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Microscopic and Histochemical Study of Odontoclasts in Physiologic Resorption of Teeth of the Polyphyodont Lizard, *Liolaemus gravenhorsti*

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ABSTRACT Using tartrate-resistant acid phosphatase (TRAP), we examined the cytodifferentiation of odontoclast cells in resorbing areas of dental tissues during the replacement of teeth in a polyphyodont lizard, *Liolaemus gravenhorsti*. We also report, by means of Lectin-HRP histochemistry, the distribution pattern of some specific sugar residues of TRAPase-positive cells. For detection of TRAPase activity, the azo dye-coupling technique was used. Lectin binding sites were demonstrated by means of specific HRP-lectins. The process of tooth resorption was divided into four stages: 1) preresorption—the wall of the dental pulp is covered with an odontoblast layer, and no TRAP-positive cells are in the dental pulp; 2) early resorption—TRAP-positive multinucleate odontoclasts are present on the dental wall, but the rest of the pulp surface is still covered with an odontoblast layer; 3) later resorption—the entire surface of the pulp chamber is lined with multinucleate odontoclasts; and 4) final resorption—the tooth has been totally resorbed. Odontoclasts are usually detached from the resorbed surface, and show signs of degeneration. Of the six lectins used, PNA, ECA, and UEA-1 bind to multinucleated but not mononuclear cells. All the remaining lectins, BS-1, RCA_{120}, and LTA showed no binding to any cells of the teeth. The significance of saccharidic moieties such as acetyl-galactosamine, acetyl-glucosamine, and fucose sugar residues is difficult to ascertain. Perhaps these oligosaccharides might be borne on molecules associated with odontoclastic resorption or associated with multinucleation of odontoclasts after attachment to the dentine surface. J. Morphol. 242:295–309, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: odontoclasts; TRAPase-positive cells; HRP-lectins; polyphyodont; lizard

Development of tooth form in nonmammalian vertebrates has received little attention. Experimental research of tooth development is largely based on mammals (especially murid rodents). The dentition of the mouse, with its highly specialized incisors, small number of teeth, absence of tooth-replacement, and distinctive molar patterns is by no means typical of mammals in general. Likewise, mammalian dentition diverges in many ways from that of other vertebrates. Thus, extrapolation from the mouse to vertebrates in general must be made with caution; it is highly desirable that results obtained from mammals should be tested on other animals (Butler, '95). With regard to tooth succession, most vertebrates are polyphyodonts, replacing teeth continuously throughout life. The search for differences and similarities between polyphyodonts and diphyodonts is very important. The odontoclasts (multinucleated cells) are

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*TABLE 1. Lectin characteristics**

Lectin	Carbohydrate binding specificity	Inhibitory sugar
Bandeireae simplifolia BS 1	αGalNac > α Gal	GalNac; D-Gal; βLactose
Arachis hypogea (peanut) PNA	Gal β1–3 Gal Nac > α and β Gal	GalNac; D-Gal; βLactose
Ricinus communis (Castor bea) RCA 120	βGal > αGal ≫ GalNAc	GalNac; D-Gal; βLactose
Erythrina cristagally (Coral tree) ECA	Gal β1–4GlcNac	GalNac; D-Gal; βLactose
Ulex europeaus (Gorse seed) UEA 1	αL-Fuc	αL-Fuc
Lotus tetragonolobus purpureas (Asparagus pea) LTA	αL-Fuc	αL-Fuc

*GalNac = N-acetyl-galactosamine; GlcNac = N-acetyl-glucosamine; Gal = galactose; Fuc = fucose.

mainly involved in resorption of dental hard tissues. They are generally considered to be the same cell type as osteoclasts that resorb bone, since both clastic cells have the same ultrastructural and functional characteristics (Tanaka et al., '90; Matsuda, '92; Sahara et al., '92, '94). Although numerous in vivo and in vitro studies have been reported concerning the origin and differentiation of osteoclasts (see reviews by Mundy and Rodman, '87; Marks and Popoff, '88), little information is available on odontoclast precursors or their differentiation into functional odontoclasts (Sasaki et al., '89a; Sahara et al., '96). TRAPase is an enzyme-histochemical marker that can be used to detect odontoclasts (Sahara et al., '96) and osteoclasts and their precursors (Minkin, '82; Hammarström et al., '83; Baron et al., '86; van de Wijngaert and Burger, '86). Lectins that bind to specific sugar residues also have been used as histochemical reagents for the demonstration of carbohydrates associated with cell surfaces, as well as associated with cytoplasmic organelles. Some studies have been performed on the characterization, distribution, and significance of glycoconjugates in the teeth of embryonic and adult animals (Meyer et al., '81; Sasano et al., '92; Lemus et al., '94, '96, '97).

In this study, using tartrate-resistant acid phosphatase (TRAP), we examined the cytodifferentiation of odontoclast cells on resorbing areas of dental tissues during the replacement of teeth in a polyphyodont lizard. In addition, we also investigated the binding pattern of specific sugar residues of (TRAPase)-positive cells using lectins conjugated to horseradish peroxidase.

MATERIALS AND METHODS

Adult *Liolaemus gravenhorsti* were collected in the foothills of the Andes mountains near Santiago, Chile. Twelve specimens were employed in this study. Lizards were killed by decapitation and their mandibular arches were dissected.

Histochemical demonstration of TRAP activity

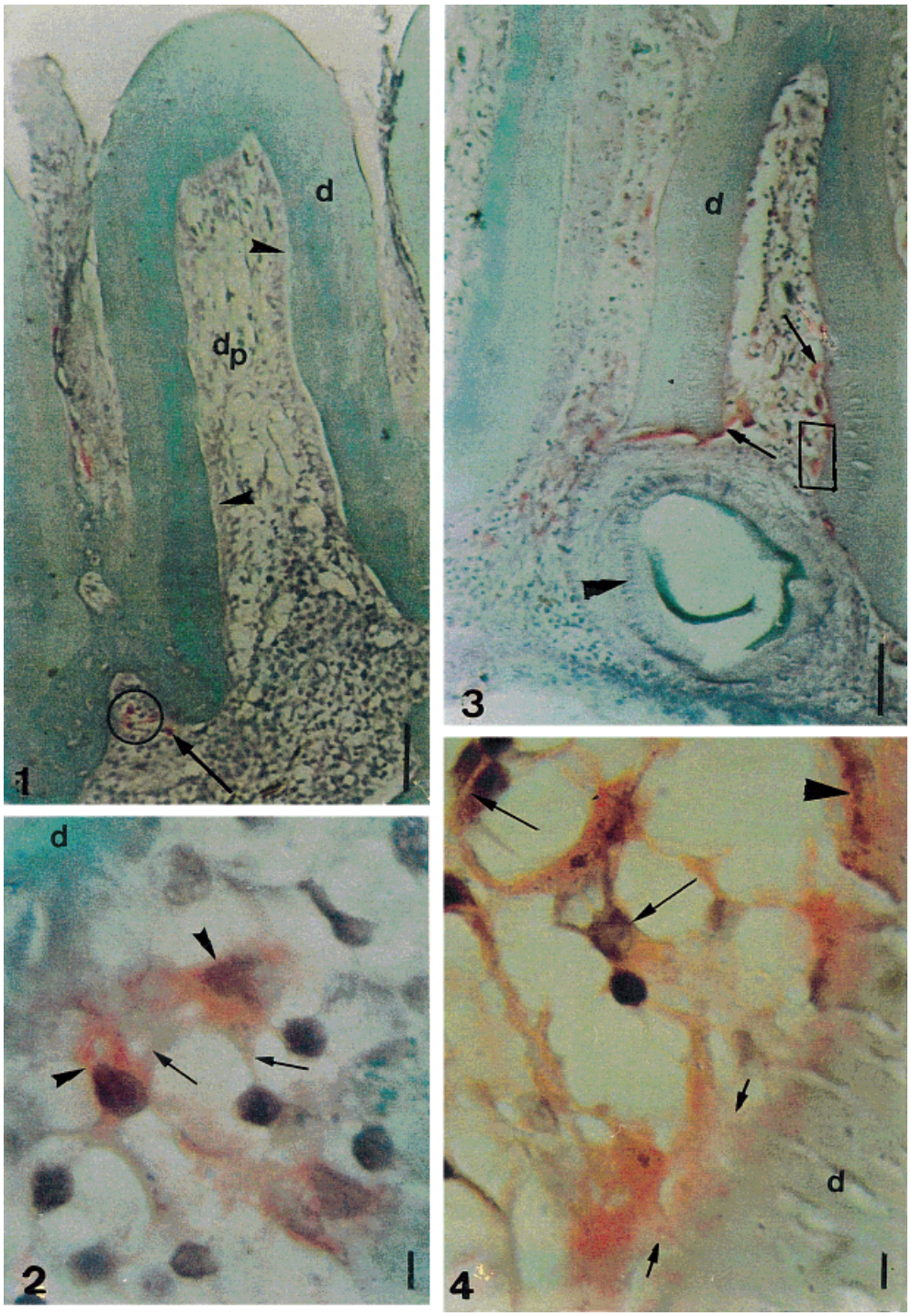
Mandibles were fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, for 12 h at 4°C. The specimens were demineralized in 10% EDTA buffered with 0.1 M sodium cacodylate, pH 7.4, for 5 days. The mandibles were dehydrated in a graded series of ethanol, cleared with xylene, and embedded in paraffin. The paraffin-embedded tissues were stored at −20°C until use. Sections were cut at 5 μm thickness and mounted on silaized glass slides. For the detection of TRAPase activity in the deparaffinized sections, an azo dye-coupling technique was used (modified from Sakakura et al., '98). The incubation medium contained 4 mg of naphthol AS-MX phosphate (N-4875; Sigma, St. Louis, MO) in 0.25 ml of N,N′-dimethyl formamide, followed by addition of

Fig. 1. *Liolaemus gravenhorsti*. Preresorption stage. TRAP-positive cells are present in the dental pulp. The dentine surface is covered with the odontoblasts layer (arrowheads). Long arrows point to TRAP-positive mononuclear cells outside the dental pulp; d, dentine; dp, dental pulp. TRAP-reaction and Meyer's hematoxylin and fast green staining. Scale bar = 150 μm.

Fig. 2. Higher magnification of the area enclosed by the circle in Figure 1. Arrowheads point to TRAP-positive mononuclear cells. These cells show long cytoplasmic cell processes (arrows); d, dentine. TRAP-reaction and Meyer's hematoxylin and fast green staining. Scale bar = 4 μm.

Fig. 3. Early resorption stage. TRAP-positive cells in dental pulp being in contact with the dentine. (arrows). Arrowhead points to tooth rudiment germ. The area indicated by the rectangle is magnified in Figure 4. TRAP-reaction and Meyer's hematoxylin and fast green staining. Scale bar = 150 μm.

Fig. 4. Mononuclear cells (long arrows) with long cytoplasmic cell processes and intense staining for TRAP fused with either mononuclear cells or multinucleate odontoclasts (arrowheads) attached to the dentine surface. Short arrows point to ruffled border; d, dentine. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 4 μm.



Figures 1–4

50 ml of 0.2 M acetate buffer, pH 5.0, containing 30 mg of Fast Red TR salt (F-8764; Sigma) as the coupling agent, and 50 mM sodium tartrate (L+) was then added to this medium. Sections were incubated in the above medium at 36°C for 15 min. After incubation, the sections were washed in running water and counterstained with Mayer's hematoxylin and fast green FCF 0.02%.

Lectin histochemistry

Jaws were fixed in Carnoy's fixative, followed by decalcification in 5% EDTA buffered with 0.01 M phosphate-buffered saline (PBS), pH 7.4, at 4°C for 3 weeks. Following ethanol dehydration, tissues were embedded in paraffin. Serial sections (5 µg) were cut parallel to the long axis of the jaw and processed for lectin histochemistry. After hydration, sections were treated with 0.3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase, rinsed in distilled water, and washed with 1% bovine serum albumin (BSA) in 0.1 M PBS, pH 7.2. The sections were then incubated for 30 min at room temperature in a series of HRP-conjugated lectins: (BS-1 *Bandeiraea simplicifolia*, PNA *Peanut agglutinin*, RCA *Ricinus communis* Castor bean, ECA *Erithrina cristagalli*, UEA-1 *Ulex europaeus agglutinin*, and LTA *Lotus tetragonolobus purpureas*). Each lectin was dissolved in 0.1 M PBS, pH 7.2, containing 0.1 M NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, and 0.1 mM $MnCl_2$. The sections were then rinsed three times in PBS and incubated for 10 min at room temperature in PBS (pH 7.0) containing 3,3′-diaminobenzidine (DAB) (25 mg/dl) and 0.003% hydrogen peroxide. The specimens were rinsed in distilled water, dehydrated using graded ethanol solutions, cleared in xylene, and mounted in Permount. The optimal concentration of each lectin (Sigma), which allowed maximum staining with minimum background, was: BS-1 20 µg/ml, PNA 20 µg/ml, RCA 20 µg/ml, ECA-1 25 µg/ml, UEA 25 µg/ml, and LTA 25 µg/ml. The sugar-binding specificity of each lectin is shown in Table 1.

Control experiments

To confirm the binding specificity of a lectin for a particular sugar, 0.1 M of an appropriate competing sugar (Table 1) was added to the solution of each lectin and allowed to react for 2 h at room temperature prior to use. The lectin N-acetyl-galactosamine (GalNAc) was used as an inhibitory sugar for BS-1, PNA, and fucose; (aL-Fuc) was used for UEA-1 and LTA. Under these conditions, either decreased staining or inhibition of staining was considered evidence of specific binding of the lectin to the carbohydrate moiety in question.

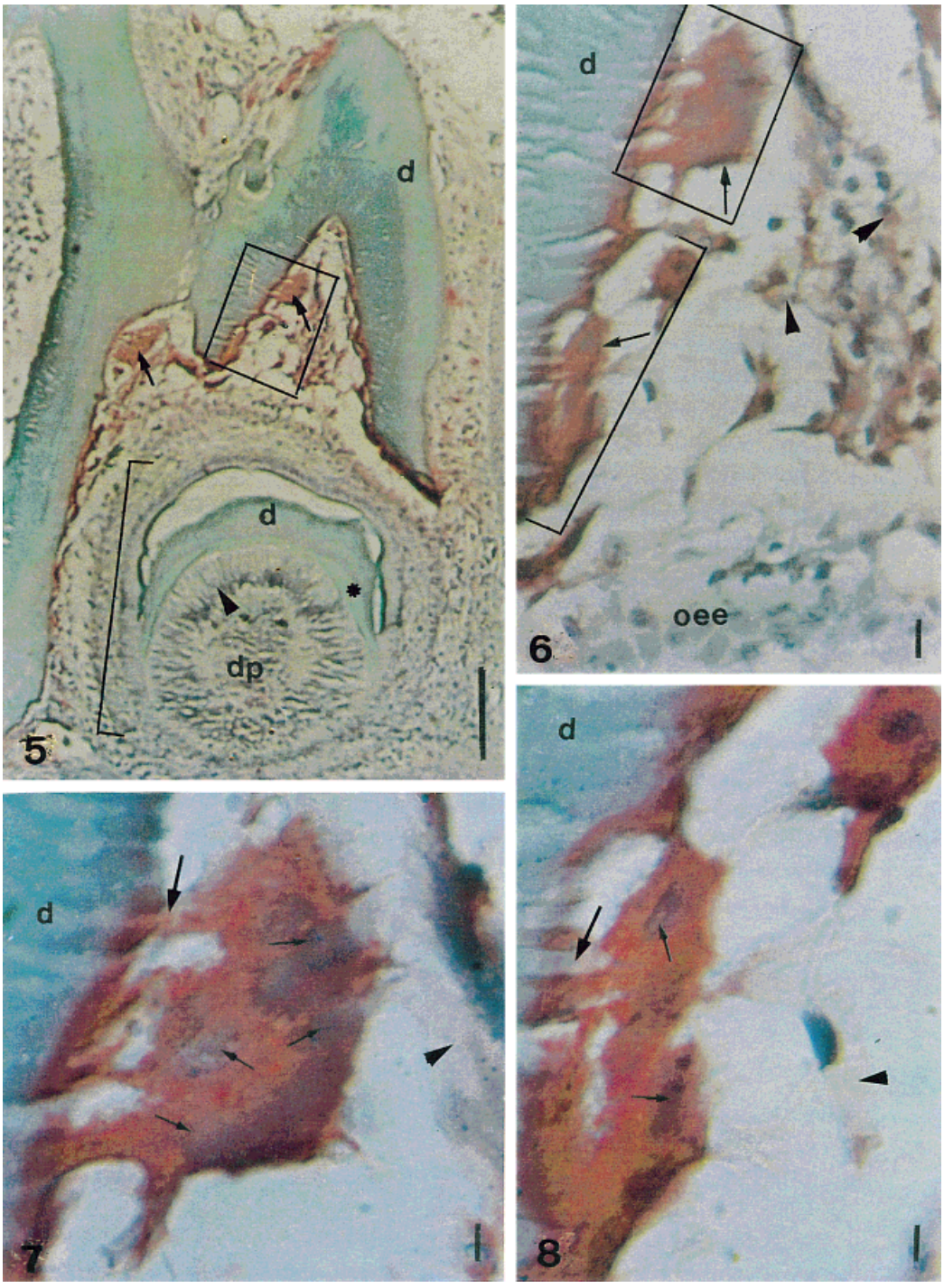
RESULTS

We divided the process of resorption into four stages by following histological criteria as previously reported by Sahara et al. ('92, '96): 1) preresorption stage—the wall of the dental pulp is covered with an odontoblast layer, and no TRAP-positive cells are present in the dental pulp; 2) early resorption stage—TRAP-positive multinucleate odontoclasts are present on the dental pulp wall, but the rest of the pulpar surface is still covered with an odontoblast layer; 3) later resorption—no odontoblast layer is present; the entire surface of the pulp chamber is lined with multinucleate odontoclasts; and 4) final resorption stage—the tooth has been totally resorbed. During the preresorption stage, staining for TRAP was negative in the dental pulp. The pulpal tissues are intact, and the dentine surface is covered with the odontoblast layer (Fig. 1). TRAP-positive mononuclear cells were detected outside the

Fig. 5. *Liolaemus gravenhorsti*. TRAP reaction of the internal resorption of lizard *L. gravenhorsti* teeth illustrating the histological characteristics in later resorption stage. Arrows point to TRAP-positive multinucleate odontoclasts. The area indicated by the bracket shows a replacement tooth. Arrowhead points to odontoblast; d, dentine; dp, dental pulp. Asterisk marks predentine. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 150 µm.

Fig. 6. Higher magnification of the areas encompassed by the rectangle in Figure 5. Arrows point to multinucleate odontoclasts that possess ruffled borders directed toward the resorbed dentine surface. Arrowheads point to TRAP-positive mononuclear cells in the pulp chamber. They are irregular in appearance, often with long cytoplasmic cell processes. Higher magnification of the areas indicated by the rectangle and by the bracket in Figure 6 are shown in Figures 7 and 8, respectively; d, dentine; oee, outer enamel epithelium. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 12 µm.

Figs. 7, 8. The TRAP-positive multinucleate odontoclasts on the dentine surface increased in cell size as well as in the number of nuclei per cell. Short arrows point to nuclei. Long arrow points to the ruffled border and their resorbing regions. Arrowheads point to odontoclasts with diffuse staining of TRAP in close proximity to the multinucleate odontoclasts; d, dentine. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 5 µm.



Figures 5–8

dental pulp. These cells are irregular in appearance, with long cytoplasmic cell processes, and stain intensely for TRAP (Fig. 2). At the early resorption stage, TRAP-positive multinucleate cells in the dental pulp are in contact with dentine, and TRAP-positive mononuclear cells are in close proximity to the multinucleate odontoclasts on the resorption surface. Intense TRAP activity is localized in the mononuclear cells as well as in the multinucleate odontoclasts (Fig. 3). In this early resorption stage, we observed that multinucleation of the odontoclasts frequently occurred on the dentine resorption surface. Mononuclear cells fused with either mononuclear cells or multinucleate odontoclasts attached to the dentine surface (Fig. 4). These multinucleate odontoclasts have ruffled borders at their resorbing regions and fuse with each other by their basolateral membranes (Fig. 4). Multinucleation was never observed between mononuclear cells in the central pulp apart from the resorption surface. Furthermore, in the early and later resorption stages, no multinucleated cells away from the resorption surface could be observed (Figs. 4–8). At the final stage of resorption, multinucleate odontoclasts showed a distinctive histological configuration. Light microscopical examination revealed that most odontoclasts at this stage were usually rounded up and appeared to be connected to the resorbed surface by only a narrow, neck-like band of cytoplasm (Figs. 9–14). Odontoclasts in the final stage of resorption showed a prominent reduction in the area of ruffled border against the resorbed dentine surface. TRAPase-positive cells were observed in lizard teeth (odontoclasts, Figs. 1–14), and in lizard bone (osteoclasts, Fig. 15).

Of the six different lectins used, only PNA, ECA, and UEA-1 showed binding to multinucleate odontoclast cells. All the remaining lectins (BS-1, RCA^{120}, and LTA) showed no binding to any region of the dental tissues. Lectin activity in all resorption stages is summarized in Table 2. PNA-HRP binding sites (affinity for Galβ1–3GalNAc and α and β Gal) were detected, especially in areas of multinucleate odontoclast cells (Fig. 16). At higher magnifications, intense staining of the cytoplasm of these cells was clearly observable (Figs. 17, 18).

After exposure of sections to PNA-HRP conjugate containing N-acetylgalactosamine (inhibitory sugar for PNA), staining was not observed in any dental components. However, when sections were examined by phase contrast microscopy, odontoclasts were observed (Fig. 19).

The lectin ECA-HRP, which binds to sugar sequences found in Gal β1–4 GlcNAc clearly showed affinity only for multinucleate odontoclast cells, mainly localized in the dental pulp adjacent to the dentin. They were irregular in appearance, often with long cytoplasmic cell processes (Fig. 20). At higher magnification, intense staining for lectin ECA-HRP was found in small granular-like structures in their cytoplasm, whereas the dental pulp cells were not detectably stained (Figs. 21, 22). Exposure of sections to ECA-HRP conjugate containing GalNAc produced an appreciable decrease in staining (Fig. 23).

UEA 1-HRP binding sites (affinity for aL-fucose) were also detected in the multinucleated cells (Fig. 24). Higher magnification showed an intense granular positivity in the cytoplasm of these cells (Figs. 25–27). After treatment with aL-fucose (inhibitory sugar for UEA 1), staining was not observed in any of the dental components.

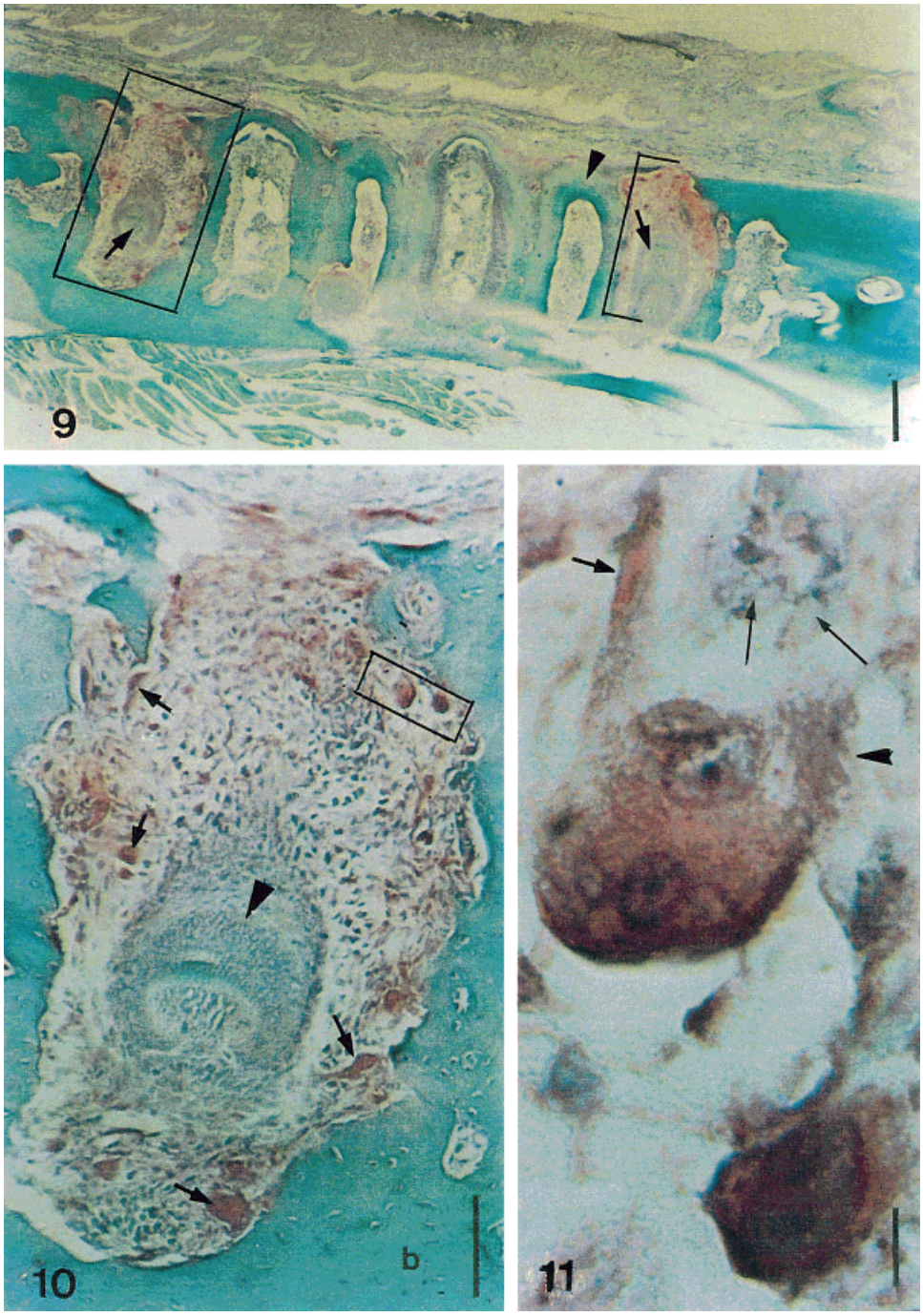
DISCUSSION

The resorption of polyphyodont lizard teeth provides us with an in vivo model system for studying odontoclast cytodifferentiation. In

Fig. 9. *Liolaemus gravenhorsti*. Final resorption stage. Light microscopic section of an adult mandible from *L. gravenhorsti*. The areas indicated by the rectangle and by the bracket show intense staining for TRAP in this stage. Arrowhead points to well-developed tooth. Arrows point to tooth rudiments that are replaced continuously throughout the life of the lizard (polyphyodont condition). TRAP reaction Meyer's hematoxylin and fast green staining. Scale bar = 350 μm.

Fig. 10. Higher magnification of the area encompassed by the rectangle in Figure 9. Most TRAP-positive multinucleate odontoclasts become detached from the resorbed surface dentine (arrows). Arrowhead points to tooth rudiment; b, bone. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 150 μm.

Fig. 11. Higher magnification of the area encompassed by the rectangle in Figure 10. The TRAP-positive multinucleate odontoclasts are round in shape and connected with the dentine surface by a narrow neck-like band of cytoplasm (short arrow). In these cells, their ruffled borders in the area facing the resorbed surface are decreased (arrowhead). Some detached odontoclasts usually showed diffuse staining of TRAP (long arrow). TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 12 μm.



Figures 9–11

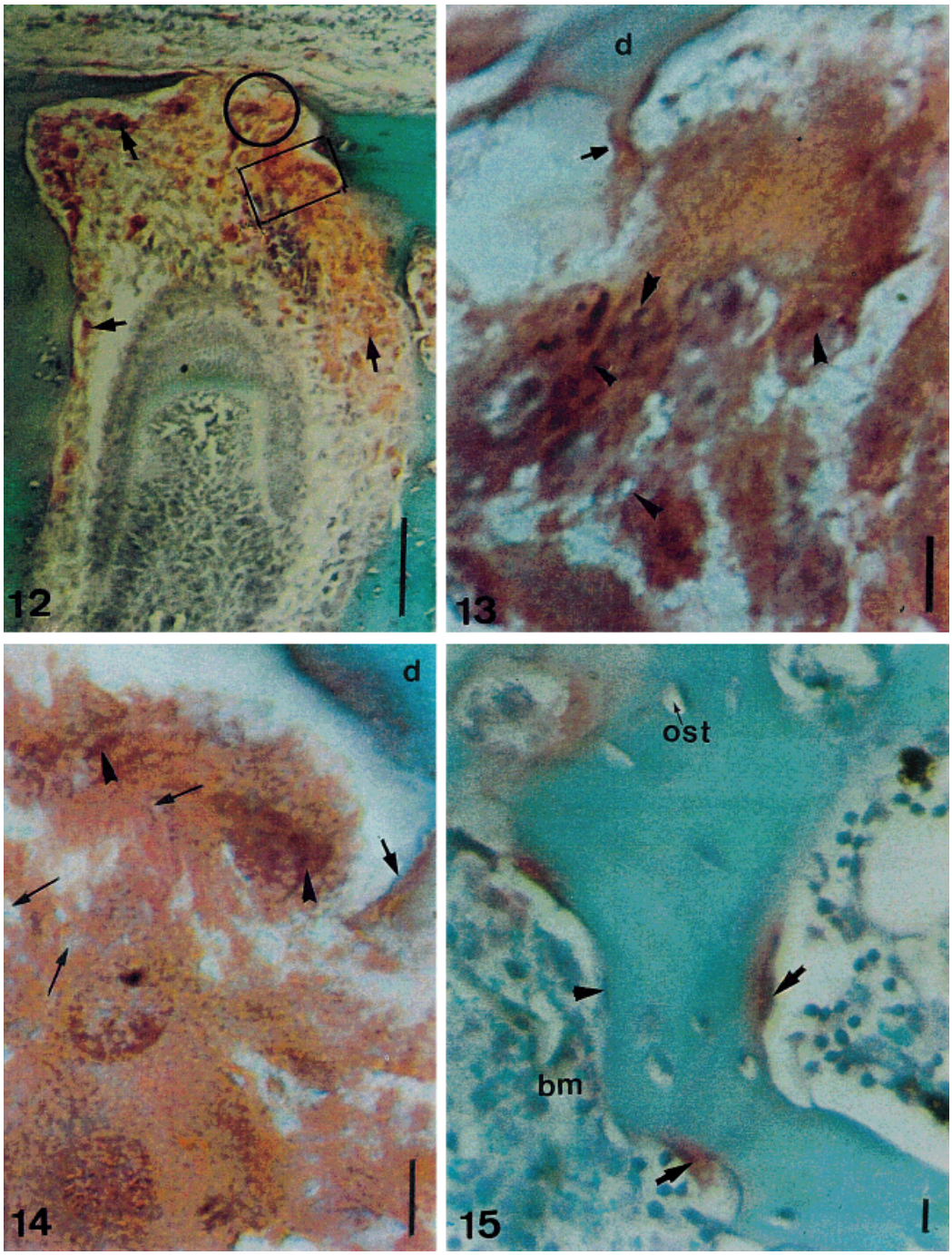


Fig. 12. *Liolaemus gravenhorsti*. Final resorption stage. Arrows point to numerous TRAP-positive odontoclasts. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 150 µm.

Figs. 13, 14. Higher magnification of the areas encompassed by the circle and by the rectangle in the Figure 12. The odontoclasts detached from the resorbed surface are connected with the dentine surface by a narrow neck like band of cytoplasm (short arrow). These cells show signs of degeneration, i.e., pycnotic nuclei (arrowheads), and numerous vacuoles in their cytoplasm (long arrows); d, dentine. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 12 µm.

Fig. 15. Light photomicrograph of a section of trabeculae of spongy bone from *L. gravenhorsti*. Arrows point to TRAP-positive osteoclasts. Arrowhead points to osteoblasts; ost, osteocytes; bm, bone marrow. TRAP reaction and Meyer's hematoxylin and fast green staining. Scale bar = 12 µm.

TABLE 2. Lectin reactivity of cells of polyphodont, Liolaemus gravenhorsti; summary of lectin binding[1]

	TPO[2]	O[3]	A[4]	DP[5]	OE[6]
BS-1	–	–	–	–	–
PNA	+++	–	–	–	–
RCA 120	–	–	–	–	–
ECA	+++	–	–	–	++
UEA 1	+++	–	–	–	++
LTA	–	–	–	–	–

[1]Evaluation of binding indicates staining intensity on a subjectively estimated scale; –, no staining; ++, moderate staining; +++, intense staining.
[2]TRAP = positive odontoclasts.
[3]Odontoblasts.
[4]Ameloblasts.
[5]Dental pulp.
[6]Oral epithelium.

fact, using TRAP activity as a histochemical marker for odontoclast differentiation, we could identify mononuclear precursor cells and could observe their morphological as well as their functional differentiation into mature multinucleate odontoclasts. Similar results have been reported by Sahara et al. ('96) in cytodifferentiation of the odontoclast prior to shedding of human deciduous teeth. On the other hand, during the process of lizard teeth odontoclastic resorption, we observed four stages by following histological criteria as previously reported by Sahara et al. ('92): 1) preresorption stage, 2) early resorption stage, 3) later resorption stage, and 4) final resorption stage.

During the preresorption stage, no TRAP-positive cell were found in the dental pulp. In the early resorption stage, two different cells types were seen in the polyphyodont lizard dental pulp: 1) TRAP-positive multinucleate odontoclasts in close proximity to the resorbed surface of the dentine, and 2) TRAP-positive mononuclear cells, somewhat removed from the resorbed surface.

Although numerous in vivo and in vitro studies have been done on the origin and differentiation of osteoclasts (see reviews by Mundy and Rodman, '87; Marks and Popoff, '88; Chambers, '89), little information is available on odontoclast precursors or their differentiation into functional odontoclasts (Lindskog et al., '87; Sasaki et al., '88b, '89a). It has been well established that multinucleate clastic cells are formed by fusion of mononuclear precursors. However, it is difficult to observe the multinucleation process forming clastic cells, especially in vivo.

Recently, Sahara et al. ('92, '96) speculated that the TRAP-positive mononuclear cells appearing in the dental pulp of human deciduous teeth prior to shedding might be precursor cells of odontoclasts. These authors reported that the ultrastructural characteristics of these mononuclear cells resemble those of preosteoclasts, mononuclear precursor cells of osteoclasts, as reported by several investigators (Ejiri, '83; Baron et al., '86; Tanaka and Tanaka, '88; Fukushima et al., '91a,b). Sahara et al. ('96) also provided the first demonstration of the ultrastructural localization of TRAP in mononuclear precursor cells. In these cells, the enzyme activity was localized along the biosynthetic pathway of the endoplasmic reticulum and Golgi lamellae and was also found in small

Fig. 16. *Liolaemus gravenhorsti*. PNA-HRP reaction. Light microscopic section of well-developed tooth and tooth rudiments at late bell stage (bracket). Cells localized in the dental pulp adjacent to the dentine display strong reaction (arrowheads). The separation between these cells and the dentine is an artifact. Odontoblasts (long arrow) and ameloblasts (a) are diffusely positive for the lectin. The area enclosed by the circle is shown in Figure 17, and the area in the rectangle is shown in Figure 18; dp, dental pulp; d, dentine. Scale bar = 90 μm.

Figs. 17, 18. The cytoplasm of multinucleate odontoclasts shows appreciable binding to PNA (arrowhead). Arrows point to nuclei. Scale bar = 4 μm.

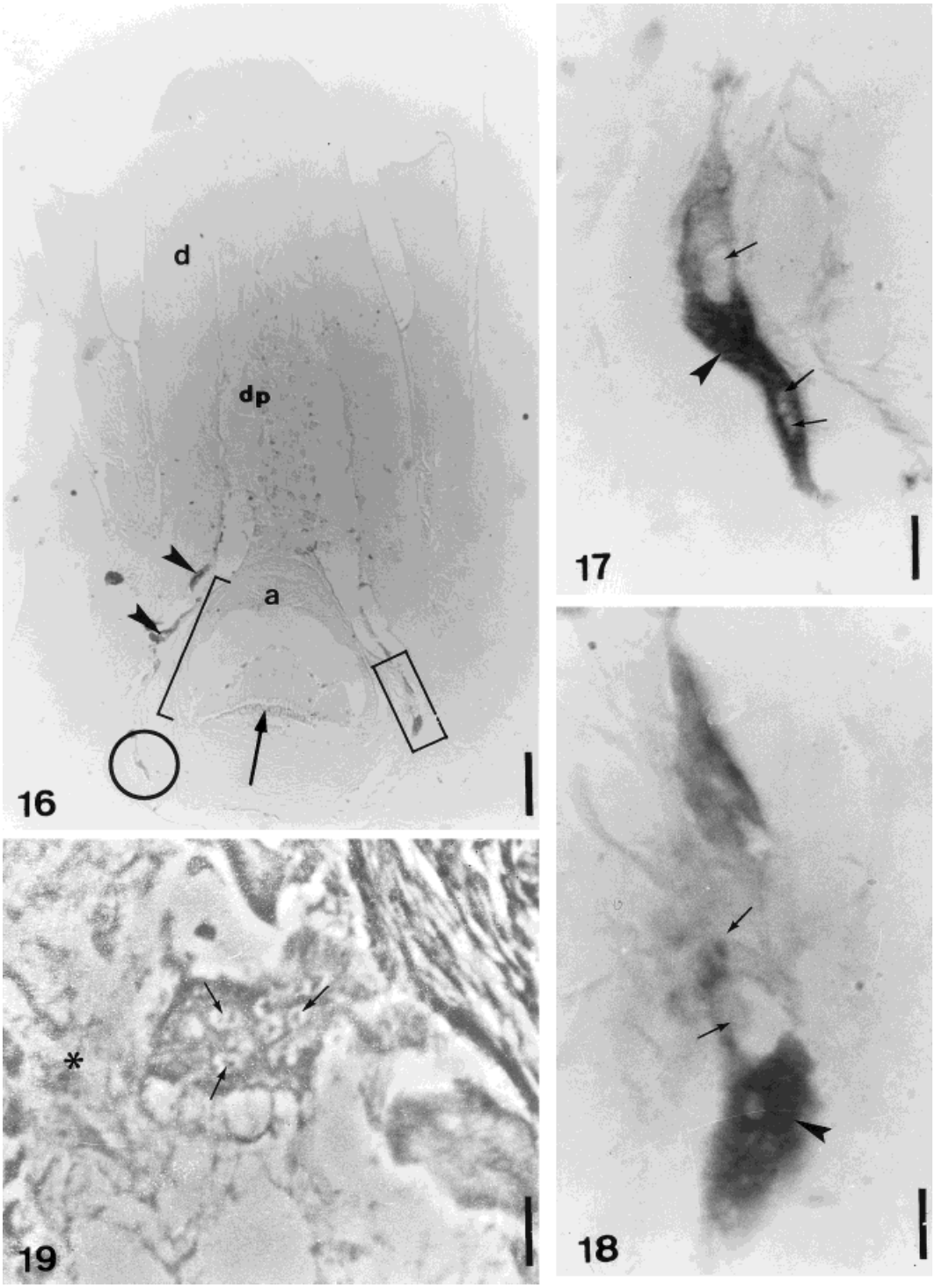
Fig. 19. Section showing PNA-binding; after N-acetylgalactosamine (GalNac) staining is not observed in any of the dental components. Arrows point to nuclei of multinucleate odontoclasts. Asterisk marks dental pulp cells. Phase contrast microscopy. Scale bar = 18 μm.

Fig. 20. *Liolaemus gravenhorsti*. ECA-HRP. A strong reaction is detectable in the cytoplasm of cells mainly localized in the dental pulp adjacent to the dentine (arrows). The area marked by the bracket shows a replacement tooth. Arrowhead points to ameloblast cells; d, dentine; oe, oral epithelium. Scale bar = 12 μm.

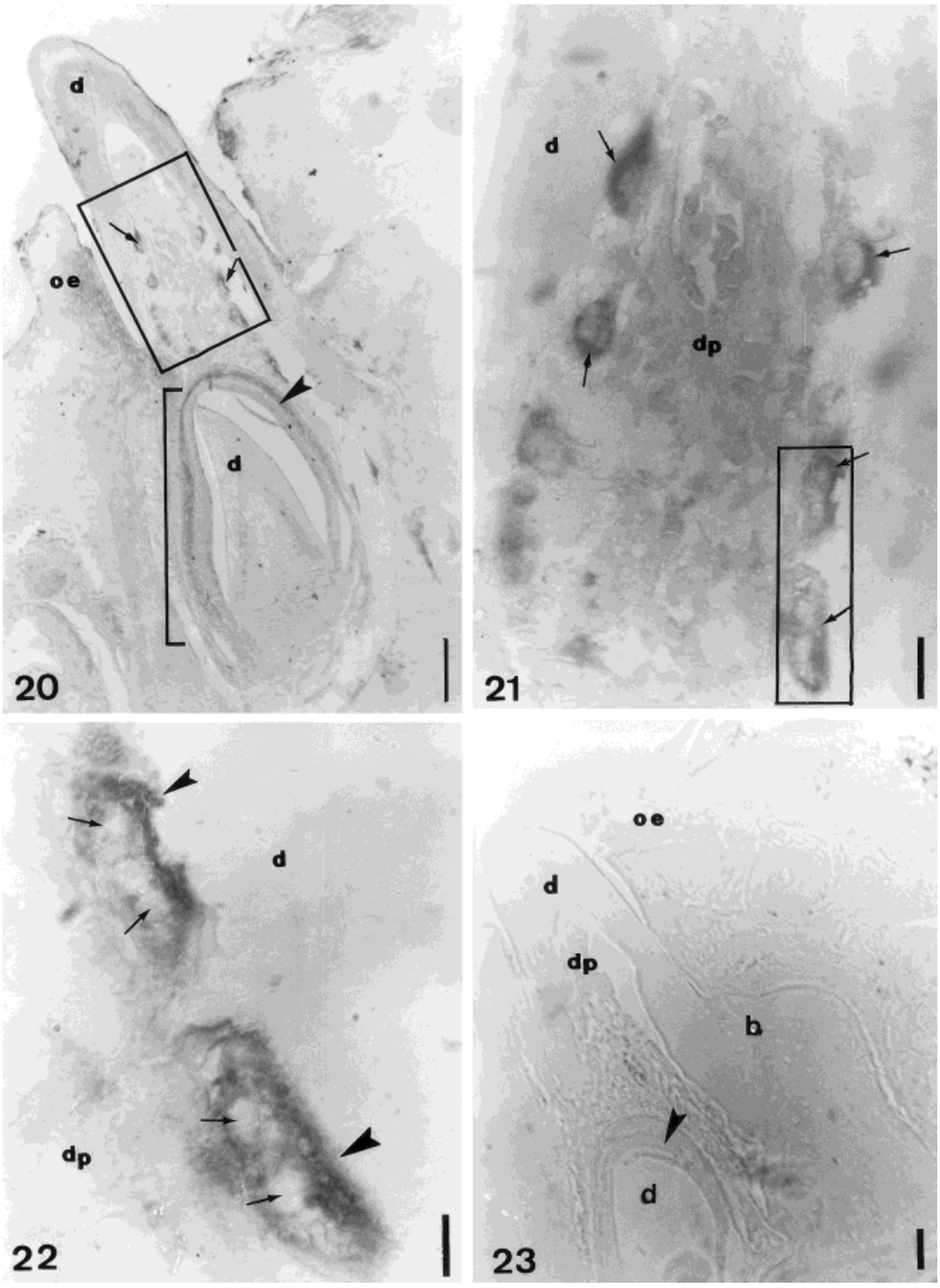
Fig. 21. Higher magnification of the area enclosed by the rectangle in Figure 20. Only the cells in the upper region of the dental pulp and adjacent to the dentine react with the lectin (arrows); d, dentine; dp, dental pulp. Scale bar = 30 μm.

Fig. 22. Higher magnification of the area enclosed by the rectangle in Figure 21. Intense granular positivity is shown in the cytoplasm of multinucleate odontoclasts. Arrowheads point to the ruffled border of odontoclasts. Short arrows point to nuclei; d, dentine; dp, dental pulp. Scale bar = 12 μm.

Fig. 23. Section showing ECA-binding after N-acetylgalactosamine (GalNac). All the positive sites described in Figures 21 and 22 are inhibited. Arrowhead points to ameloblast; d, dentine; dp, dental pulp; b, bone; oe, oral epithelium. Scale bar = 36 μm.



Figures 16–19



Figures 20–23

primary lysosomes. In mononuclear preosteoclasts, lysosomal enzymes, such as β-glycerophosphate, arylsulfatase, and acid p-nitrophenyl phosphatase, were also localized in every compartment of the biosynthetic pathway (Baron et al., '86; Akisaka et al., '89). From the ultrastructural and cytochemical similarity between mononuclear precursor cells of odontoclasts and osteoclasts, it appears that, like osteoclasts, odontoclasts are derived from hematopoietic stem cells and arrive in the pulp chamber via the circulation (see Sahara et al.,'96). Thus, in the lizard *Liolaemus gravenhorsti* the initial appearance of TRAP-positive mononuclear cells in the dental pulp suggests that they also might be precursor cells of the odontoclasts.

Sahara et al. ('96) examined cytodifferentiation of odontoclasts during resorption prior to shedding of human deciduous teeth by means of histochemical activity (TRAP) and cytochemical techniques (electron-microscopic enzyme). These authors observed that the membrane specialization of odontoclasts, i.e., development of a clear zone and ruffled border, is induced following their contact with the resorption surface, and that the multinucleation of odontoclasts takes place only after their attachment to the resorption surface. Our observations also demonstrate that multinucleated odontoclasts were always detected on the dentine surface, and they excavated resorption lacunae in the dentine. Mononuclear odontoclasts with a well-developed clear zone and ruffled border were not observed in lizard deciduous teeth. With regard to the membrane specialization of lizard odontoclasts, ruffled borders were usually found in odontoclasts that had few nuclei during the early resorption stage, but many nuclei during later and final resorption stages.

Sahara et al. ('96) reported a similar pattern of ruffled borders in odontoclasts of human deciduous teeth and suggested that membrane specialization and multinucleation of odontoclasts would take place rapidly or simultaneously after the attachment to the predentine surface, since the initial ruffled border was usually found in odontoclasts that had two or three nuclei. However, mononuclear odontoclasts with well-developed clear zones and ruffled borders were occasionally observed on the predentine surface, and they excavated resorption lacunae in the predentine.

These results suggest that in vivo the multinucleation appears not to be an indispensable phenomenon for the membrane specialization of odontoclasts, as reported by Prallet et al. ('92) for osteoclasts. However, the relative frequency of such cells and the distribution of the number of nuclei, including mononuclear cells, have not been elucidated. For example, Domont et al. ('97) reported the presence of mononuclear osteoclasts and odontoclasts with ruffled borders in human deciduous teeth. These authors studied mononuclear odontoclast participation in tooth resorption and the distribution of nuclei in these teeth. TRAP activity was detected in both multinucleated and mononuclear odontoclasts. Only 2.9% of odontoclast were mononuclear and 93.8% had 10 or fewer nuclei.

The fate of multinucleate clastic cells (osteoclasts and odontoclasts) after the termination of their resorptive function is still open to question (Baron et al., '86; Zamboni-Zallone and Teti, '81; Liu et al., '82). We found that at the final resorption stage of internal resorption of lizard teeth, odontoclasts detached from the resorbed surface. These odontoclasts showed signs of degeneration, i.e., pycnotic nuclei and numerous vacu-

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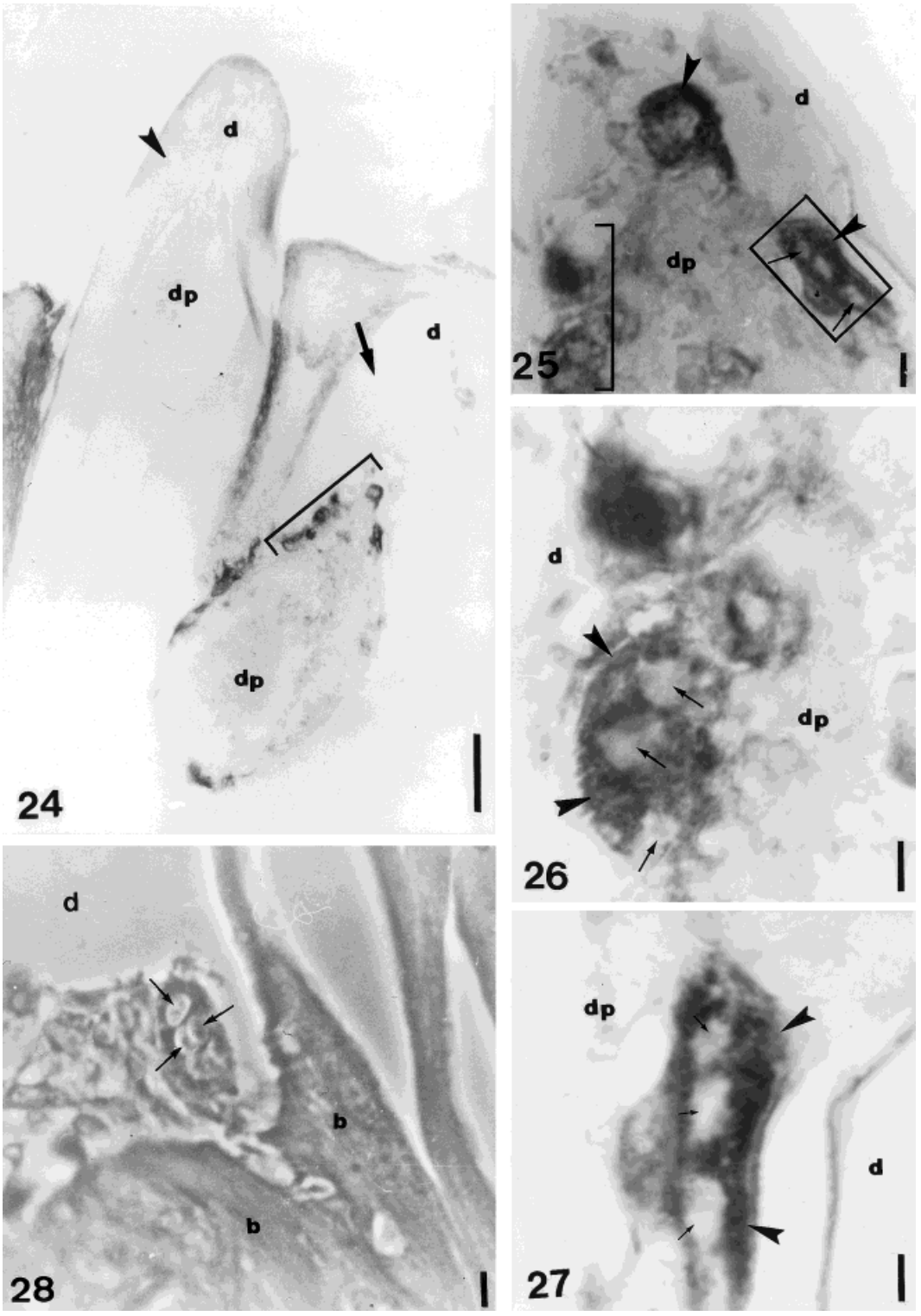
Fig. 24. *Liolaemus gravenhorsti*. UEA-HRP reaction. Photomicrograph of frontal section of mandible showing some in situ histological characteristics of the region. Arrowhead points to longitudinal section of adult teeth. Arrow shows tooth rudiments, which are replaced continuously throughout the lizard's life (polyphyodont condition). The area encompassed by the bracket is shown in Figure 25; dp, dental pulp; d, dentine. Scale bar = 10 μm.

Fig. 25. Some cells display a strong reaction with the lectin (arrowheads); d, dentine; dp, dental pulp. The area encompassed by the bracket is shown in Figure 26; the area encompassed by the rectangle is shown in Figure 27. Scale bar = 4 μm.

Fig. 26. A strong reaction is shown by the multinucleate odontoclasts (arrowheads). Arrows point to nuclei. Scale bar = 8 μm.

Fig. 27. Intensely positive granular material is observable with UEA-HRP in the cytoplasm of the multinucleate cells (arrowheads). Arrows point to nuclei; d, dentine; dp, dental pulp. Scale bar = 8 μm.

Fig. 28. UEA-binding after α-L-fucose staining is not observed in any of the dental components. Arrows point to nuclei of multinucleate odontoclasts; d, dentine; b, bone. Phase contrast microscopy. Scale bar = 10 μm.



Figures 24–28

oles in their cytoplasm. Sasaki et al. ('89a) and Sahara et al. ('96) reported a similar degenerative process in odontoclasts in physiological root resorption of kitten and human deciduous teeth.

Of the six lectins used, PNA, ECA, and UEA-1 bound to multinucleated cells from *Liolaemus gravenhorsti,* but not to mononuclear cells. All the remaining lectins, BS-1, RCA^{120}, and LTA showed no binding to any cells regions of the lizard teeth. Lemus et al. ('94) previously reported that sugar residues recognized by WGA (sialic acid and acetylglucosamine) and by ConA (α-D-mannose and α-D-glucose) are widely distributed in odontoblasts and ameloblasts during tooth development in adults of the same species. The significance of saccharidic moieties such as acetyl-galactosamine, as well as acetylglucosamine and fucose, sugar residues recognized by PNA, ECA, and UEA-1, respectively, is difficult to ascertain. Perhaps these oligosaccharides might be borne on molecules associated with odontoclastic resorption or associated with multinucleation of odontoclasts after the attachment to the dentine surface in this polyphyodont species.

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