Reopening of Fused Palatal Shelves

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The formation, approximation, and subsequent fusion of the secondary palate follow an important series of consecutive morphological and biochemical events. The final stage, properly called fusion, begins with an alteration in the marginal cells of the epidermis (*4*) which then fuse at the medial interfaces of the palatal processes. Subsequently, the laminated partition undergoes a process of degenerescene presumably initiated by autolysis and later accompanied by phagocytosis. Mesodermal penetration and coalescence complete the series of events. However, considerable controversy has centered on the exact mechanism responsible for cleft palate formation. Veau (*5*), re-echoing previous doubts, proposed that rupture or degeneration of imperfectly fused epithelial tissue without mesodermal penetration may result in cleft formation. Although postclosure clefts have been essentially ruled out by animal experimentation, any implied carryover of this concept into humans has been challenged by Kitamura (*2*) who has strongly suggested that cleft formation in humans may occur after mesodermal penetration of the epithelial raphe.

The present report illustrates for the first time reopening of fused epithelial interfaces in the palatal shelves of two 16½-day A/Jax mouse embryos. The process of reopening is accompanied by a malpighian-like differentiation of the epithelial cells at the zone of fusion.

Methods and Materials

The embryos described here were a part of a sample in a histochemical investigation of palatal closure in A/Jax mice. The specimens were prepared for enzyme (acid phosphatase) studies with the technique of Barka and Anderson (*1*). They were fixed in a formol-calcium solution, pH 7.2; washed in a gum-sucrose solution; frozen at −70°C on solid CO_2; and cut at 8 microns on a cryostat maintained between −18° and −20°C. Whole embryo heads were sectioned along the entire length of the palate and incubated for 1 hour in a freshly-prepared naphthol AS-TR phosphate medium, pH 5.0. The sections were counterstained with a phosphate-buffered methyl green solution, pH 4.0, for 30 seconds.

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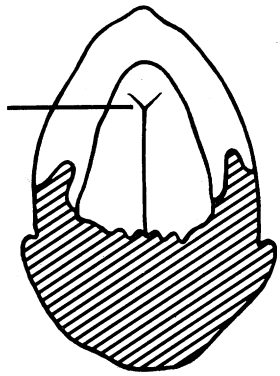

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FIGURE 1. A schematic drawing showing the area from which those sections in Figures 2, 3, 4, and 5 were taken.

Observations

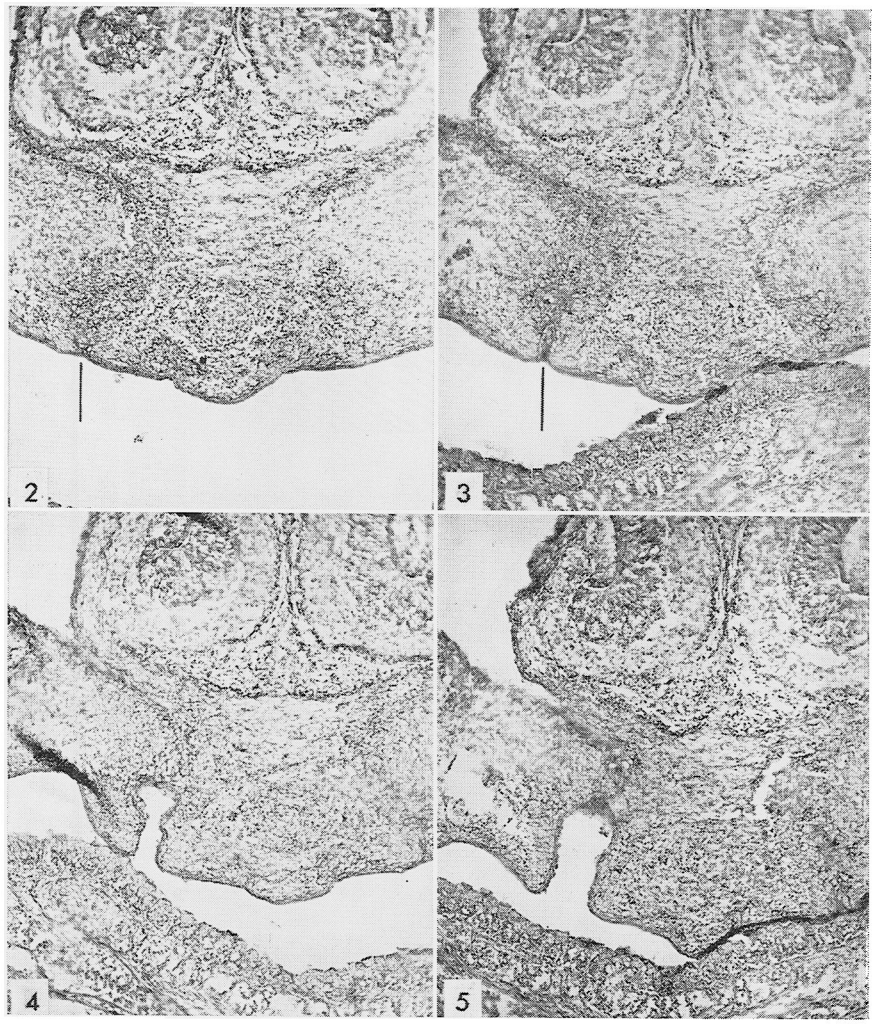
The descriptions in this report represent only several of the serial coronal sections taken from the anterior portion of the secondary palates of two embryos. The sections presented are those which best represent the phenomenon of reopening observed in these specimens. Two schematic drawings (Figures 1 and 6) indicate the regions from which the coronal sections were removed for examination from each embryo.

NORMAL MOUSE EMBRYO, 16½ DAYS. In this specimen, fusion was seen along the entire length of the palate and mesenchymal contact had occurred throughout the secondary palate (true fusion). Fusion at epithelial interfaces was also present between the lateral palatal processes and the lateral margins of the medial nasal process (zone of septopalative closure).

In this region, the most anterior section (Figure 2) showed, in addition to epithelial fusion, mesodermal penetration of the right partition and an almost imperceptible indentation of the left seam (shown by an arrow). No mesenchymal contact had occurred on the left side. In Figure 3, a slight indentation (arrow) was observed in the left partition on the oral surface while in Figures 4 and 5, reopening was readily seen. In the last two sections (Figures 4 and 5), the oral epithelium was no longer continuous and morphological changes similar to differentiation of mucous membranes occurred at the superior aspect of the defect. The epithelial cells in this region showed malpighian-like differentiation and proliferation. Above the defect (Figure 5), the mesoderm of the lateral palatal process and the medial nasal process made contact indicating that true fusion had occurred in this region.

SPONTANEOUS CLEFT MOUSE EMBRYO, 16½ DAYS. This embryo possessed a unilateral cleft lip on the left and an incomplete, bilateral cleft of the secondary palate.

For a short distance posteriorly, the secondary palate exhibited fusion



FIGURES 2, 3, 4, 5. All sections represent the anterior palatal region generally referred to as the septopalatal zone of closure. These sections are sequentially arranged anteroposteriorly. A slight indentation in the epithelial partition is apparent in Figure 2. In Figure 3, the indentation in the oral mirosa has progressed and the condensation of epithelial cells indicates an alteration in epithelial cell morphology. In Figures 4 and 5, the partial cleft and epithelial changes are shown.

between the right palatal shelf and the nasal septum (Figure 6). In the most anterior section (Figure 7), fusion at epithelial interfaces without mesodermal penetration was observed. However, in the nasal epithelium, above the line of fusion (arrow), the epithelial cells showed epithelial differentiation similar to that seen in the defect of the normal mouse embryo described above. Reopening of epithelial interfaces on both the oral and nasal surfaces at the line of fusion has progressed (arrows, Figures 8 and

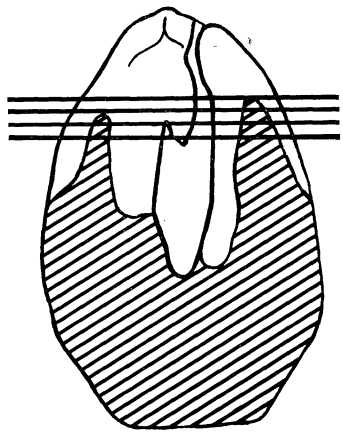


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FIGURE 6. A schematic drawing showing the region of the head through which those sections in Figures 7, 8, 9, and 10 were removed.

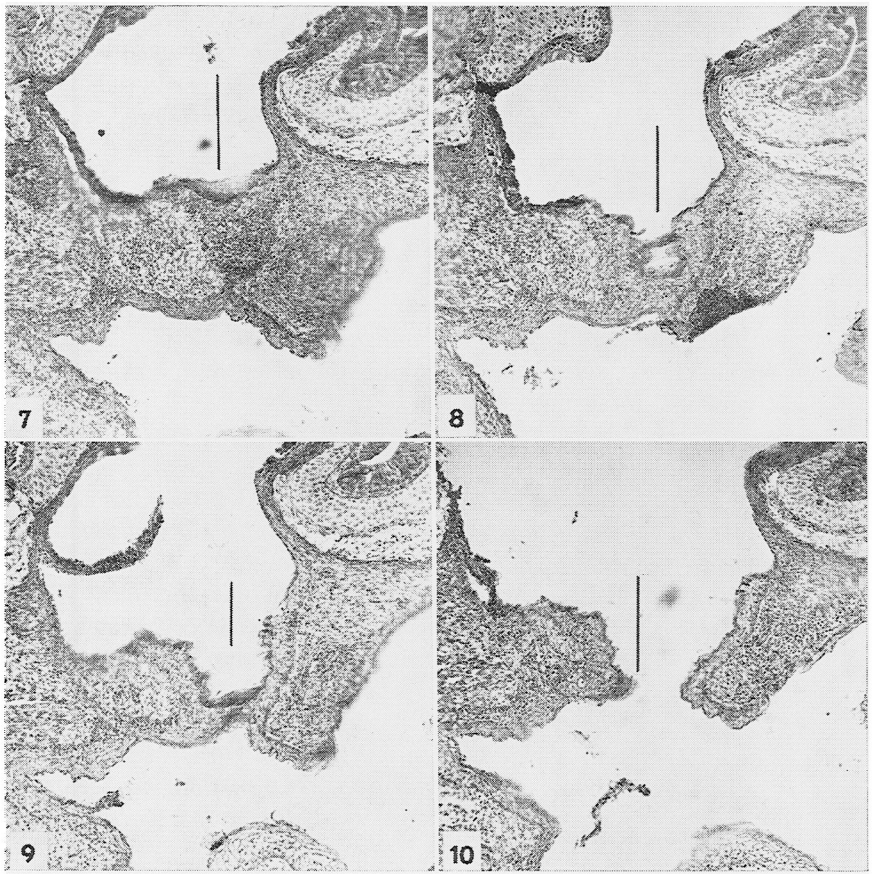
9). The nasal cavity side apparently reopened more rapidly than did the oral cavity side. Complete separation of the right side from the nasal septum ultimately resulted (Figure 10). In the most anterior region of the cleft (Figure 10), the right palatal process exhibited epithelial differentiation at its medial extremity (arrow).

Discussion

The controversy which surrounds the pathogenesis of cleft palate has existed since the nineteenth century. The evidence which this report provides tends to support the hypothesis that some clefts may be the result of a reopening of epithelial interfaces. However, these data do not lend support to the theory that proliferation of mesoderm is a causal factor of mesenchymal penetration of the laminated epithelial partition. In fact, the hypothesis that imperfectly-fused epithelial layers rupture to form the defect also does not receive support from these data. Rather, it would appear that the unusual malpighian-like differentiation of epithelial cells similar to that which occurs in the rupture (reopening) of the eyelids (*3*) appears to be mainly responsible for the phenomena observed here. The epithelium in the zone of fusion does not normally undergo differentiation of this type at this stage of development.

The apparent lack of acid phosphatase activity in the epithelial cells in this region has been noted by this investigator in previous experiments. However, the absence of hydrolytic activity in the fused epithelial does not negate the possibility that autolysis or autophagy is an important mechanism which disrupts the laminated partition between the fused processes. In fact, the degenerescence of the raphe appears to be dependent upon the timely appearance of hydrolytic enzymes in the fused epithelia.

The observations reported here are not the usual mechanical and bio-



FIGURES 7, 8, 9, 10. All sections represent the anterior region of the palate, arranged anteroposteriorly. These sections indicate the progressive reopening of the fused portion of the secondary palate in the septopalatal zone of closure. The epithelial changes can be observed during reopening.

logical events normally associated with cleft formation in mouse embryos since most of these clefts occur because the shelves do not approximate. However, differentiation of the epithelium does occur and plays a determining role in the separation of other anatomical structures such as the eyelid (*3*).

These data are the first, to the knowledge of this investigator, to indicate that some clefts of the secondary palate may be the result of incomplete epithelial fusion coupled with a subsequent reopening of the fused epithelial walls.

Summary

Two cases of incomplete cleft palate in littermate A/Jax mouse embryos were examined histologically. The data indicate that postfusion

reopening had occurred at epithelial interfaces between the lateral palatal process and the medial nasal process. An abnormal persistence of the epithelial wall, as well as a malpighian-like differentiation between its two slopes, appears to have originated the malformations observed in these embryos.

Résumé

Cet article rend compte de l'examen histologique de fissures palatines incomplètes chez deux embryons de souris A/Jax. Les images observées indiquent la possibilité d'une réouverture du palais aprés fusion. Le processus pathologique semble avoir eu pour origine la persistance anormale su mur épithelial et l'avènement d'une différenciation malpighienne entre les deux versants de ce mur.

Zusammenfassung

Dieser Artkel befasst sich mit der histologischen Untersuchungen von unrollkommenen Gaumenspalten in zwei A/Jax Mausembryos. Beobachtungen zeigten, dass Möglichkeit einer Wiederöffnung des Gaumens nach Verschmelzung besteht. Das annormale Beharren der epithelen Wand, sowie eine malpighian-ähnliche Differenzierung zwischen den beiden Spalten scheint der Grund für die beobachtete Missbildung in beiden Embryos zu sein.

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