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Original Article

Mouse molar morphogenesis revisited by three dimensional reconstruction. I. Analysis of initial stages of the first upper molar development revealed two transient buds

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ABSTRACT Early stages of tooth development in the maxillary cheek region in the mouse were investigated by combined analysis of histological sections, computer assisted 3D reconstructions and morphometry. In ED 12.5 embryos, 3D reconstructions revealed an accessory epithelial bud (R1) and a large bud (R2), which appeared as a single bud-shaped epithelium in frontal sections. This developmentally most advanced dental epithelium in the mouse embryonic maxilla until ED 13.5, generally considered as the bud of the first molar, regressed during later development. Meanwhile the bud and cap of the first upper molar originated more posteriorly, from ED 13.5. The regression of R1 and R2 was associated with epithelial apoptosis. Apoptotic cells and bodies were apparent on sections in the R1 epithelium from ED 12.5. The R2 epithelium maintained the large bud-shaped appearance on sections, representing the largest part of the dental epithelium in the maxillary cheek region until ED 14.0; apoptoses were detected there as late as from ED 13.5. During regression, the R2 rudiment was transformed into the medial and lateral epithelial ridges, posteriorly in continuity with the arising cap of the first molar. The reduced R1 epithelium seemed to contribute to the medial ridge. These results should be taken into consideration in the interpretation of early odontogenesis in the upper jaw in the mouse. The interesting problem of the identification of tooth homology of the rudiments should be elucidated by further comparative morphological and paleontological investigations.

KEY WORDS: *mouse, tooth, development, molar, three dimensional, apoptosis*

Introduction

In order to understand morphogenesis, molecular data have to be correlated with indisputable morphological data. Edelman (1992) made an attempt to link animal form and tissue pattern to a specific molecular regulatory program and formulated the morphoregulation hypothesis. This hypothesis links genetic control and cellular driving forces (division, movement, adhesion and death of cells) to the spatial-dependent expression of morphoregulatory molecules (cell adhesion molecules, substrate adhesion molecules and cell junctional molecules) in tissues. This hypothetical scheme allows the ranking of a large number of molecules putatively implicated in morphogenesis into function specific classes and enhances understanding of shape formation including changes at various levels of biological organization. Edelman (1992) stressed that now "...even tighter bonds must be made between classical anatomical approaches and molecular physiology and molecular genetics. This rapprochement must be considered at all anatomical scales...".

Considerable data has accumulated from both *in vivo* and *in vitro* studies suggesting various extracellular and cellular factors to play important roles during odontogenesis (for recent reviews see Ruch, 1995a; Sharpe, 1995; Thesleff, 1995). Odontogenesis in the mouse is the model most frequently used to analyze mechanisms regulating spatial distribution of odontogenic cells and tooth class specific histo-morphogenesis (Ruch, 1995b). Results of such studies have been correlated with classical morphological knowledge on tooth development, achieved mostly from histological sections (Gaunt, 1956; Cohn, 1957; Pourtois, 1961). Only limited data are available on the spatial arrangement of the tooth primordia and its dynamic changes with time: Gaunt (1955, 1966) employed the graphic method combined with hand-made 3D models to document molar morphodifferentiation of the dental papilla and formation of a dental lamina for secondary teeth. Knudsen (1965)

Abbreviations used in this paper: 3D, three dimensional; ED, embryonic day; wtc, weight class.

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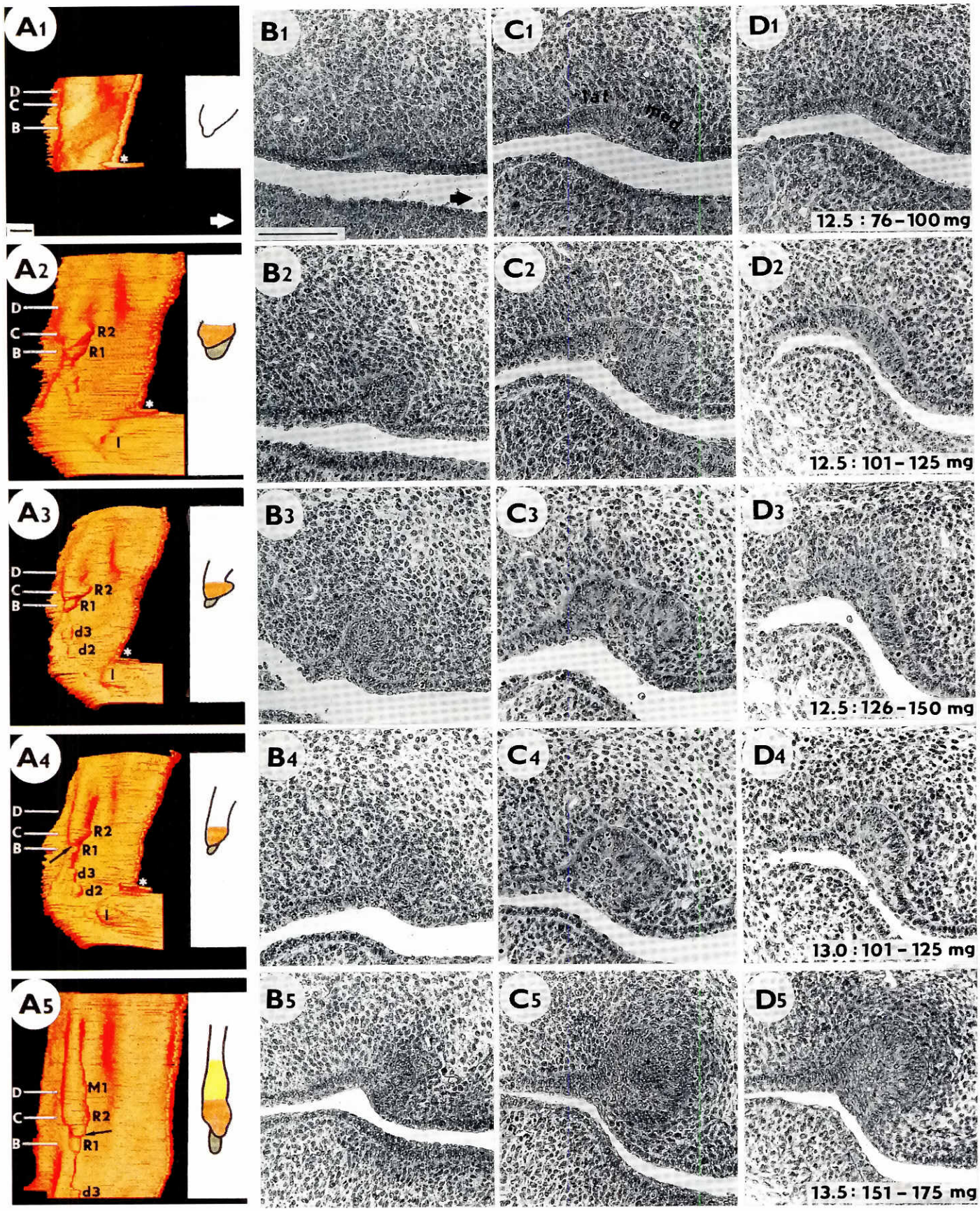
A1
D
C
B
B1
C1
D1
12.5 : 76–100 mg
A2
D
C
B
R2
R1
l
B2
C2
D2
12.5 : 101–125 mg
A3
D
C
B
R2
R1
d3
d2
l
B3
C3
D3
12.5 : 126–150 mg
A4
D
C
B
R2
R1
d3
d2
l
B4
C4
D4
13.0 : 101–125 mg
A5
D
C
B
M1
R2
R1
d3
B5
C5
D5
13.5 : 151–175 mg

made wax models of the incisor at bud and cap stages. The early development of the upper incisor and diastema region has been correlated in histological sections and computer-assisted 3D reconstructions (Peterková *et al.*, 1993b, 1995).

Knowledge on the spatial arrangement of a developing organ appeared to be essential for the understanding of development as a sequence of multifactorial interactions (Radlanski, 1995). Validity of descriptive data provided by morphological approaches is a prerequisite for correct correlation of molecular data with morphogenesis. The hitherto descriptive morphological knowledge, derived mostly from tissue sections, should thus be revisited and extended employing the technical advantages of modern computer assisted 3D reconstruction.

In the present study, the combined histological and computer-assisted 3D reconstruction analysis of the upper molar area was performed in mouse embryos with the aim of providing knowledge on the spatial arrangement of the upper molar epithelium at early stages. The fate of the large bud-shaped epithelium, generally considered to be the first molar primordium in day 12 embryos, was followed until day 14. Surprisingly, this epithelium appeared to be comprised of two distinct buds, which regressed during development of the enamel cap of the real first molar beyond it.

Results

Histological aspects

In frontal sections, the stages of dental development were determined according to the following criteria:

Dental epithelial thickening differed from the adjacent oral epithelium by comprising more layers of deeper columnar cells with the prevalent orientation of the long axes of their nuclei perpendicular to the basement membrane. At the oral surface, 1-2 layers of flat peridermal cells were present, as elsewhere (Fig. $1D_1$,D_2).

Dental lamina was formed by a fold of the thick stratum of the columnar cells with the prevalent orientation of the long axes of their nuclei perpendicular to the basement membrane. The fold groove was filled with accumulation of smaller cells. On the mesenchymal face, the medial and lateral slopes of the fold showed an angle equal to or larger than 90° with the adjacent epithelium (Fig. $1D_3$,D_4).

Dental bud comprised the stratum of larger (mostly columnar) cells at the basement membrane and smaller cells in the center. At least one of its medial and lateral mesenchymal faces exhibited a protrusion showing an angle smaller than 90° with the mesenchymal face of the adjacent epithelium (e.g. Fig. $1C_{2\text{-}5}$ and $1D_5$).

Morphogenesis

In the developmentally least advanced embryos at ED 12.5 (wtc. 76-100 mg), a low and large elevation with a narrow anterior extension was observed in the posterior part of the upper jaw oral epithelium (Fig. $1A_1$). On frontal sections, the dental lamina folded into the mesenchyme (Fig. $1B_1$). The dental lamina decreased posteriorly, giving rise to a large bifurcated invagination (Fig. $1C_1$). Behind this, only the epithelial thickening was present (Fig. $1D_1$). In developmentally more advanced specimens, a large dental bud (R2) was formed (Fig. $1A_2$,C_2 and $1A_3$,C_3). Its medial protrusion showed similarities to the medial elevation of the large bifurcated invagination at the earlier stage (compare Fig. $1C_2$ with $1C_1$). Behind the R2 bud, an epithelial thickening (Fig. $1D_2$) and later a dental lamina (Fig. $1D_3$) were present. Only the epithelial thickening could be detected in the most posterior part of the upper jaw.

The accessory bud (R1) was adjacent anteromedially to the R2 bud (Fig. $1A_2$,B_2 and $1A_3$,B_3). The accessory bud R1 appeared like a miniature of the posterior large one separated by a shallow groove (Fig. 3). From ED 12.5 (wtc. 101-125 mg), apoptotic cells and bodies were found in the R1 epithelium (Fig. 4A). On frontal sections, the mesenchymal cells were concentrically arranged around the accessory bud R1 at ED 12.5 (Fig. $1B_2$,B_3).

During ED 13.0 and 13.5, the cross-sectional area of the R1 epithelium reduced on frontal sections (Fig. $1B_4$,B_5), while this epithelium elongated anteriorly (Fig. $1A_4$,A_5). Posteriorly, the dental epithelium achieved a cylinder-like shape in 3D reconstructions, whose posterior end gradually elongated. The R2 bud became the anterior swelling of this structure, remaining the prominent part of the dental epithelium (Fig. $1A_4$,A_5). The conical end of the anterior swelling elongated anteriorly, and seemed to incorporate the posterior part of the R1 epithelium (Fig. $1A_5$). In sections, a well-formed lamina was present at ED 13.0 in the middle segment of the antero-posterior course of the dental epithelium, behind the anterior swelling (Fig. $1D_4$). In this place, a new swelling appeared on the epithelial cylinder at ED 13.5 (Fig. $1A_5$), reflecting initiation of the first molar bud (Fig. $1D_5$). Posteriorly, the height of the dental epithelium gradually decreased forming the dental lamina and, close to the isthmus faucium, the epithelial thickening.

Apoptosis remained present in the R1 epithelium and could also be detected amongst the R2 epithelial cells close to the oral cavity from ED 13.0. A significant number of apoptotic cells and bodies were found on sections at the top and amongst adjacent internal cells of the R2 bud at ED 13.5 (Fig. 4B). A distinct concentric arrangement of the adjacent mesenchymal cells was observed around the R2 epithelium from ED 13.0 (Fig. $1C_4$), and around the bud-shaped epithelium of the first molar from ED 13.5 (Fig. $1D_5$).

The R2 epithelium increased its height (Fig. $2C_1$, C_2 and lateral views on 3D reconstructions – data not shown) and remained the prominent part of the dental epithelium before ED 14.0 (Fig. $2A_1$) in spite of the presence of apoptoses. The anterior cone was well-detected (Fig. $2A_1$,B_1,A_2,B_2). At ED 14.0, a distinct individualization of the first molar anlage was apparent posteriorly (Fig. $2A_2$,D_2). From ED 14.0, the R2 epithelium started to reduce, more at its lateral side ($2A_3$), and achieved a form of two epithelial ridges at ED

Fig. 1. 3D reconstructions of the right upper jaw epithelium and schematic interpretation of the arrangement of the dental epithelium in the cheek region. *The mesenchymal face is represented in the aerial view (A_2,A_5) or antero-medial view (A_1,A_3,A_4); the anterior part is at the bottom of each picture. R1, bud-shaped or reduced epithelium of the accessory rudiment (green); R2, bud-shaped epithelium of the large rudiment (orange); M1, bud-shaped epithelium of the first molar anlage (yellow). The uncoloured part of the demarcated area of the dental epithelium in the scheme corresponds to the dental lamina morphology in sections. I, the incisor anlage; d2 and d3, the second and third diastemal dental rudiment (Peterková et al., 1995), respectively. Slim black arrow points to the anterior cone of the dental epithelium. Star indicates the position of the primary choana. The dental epithelium in frontal histological sections in the antero-posterior direction ($B_{1\text{-}5}$, $C_{1\text{-}5}$, $D_{1\text{-}5}$) at respective levels, is indicated by white bars* **(B,C,D)** *in the related 3D reconstructions. med and lat, medial and lateral protrusion respectively, of the large epithelial invagination. Age in ED and weight in milligrams (mg) of the embryos are shown. Large arrow points medially. Bar, 100 μm.*

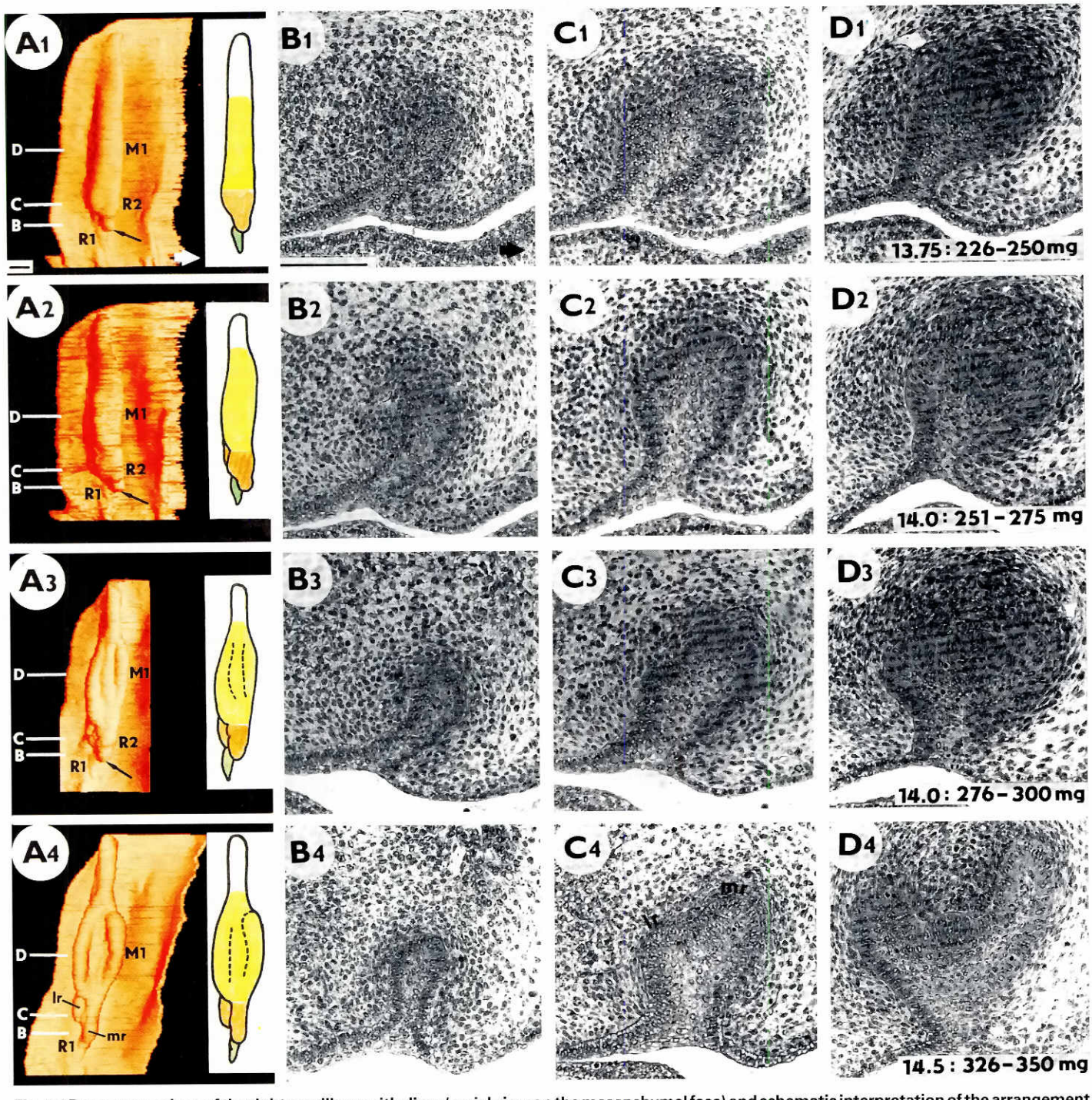

Fig. 2. 3D reconstructions of the right maxillary epithelium (aerial view on the mesenchymal face) and schematic interpretation of the arrangement of the dental epithelium; *the anterior part is at the bottom of each picture (A_{1-4}). Green, reduced epithelium of the accessory rudiment (R1). Orange, bud-shaped epithelium of the large rudiment (R2), which started to transform at ED 14.0 (A_3) into the medial (mr) and lateral (lr) epithelial ridges distinct at ED 14.5 (A_4). Yellow, late bud-shaped epithelium (A_1,A_2) or cap (A_3,A_4) of the first molar (M1). The uncoloured area in the scheme (A_1,A_2) corresponds to the dental lamina morphology in sections. The boundary of the first molar late bud (A_2) or cap (A_3,A_4) with the R2 epithelium was determined according to difference in morphology on histological sections. The boundary could only be estimated at ED 13.75 (A_1). Slim black arrow points to the anterior cone. The dental epithelium in frontal histological sections in the antero-posterior direction (B_{1-4}, C_{1-4}, D_{1-4}) at respective levels, is indicated by white bars* **(B,C,D)** *in the related 3D reconstructions. Age in ED and weight in milligrams (mg) of the embryos are shown. Large arrow points medially. Bar, 100 µm.*

14.5 (Fig. 2A_4,C_4). During this process, a sagittal furrow arose on the epithelial convexity, separating both ridges (Fig. 2A_4,C_4). In the posterior direction, the ridges were in continuity with the anterior end of the forming cap of the first molar. The medial ridge was higher and extended more anteriorly than the lateral one; it included the former anterior cone of the dental epithelium and

seemed to incorporate the remnant of the R1 epithelium during its anterior elongation (Fig. $2A_4$).

Morphometry

All curves documenting the antero-posterior changes in the cross-section area of the dental epithelium (Fig. 5) exhibited a peak in their anterior segment within ED 12.5-14.5. This peak reflected the existence of the large tooth bud R2 in the cheek region at early stages and was dominant on the curves before ED 13.5. Due to its smaller size and position, the accessory bud R1 did not give rise to an apparent accessory peak on the anterior slope of the dominant peak. During further development, the anterior peak amplified, reflecting increase in size of the R2, which became the anterior swelling of the dental epithelium in the maxillary cheek region within ED 13.0-14.0. From ED 14.0, the anterior peak decreased, as a result of regression of the R2 epithelium and formation of the medial and lateral ridges anterior to the first molar cap. The anterior extension of the anterior slope of the peak reflected the elongation of both the anterior cone of the dental epithelium at ED 13.0-14.0 and the medial ridge at ED 14.5 (compare Fig. 5 with Figs. 1 and 2).

At ED 13.5, a prominent posterior peak arose, corresponding to the initiation of formation of the first molar bud. From ED 13.5, the epithelium behind the anterior swelling rapidly increased its cross-sectional area, reflecting development of the first molar anlage, clearly seen at ED 14.5 (Fig. 5).

Discussion

The present data demonstrate that the classical description of initial upper first molar development in mouse is misleading.

Terminology related to the structures derived from "two-dimensional" sections does not fit with their form revealed by three-dimensional reconstructions. In spite of a bud-shaped appearance of the dental epithelium on frontal sections of the maxillary cheek region within ED 12.5-14.0, a substantial difference was found in its spatial arrangement during this period (Figs. 1 and 2). Both analysis of 3D representations and their careful backward correlation with original sections led to the understanding of the real form of developing structures in space and their dynamic changes in time.

It has been generally assumed that the large epithelial bud in the maxilla of day 12 mouse embryos represents the primordium of the first molar and is later transformed into the first molar enamel cap. The present data revealed that this structure comprised two rudimental buds: an accessory bud R1 and a large bud R2. Due to their respective positions, their individuality can be hardly detected on frontal sections: the accessory bud R1 appears as the anterior part of the large bud R2. These rudiments regressed, giving rise to two epithelial ridges, while the first molar bud and cap originated more posteriorly. The epithelial ridges were in continuity posteriorly with the anterior end of the epithelial cap of the first molar. The ridges are still very apparent at the bell stage and might be involved in secondary anterior elongation of the first molar enamel organ (Lesot *et al.*, in this issue).

Our morphological data do not support the interpretation of the first molar development provided by MacKenzie *et al.* (1992) and Sharpe (1995). MacKenzie *et al.* (1992) documented in day 11.5 mouse embryos a dental placode, which is incorporated into the buccal aspect of the external enamel epithelia of the invaginating

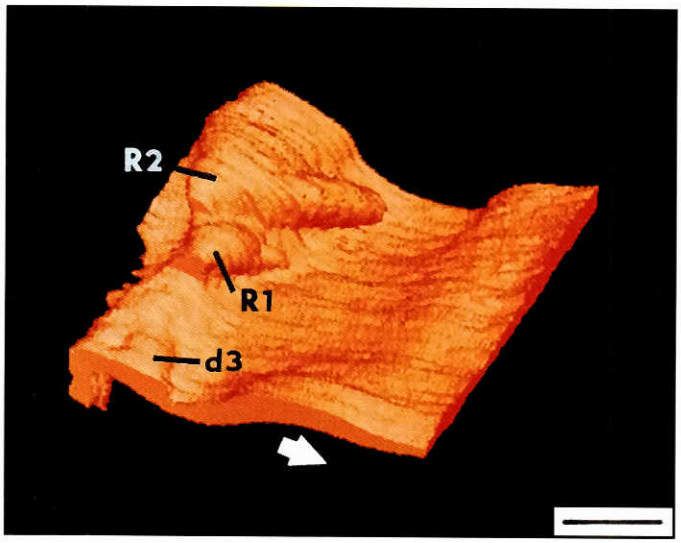

Fig. 3. Mesenchymal face of the accessory rudiment (R1) and the large rudiment (R2) *at ED 12.5, wtc. 126-150 mg, in a 3D reconstruction viewed from the antero-medial side. d3, third diastemal rudiment. Large arrow points medially. Bar, 100 μm.*

bud of both the upper and lower first molars. This site of placodal incorporation has been assumed to correspond later to the site of the enamel navel connected by the enamel septum with the enamel knot in the molar cap (MacKenzie *et al.*, 1992; Sharpe, 1995). According to our present results, however, the arrangement of the dental epithelium, as documented for the first molar in 11.5 day embryos by MacKenzie *et al.* (1992), corresponded to the site of forming epithelial ridges in front of the first molar cap at ED 14.0-14.5.

Not only progressive, but also regressive processes participated in positional determination and early development of the first upper molar in the mouse. At early stages, both the progression of the dental epithelium in the maxillary cheek region and regression in its anterior part (including R1 and R2 rudiments) occurred in the antero-posterior direction. The apoptotic cells and bodies first appeared in the young bud R1 at ED 12.5, synchronously with those observed in the diastemal rudiments (Turecková *et al.*, 1996). The apoptotic process in the rudiment R2 started one day later, affecting its epithelium at the well-formed bud stage. During further development, the apoptotic activity culminates in the anterior part of the dental epithelium in the maxillary cheek region. As a result, this epithelium reduced, which appears to play an important role in anterior delimitation of the first molar cap (Lesot *et al.*, in this issue).

The present morphological data should be taken into consideration in the interpretation of results of molecular studies on the maxillary tooth development in day 11-13 mouse embryos. The expression of *msx-1*, *msx-2* and *BMP-4* has been documented in the developmentally most advanced part of the maxillary dentition at early stages and related to the first molar anlage (MacKenzie *et al.*, 1991, 1992; Vainio *et al.*, 1993; Thesleff *et al.*, 1995). Our data demonstrated that this developmentally most advanced part does not correspond to the first molar primordium (transformed later in the cap), but to that part, which will regress in the presence of apoptosis. From this point of view, our previous *in situ* hybridization data (Turecková *et al.*, 1995) need also to be re-evaluated (Lesot

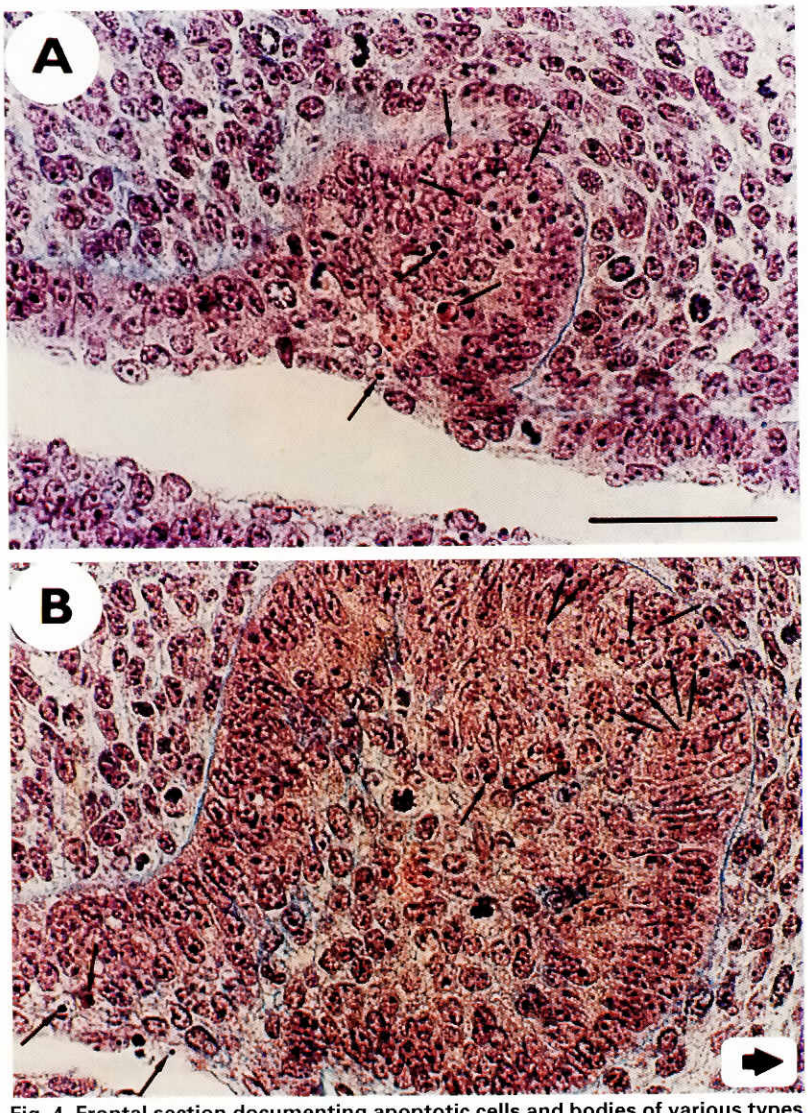

Fig. 4. Frontal section documenting apoptotic cells and bodies of various types *(slim arrows) in the accessory rudiment R1 at ED 12.5, wtc. 101-125 mg* **(A)** *and in the large rudiment R2 at ED 13.5, wtc. 151-175 mg* **(B).** *Large arrow points medially. Bar, 50 μm.*

et al., in this issue). Similar re-assessment of our former morphological data (especially Peterková, 1985; Peterková *et al.*, 1995; Turecková *et al.*, 1996) shows that what has been considered as the first upper molar at early stages, corresponds mostly to the R1-R2 rudiments. Comparison of the mouse molar development in the upper jaw with the mandible, which is more often used in tooth developmental studies, is necessary.

In rodents, the dental lamina or even rudimental primordia of the missing teeth have been reported in the prospective diastema in Sciuridae and Cavidae (Freund, 1892; Adloff, 1898; Tims, 1901; Luckett, 1985). The tooth vestiges are more often present in the upper jaw, which seems to retain the more primitive condition, than the lower one in rodents (e.g. Tims, 1901; Luckett, 1985; Peterková 1995). In Muridae, 3 distinct rudimental buds (D1,D2 and D3) have been documented in the prospective upper diastema region of mouse embryos (Peterková *et al.*, 1995). The epithelial apoptosis appears to be responsible for their extinction (Turecková *et al.*, 1996). Now, two further distinct transitory buds R1 and R2 were demonstrated in front of the upper first molar bud and arising cap. Correlation of the temporospatial pattern of gene expression (Turecková *et al.*, 1995) with the distribution of cell death suggests a regulatory role of BMP-4 in apoptosis during regression of antemolar rudiments in mouse (Turecková *et al.*, 1996; Lesot *et al.*, in this issue). There is a well-supported example among other metameric structures documenting participation of the BMP-4 regulated apoptosis in segment specific depletion of neural crest in rhombomeres 3 and 5 of the chick embryo. As a result, the discrete streams of neural crest cells, migrating into the branchial region, are sculptured (Graham *et al.*, 1994). According to Saunders (1966), cell death is the usual method to eliminate phylogenetic vestiges – phylogenetic death. The antemolar dental rudiments in mouse may represent repeated ancestral teeth, which are suppressed by apoptosis.

The correct identification of the first molar is critical for the determination of the number and nature of the anterior maxillary teeth (Luckett, 1993a). Therefore, the tooth homologies of the maxillary dental rudiments in mouse should be related to the identification of homologies of the maxillary functional teeth. In comparison with the eutherian general dental formula, where 3 incisors, 1 canine, 4 premolars and 3 molars are present in each dental quadrant, in mouse only one incisor exists separated by a toothless diastema from the group of 3 molariform teeth. It is generally supposed that the latter teeth in mouse correspond to the three (permanent) molars of other mammals. The mammalian molars are defined as the posterior members of deciduous dentition without successors (Miles and Poole, 1967). A "successor" dental lamina, however, has been found during development of the molars in mouse (Gaunt, 1966) and also in man (Ooë, 1979). The first (permanent) molar in primates, including man, has been hypothetically identified as the fourth unreplaced premolar (Bolk, 1914; Adloff, 1916). The same has been proposed in rodents by the so-called premolar theory (for review see Wilson, 1956). A new scheme for the identification of tooth homologies in mammals has been suggested by Osborn (1978) on the basis of the dental formula of the common ancestors to both Eutheria and Metatheria, which possessed 5 premolars and 3 molars. According to this scheme, the first, second and third molar in muroids presumably correspond to the unreplaced fourth and fifth premolar and to the first molar, respectively, of the ancestral dental formula (Osborn, 1978). For further problems and hypotheses related to the identification of premolar and molar tooth homology in mammals, see the recent detailed survey provided by Luckett (1993b). Several possibilities thus exist as to how to interpret the dental rudiments in the upper jaw in the mouse, depending on the interpretation of the mouse functional molars. On the basis of the present data, however, any attempt would be purely speculative.

The early tooth development and determination of the functional dental pattern in the upper jaw in the mouse appeared to be more complicated and dynamic than previously expected. Coor-

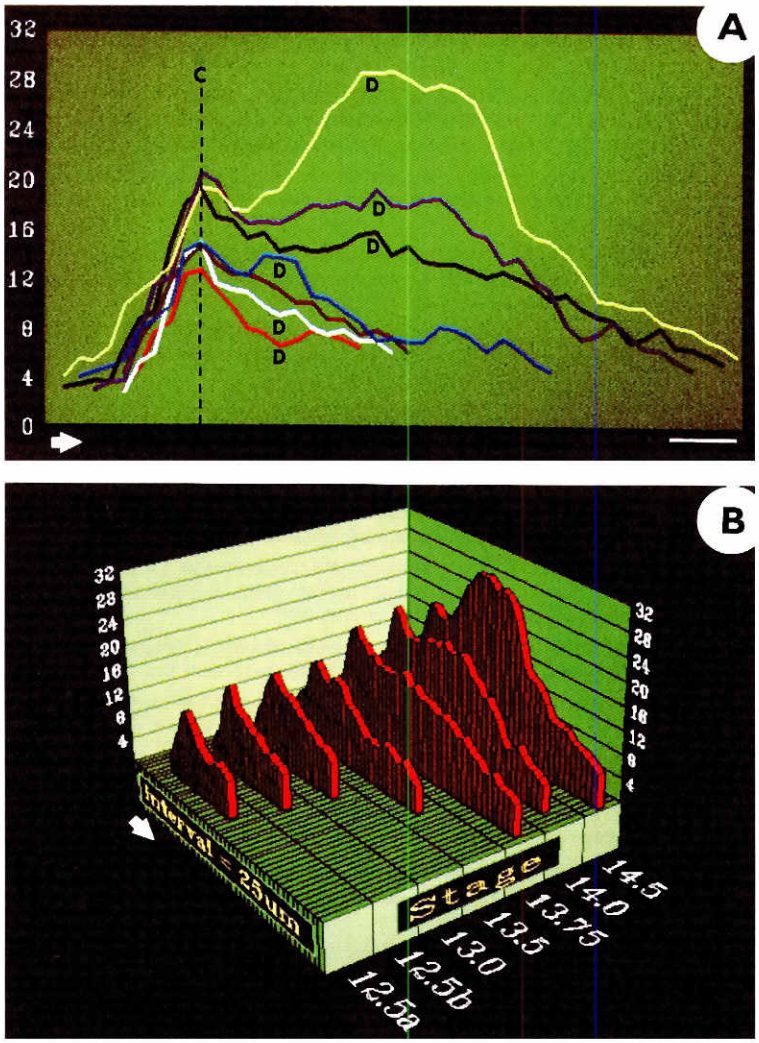

Fig. 5. Morphometric evaluation of the dental epithelium. (A) *Anteroposterior size changes of the cross-sectional area in square micrometers x1000 of the upper dental epithelium at ED 12.5a, wtc. 101-125 mg (red), 12.5b, wtc. 126-150 mg (white), 13.0, wtc. 126-150 mg (brown), 13.5, wtc. 151-175 mg (blue), 13.75, wtc. 226-250 mg (black), 14.0, wtc. 251-275 mg (violet) and 14.5, wtc. 326-350 mg (yellow). The curve position was standardized according to their anterior peak (dashed line). The position of the histological sections documented in Figures 1 and 2 at levels C and D are indicated by a dashed line C (±2 sections) and by D, respectively, on curves of the corresponding embryos. Stages 13.0 (wtc. 101-125 mg) and 14.0 (wtc. 276-300 mg) were omitted because of overlapping of curves. Note the growth of the dental epithelium propagating in the antero-posterior direction at early stages. Large arrow points posteriorly. Bar, 100 µm.* **(B)** *The same curves represented in the three dimensional graph. Large arrow points posteriorly.*

dinated studies including comparative morphology, molecular investigations and paleontological aspects should lead to the elucidation of the morphogenetic mechanisms and regulatory factors participating in patterning and morphogenesis of functional teeth in dentition.

Materials and Methods

ICR female mice were mated overnight and the midnight before the morning detection of the vaginal plug was considered as the start of prenatal development: the embryonic day (ED) 0.0. This means that for example day 12 embryos (harvested at noon of day 12 when the day of vaginal plug indicates day 0) corresponded to ED 12.5 embryos here. Embryos and fetuses of at least 3 pregnant mice were harvested in 12 h intervals (at noon and midnight) within ED 12.5-14.5. In addition, one intermediate stage (ED 13.75) was included. As morphological criteria of embryo staging (Grüneberg, 1943; Theiler, 1972; Kaufman, 1994) appeared to be too crude for a more detailed developmental study, weight of specimens was used for more precise specification of their chronological stage (Peterková *et al.*, 1993a, for details).

Histology

For histological processing, one specimen was used from each of the weight classes present at each of the chronological stages. 5 µm serial frontal sections were prepared from heads fixed in Bouin-Hollande solution and embedded in paraffin. The sections were stained with alcian blue-hematoxylin-eosin. The apoptotic cells and bodies were detected by morphological criteria (Kerr *et al.*, 1995; Turecková *et al.*, 1996). The apoptotic nature of such structures has been confirmed by the TUNEL method elsewhere (Turecková *et al.*, 1996; Lesot *et al.*, in this issue).

3D reconstructions

Six specimens at ED 12.5, 2 specimens at each ED 13.0 and 14.0, and 1 specimen at remaining stages were investigated. Serial drawings of the right maxillary oral epithelium (delimited medially by the edge of the palatal shelf and laterally by the inflection of the vestibulum oris) were made at a magnification 280x or 320x, using a Wild-Leitz Orthoplane or a Zeiss Jenaval microscope, equipped with a drawing chamber. The serial drawings were digitalized by a Hamamatsu C2400 camera connected to a digital imaging system, using the best fit procedure (Gaunt and Gaunt, 1978). A real time superposition allowed correlation of successive images (Olivo *et al.*, 1993). Software used for image acquisition and treatment was specifically developed and adapted for this work. The generation of 3D images was performed using a volume rendering program (Sun Voxel, Sun Microsystems). The mesenchymal side of the oral epithelium was represented under antero-lateral, antero-medial, postero-lateral, postero-medial, lateral, medial and aerial views.

Morphometry

The measurements were performed in 2 embryos at each ED 12.5, 13.0 and 14.0, and in 1 specimen at each ED 13.5, 13.75 and 14.5. The cross-sectional area of the dental epithelium in the right maxillary cheek region was measured in each 5th drawing (i.e. in 25 µm intervals) using a planimeter (REISS). The medial and lateral demarcations of the measured area were determined in the place where thickness of the dental epithelium decreased to the thickness of the adjacent oral epithelium. The measurements were not performed in the most posterior maxillary region, as the demarcations were not distinct there. The values in square micrometers were plotted graphically (Fig. 5).

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