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Spontaneous complete clefting of the palates in a mouse fetus: A study by scanning electron microscopy and serial section reconstruction

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Summary. A 15 day mouse fetus having spontaneous complete clefting of the primary and secondary palates was studied in comparison with its normal litter mates and with normal 14 day fetuses.

Specimens were studied by scanning electron microscopy at various stages of microdissection, by light microscopy of thin serial sections and by serial section reconstruction of the anterior chondrocranium of the clefted specimen and one of its normal litter mates.

Differentiation of tooth and bone tissue was slightly retarded in the clefted fetus but paranasal and oral landmarks, though distorted, were present. The clefted fetus had a smaller angle between cranial base and nasal capsule and a marked discontinuity between the primary and secondary palates. Cell surfaces on the medial edge of the secondary palate in the clefted fetus resembled cell surfaces of oral areas that do not normally fuse, i.e. they are polygonial, flat and bear few surface projections in constrast to the normal 14 day condition where these cells are spindle shaped, convex and have many microvilli.

The observations support the concepts that clefting of the secondary palate is consequential to clefting of the primary palate, that maldevelopment of neural crest mesenchyme is not necessarily a contributing factor, that clefting of the primary and secondary palates is associated with a shorter anterior-posterior dimension of the head and that when fusion of palatal shelves fails to occur the cells of the medial edges modulate in the direction of a generalized type of surface epithelium.

Key words: Mouse fetus - Palatal cleft - Cell surface - Chondrocranium - SEM

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Introduction

The occurrence of a single fetus with cleft palate and double cleft lip in an otherwise normal litter of eight 15-day *post-coitum* mice has provided an opportunity of comparing the anatomy of a spontaneously clefted individual with its unaffected littermates. Other normal fetuses at relevant stages of development were also examined.

Materials and methods

The specimens were obtained from random-bred Swiss-Webster mice. Fertilization time was detailed according to the protocol of Long et al. (1974). The dams were killed by cervical dislocation on the 14th or 15th day of gestation and the gravid uteri were placed in isothermal Ringer's solution. Besides the one 15-day clefted specimen, four unaffected fetuses from the same litter and two from another 15-day litter were taken, as well as four normals from two 14-day litters. The fetal heads were fixed in glutaraldehyde, following with osmium tetroxide by standard procedures (Tamarin and Boyde, 1977). Some heads (including the clefted specimen) were prepared for scanning electron microscopy (SEM) by critical-point drying with Freon. Others were embedded in araldite for serial sectioning at 2 μm and staining with azure II and methylene blue (Richardson et al., 1960). For SEM, heads were mounted on aluminum stubs and sputter-coated with gold-pladium in an Argon atmosphere. Micrographs were taken with an ETEC at 20 kV using apertures of 100 or 200 μm.

The clefted fetus was dissected, recoated and sequentially examined by SEM a number of times. Subsequently the remaining part of this specimen was immersed in fluid araldite for 48 hours then polymerized at 60° for 24 hours. It was serially sectioned at 2 μm for light microscopy. The chondrocranium of the clefted

specimen (left half) and of a littermate (left half) were reconstructed at X 40 from camera lucida drawings of every 5th section. Registration was provided by grooves cut in the sides of the araldite blocks. The magnification of illustrations is given as "picture width"(PW).

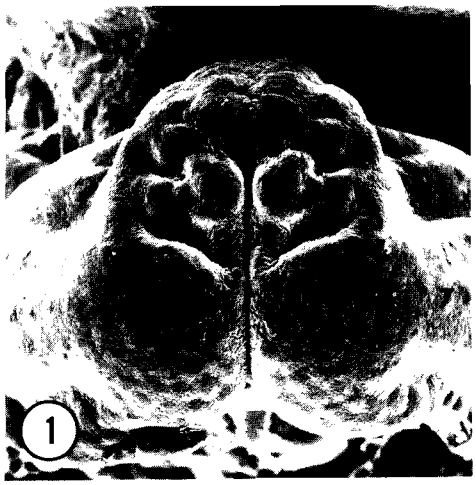
Results

The clefted specimen is compared with a litttermate in frontal view in Figs. 1 and 2. In the abnormal fetus (Fig. 2) bilateral labial clefts separate the medial nasal prominences. The seven swellings bordering each labial cleft have counterparts in the normal littermate (Fig. 1).

The normal 14-day palate is compared with the clefted 15-day specimen, and with a normal 15-day littermate in Figs. 3, 4, and 5. The palatal shelves of the clefted fetus resemble the 14-day picture, with one obvious difference: they have remained entirely separate from the primary (premaxillary) palate. In the normal 15-day littermate the shelves are in apposition; a later dissection of this specimen in the frontal plane showed a fused epithelial layer between the two shelves (Fig. 6).

The reconstructed chondrocranium of the abnormal fetus is compared with that of a littermate in Figs. 7 and 9 and paramedian sections of the two are shown in Figs. 11 and 12. Figs. 8 and 10 are enlargements of their incisor tooth germs. It is clear that the entire nasal capsule (including the nasal septum) of the clefted specimen is relatively erect with reference to cranial base. The palatal shelf is also closer to the roof of the nasal chamber. It should be pointed out that even in the normal mouse the nasal septum fuses only with the primary palate (Harris, 1967).

Cell surface characteristics in the oral region were similar in the normal and clefted 15-day specimens, both contained flattened cells with dispersed central and clustered peripheral microvilli. This pattern was also present along the medial edge of the palatal shelves of the clefted fetus (Fig. 13), whereas the corresponding cells of a normal 14-day fetus (Fig. 14) were convex, had ellipsoidal cell outlines and many more centrally located microvilli with no peripheral clustering.



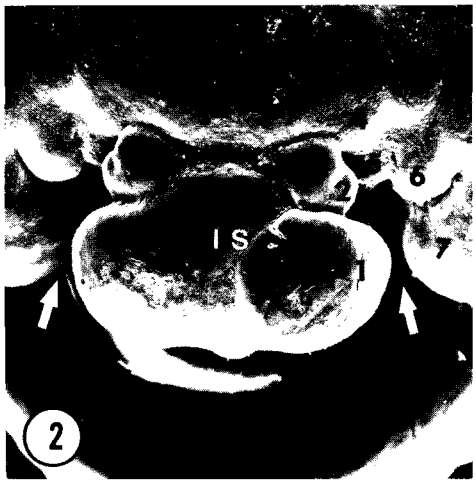


Fig. 1. (PW = 1.9 mm) Front-facial view of snout of a normal 15-day mouse fetus. The numbers indicate homologous landmarks as shown in Figure 2. Note the presence of the midsagittal groove.

Fig. 2. (PW = 1.5 mm) Front facial view of clefted specimen. The intermaxillary segment, double cleft (arrows) and paranasal tubercles (numbered) should be compared with the normal (Fig. 1). Note the absence of a midsagittal grooove. IS = intermaxillary segment.

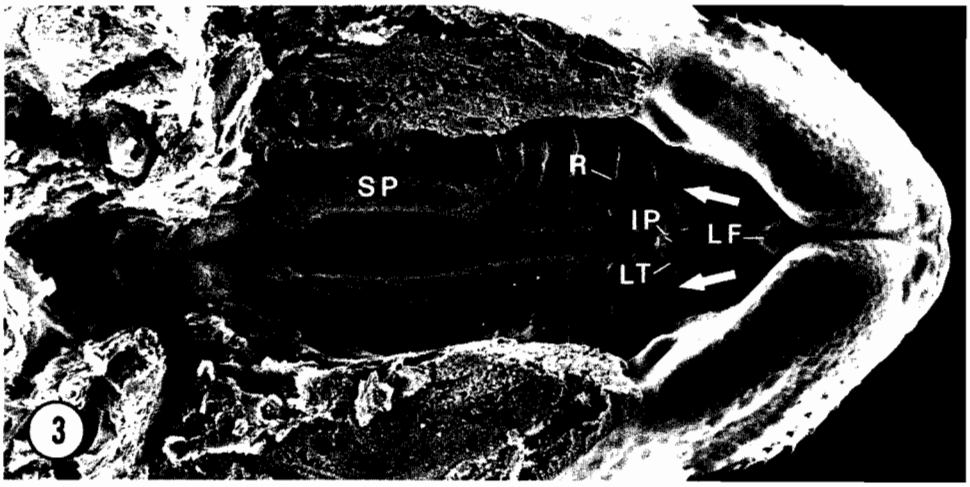


Fig. 3. (PW = 5.0 mm) Oral aspect of palate in a normal 14-day mouse fetus. The letters indicate comparable landmarks in the clefted (Fig. 4) and normal (Fig. 5) 15-day specimens. Arrows indicate the zone of fusion between the primary and secondary palates. IP = incisive papilla, L = lip, LF = labial frenum, LT = lateral tubercles, R = ruga, SP = secondary palate (these abbreviations also apply to other figures).

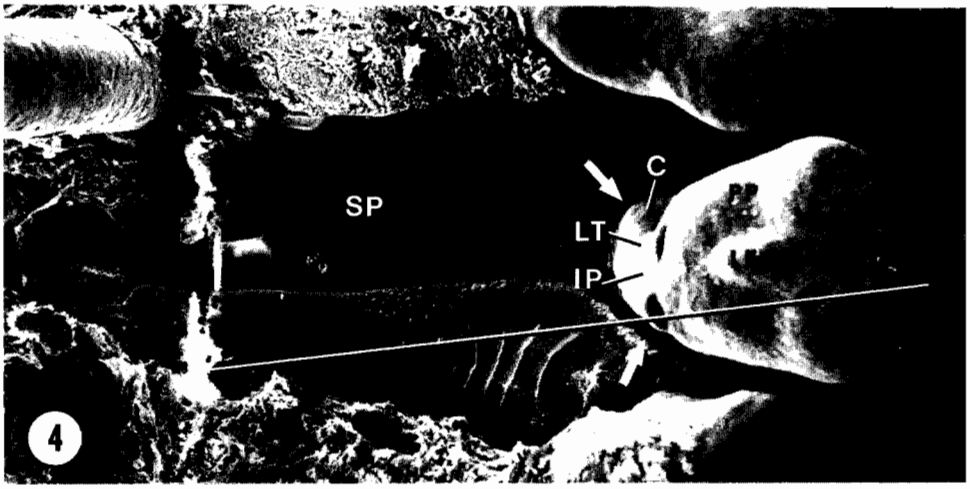


Fig. 4. (PW = 4.2 mm) Oral aspect of palate in the clefted 15-day fetus. This is the same specimen as in Figure 1 but with the mandible removed. Note that the primary palate is completely separated from the secondary palate (arrows). The line through the specimen indicates the orientation of the histologic section in Figures 8 and 11. C = columella, PP = primary palate.

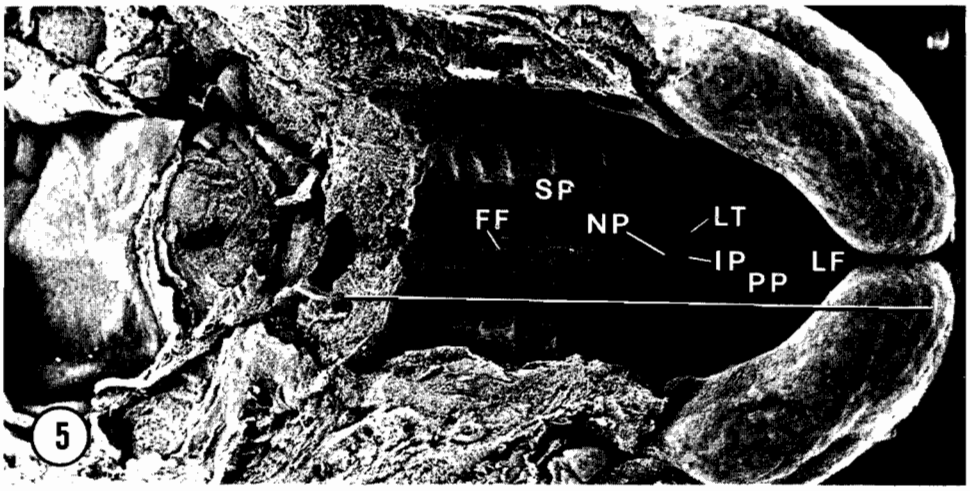


Fig. 5. (PW = 4.3 mm) Oral aspect of palate in a normal litter mate of the specimen shown in Figure 4. Compare the landmarks and note that the shelves of the secondary palate, though united are still separated by a midsagittal furrow extending caudally from the patent nasopalatine canal. The line through the specimen indicates the orientation of the histologic section in Figures 10 and 12. FF = fusion furrow, NP = nasopalatine canal.

Fig. 6. (PW = 0.5 mm) This is a view of the secondary palate of a normal 15-day fetus which was cut in a frontal plane just posterior to the naso-palatine canal. At this stage the shelves are connected only by the fusion of epithelium. The fusion furrow on the oral surface is deeper than on the nasal surface. Note that there is no nasal septum in contact with the secondary palate. C = columella, NC = nasal chamber, OC = oral chamber.

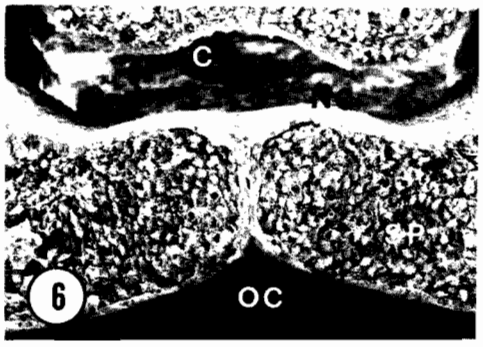
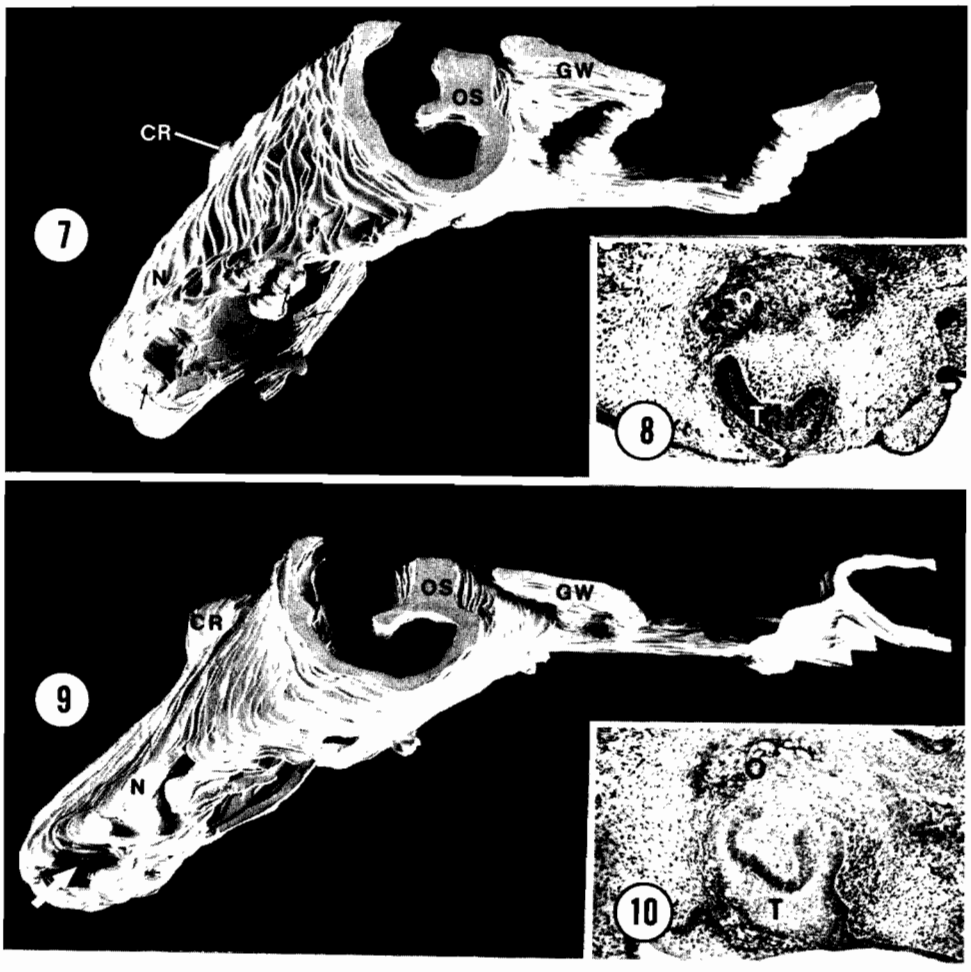


Fig.7 (PW = 6.7 mm) Serial section reconstruction of the left half of the chondrocranium of the clefted 15-day mouse fetus. Various anatomic landmarks are labeled: the large arrow indicates the abnormally large opening of the nasal capsule; small arrows indicate cartilaginous tubercles. Compare with Figure 9 and note the more erect relationship of the nasal capsule to the cranial base. CR = crista galli, GW = great wing of sphenoid, N = nasal capsule, OS = orbitosphenoid.

Fig. 8 (PW = 0.9 mm) Incisor tooth germ and osteogenic blastema of clefted specimen. The boxed area in Figure 11 shows site of this micrograph. O = osteogenic blastema, T = tooth germ.

Fig. 9 (PW = 6.1 mm) Serial section reconstruction of the left half of the chondrocranium of a normal 15-day mouse fetus. The large arrow indicates the antrum of the nasal capsule, compare with Figure 7. Note the relatively large angle formed between the cranial base and the nasal capsule. (See Figure 7 for abbreviations.)

Fig. 10 (PW = 1.1 mm) Incisor tooth germ and osteogenic blastema of normal 15-day specimen. Morpho- and cytodifferentiation are slightly more advanced as compared to Figure 8 but there are no significant differences between the two specimens. The boxed area in Figure 12 shows site of this micrograph.



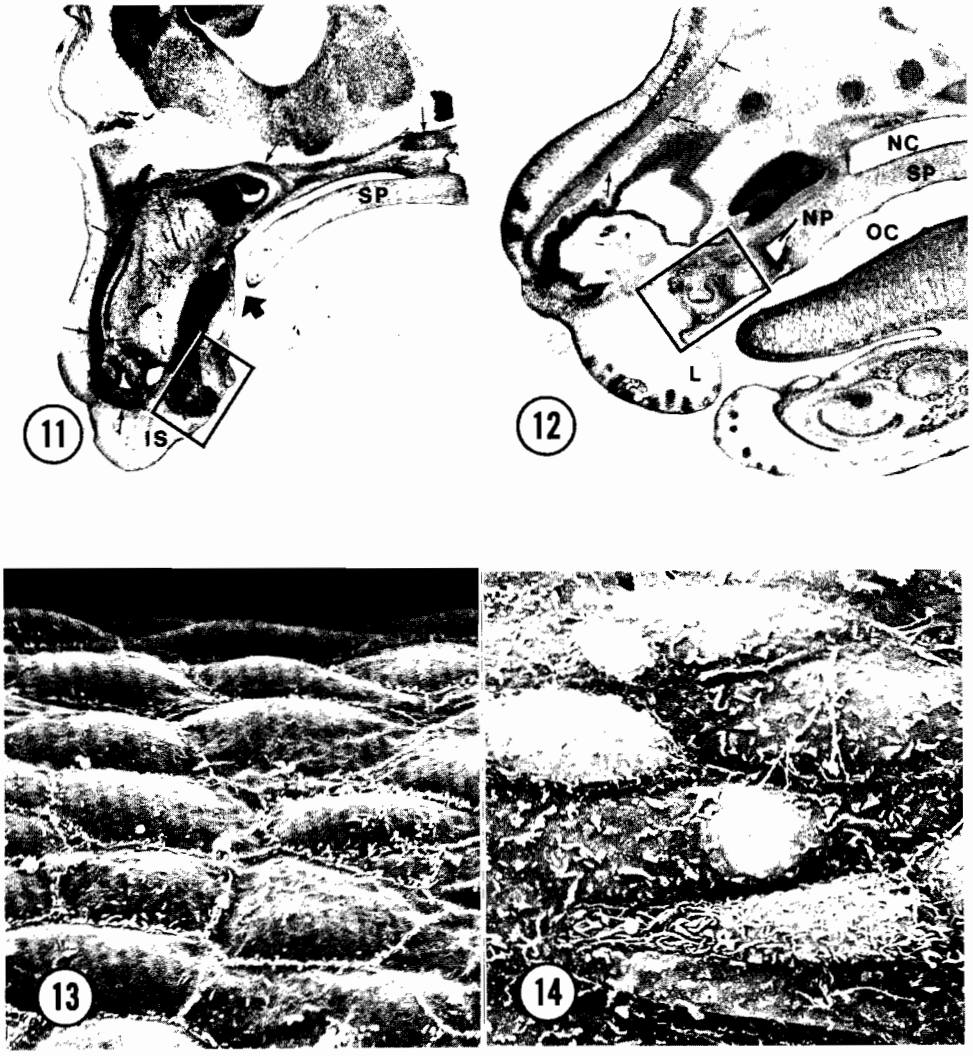


Fig. 11. (PW = 5.2 mm) Saggital section of the head of the clefted 15-day mouse fetus; See transecting line in Figure 4 for orientation. Cartilage (chrondrocranium) is indicated by dark grey (small arrows). Discontinuity between the primary and secondary palate is indicated by large arrow. Note the absence of a definitive lip; compare with Figure 12. The boxed area is site of Figure 8. IS = intermaxillary segment, SP = secondary palate.

Fig. 12. (PW = 3.6 mm) Sagittal section of the head of a normal 15-day mouse fetus; see transecting line in Figure 5 for orientation. Observe the continuity between the primary and secondary palates. The boxed area is site of Figure 10. L = lip, NC = nasal chamber, NP = nasopalatine canal, OC = oral chamber.

Fig. 13. (PW = 37 μm) Epithelial surface of medial edge of palatal shelf in clefted 15-day fetus. This pattern is indistinguishable from that found in normally nonfusing oral areas in 14-and 15-day fetuses.

Fig. 14. (PW = 37 μm) Epithelial surface of medial edge of palatal shelf in normal 14-day fetus.

Discussion

The clefted 15-day fetus was less advanced than the normal 14-day fetus in that its palatal shelves were not connected to the primary palate. This finding suggests that clefting of the secondary palate may have been preconditioned by this prior failure; an interpretation in accord with Trasler and Fraser (1963), who concluded that nonclosure of the secondary palate in mice could be consequential to abnormal development of the primary palate. Fraser (1970) also presented statistical evidence from human population studies, to show that complete clefting of the secondary palate occurs in 86% of infants having a bilateral clefting of the primary palate.

In the clefted specimen, the facial aspect of the nasal antrum was greatly distorted, and the normal midfacial groove was absent. The fact that the tubercles normally associated with the nasal conchae were exteriorized suggests that the normal constraints were severely modified by the failure of palatal fusion, but that the capacity for differentiation of specific components had not been eliminated.

The differences between the incisor tooth germs in the normal and abnormal 15-day fetuses (Figs. 11, 13) most likely reflect a disparity in 'developmental age' (a normal occurrence in litter mates; Otis and Brent, 1945) rather than an intrinsic developmental aberrancy. Both tooth germs appeared normal (Gaunt, 1964), but that of the normal 15-day fetus was slightly more advanced. The status of intramembranous bone development in the clefted specimen also appeared normal, though slightly retarded. These observations argue that teratogenesis, in this instance, was probably not related to a faulty development of neural crest mesenchyne; for if this were the case, odontogenesis and cephalic osteogenesis and chondrogenesis would have been severely affected (Horstadius, 1950; Johnston and Listgarten, 1972).

The epithelial surface characteristics associated with the zones of normal palatal fusion are compatible with the transmission EM descriptions of Farbman (1966) and of Green and Pratt (1973), and with the SEM observations of Waterman et al. (1973). From the present study it appears that, if palatal shelf contact fails, the cells along the medial edges modulate rapidly in the direction of a generalized type of oral epithelium. The failure of fusion could also account for the relatively short anteroposterior dimension of the clefted fetus, because changing growth rates can be closely dependent on new phases of differentiation and can be manifested as gross anatomic changes within a half day (Groedbloed, 1977).

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References

Farbman A.I. (1968). Electron microscope study of palate fusion in mouse embryos. Dev. Bio. 18,93-116.

Fraser F.C. (1970). The genetics of cleft lip and cleft palate. Am. J. Hum. Genetics 22,336-352.

Gaunt W.A. (1964). The development of the teeth and jaws of the albino mouse. Acta Anat. 57,115-151.

Goedbloed J.F. (1977). Embryonic and postnatal growth of rat and mouse: V. Prenatal growth of organs and tissues. Acta Anat. 98,162-182.

Greene R.M. and Pratt R.M. (1976). Developmental aspects of secondary palate formation. J. Embryol. Exp. Morph. 36,225-245.

Harris J.W.S. (1967). Experimental studies on closure and cleft formation in the secondary palate. Sci. Basis Med. (Ann. Review) 356-370.

Hörstadius S. (1950). The Neural Crest. Oxford University Press. London.

Johnston M.C. and Listgarten M.A. (1972). Observation on the migration, interaction and early differentiation of orofacial tissues. In: Developmental Aspects of Oral Biology. Slavkin H.C. and Bavetta L.A. (eds). Academic Press. N.Y. Ch. 4,53-80.

Long S.V., Larsson K.S. and Lohmander K.S. (1974). Cell proliferation in the cranial base of A/J mice with 6-AN-induced cleft palate. Teratology 8,127-138.

Otis E.M. and Brent R. (1954). Equivalent ages in mouse and human embryos. Anat. Rec. 120,33-64.

Richardson K., Jarrett L. and Finke E. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35,313-323.

Tamarin A. and Boyde A. (1977). Facial and visceral arch development in the mouse embryo: a study by scanning electron microscopy. J. Anat. 124,563-580.

Trasler D.G. and Fraser F.C. (1963). Role of the tongue in producing cleft palate in mice with spontaneous cleft lip. Develop. Biol. 6,45-60.

Waterman R.E., Ross I.M. and Meller S.M. (1973). Alterations in the epithelial surface of A/Jax mouse palatal studies prior to and during palatal fusion: a scanning electron microscopic study. Anat. Rec. 176,361-376.

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