Cell Tissue Res (1999) 297:271–281

REGULAR ARTICLE

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Developmental regulation and ultrastructure of glycogen deposits during murine tooth morphogenesis

Received: 9 November 1998 / Accepted: 17 January 1999

Abstract The distribution and ultrastructure of glycogen deposits were investigated in the murine tooth germ by histochemical periodic acid-Schiff (PAS) staining and transmission electron microscopy. Lower and upper first molars were examined in mouse embryos at embryonic days 11.5–17 (E11.5–E17) and in 2-day-old postnatal (P2) mice. The oral and dental epithelia and the mesenchymal cells were generally PAS-positive during tooth morphogenesis. PAS-negative cells were present at E13 in the distal tip of the tooth bud epithelium and in the contacting mesenchyme, and this complete lack of PAS reactivity continued in the dental papilla mesenchyme and inner enamel epithelium during the cap and bell stages. The lack of glycogen deposits in the interacting epithelium and mesenchyme during early morphogenesis may be associated with their demonstrated high signaling activities. Mesenchymal cells in the dental follicle consistently possessed small clusters or large pools of glycogen, which disappeared by P2. Since an intense PAS reaction was seen in mesenchymal cells at future bone sites, the glycogen in the dental follicle cells may be associated with their development into hard-tissue-forming cells. Ultrastructural observation of the enamel organ cells from the cap to early bell stages (E14–E15) revealed the occurrence of glycogen pools, which were associated with the Golgi apparatus and with vesicles having amorphous contents. Glycogen particles were also occasionally present inside vesicles or in the extracellular matrix. These may be associated with the exocytosis of glycosaminoglycan components into extracellular spaces and the formation of the stellate reticulum.

Key words Dental follicle · Dental papilla · Enamel organ · Glycogen · PAS reaction · Tooth development · Epithelial-mesenchymal interactions · Mouse (CBA × NMRI)

This work was supported by a grant from the Finnish Academy and a Grant-in-Aid for Scientific Research (no. 10671696) from the Ministry of Education, Science, Sports, and Culture, Japan

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Introduction

Teeth develop as epithelial appendages in the branchial arches. Their developmental anatomy is well known, and the molecular basis of tooth development has been elucidated to a significant degree during the last decade (Thesleff and Nieminen 1996). The distribution patterns of more than one hundred molecules are available from the WWW-database (http://honeybee.helsinki.fi/toothexp); this information can be used to analyze correlations between the expression of various molecules. Signaling molecules in the sonic hedgehog (Shh), fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and Wnt families appear to regulate the early steps of tooth morphogenesis, and some transcription factors associated with these pathways have been shown to be necessary for tooth development (Thesleff and Sharpe 1997).

Animal cells, especially liver and muscle cells, store carbohydrate in the form of glycogen. This is depolymerized to yield glucose, which is a major energy source for metabolic processes (Fawcett 1994). Glycogen, which is known to occur widely in embryonic tissues, is thought to be indicative of relatively anaerobic conditions (Ten Cate 1962). Periodic acid-Schiff (PAS)-positive polysaccharides have been localized in fetal dental tissues by histochemistry at the level of the light microscope (Horrowitz 1942; Bevelander and Johnson 1950; Ten Cate 1962; Mattiessen 1963; Nozue 1973). However, little attention has been paid to glycogen distribution in early developing tissues, either dental or other tissues, during the last 25 years. Although glycogen has been re-

ported to occur in the oral epithelium, dental lamina, enamel organ, dental pulp, and dental follicle in earlier studies, there has been controversy over its localization, and no ultrastructural features of glycogen deposits have been reported.

The precursor cells of osteoblasts possess accumulations of glycogen during fetal (Cabrini 1961; Scott and Glimcher 1971) and experimentally induced bone (Decker et al. 1995) formation, and the loss of glycogen in the differentiated osteoblasts has been related to calcification (Harris 1932). On the other hand, skate inner dental epithelial cells have been reported to possess large pools of intracellular glycogen, which appears to be associated with the exocytosis of glycogen toward the forming enameloid matrix (Prostak and Skobe 1988). Glycogen has also been localized in cartilage cells and in the epithelium (Symons 1965; Greenspan and Blackwood 1966; Silbermann and Frommer 1974; Morita 1982; Baba 1993).

As the functional significance of glycogen in development has remained undetermined, this study aims to elucidate its role in tooth morphogenesis by analyzing the distribution and ultrastructure of glycogen-loaded cells during tooth development. Interestingly, we have observed that the epithelial and mesenchymal cells that interact actively show no PAS reaction during early tooth morphogenesis. We suggest that the total lack of glycogen deposits is associated with the high signaling activities of the cells.

Materials and methods

The lower first molars and mandibles were dissected from embryonic day 12 (E12; dental lamina invagination), E13 (bud stage), E13.5 (late bud stage), E14 (cap stage), E14.5 (late cap stage), E15 (early bell stage), and E17 (bell stage) mouse embryos (CBA × NMRI; vaginal plug = day 0) and fixed in 4% paraformaldehyde plus 2.5% glutaraldehyde in 0.05 M phosphate buffer for 3–4 h at 4°C. E11.5 whole embryos and E13 whisker follicles with surrounding tissue were fixed as above. Two-day postnatal (P2) mice were perfused through the ascending aorta with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) under deep anesthesia by inhalation of 3% Halothane gas. The maxillae were removed *en bloc*, immersed in the same fixative for an additional 4 h, and decalcified in 5% ethylene diamine tetraacetic acid for 1 week at 4°C. The tissues were dehydrated through a graded series of ethanol and embedded in paraffin. Serial frontal sections of the lower first molars and mandibles and sagittal sections of the upper first molars were cut at 5 µm and stained with hematoxylin-eosin (H-E) and PAS for light microscopy. For observation of the E11.5 whole embryos, both frontal and sagittal serial sections were prepared and stained with H-E and PAS. For observation of the whiskers, horizontal sections of maxillae were prepared and stained in the same manner. Some PAS-stained sections were counterstained with hematoxylin, and some were subjected to digestion with amylase (37°C, 1 h) as a control procedure for the demonstration of glycogen.

For electron microscopy, the dissected tooth germs were immersed in the same fixative for 3–4 h. They were then post-fixed in 1% osmium tetroxide for 1 h, dehydrated through a graded series of ethanol, and embedded in LX-112. Ultrathin sections were prepared on a Reichert Ultracut-E ultramicrotome (Reichert-Jung, Vienna, Austria) with a diamond knife, and double-stained with uranyl acetate and lead citrate. They were examined in a Jeol JEM 1200EX (Jeol, Tokyo, Japan) transmission electron microscope.

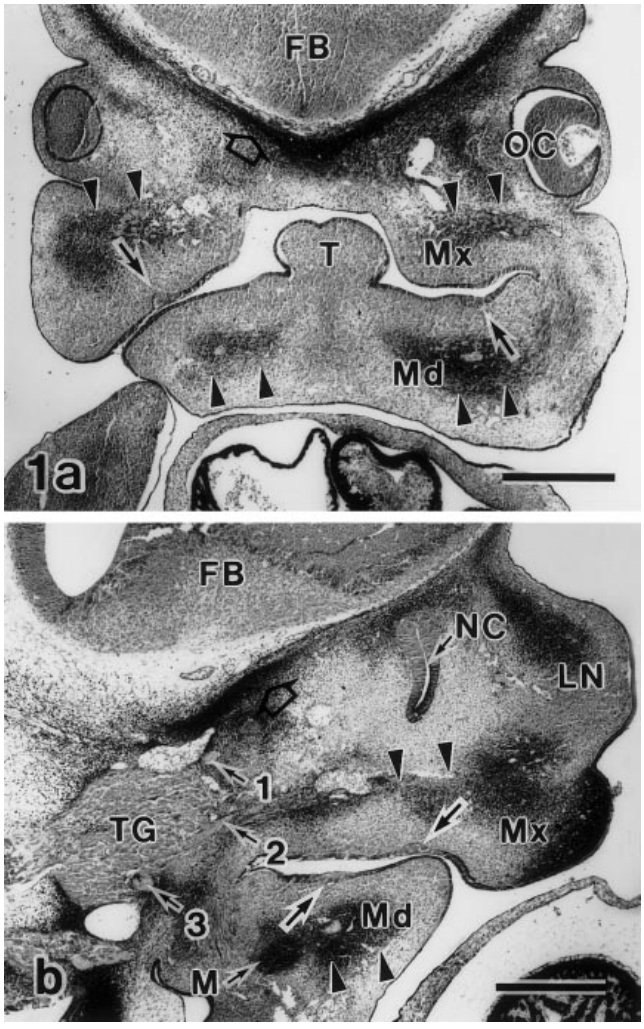
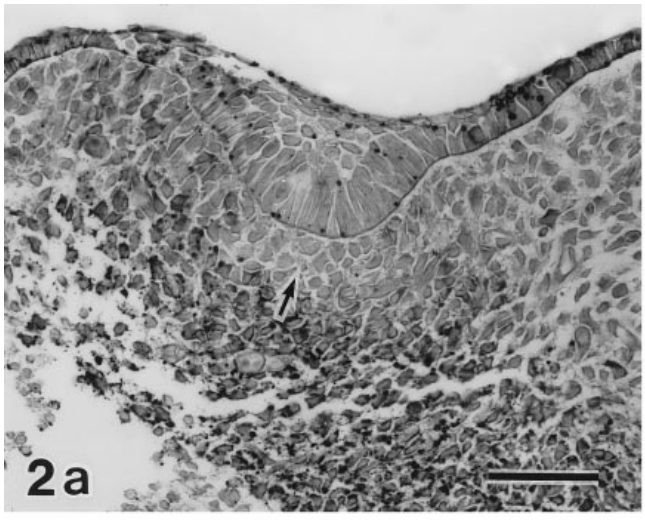


Fig. 1a, b PAS reaction with no counterstaining of frontal (**a**) and sagittal sections (**b**) of E11.5 mouse heads. PAS-positive cells (*arrowheads*) are associated with the trigeminal nerves, i.e., the ophthalmic (*1*), maxillary (*2*), and mandibular nerves (*3*), in the maxillary (*Mx*) and mandibular processes (*Md*). An intense PAS reaction is observed in the frontal end of the maxillary process, a part of the lateral nasal process (*LN*), and the cranial basal region (*open arrow*) beneath the forebrain (*FB*). The nerves can be easily recognized by their negativity in the PAS-stained sections. Note that Meckel's cartilage primordium (*M*) shows a PAS-positive reaction. *Arrows* Budding dental epithelium (*NC* nasal cavity, *OC* optic cap, *T* tongue, *TG* trigeminal ganglion). *Bar* 400 µm

Results

Light-microscopic observations

Glycogen appears as pink deposits after histochemical PAS staining. PAS-positive cells were observed in the mandibular arch and in dental tissues at all observed stages of tooth development, and their distribution appeared to be developmentally regulated. Frontal and sagittal sections of E11.5 mouse embryonic heads showed that PAS-positive cells were associated with the trigeminal nerves in the maxillary and mandibular processes,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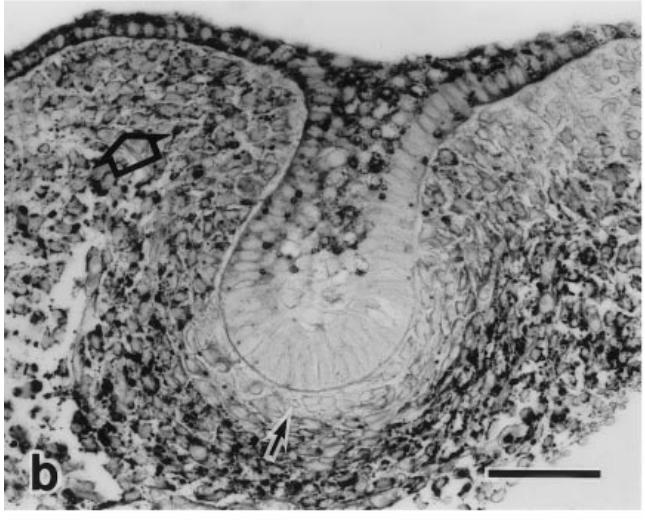
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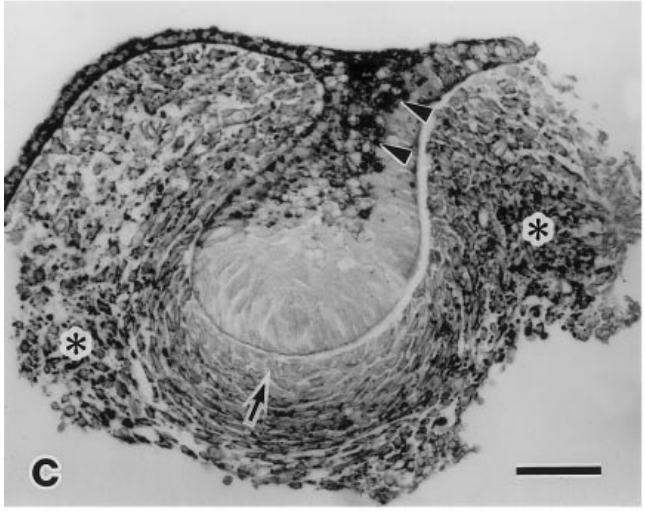
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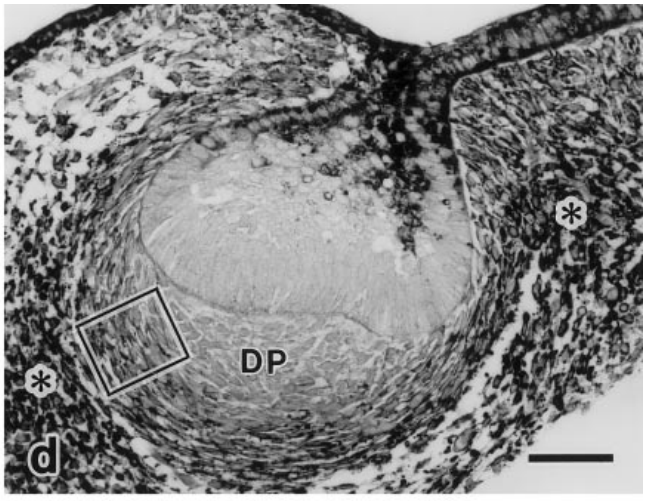
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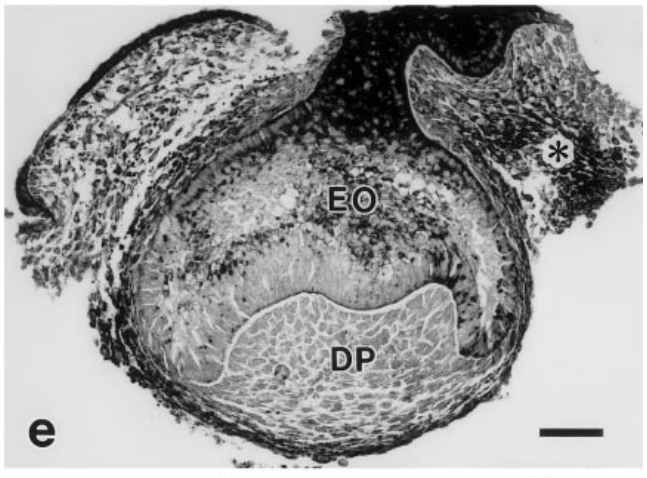
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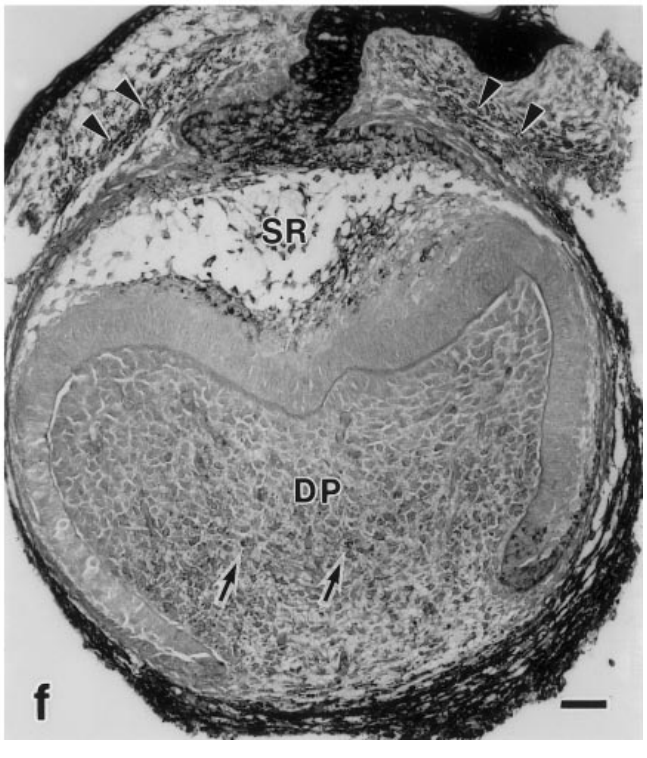
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but the trigeminal ganglion was PAS-negative. An intense PAS reaction was seen in the cranial base and Meckel's cartilage primordium. An intense reaction was also recognizable in the anterior tip of the maxillary process and in a part of the lateral nasal process (Fig. 1).

At E11.5, most of the oral epithelium was PAS-positive, but the thickened dental epithelium was almost devoid of PAS reaction. The subepithelial mesenchymal tissue, including the presumptive dental mesenchyme, showed no PAS positivity (Fig. 1). In E12 embryos, a stage by which time the invagination of the dental epithelium had proceeded, this epithelium remained PAS-negative. The condensed dental mesenchyme was also PAS-negative, but the mesenchyme surrounding the dental mesenchyme was PAS-positive (Fig. 2a).

From the bud to bell stages (E13–E17), the condensed dental papilla mesenchyme and the adjacent dental epithelium remained remarkably PAS-negative (Fig. 2b–f). Scattered PAS-positive cells were seen in the dental papilla cells at the bell stage (E17; Fig. 2f). PAS-positive cells were consistently seen in the dental follicle and in the surrounding mandibular arch mesenchyme (Figs. 2b–f, see also Fig. 4a). In the dental follicle, however, PAS positivity was more restricted by E17 (Fig. 2f), and no reaction was seen postnatally (Fig. 3). The mandibular arch mesenchyme surrounding the tooth germ continued to show intense PAS positivity; it appeared to be associated specifically with the formation of bone tissue (Figs. 2, 3, 4c–e).

Epithelial cells in the oral mucosa and in the dental lamina connecting the tooth germ to the oral mucosa

◀ **Fig. 2** PAS reaction with no counterstaining of tooth germs (**a** E12, **b** E13, **c** E13.5, **d** E14, **e** E15, **f** E17). **a** PAS-positive cells are distributed in the mandibular arch mesenchyme and oral epithelium. The invaginating dental epithelium and the condensed dental mesenchyme (*arrow*) are negative. **b** At the bud stage, the dental mesenchyme just beneath the tip of the tooth bud (*arrow*) shows no PAS-positive reaction, but the more peripheral dental mesenchymal cells, which differentiate into dental follicle, are PAS-positive. PAS-positive cells are also observed in the oral epithelium and the dental lamina. The non-dental mesenchyme (*open arrow*) beneath the oral epithelium is also PAS-positive. **c** At the late bud stage, the distribution pattern of the PAS-positive cells is similar to that in the previous stage. The dental lamina (*arrowheads*) and the mandibular arch mesenchyme exhibit an increase in the intensity of their PAS reactions. The future dental papilla cells facing the tip of the dental epithelium show no PAS-positive reaction (*arrow*). Note the intense PAS reaction in the mesenchyme on the *right* and *left* sides (*asterisks*) of the germ. **d** At the cap stage, PAS-positive cells are present in the dental follicle and epithelium. The dental papilla (*DP*) shows no PAS positivity. The osteogenic jaw mesenchyme surrounding the tooth germ is intensely PAS-positive (*asterisks*). The *boxed area* is shown at higher magnification in Fig. 4a. **e** At the early bell stage, the distribution pattern of PAS-positive cells is essentially the same as that in the previous stage. The oral epithelium and the dental lamina show an increased PAS reaction. The enamel organ (*EO*) is also PAS-positive. **f** At the bell stage, some dental follicle cells start to be PAS-negative. The dental follicle cells toward the oral cavity still show PAS reaction (*arrowheads*). The cells of the stellate reticulum (*SR*) and some cells (*arrows*) in the dental papilla (*DP*) are PAS-positive. In these micrographs and in Fig. 4, the *left* side corresponds to the lingual side, and the *right* is the buccal side. *Bar* 50 μm

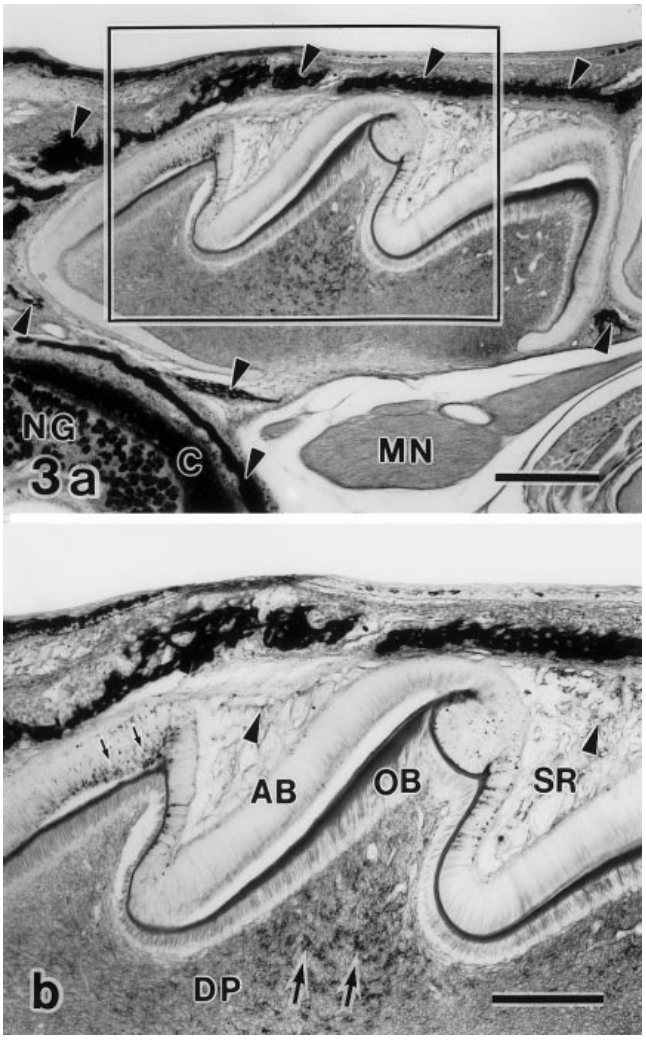
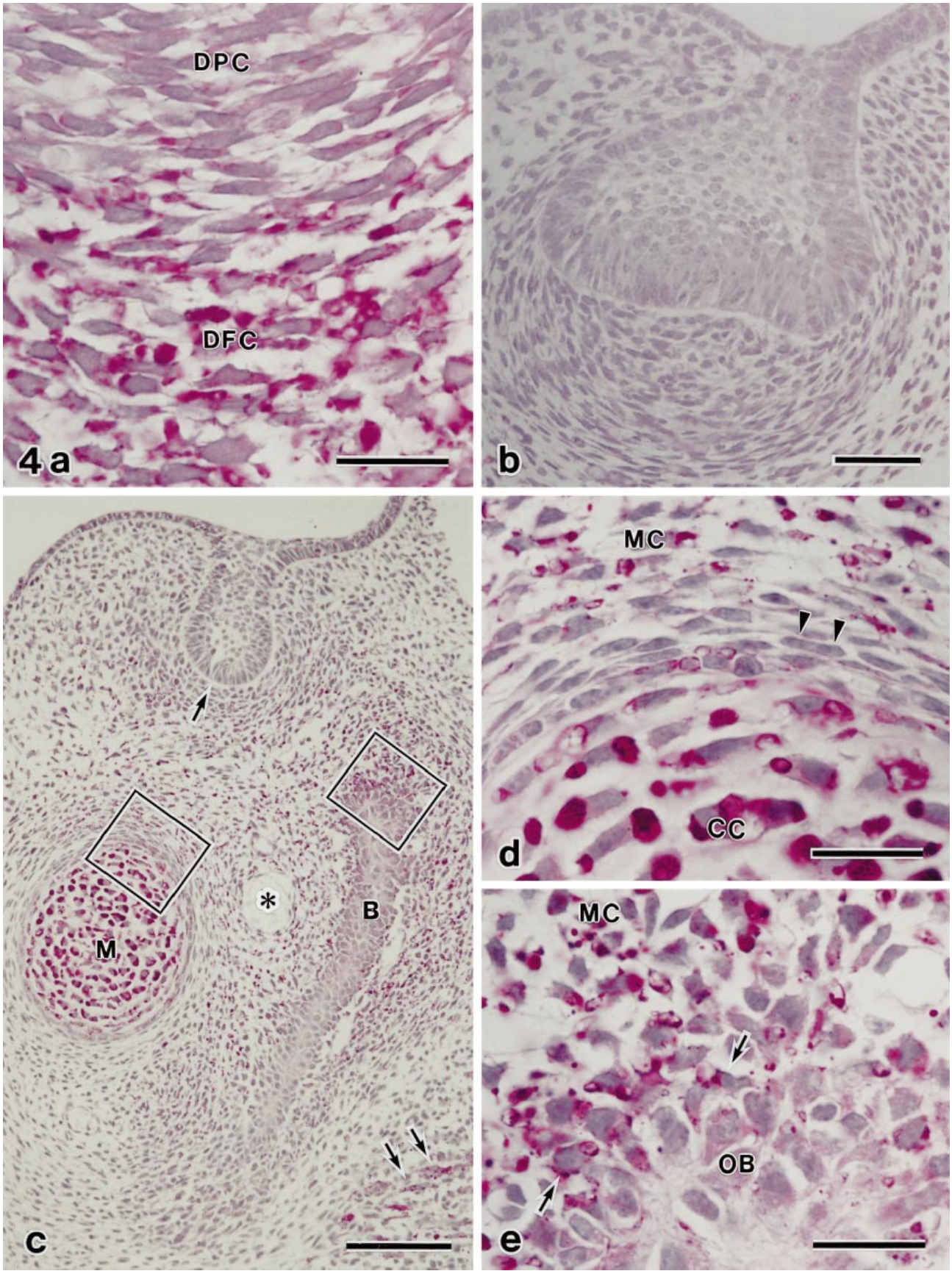


Fig. 3a, b PAS reaction with no counterstaining of sagittal sections of the P2 maxillary first molar. **a** The first molar is surrounded by the alveolar bone (*arrowheads*), which shows a PAS-positive reaction. PAS-positive cells have disappeared from the dental follicle. The oral epithelium is still PAS-positive, except for the area covering the first molar. The nasal glands (*NG*) and cartilage (*C*) also exhibit PAS-positive reaction (*MN* maxillary nerve). **b** Higher magnification of the *boxed area* in **a**. PAS-positive cells are recognizable in the ameloblasts (*small arrows*), stellate reticulum (*arrowheads*), and dental pulp (*arrows*). The dentin and enamel matrixes are also PAS-positive (*AB* ameloblasts, *DP* dental pulp, *OB* odontoblasts, *SR* stellate reticulum). *Left* Mesial side, *upper* oral side. *Bars* 300 μm in **a**, 200 μm in **b**

Fig. 4 PAS reaction with hematoxylin counterstaining in the mesenchymal tissues (**a, b** E14, **c–e** E13). **a** Higher magnification of the *boxed area* in Fig. 2d. Dental follicle cells (*DFC*) possess large PAS-positive granules, whereas dental papilla cells (*DPC*) do not contain PAS-positive granules. **b** A control section (subjected to digestion with amylase before the PAS reaction) is negative. **c** PAS reaction in a transverse section of an E13 mandible. PAS-positive cells are seen in the mesenchyme surrounding the tooth germ (*arrow*), in the bone matrix (*B*), and in Meckel's cartilage (*M*). Myoblasts (*double arrows*) are also PAS-positive. *Asterisk* Lower alveolar nerve. **d** Higher magnification of the *boxed area* (*left*) in **c**. Chondrocytes (*CC*) possess large intense PAS-positive granules, whereas cells in the perichondrium (*arrowheads*) show no PAS reaction. **e** Higher magnification of the *boxed area* (*right*) in **c**. Although precursor cells (*arrows*) of osteoblasts contain intense PAS-positive granules, definitive osteoblasts (*OB*) do not exhibit such an intense PAS reaction (*MC* undifferentiated mesenchymal cells). *Bars* 20 μm in **a**, 50 μm in **b**, 100 μm in **c**, 20 μm in **d**, 20 μm in **e** ▶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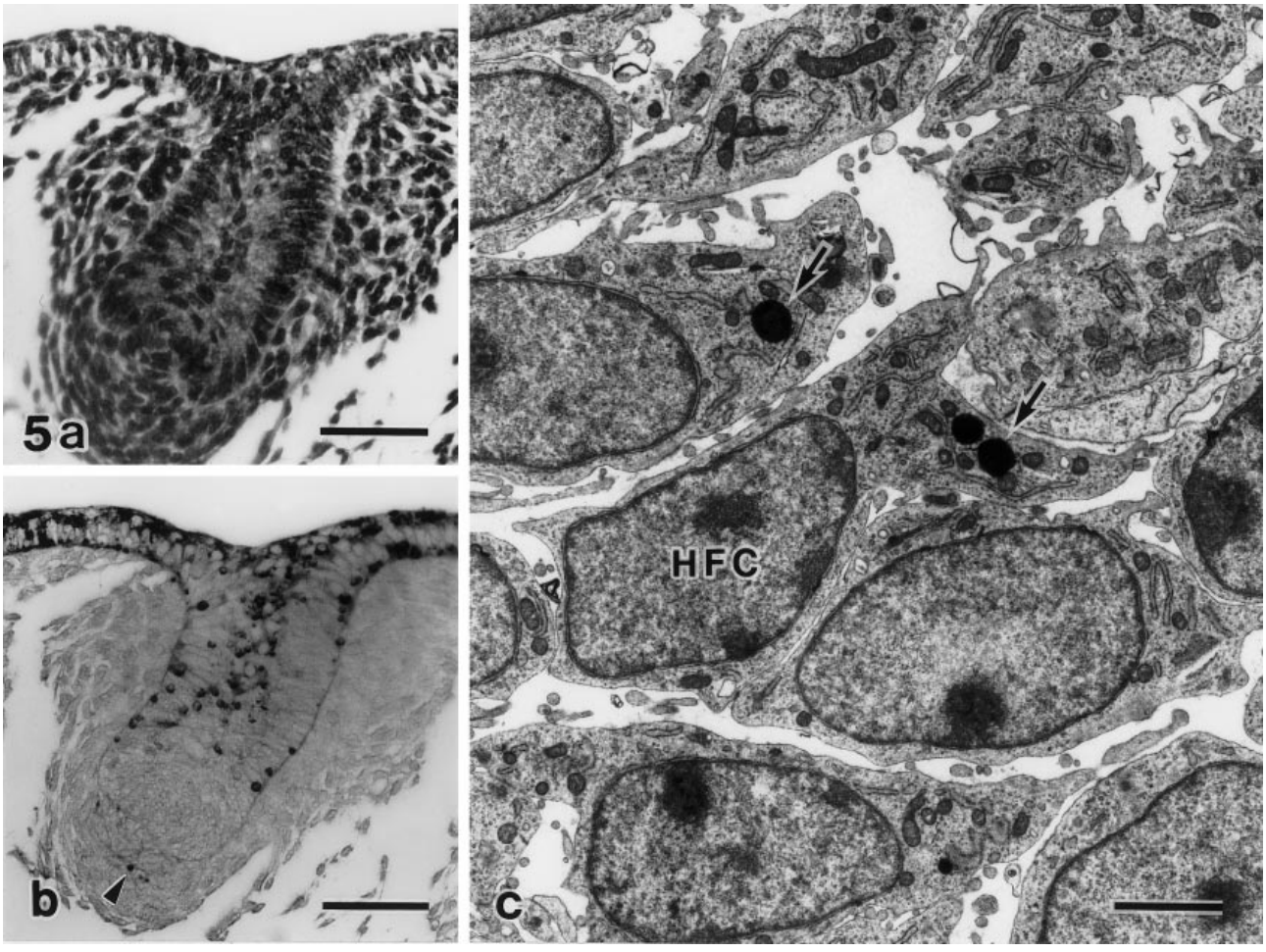


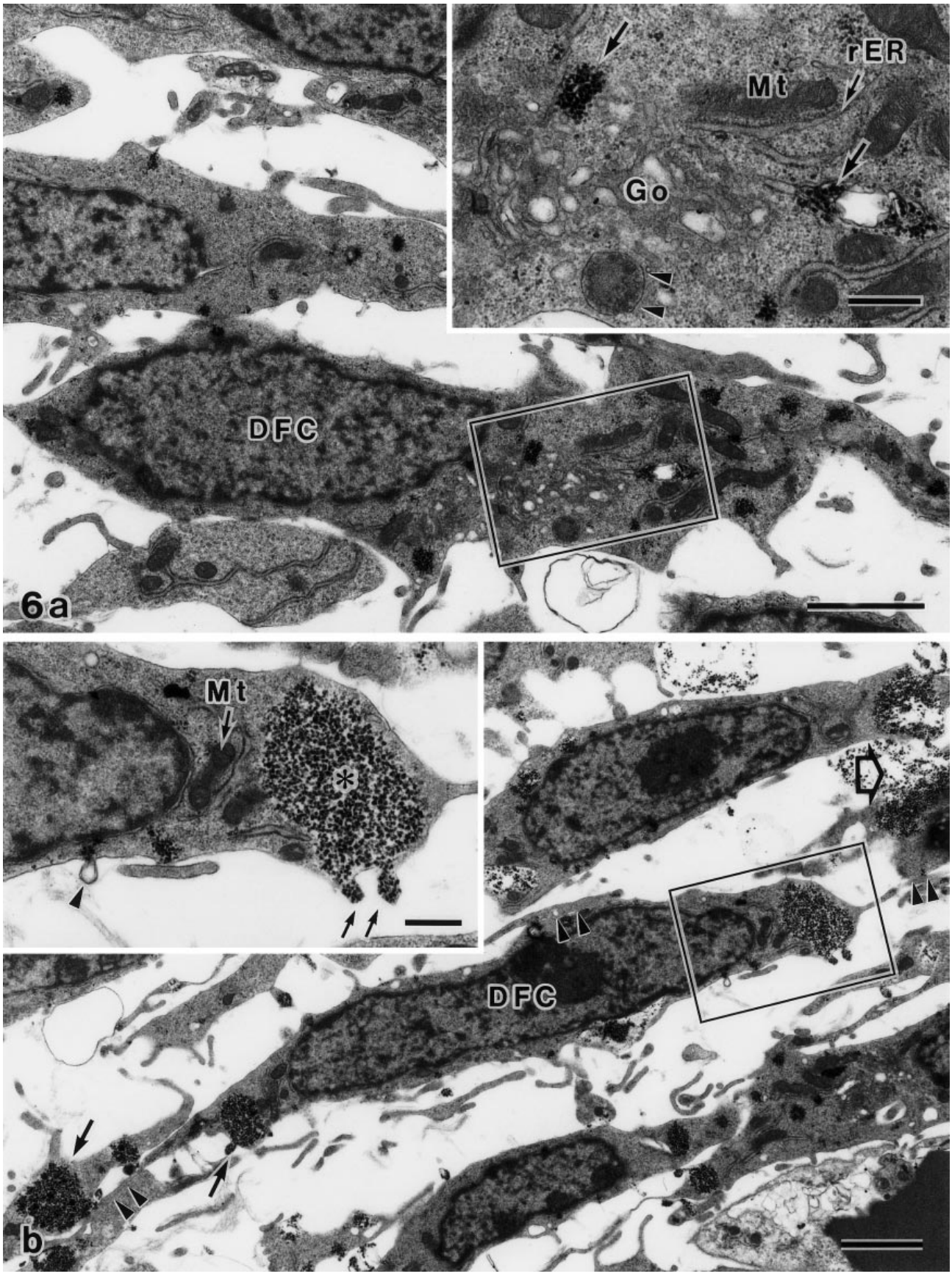
gradually gave a more intense PAS reaction (Fig. 2). Although the tip of the dental epithelium contained no PAS-positive cells between the bud to cap stages (E13–E14), the stellate reticulum cells inside the enamel organ gradually showed increasing PAS positivity from the early bell stage onwards (Fig. 2). Postnatally, the oral epithelium was still PAS-positive, except for the area overlying the molars. The bone, dentin, and enamel matrix exhibited a PAS-positive reaction, in addition to the nasal glands and cartilage. PAS-positive cells were recognizable in the ameloblasts, stellate reticulum, and dental pulp (Fig. 3). Control sections, which were subjected to digestion with amylase before the PAS reaction, showed no PAS positivity (Fig. 4b).

The mesenchymal cells surrounding the bone matrix gave a positive PAS reaction. Chondrocytes possessed large intense PAS-positive granules, whereas the perichondrium showed no PAS reaction. Osteoblast precursors and undifferentiated mesenchymal cells contained intense PAS-positive granules (Fig. 4c–e). However, differentiated osteoblasts did not present an intense PAS reaction (Fig. 4e). Myoblasts were intensely PAS-positive (Fig. 4c).

The mesenchymal follicle cells around the developing (E13) whiskers were PAS-negative, except for a faint reaction of the hair papilla mesenchyme. The budding epi-

Fig. 5a–c Horizontal sections of the E13 whisker. **a** H-E staining. The morphology of the budding epithelium is similar to that of the oral epithelium at E13 (Fig. 2b). **b** PAS reaction with no counterstaining. The invaginating whisker epithelium and the skin epithelium shows a PAS-positive reaction, but the tip of the epithelial bud is negative. Some hair papilla mesenchymal cells (*arrowhead*) beneath the budding epithelium are also PAS-positive. **c** Electron micrograph of hair follicle cells (*HFC*) of the whisker bud. They contain lysosome-like vesicles (*arrows*) in addition to the Golgi apparatus, rough-surfaced endoplasmic reticulum, and mitochondria. *Bars* 50 μm in **a**, 50 μm in **b**, 2 μm in **c**

Fig. 6a, b Electron micrographs of glycogen-loaded dental follicle cells at E13.5. **a** Dental follicle cells (*DFC*) contain clusters of glycogen in the cytoplasm. *Inset* Higher magnification of the *boxed area* in **a**. Glycogen particles (*arrows*) are associated with the mature Golgi apparatus (*Go*) and rough-surfaced endoplasmic reticulum (*rER*). The glycogen particles are aggregated into a rosette pattern consisting of spherical particles ranging from 20 nm to 40 nm in diameter. *Arrowheads* indicate a multivesicular body. **b** Dental follicle cells (*DFC*) contain pools of glycogen (*arrows*) in the cytoplasm. They possess many fine cell processes that contact each other (*double arrowheads*). The outflow of glycogen (*open arrow*) is recognizable in the extracellular matrix. *Inset* Higher magnification of the *boxed area* in **b**. Apocrine processes (*arrowhead*), some of which include glycogen granules (*double arrows*), are observed on the cell surface (*Mt* mitochondria). *Bars* 2 μm in **a** (500 nm in *inset*), 2 μm in **b** (500 nm in *inset*) ▶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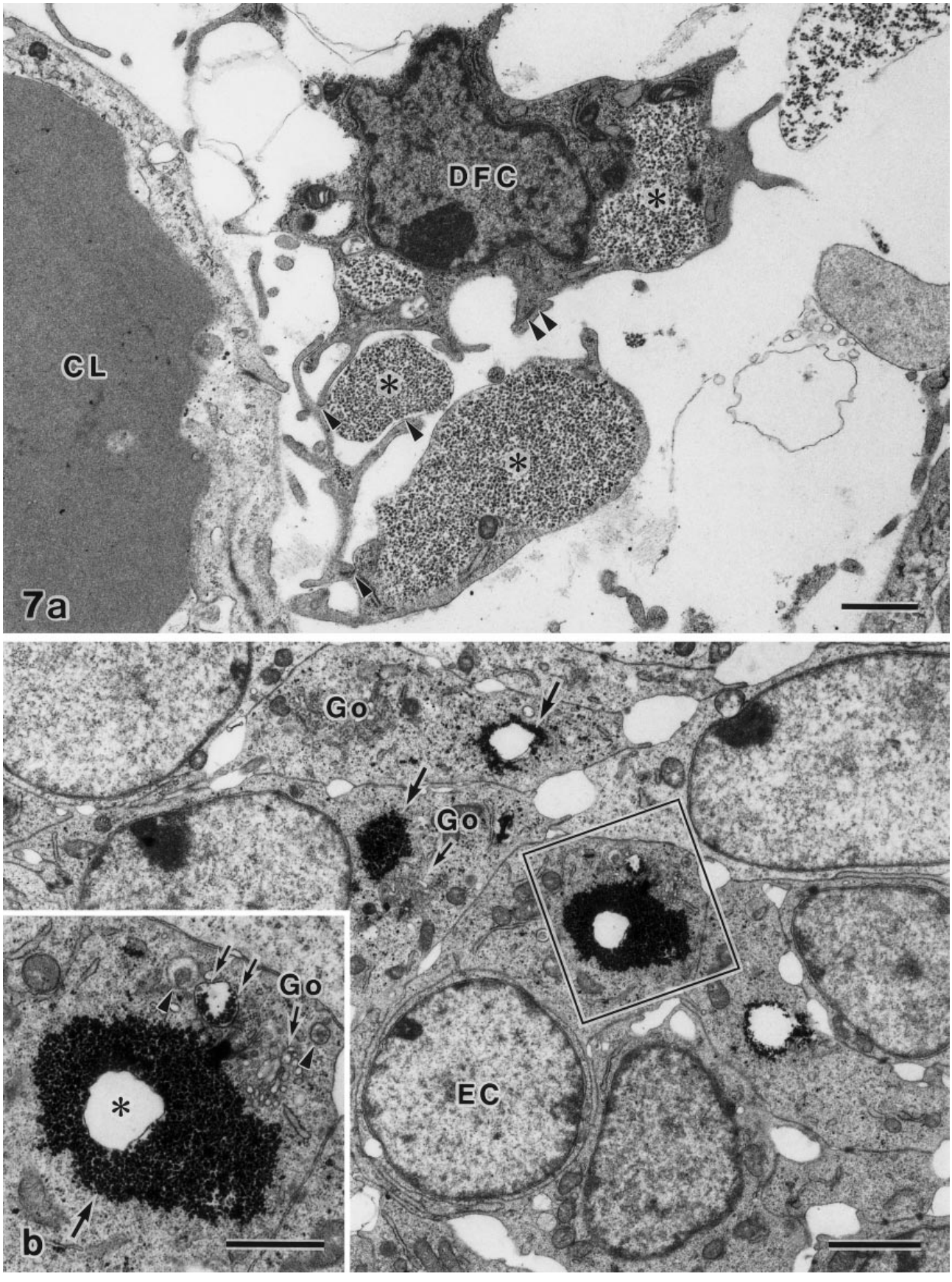
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thelium showed a similar phenomenon as the dental bud, i.e. the cells at the tip of the epithelium were PAS-negative (Fig. 5a, b).

Electron-microscopic observations

Under the electron microscope, dental follicle cells corresponding to the PAS-positive area possessed many clusters of electron-dense granules in their cytoplasm (Fig. 6a). Glycogen particles were sometimes associated with the mature Golgi apparatus, rough-surfaced endoplasmic reticulum, and vesicles, and mitochondria were often situated nearby. These glycogen particles exhibited a rosette pattern and could be classified as "α particles". Capillaries were sometimes seen in the vicinity of dental follicle cells containing large pools of glycogen in their cytoplasm (Figs. 6b, 7a). Occasionally, an outflow of glycogen was recognizable in the extracellular matrix. Apocrine processes, some of which included glycogen granules, were observed at the cell surface (Fig. 6b). At the bell stage (E15), the cytoplasm of enamel organ cells corresponding to the PAS-positive sites possessed pools of glycogen granules that were occasionally associated with the Golgi apparatus and the vesicles containing amorphous contents (Fig. 7b). The pools of glycogen often included intracellular canaliculi. Glycogen granules were also recognizable inside the vesicles or in the extracellular space.

The mesenchymal follicle cells around the developing (E13) whiskers possessed neither clusters nor pools of electron-dense granules in the cytoplasm. They instead contained lysosome-like vesicles in addition to the Golgi apparatus, rough-surfaced endoplasmic reticulum, and mitochondria (Fig. 5c).

Discussion

Histochemical PAS staining has demonstrated developmentally regulated patterns of glycogen-loaded cells during tooth morphogenesis. The observed distribution patterns of PAS-positive cells in developing teeth is essentially in accordance with previous studies (Horrowitz 1942; Bevelander and Johnson 1950; Ten Cate 1962; Mattiessen 1963; Nozue 1973). However, we have been able to present a more complete picture of glycogen distribution during tooth morphogenesis, because we have examined dissected dental tissues rather than whole mandibles or heads as was the case in all earlier investigations.

Interestingly, we have observed a complete absence of glycogen deposits during the active phase of tooth morphogenesis in the dental epithelium and underlying mesenchyme. Tooth morphogenesis and the differentiation of the various cell lineages of the tooth are regulated by epithelial-mesenchymal interactions (Thesleff and Nieminen 1996; Thesleff and Sharpe 1997). The budding dental epithelium induces condensation in the surrounding mesenchymal cells, which are destined for the dental mesenchymal lineage. These mesenchymal cells regulate subsequent epithelial morphogenesis and form the dental papilla and dental follicle. The dental mesenchyme is strikingly devoid of PAS reaction at E12, by which time it has been induced by the epithelium, and the lack of glycogen continues in the condensed dental mesenchyme at the bud stage and in the dental papilla during the cap and bell stages. In the dental epithelium, the lack of PAS reaction is striking initially in the thickened epithelium, then in the epithelial cells at the tip of the dental bud, and subsequently in the inner enamel epithelium, whereas the dental lamina and, later, the stellate reticulum cells in the enamel organ are rich in glycogen deposits. These observations indicate that the glycogen content of the cells in both the dental epithelium and mesenchyme is associated with their interactions, i.e., the interacting cells are devoid of glycogen granules.

The epithelial-mesenchymal interactions in the developing tooth are associated with numerous changes in the molecular composition of the interacting cells (Thesleff and Nieminen 1996; see also WWW-database http://honeybee.helsinki.fi/toothexp). In particular, large numbers of molecules functioning in the regulatory signaling networks are expressed by the interacting tissues. Many of them show a clear co-expression with glycogen-free areas in the epithelium and mesenchyme during early morphogenesis, but no individual molecule so far analyzed has shown an exact co-distribution. A number of signaling molecules is expressed at the tip of the tooth bud in the enamel knot, which is a putative signaling center. These include Shh and several Wnts, BMPs, and FGFs, and their expression domains in the epithelium overlap with the glycogen-free area (Vaahtokari et al. 1996). The heparan-sulfate proteoglycan syndecan-1 and epidermal growth factor receptors are also expressed in the PAS-negative areas both in the epithelium and mesenchyme (Partanen and Thesleff 1987; Thesleff et al. 1988). It can therefore be speculated that the lack of glycogen deposits in the interacting cells is associated with their high signaling activities. Such an idea is also supported by the observation, in the whisker follicles, that no PAS positivity is present in the tip of the whisker bud. This also represents a site of high signaling activity.

The role of the notable content of glycogen granules in the dental follicle is not known at present. We have

◀ **Fig. 7** Electron micrographs of glycogen-loaded dental follicle cells at E14 (**a**) and enamel organ cells at E15 (**b**). **a** Dental follicle cells (*DFC*) in the vicinity of the capillary lumen (*CL*) contain large pools of glycogen (*asterisks*). They possess many fine cell processes that contact each other (*arrowheads*). **b** Epithelial cells (*EC*) contain glycogen granules (*arrows*) that are frequently associated with the Golgi apparatus (*Go*) in the cytoplasm. *Inset* Higher magnification of the *boxed area* in **b**. Vesicles with amorphous contents are observed (*arrowheads*). Note the glycogen granules inside the vesicle or in the extracellular space (*double arrows*). The pools of glycogen are often associated with the intracellular canaliculus (*asterisk*) (*Go* Golgi apparatus). *Bars* 1 μm in **a**, 2 μm in **b** (1 μm in *inset*)

shown that, in the developing whiskers, the mesenchymal follicle is remarkably negative in PAS reaction, although the follicle morphologically resembles that of the tooth germ. Thus, the glycogen granules in the tooth have functions that are not shared between the tooth and whisker. It can be postulated that the glycogen in dental follicle cells has similar functions as in preosteoblasts and that it is associated with the capacity of dental follicle cells to develop into hard-tissue-producing cells, namely cementoblasts forming cementum and osteoblasts forming alveolar bone. Since the dental follicle is well vascularized, glycogen-loaded dental follicle cells are occasionally situated in close proximity to capillaries, and it is thus improbable that they are subjected to anaerobic conditions. Large pools of glycogen in chondrocytes and in the oral epithelium have been thought to result from anaerobic conditions, since both tissues have a poor vascular supply (Morita 1982).

Large amounts of glycogen deposits have also been observed in the stellate reticulum cells in the center of the epithelial enamel organ. These cells synthesize and secrete glycosaminoglycan into the extracellular compartment between the epithelial cells (Ten Cate 1998). The exocytosis of glycogen into the extracellular matrix has been previously demonstrated in skate inner dental epithelial cells (Prostak and Skobe 1988). Glycosaminoglycans are hydrophilic and may contribute to the increase in the extracellular space, resulting in the formation of the stellate reticulum (Ten Cate 1998). In this study, glycogen granules have been shown to be associated with the Golgi apparatus and with vesicles containing amorphous material and are recognizable either inside vesicles or as free aggregates in the extracellular matrix. In addition, pools of glycogen have commonly been seen around intracellular canaliculi in the enamel organ cells, probably indicating the passage of secreted glycosaminoglycan components into the extracellular spaces.

We have shown that, in the embryonic head at E11.5, PAS positivity is associated with early cartilage and bone formation. The mesenchymal condensates forming cartilages in the cranial base and Meckel's cartilage are intensely PAS-positive. Meckel's cartilage (but not the perichondrium) remains PAS-positive, whereas definitive osteoblasts become depleted of glycogen. These findings are in accordance with previous reports on fetal ossification (Cabrini 1961; Scott and Glimcher 1971). It has been proposed that large accumulations of glycogen particles characterize osteoprogenitor cells and that they may be associated with the production of the bone matrix or serve as a general metabolic energy source for the cytodifferentiation of the osteoblastic cells (Scott and Glimcher 1971; Takahashi et al. 1986; Decker et al. 1995).

We have noticed an association between the development of innervation and PAS positivity. In the head at E11.5, the tissues surrounding the trigeminal nerve tract are intensely PAS-positive. Furthermore, during tooth morphogenesis, mesenchymal PAS positivity is correlated with the onset of tooth innervation. The dental follicle is innervated first, starting at the cap stage, and the dental papilla becomes innervated postnatally (Fristad et al. 1994; Luukko 1997) at the time when PAS positivity appears in this tissue. Thus, PAS positivity may be associated with the expression of the nerve growth factor, as the observed distribution pattern of PAS-positive cells is correlated closely with the reported expression of this factor, which has been suggested to be involved in the guidance of trigeminal axons to embryonic and postnatal teeth (Luukko et al. 1997).

Acknowledgments The authors cordially thank Ms. Riikka Santalahti, Institute of Biotechnology, University of Helsinki, for her skillful technical assistance, and Mr. Masaaki Hoshino and Mr. Kiichi Takeuchi, Second Department of Oral Anatomy, Niigata University School of Dentistry, for their excellent histological and photographic assistance.

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