Width of the Craniofacial Complex During Formation of the Secondary Palate

G. R. SMILEY, D.D.S. , M.Sc.
R. J. VANEK,* D.D.S.
A. D. DIXON, M.D.S., Ph.D., D.Sc

Attempts to elucidate the formation of the secondary palate have involved extensive use of teratological methods. To date there have been few cephalometric studies of the growth of the fetal craniofacial complex aimed at a better understanding of the interrelationship of various growth parameters during closure of the secondary palate. Zeiler, Weinstein, and Gibson (*18*) found a differential growth spurt of the mandible, relative to the maxilla, prior to and during palatal closure in the rat, which was also observed in mice by Hart, Smiley, and Dixon (*7*). Increase in length of the mandible *per se* was essentially linear during shelf movement and the vertical dimension, which is an indicator of mandibular position and the degree of cranial base extension, may be more significant than mandibular length as a prerequisite for palatal shelf movement (*7*). Studies by Harris (*5, 6*) using mixed species and strains of rodents, as well as cortisone-treated mice, revealed that the degree of cranial base flexion could restrict palatal movement at the critical time for palatal closure. In another growth study, Deuschle and Kalter (*3*) reported that the mandibles of neonatal mice with cortisone-induced clefts of the palate were larger than those of the normal offspring, while Schwartz and Chaudhry (*11*) observed shorter mandibles in similar experimental animals.

Linear and angular measurements of the length and height of the craniofacial complex during closure of the secondary palate have seldom been examined and there have been no detailed metrical studies on craniofacial width. The purpose of the present investigation was to carry out a quantitative appraisal of maxillary arch width, nasal septal height and septal width prior to, during and after closure of the secondary palate.

Methods

Mice of the A/Jax strain, which produce spontaneous clefts of the lip and palate, were bred on Monday and Friday from 9:00 a.m. to 12 noon.

The authors are affiliated with the Dental Research Center and Department of Orthodontics, School of Dentistry, University of North Carolina.
This investigation was supported by USPHS Research Grant DE-02668, National Institute of Dental Research.
* Senior Pfeiffer Dental Research Fellow.

Gravid females were sacrificed at 10:30 a.m. on days 13–16 of gestation, counting the day of copulation as day zero. The uterine horns were removed by Caesarean section and fixed in Bouin's solution. After thorough fixation the fetuses were removed from the uterus and their chronological age was compared with morphological features to obviate any gross errors in aging. The fetuses were decapitated and the heads processed for paraffin embedding. After careful orientation in the coronal plane, the heads were serially sectioned at 10 microns and stained with hematoxylin and eosin. To insure that true coronal sections were used in our measurements only those fetuses that showed both optic nerves attached to the retinae within a group of 5 serial sections were studied. The total number of serial sections from the full length of the secondary palate was counted and sections representing the junction of the anterior-middle and middle-posterior thirds of the secondary palate were selected. The two representative sections were taken from 13–16 day old, normal and bilateral cleft lip uterinemates. Standardized photographic enlargements (×33) were made for tracing onto acetate matte paper. Intermaxillary arch width was measured by drawing a line between the oral aspect of the dental laminae of the maxillary molar tooth buds, which were the most stable and clearly defined landmarks in the upper jaw at all ages (Figure 1). Height of the nasal septum was measured by reference to the cartilaginous nasal septum from the point on the dorsal surface of the septum in the midline to its ventral tip (Figure 1). Width of the nasal septum was measured at its greatest dimension below the ventral edge of the cartilaginous nasal septum (Figure 1).

The sample size in each age group was a minimum of ten spontaneous bilateral cleft lip specimens and a similar number of normal fetuses found immediately adjacent to the cleft specimens in the uterine horn. The data were analyzed by methods of multivariate analysis of variance which included adjustments for litter effects. All tests of significance reported were based on F-statistics as applied to either the response variables themselves or suitable linear combinations. The sources of variation which were investigated by this approach were the effects of age in days, normal versus cleft, and the interaction of age with normal versus cleft. Suitable single degree of freedom contrasts were extracted separately for normal and cleft animals to test hypotheses pertaining to the linearity of the growth trend as well as to the location of plateaus. This was done by comparing the successive differences between 13 and 14 days, 14 and 15 days, 15 and 16 days with each other and with zero for each response variable.

Results

A/Jax mice bred under controlled conditions had vertically-oriented palatal processes on day 14 and by day 15 all normal fetuses used in this study had horizontal palatal processes. The critical time for movement of the palatal processes occurred just prior to the fifteenth day of gestation

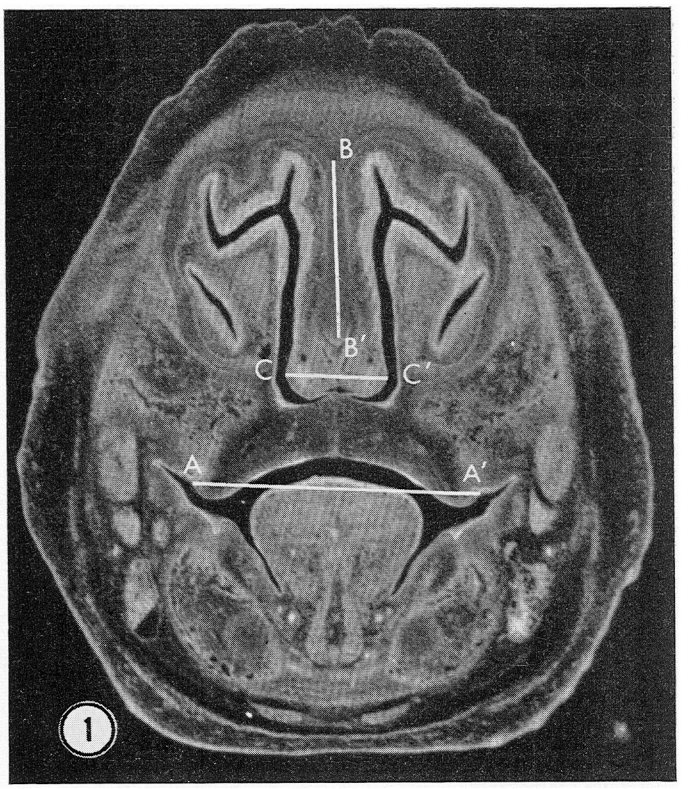

FIGURE 1. Coronal section through the junction of the anterior and middle thirds of the secondary palate in a 15 day normal A/Jax fetus illustrating the measurements used. Maxillary arch width: between the oral aspect of the right and left maxillary molar dental laminae (A-A'). Cartilaginous nasal septal height: from the point on the dorsal surface of the septum in the midline (B) to its ventral tip (B'). Nasal septal width: from its greatest dimension below the ventral edge of the cartilaginous nasal septum, (C-C').

since a few of our 15 day fetuses exhibited palatal processes in intermediate stages of palatal closure. Statistically, intermaxillary arch width in normal fetuses was not significantly different between the anterior and posterior regions at any time studied, although the posterior width was greater at all ages (Figure 2). The growth trend for both regions was non-linear and a statistically significant plateau occurred during the critical time for closure.

A non-linear trend for intermaxillary arch width in the spontaneous cleft fetuses also occurred, but there was no plateau during the time of closure (Figure 2). Both normal and cleft fetuses demonstrated a similar relationship of intermaxillary arch width between anterior and posterior regions at 13 days. By 14 days, just prior to palatal closure, the anterior-posterior width relationship in cleft fetuses was reversed, becoming greater

anteriorly. The difference in width between anterior and posterior regions in cleft fetuses was not statistically significant at any age studied.

There were no statistically significant differences between normal and cleft animals in the anterior region prior to palatal closure 13–14 days (Figure 3). However, during and after closure of the secondary palate

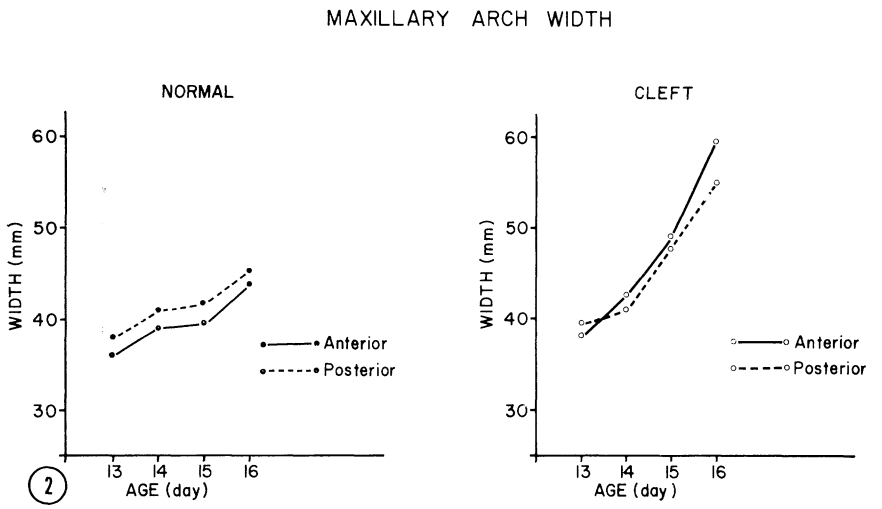

FIGURE 2. Anterior and posterior maxillary arch width is compared within each fetus. Note the plateau between the 14–15 day in the normal fetuses. Reversal of normal arch width relationship is demonstrated in the cleft fetuses.

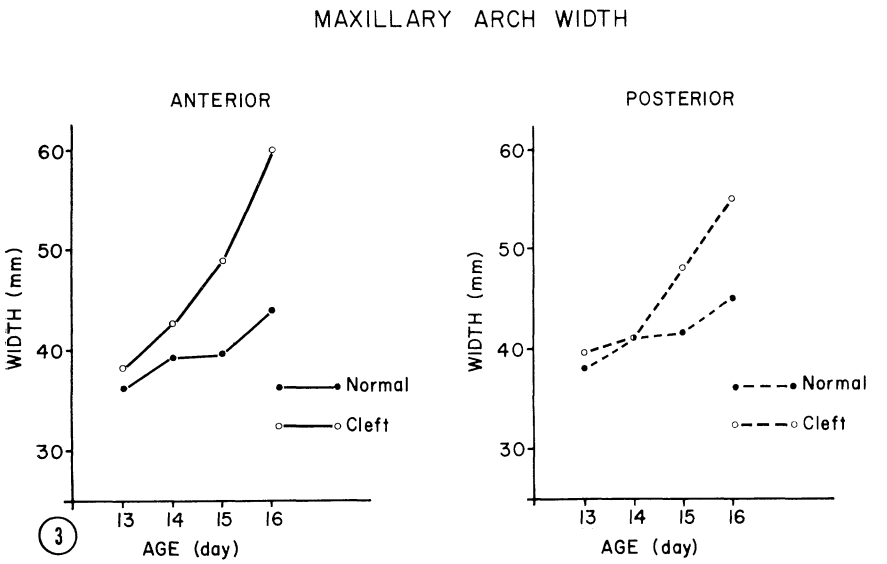

FIGURE 3. Comparison of anterior and posterior arch widths in normal and cleft fetuses. Both anterior and posterior widths show the same trend, with a significant difference being reached during and after the critical time for palatal closure.

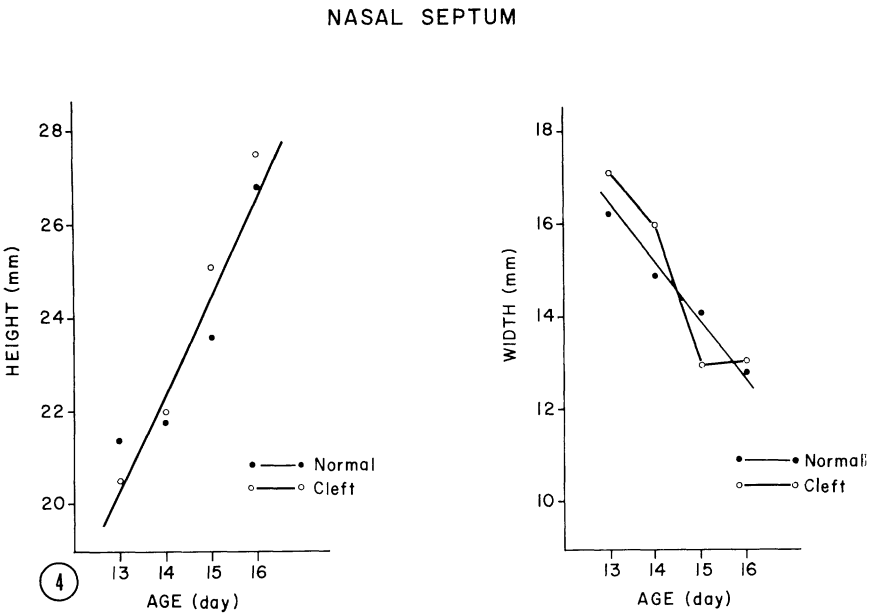

FIGURE 4. Normal septal height in the anterior region is linear and there is no significant difference between normal and cleft specimens. Nasal septal width in the anterior region shows a decreasing linear trend in normal fetuses while there is a significant decrease in width during the time of palatal closure in the cleft animals.

maxillary arch width was significantly greater in the cleft fetuses. The posterior third of the secondary palate demonstrated the same trend as the anterior third (Figure 3). The difference in width was less posteriorly, especially before palatal closure.

The height of the cartilaginous nasal septum with respect to the anterior third of the secondary palate showed a linear increase, with no statistically significant difference between normal and cleft specimens throughout the time span studied (Figure 4). Change in width of the nasal septum at the anterior third of the secondary palate showed a linear trend for the normal A/Jax fetuses while for the spontaneous cleft specimens it was non-linear (Figure 4). During the time of palatal closure a constant decrease in width was noted in normal fetuses, while in the cleft group a statistically significant decrease was observed. The range of dimensional change was very small for the septal measurement when compared to arch width. Nasal septal width at the anterior third of the secondary palate was the only parameter that decreased with age.

Discussion

The results of this study indicated that there was a differential rate of growth in width of the maxillary arch during formation of the secondary palate. A significantly smaller increase in width took place during the

time of palatal shelf movement in normal A/Jax mouse fetuses compared with the rate of increase before or after closure. Although excessive width of the head has been proposed as a factor in cleft palate etiology (*4*) we are not aware of any quantitative studies of intermaxillary width during formation of the secondary palate. The data presented in this study suggest this explanation could easily become a reality, if the growth processes were atypical and maxillary arch width increased at the same rate during the critical time for palatal closure as before and after this event. The formation of a cleft palate due to excessive maxillary width could take place up to the time both palatal processes become horizontal provided they have not fused in the midline. Subsequently, post-fusion rupture (*8*) or abnormal epithelial breakdown prior to adherence (*12*) could be factors contributing to cleft formation. Callas and Walker (*2*), who used X-irradiation to induce cleft palate, observed that the ratio of palate width to head width increased as palatal closure progressed and attributed this change to the shelves going from a vertical to a horizontal position. Even though morphological criteria other than chronological age were used in their study, another possible explanation for the increased ratio could be due to the plateau for width of the maxillary arch observed in our study (Figures 2 and 3). This period of stasis may be very important for it may be one mechanism which permits the palatal processes to remain in contact at the midline, during the time when tissue changes result in epithelial adherence. Since movement of the palatal shelves in A/Jax fetuses occurs just prior to the 15th day of gestation, after which there is a significant increase in intermaxillary arch width, it is easier to understand how a retardation in shelf movement due to cortisone (*17*), hypervitaminosis A (*16*), and X-irradiation (*2*) could result in a cleft palate. Studies are now being conducted in our laboratory on C57BL mice which have a low frequency of cleft palate with cortisone treatment to see if their growth trend is similar to the A/Jax strain. The palate in C57BL mice closes earlier and the delay in shelf movement may not be as significant, if their growth trend is similar to A/Jax mice, since closure begins in the earlier part of the plateau between the 14th and 15th day (Figure 2). Thus, the palatal processes would have a longer time to make epithelial contact before the increased rate of growth occurs.

In the fetuses with a spontaneous bilateral cleft lip, width of the maxillary arch continued to increase at a greater rate during and after the normal time for palatal closure. Undoubtedly the influence of the cleft lip increased the potential for unrestricted growth and/or lateral displacement of the maxillary arch. The observation that increase in width does not manifest itself until after the 13th day of gestation could be due to the fact that the face has just completed its formation by the 12th day of gestation in A/Jax mice (*13*) and the cleft condition has not had time to take effect. The effects of muscular imbalance in newborn humans with cleft lip and palate have been pointed out (*10*) and our study shows that

this imbalance probably manifests itself very early, prior to palatal closure in mouse fetuses with cleft lip.

The present study did not show any significant difference in nasal septal height in the anterior third of the secondary palate, in contrast to the finding that the nasal septum was significantly shorter in spontaneous bilateral cleft fetuses (*1*). Nasal septal width in the anterior third of the secondary palate decreased with age and the decrease was more pronounced during the critical time for palatal closure. It appears that the significant decrease in septal width was due to the non-fusion of nasal septum to the secondary palate, which occurs in normal animals. No enlargement of the septum was measured that could cause abnormal tongue position. Although the tongue in many of our cleft specimens was behind the primary palate, as shown by Trasler (*14*), this could have been due to the tongue being arched high into the nasal cavity making it shorter as a result, rather than the cause, of the cleft palate.

Although our study has indicated that intermaxillary width increased in cleft lip fetuses prior to closure of the palatal shelves, the palatal processes can sometimes fuse in the midline if they can reach their horizontal position before maxillary width becomes too great (*1*). Furthermore, Pourtois (*9*) has shown in an *in vitro* system that the palatal processes from spontaneous cleft fetuses have the potential for mesenchymal fusion. Even though the importance of the relationship between angulation of the cranial base and palatal closure has been discussed (*5, 6, 15*), Babula, Smiley and Dixon (*1*) found no significant difference in the cranial base angle between spontaneous bilateral cleft A/Jax mice and their normal uterinemates during formation of the secondary palate. All of the cleft specimens used in this study had vertical palatal processes and other investigations in our laboratory show that the majority of bilateral and unilateral spontaneous cleft lip fetuses have vertical palatal processes. Studies are now being conducted in an attempt to determine whether the "growth" processes discussed in the present paper which become atypical prior to the time of palatal closure in the cleft animals could influence the movement of the palatal processes.

Summary

Width of the craniofacial complex during formation of the secondary palate in normal and spontaneous bilateral cleft A/Jax mice fetuses was investigated. Histological sections in the coronal plane from the anterior and posterior regions of the secondary palate were photographically enlarged for cephalometric tracing during the 13–16 days of gestation. Statistically there was a significant difference in the anterior and posterior intermaxillary arch width between normal and cleft fetuses, while the difference was not significant, between anterior and posterior dimensions within each fetus. In normal specimens there was a significant growth plateau in intermaxillary width during the critical time of palatal closure,

while in cleft fetuses this dimension increased steadily. Nasal width in the anterior region significantly decreased in the cleft fetuses only during the critical time for palatal closure. Septal length increase in the anterior region was not significantly different between the normal and cleft specimens. The implications of these findings are discussed with respect to the etiology of cleft palate.

Acknowledgements: The authors are indebted to Fred Six, Research Assistant, and G. G. Koch, Assistant Professor, Department of Biostatistics, School of Public Health, for their statistical assistance.

reprints: *Dr. Gary R. Smiley*
Dental Research Center
University of North Carolina
Chapel Hill, North Carolina 27514

References

1. Babula, W. J., Jr., Smiley, G. R., and Dixon, A. D., The role of the cartilaginous nasal septum in midfacial growth. *Amer. J. Orthodont., 58,* 250–263, 1970.
2. Callas, Gerald, and Walker, B. E., Palate morphogenesis in mouse embryos after X-irradiation. *Anat. Rec., 145,* 61–71, 1963.
3. Deuschle, F. M., and Kalter, Harold, Observations on the mandible in association with defects of the lip and palate. *J. dent. Res., 41,* 1085–1095, 1962.
4. Fraser, F. Clarke, Gene-environment interactions in the production of cleft palate, in Nishimura, H. and Miller, J. R., eds. Methods for Teratological Studies in Experimental Animals and Man. Igaku Shoin Ltd., Tokyo, 1969, pp. 34–39.
5. Harris, J. W. S., Oligohydramnios and cortisone-induced cleft palate. *Nature, 203,* 533–534, 1964.
6. Harris, J. W. S., Experimental studies on closure and cleft formation in the secondary palate, *The Scientific Basis of Medicine Annual Reviews 1967,* Chapter XX, 354–370.
7. Hart, J. C., Smiley, G. R., and Dixon, A. D., Sagittal growth of the craniofacial complex in normal embryonic mice. *Arch. oral Biol., 14,* 995–997, 1969.
8. Kitamura, Hironori, Epithelial remnants and pearls in the secondary palate in the human abortus: A contribution to the study of the mechanism of cleft palate formation. *Cleft Palate J., 3,* 240–257, 1966.
9. Pourtois, Michel, Influence of cleft lip upon palatal closure in A/Jax mice. *Cleft Palate J., 4,* 120–123, 1967.
10. Pruzansky, Samuel, Description, classification, and analysis of unoperated clefts of the lip and palate. *Amer. J. Orthodont., 39,* 590–611, 1953.
11. Schwartz, D. M., and Chaudhry, A. P., Planimetric studies of mandibles in A/Jax mice born with cortisone-induced cleft palates. *J. dent. Res., 47,* 725–732, 1968.
12. Smiley, G. R., Fine structure of mouse embryonic palatal epithelium prior to and after midline fusion. *Arch. oral Biol., 15,* 287–296, 1970.
13. Trasler, D. G., Pathogenesis of cleft lip and its relation to embryonic face shape in A/J and C57BL mice. *Teratology, 1,* 33–49, 1968.
14. Trasler, D. G., and Fraser, F. C., Role of the tongue in producing cleft palate in mice with spontaneous cleft lip. *Develop. Biol., 6,* 45–60, 1963.
15. Verrusio, A. C., A mechanism for closure of the secondary palate. *Teratology, 3,* 17–20, 1970.
16. Walker, B. E., and Crain, Burton, Jr., Effects of hypervitaminosis A on palate development in two strains of mice. *Amer. J. Anat., 107,* 49–58, 1960.
17. Walker, B. E., and Fraser, F. C., The embryology of cortisone-induced cleft palate. *J. embryol. exp. Morph., 5,* 201–209, 1957.
18. Zeiler, K. B., Weinstein, S., and Gibson, R. D., A study of the morphology and the time of closure of the palate in the albino rat. *Arch. oral Biol., 9,* 545–554, 1964.