apparent lack of osteoclasis.

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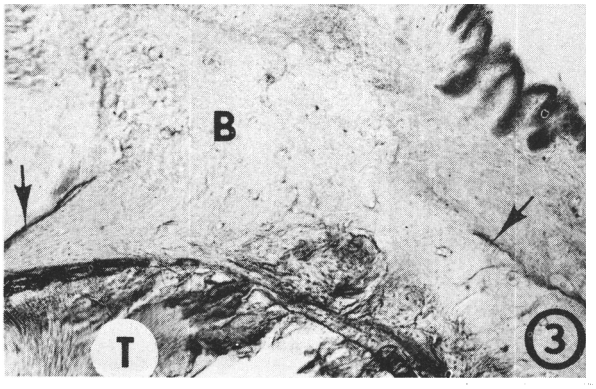

FIG. 3. The distribution of acid phosphatase activity in a section of "tl" rat mandible. Although enzyme activity is evident along portions of the periosteal surface (arrows), osteoclasts are not observed. B = alveolar bone, T = unerupted tooth (×60).

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