PRE-NATAL DEVELOPMENT OF THE MOUSE INCISOR

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Pregnant Swiss albino mice were sacrificed each day from day 12 to day 20 post-fertilization. Heads of fetuses were prepared for histological study of the developing incisor teeth. The stages of differentiation were described from the first appearance of the incisor anlage in the embryo to the end of gestation.

The mouse possesses a monophyodont dentition consisting of one incisor and three molar teeth per quadrant. Both incisor and molar development begin during the latter half of the 21-day gestation period (1,2). Embryonic molar development in the mouse has been described (1,3), however accounts of embryonic incisor development have appeared as isolated stages usually characterized by comparative descriptions of control teeth with various abnormal patterns of development induced by a variety of experimental procedures (4,5,6). Knudsen (7,8,9) has previously described normal prenatal incisor development from day 15 through day 18 post-fertilization in control mice during investigations concerning the effects of hypervitaminosis A on embryonic incisor development in the mouse. A brief description of normal incisor development in the mouse from day 13 through day 18 post-fertilization recently appeared (10).

Descriptions of normal embryonic development of the mouse incisor currently available, however, have not presented the continuity necessary for a full appreciation of the sequential stages of differentiation of this structure. Furthermore, with increasing use of the embryonic dental organ of the mouse for studies concerning teratogenic effects on pre-natal dental development, knowledge of the normal pattern of the differentiation sequence of this structure is important for meaningful interpretations. The purpose of this report is to trace the salient morphological features of the mouse incisor from its first appearance in the embryo to the end of gestation.

METHODS

Female Swiss albino mice were placed with males at midnight and six hours later the males were removed. The females were then assumed to be pregnant and in the first day of gestation. Among females which proved to be pregnant, five were sacrificed each day from day 12 to day 20 post-fertilization. The fetuses were decapitated and their heads were fixed in Bouin's solution and embedded in paraffin. Heads were sectioned at 10 microns in both frontal and horizontal planes. Sections were stained with hematoxylin and eosin. Photomicrographs were taken of those sections which appeared to best represent the particular stage of development at each day of sacrifice.

RESULTS AND DISCUSSION

Incisor teeth in the mouse make their first appearance at day 12 post-conception as a proliferation of oral ectoderm into underlying mesenchyme in specific areas of future dental development (Fig. 1). The

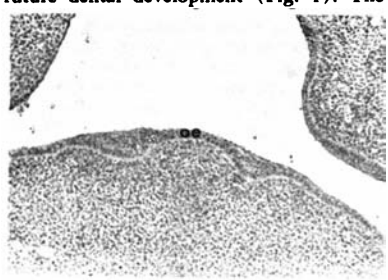

FIGURE 1. Photomicrograph of a frontal section of a mouse embryo head (12 day post-conception) indicating lower incisor anlages proliferating from oral ectoderm (oe). H&E stain. X100.

oral epithelium consists of several layers of cuboidal cells separated from underlying mesenchyme by a basement membrane. Initiation of incisor dental development is marked by a more rapid division of certain cells in the basal layer of the oral ectoderm as compared to adjacent cells resulting in an epithelial thickening of 5-7 cell layers in

the anterior portion of the oral cavity. The structure represented by the localized stratification of oral ectoderm appears to correspond to a similar structure in human dental development which is termed the dental lamina (11).

At day 13 post-conception, the incisor anlage is characterized by further growth into the underlying mesenchymal tissue and appears in both frontal and longitudinal sections as a rounded structure attached to the oral ectoderm (Fig. 2). Cells forming

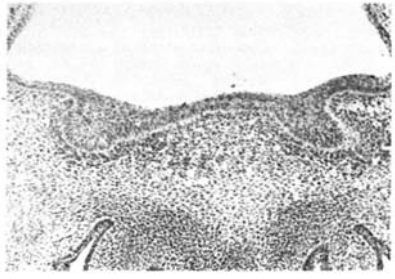

FIGURE 2. Photomicrograph of a frontal section of a mouse embryo head (13 day post-conception) showing deeper proliferation of lower incisor buds into underlying mesenchyme. H&E stain. X100.

the circumference of the structure appear to be a continuous extension of the basal layer of the oral ectoderm. Mesenchymal cells surrounding the incisor bud are more deeply stained and are separated from the dental structure by lighter-stained basement membrane. The lighter stained mesenchymal tissue is marked by an increased vascularity over the previous day of development. Mitotic figures are especially prominent in rapidly dividing cells of the bud-shaped structure.

Unequal growth of the incisor bud can be seen by day 14 post-conception in a hollowing out of the basal portion of the structure producing an almost symmetrical concavity facing the mesenchyme in a posterior and medial direction (Fig. 3). The structure resembles the "cap" stage of the previously described molar development (1) as its margin is characterized by cuboidal cells continuous with the basal layer of the oral ectoderm. The cells form a sharp turn at the rim of the "cap" and continue as a layer of tall cells completing the

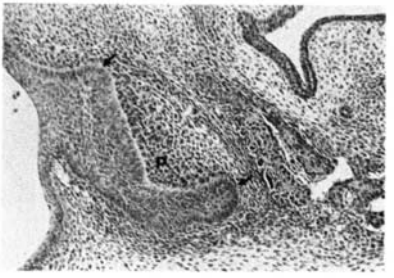

FIGURE 3. Photomicrograph of a frontal section of a mouse upper incisor (14 day post-conception) showing the medially directed concavity of the dental organ, cervical loop formation (arrows), and dental papilla (p). H&E stain. X130.

inner lining of the concavity. Using human dental terminology, the cells of the inner concavity would be termed the inner enamel epithelium (11). Cells forming the rim of the concavity would represent the cervical loop and the cell layer connecting the loop with the oral ectoderm would be termed the outer enamel epithelium (11). The mesenchymal tissue area partially enclosed by the structural concavity appears darker stained and more densely packed than outlying mesenchyme and in human terminology is referred to as the dental papilla (11). Dark-stained mesenchymal cells also are evident around the periphery of the dental organ adjacent to the outer enamel epithelium and appear to correspond to a similar structure in human development which is termed the dental sac (11). Capillaries are seen in the mesenchymal tissue as well as the beginning of bone formation.

By day 15 post-conception, the margins formed by the transition of the inner enamel epithelium have continued proliferation into the underlying mesenchymal tissue along with a deeper invagination of the dental organ to form a structure resembling a bell (Fig. 4). The outer enamel epithelial layer remains connected to the oral ectoderm. In longitudinal sections, cells of the outer enamel epithelial layer appear as columnar types on the lingual surface of the structure, while cells of the labial outer enamel epithelial layer appear cuboidal and form a much longer extension from the oral ectoderm due to the angle in which the dental organ is positioned with respect to

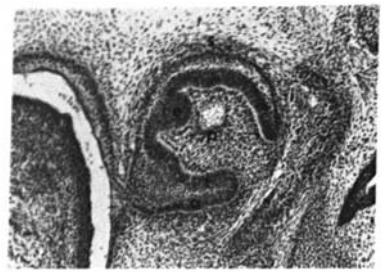

FIGURE 4. Photomicrograph of a longitudinal section of a mouse upper incisor (15 day post-conception) showing enamel knot (e), inner (i) and outer (o) enamel epithelium, dental sac (s), dental papilla (p), and cervical loop. Top surface of structure is labial. H&E stain. X100.

the plane of the jaws. The area between inner and outer epithelium remains densely packed with dark-stained cells except in the labial region. Here, cells are less dense in distribution, are "star-shaped" with long processes, and appear similar to cells of the stellate reticulum of human development (11). Cells of the inner enamel epithelium are dark-stained, and columnar in appearance with many mitotic figures at various levels. Cells at the apex of the concavity formed by the inner enamel epithelium appear as a knob-like elevation into the interior of the "bell" bounded by grooves on either side. This structure corresponds to the enamel knot in human dental development (11). Cells of the dental papilla are dark-stained, and still separated from the inner enamel epithelium by a light-stained basement membrane. These cells, however, appear less densely packed than in previous stages and the area has become increasingly vascularized. The labial cervical loop is marked by an abrupt transition from a columnar inner enamel epithelial cell layer to a cuboidal outer enamel epithelial cell layer. This transition is not evident in the lingual cervical loop. Mesenchymal cells comprising the dental sac which surrounds the outer enamel epithelium still retain their dark stained appearance and this area is characterized by an increased presence of capillaries. Bone formation is also evident at this stage.

At day 16 post-conception, the bell-shaped dental organ has increased in length due mainly to proliferation in a posterior direction of the labial cervical loop (Fig. 5). The inner enamel epithelium is comprised of tall columnar cells characterized by mitotic figures and separated from the adjacent dental papilla by a light-stained area which represents the future dentino-enamel junction. The sharp, labial cervical loop still is marked by the sudden transition of columnar to cuboidal cell types. Loosely-packed stellate appearing cells occupy most of the area between the inner and outer enamel epithelial layers. The dental organ still retains its attachment to the oral ectoderm. With increased capillary infiltration, cells of the dental sac and dental papilla become less densely packed.

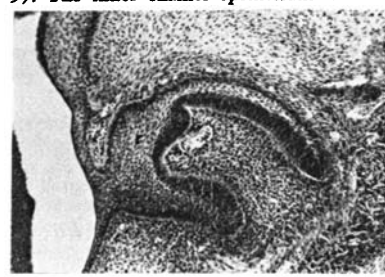

FIGURE 5. Photomicrograph of a longitudinal section of a mouse upper incisor (16 day post-conception) showing deeper proliferation of cervical loop, increased vascularity of dental sac and dental papilla, and stellate reticulum (r). Top surface of structure is labial. H&E stain. X100.

At day 17 post-conception, the structure of the developing dental organ has changed little except for an increase in length as

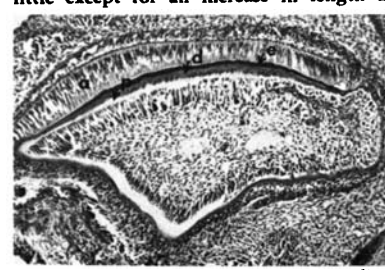

FIGURE 6. Photomicrograph of a longitudinal section of a mouse upper incisor (17 day post-conception) showing increased length of dental organ, stratum intermedium (arrow), and odontoblasts (o). Top surface of structure is labial. H&E stain. X100.

the margins formed by the cervical loops have pushed deeper into the mesenchyme in a generally posterior direction and at an angle to the plane of the oral ectoderm (Fig. 6). Lingual and labial grooves on either side of the enamel knot still are evident and vascularized dental papilla fills the inside of the bell-shaped structure. Dark-stained cells of the dental papilla are now aligned opposite the light-stained dentino-enamel junction. These cells represent the future odontoblasts which will secrete dentin. Columnar cells of the inner enamel epithelium which represent the future enamel secreting ameloblasts are bordered on the labial surface by a thin layer of flattened cells which correspond to the stratum intermedium of human development (11). The highly vascularized dental sac has become less prominent.

By day 18 post-conception, the dental organ has lost its connection to the oral ectoderm (Fig. 7). The structure has increased even further in length as the cervical loop has continued to proliferate posteriorly. Labial odontoblasts have differentiated into tall columnar cells and are secreting pre-dentin. The stratum intermedium is especially prominent on the labial surface as a border of cuboidal cells adjacent to the differentiating ameloblasts. Remnants of the dental sac and outer enamel epithelium are present and bone is beginning to encircle the structure. Vascularized dental papilla which represents the future dental pulp still completely fills the concavity of the bell-shaped structure. The apex of the dental organ has assumed a more pointed shape as future development in this area will produce an incisal edge.

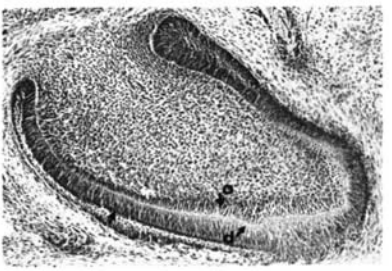

FIGURE 7. Photomicrograph of a longitudinal section of a mouse upper incisor (18 day post-conception) showing differentiated odontoblasts (o), pre-dentin formation (p), stratum intermedium (arrow), and developing ameloblast layer on labial surface (a). H&E stain. X100.

At day 19 post-conception, the dental organ has retained the general structural pattern established in previous days of development (Fig. 8). Odontoblast cells opposite the labial ameloblast area continue their secretion of pre-dentin which is bordered by a layer of dark-stained mature dentin.

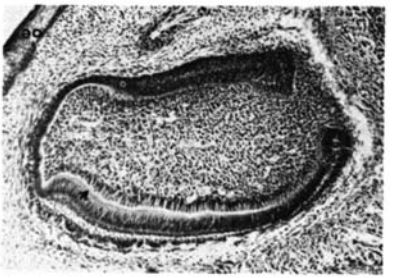

FIGURE 8. Photomicrograph of a longitudinal section of a mouse upper incisor (19 day post-conception) showing labial dentin, oral ectoderm (oe), stratum intermedium, and dentin formation (arrow). Top surface of structure is labial. (The structure appears proportionately smaller due to the angle of the section). H&E stain. X100.

Day 20 post-conception is marked by the beginning of enamel secretion by the labial ameloblasts which have become considerably taller in appearance than previously (Fig. 9). The apparent relationship be-

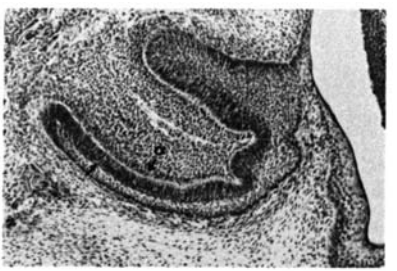

FIGURE 9. Photomicrograph of a longitudinal section of a mouse upper incisor (20 day post-conception) showing differentiated labial ameloblasts (a), enamel (e), mature dentin (d), and pre-dentin (p). (The structure appears proportionately smaller due to the angle of the section). H&E stain. X100.

tween ameloblast differentiation and the transition of the stratum intermedium from flattened to cuboidal appearing cells previously was noted in the mouse molar (1, 12). In the mouse incisor, the stratum intermedium bordering the lingual ameloblast layer does not undergo this transition which results in a tooth with an enamel covering on the labial surface only. Both labial and lingual odontoblasts are secreting a predentin layer which is bordered by a layer of dark-stained mature dentin. The structure continues to elongate as hard tissues are being secreted by the odontogenic cells and by the end of gestation the general pattern of the adult incisor in the mouse is established.

The time of occurrence and structural features of those stages of incisor development previously reported appear to agree with the corresponding stages observed in this study. In addition, previously undescribed stages of the prenatal development of the mouse incisor were described. The similarity in the developing structures of the prenatal mouse incisor and the developing human dentition suggests that the mouse can serve as an excellent experimental animal for investigations concerning dental embryology.

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