The influence of the epithelium on palate shelf reorientation

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SUMMARY
The intrinsic forces necessary for directing the reorientation of the secondary palate appear to reside in the anterior two thirds of the palate or presumptive hard palate. The hard palate could reorient regardless of whether it was intact or separated from the posterior third or presumptive soft palate. The soft palate could only reorient if the palate shelves are left intact. These intrinsic forces, within the hard palate, may be mediated by the mesenchymal cells, their extracellular matrix, or the epithelium surrounding the shelves. This latter possibly was tested by removing the epithelium, from either the presumptive oral or nasal surface followed by measurement of reorientation in vitro. Only after removal of the oral epithelium was a significant inhibition in reorientation observed. The treatment used to remove the epithelium, EDTA and scraping, was shown to remove 41% of the oral epithelium leaving the majority of the basement membrane intact. The observed inhibition of reorientation did not appear to be a consequence of wound healing. Creation of wounds twice the area that was observed after treatment with EDTA and scraping inhibited reorientation minimally. These results suggest that the epithelium and particularly the anterior oral epithelium plays a major role in the reorientation of the murine secondary palate.

INTRODUCTION
One aspect of the morphogenesis of the mammalian secondary palate involves the reorientation of the two apposing palate shelves. The shelves move from a vertical position on either side of the tongue to a horizontal position above the tongue separating the oral and nasal cavities. The process of reorientation has been studied in vitro using murine embryos (Brinkley, 1975; Wee et al. 1976). Foetal mouse heads cultured following removal of the tongue and brain showed similar reorientation to that observed in situ (Brinkley et al. 1975, 1978). These results suggest that structures such as the tongue and brain as well as circulation are unnecessary for the process of reorientation. The observations are consistent with an intrinsic shelf force directing reorientation (Walker & Fraser, 1956). The intrinsic components in the shelves which could be involved in directing this morphogenesis may be associated with the mesenchyme and its extracellular matrix, or the epithelium surrounding the shelves (Larsson, 1960; Pourtois, 1972; Pratt et al. 1973; Babiarz et al. 1975; Ferguson, 1977; Brinkley, 1980). The epithelium is of particular interest because epithelia define the shape and structure

Key words: palate, epithelium, reorientation, mouse embryo.
of tissues and appear to be morphogenetically active in a number of developing systems (Grobstein, 1967; Karfunkel, 1974; Brady & Hilfer, 1982; Brun & Garson, 1983). The evidence to support a role for the epithelium in palate shelf reorientation is still inconclusive. Epithelial cell shape, distribution and/or layering changes, as well as rugae development and an increase in mitotic figures in the presumptive oral epithelium do correlate with the timing of reorientation (Brinkley, 1984; Luke, 1984). What is uncertain is whether these changes are necessary for reorientation to occur.

In the present study we examined the effects of epithelial removal on reorientation in vitro to determine more directly whether the epithelium plays a role in this process. Preliminary observations indicated that disruption of the presumptive oral surface could inhibit reorientation in vitro (Bulleit & Zimmerman, 1984). The results presented in this paper substantiate those findings and demonstrate that removal of the epithelium on the anterior oral aspect of the palate and not wounding is primarily responsible for the observed inhibition. These findings are consistent with the epithelium, in particular the anterior oral epithelium, directing secondary palate morphogenesis.

MATERIALS AND METHODS

Animals and culture system

A/J mice (Jackson Laboratories) were used in these studies. We have also used CFW mice (Charles River), a random-bred strain, and have obtained similar results. Matings were carried out by placing individual females with males overnight. The presence of a vaginal plug the following morning was taken as evidence for pregnancy and designated day zero. On day 14 of gestation animals were sacrificed by cervical dislocation and gravid uteri were placed on ice. This is approximately 24 h prior to in situ shelf reorientation (Andrew et al. 1973). Embryos were removed and examined, those with cleft lip were eliminated. The remaining embryos were staged using a morphologic rating system described by Walker & Crain (1960). Embryos with a rating of 5–7 were used for culture.

Heads were removed followed by excision of mandibles and tongues. This allowed access to palates for mechanical manipulation of the epithelial layers. A cut was also made down the midline of the cranium which allowed better access of medium to tissues. For most experiments a transverse cut was made in the palate two thirds the distance from the anterior end to separate the presumptive hard and soft palate (Diewert, 1978). This is indicated in Fig. 1 by the dashed line (H/S). Heads were then placed in 15 × 45 mm vials containing 3 ml Dulbecco's Modified Eagles Medium (DMEM, Gibco) and 10% foetal bovine serum (FBS, M.A. Bioproducts). The vials were gassed with a mixture of 95% O₂, 5% CO₂ and sealed with rubber stoppers. The vials were placed in a rotating culture apparatus (approximately 30 r.p.m.) for up to 6 h. In one set of experiments cultures were maintained for 24 h.

Epithelial removal and wounding

Heads were treated prior to culture in phosphate-buffered saline (PBS) without Ca²⁺ and Mg²⁺ containing 10 mM-EDTA for 10 min at 37°C. The tongue remained between the palate shelves during this treatment. Following treatment, the palates on either the presumptive oral or nasal surface were scraped using the edges of finely sharpened curved forceps to tease the epithelium from the underlying basement membrane.

Wounding of the palate surface was performed by making a tear in the oral surface running the length of the hard palate and projecting into the underlying mesenchyme. This was accomplished using the sharpened point of a pair of forceps. Following epithelial removal or
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Fig. 1. The dashed line H/S indicates the transverse cut made in the palate shelves to separate the presumptive hard (H) and soft (S) palate. The solid lines indicate where razor blade sections were cut to measure anterior (A), mid (B) or posterior (C) PSI.

wounding the tongue was removed and the presumptive hard and soft palate were separated. The heads were then cultured as described previously.

Quantitation of reorientation in vitro

The amount of reorientation occurring during culture was measured using palate shelf index, PSI (Wee et al. 1976). Following culture, heads were fixed in Bouin’s solution for 24 h. Fixed heads were cut coronally using a razor blade. Razor blade sections were made by cutting the palate shelves in the middle of each third of the palate, allowing for measurement of anterior, mid, and posterior PSI, as indicated in Fig. 1 by the lines A, B, and C respectively. The transverse sections were placed on end and visualized using a dissecting microscope. Values of 1–5 were assigned to express the degree of palate shelf reorientation. A number of 1 was assigned for a completely vertical and a 5 for a completely horizontal palate. Numbers 2, 3 or 4 were designated intermediate elevations of one-quarter, one-half or three-quarters of the way to the horizontal position. Experimental results were expressed as mean PSI ± standard error. Statistical analysis of the difference between the mean PSI of treatment groups and their respective controls was performed using a two-tailed Student’s t-test. The number of palate shelves evaluated in each treatment group is indicated in the tables.

Scanning electron and light microscopy

For SEM heads were fixed in 3% glutaraldehyde in 0.163 M-sodium cacodylate buffer for 4–5 h at 25°C followed by washing in buffer. The heads were then dehydrated in a graded series of ethanol (30–100%) and critical-point dried using carbon dioxide as a transitory fluid. Thereafter heads were mounted, coated with gold, and examined using a scanning electron microscope (ETEC Autoscan).

For light microscopy, heads were fixed in modified Karnofsky’s fixative, as modified by Singley & Solursh (1980). Isotonic sodium phosphate buffer (0.2 M) was used instead of sodium cacodylate buffer. Heads were fixed overnight followed by dehydration in a graded series of ethanol (50–100%) with a final passage through 100% propylene oxide. The heads were then embedded in Polys/Bed 812 (Polyscience, Inc.). Sections were cut at 1–2 μm, stained in Richardson’s stain (Richardson et al. 1960) and examined with a Zeiss photomicroscope.

Immunofluorescence

Heads were fixed for 4 h in Hank’s balanced salt solution without Ca²⁺ and Mg²⁺ containing 2% paraformaldehyde and 0.32 M-sucrose. Heads were removed and dehydrated through a graded series of ethanol (50–100%) followed by two changes of xylene. The heads were then embedded in paraffin. Sections were cut at 6 μm, placed on glass slides and rehydrated prior to antibody staining. Slides were incubated sequentially with rabbit antiserum and rhodamine-conjugated goat anti-rabbit IgG diluted from 1:50 to 1:100. Rabbit antisera to laminin (Engvall
Morphometric analysis

Palates were analysed morphometrically. The degree of epithelial removal was quantitated from scanning electron micrographs of heads treated with EDTA and scraping of the oral surface. From these micrographs three different areas on the oral surface were defined and measured using computer-assisted image analysis (Videoplan, Zeiss). The areas measured were (1) total oral surface of the palate, (2) area of epithelial removal, (3) area in which the basement membrane had been damaged exposing the underlying mesenchyme, defined as the area of wounding. Thirty palates were measured in this experiment and the mean, standard error, and percent of total oral surface were determined for each defined area.

RESULTS

Reorientation of segmented palate shelves in vitro

The separation of the presumptive hard and soft palate allowed reorientation of the hard palate to occur while reorientation of the soft palate was inhibited. The measurement of palate shelf index showed that in fact the anterior and mid palate were enhanced in their degree of reorientation following separation of the palate shelves (Table 1). This was observed at all times of culture. Particularly apparent was a 0.6 PSI unit difference following 1 h of culture between controls and segmented palates in both anterior and mid palate. By 6 h of culture the anterior palate had reached a PSI of 4.5 or 90% movement toward the horizontal position. The mid palate had not yet reached the same degree of movement (75% of horizontal). There was also observed in both anterior and mid palate a reduced gap between the separated palate shelf compared to the control shelf (Fig. 2A,B,D,E). In contrast the posterior palate was inhibited from reorienting following separation of the hard and soft palate. By 6 h of culture there was a

<table>
<thead>
<tr>
<th>Palate segment measured</th>
<th>Protocol</th>
<th>Time in culture (h)</th>
<th>PSI ± S.E.M. (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anterior</td>
<td>Control</td>
<td>2.4 ± 0.09 (28)</td>
<td>3.4 ± 0.09 (30)</td>
</tr>
<tr>
<td></td>
<td>H/S</td>
<td>–</td>
<td>4.0 ± 0.08 (34)</td>
</tr>
<tr>
<td>Mid</td>
<td>Control</td>
<td>1.9 ± 0.05 (28)</td>
<td>2.6 ± 0.09 (30)</td>
</tr>
<tr>
<td></td>
<td>H/S</td>
<td>–</td>
<td>3.2 ± 0.09 (34)</td>
</tr>
<tr>
<td>Posterior</td>
<td>Control</td>
<td>1.1 ± 0.07 (28)</td>
<td>1.4 ± 0.09 (30)</td>
</tr>
<tr>
<td></td>
<td>H/S</td>
<td>–</td>
<td>1.2 ± 0.06 (36)</td>
</tr>
</tbody>
</table>

H/S: palate shelves with hard and soft palate separated.
Different from control values:

- \(^a\) \(P < 0.0001\)
- \(^b\) \(P < 0.001\)
- \(^c\) \(P < 0.02\)
- \(^d\) \(P < 0.05\)
difference of 0.6 PSI unit between controls and segmented palate shelves (Table 1). In comparison, segmented palates had a larger gap between the posterior shelves and they appeared more vertical (Fig. 2C,F). When heads were cultured for 24 h, the anterior palate could fully reorient and fuse, while the soft palate was still markedly inhibited. However some movement of the soft palate toward the horizontal position took place (Fig. 3B). In contrast, when the hard and soft palate were left intact both regions of the palate could reorient and fuse (Fig. 3A).

In all further experiments, culture periods of 24 h were not employed because of poor survival of the inner cranial tissue. Cultures were maintained for 1 to 6 h following separation of the hard and soft palate. In this way the effect of epithelial removal on reorientation of the hard palate could be examined separate from influences of the soft palate.

Fig. 2. Coronal section of palate shelves following 6 h of culture (×60). Shelves were cultured with the full shelf intact (A,B,C) or following separation of the presumptive hard and soft palate (D,E,F). Sections were made in the anterior (A,D), mid (B,E) or posterior (C,F) palate. Bar equals 100 µm.
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Measurements of PSI showed no consistent significant difference in the degree of reorientation from control values following treatment with EDTA alone or EDTA followed by scraping of the nasal surface. EDTA alone inhibited reorientation to a small degree which was only significant in the anterior palate following 3 h of culture. Nasal epithelial disruption showed inhibition only in the mid palate after 1 h of culture.

In contrast, treatment with EDTA followed by scraping of the oral surface significantly inhibited palate shelf reorientation when compared to control or EDTA treatment alone. This result was observed in both the anterior and mid palates at all time intervals measured (Table 2). However the anterior palate appeared to be more affected by this treatment. The inhibition did not appear to be specific to the A/J strain of mouse. CFW, a random bred strain of mouse, showed a similar pattern of inhibition. When treated with EDTA alone the PSI in the anterior end was 4.5 ± 0.11 and the mid palate was 3.7 ± 0.11 after 6 h of culture. In contrast, if they were treated with EDTA followed by scraping of the oral epithelium, inhibition was observed in both anterior (PSI = 3.0 ± 0.10) and mid (2.7 ± 0.13) palates. In all of these studies the posterior or soft palate did not reorient and was unaffected by these treatments.

The morphology of the palate shelves after 6 h of culture in control, EDTA treatment alone, or EDTA treatment followed by nasal epithelial disruption, was very similar (Fig. 4B–D). After nasal surface disruption, removal of the epithelium was observed as well as some minor disruption of the underlying
structures (Figs 4D, 5C). In contrast the morphology of palate shelves treated with EDTA followed by disruption of the oral epithelium was altered (Fig. 4E). The shape of the palates appeared expanded compared to controls or EDTA treatment alone. Also observed was an increase in the gap between the two apposing palate shelves. At higher magnification, the oral surface was devoid of epithelium and the mesenchyme directly beneath this removed epithelium appeared relatively undisturbed (Fig. 5D).

Analysis of epithelial removal

The degree of epithelial removal and wounding following treatment with EDTA and scraping was quantitated using computer-assisted image analysis. It was observed that on the average 41% of the oral epithelium had been removed while only 4% of the oral surface showed evidence of wounding (Table 3). Scanning electron micrographs showed areas in which the epithelium was still intact and other areas where the epithelium had been teased away exposing a non-cellular structure (Fig. 6A,B). In some places the non-cellular structure had been torn away to expose underlying mesenchymal cells, which indicate some wounding (Fig. 6B). These observations suggested that the non-cellular structure was basement membrane apposed between epithelium and mesenchyme. This

Table 2. The effect of EDTA treatment and mechanical disruption of the palate epithelium on reorientation of palate shelves in vitro

<table>
<thead>
<tr>
<th>Palate segment measured</th>
<th>Treatment</th>
<th>PSI ± S.E.M. (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Anterior</td>
<td>Control</td>
<td>2.4 ± 0.09 (28)</td>
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<tr>
<td></td>
<td>EDTA</td>
<td></td>
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<tr>
<td></td>
<td>EDTA,N/E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EDTA,O/E</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>Control</td>
<td>1.9 ± 0.05 (28)</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EDTA,N/E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EDTA,O/E</td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>Control</td>
<td>1.1 ± 0.07 (28)</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td></td>
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<tr>
<td></td>
<td>EDTA,N/E</td>
<td></td>
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<td></td>
<td>EDTA,O/E</td>
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</table>

Palate shelves were pretreated with 10 mM-EDTA for 10 min. Mechanical disruption was performed by scraping the presumptive nasal (N/E) or oral (O/E) surface with finely sharpened forceps. Following this treatment the palate shelves were separated into hard and soft palate and cultured in DMEM plus 10% FBS.

Different from values for EDTA treatment alone:

- \( P < 0.0001 \)
- \( P < 0.001 \)
- \( P < 0.005 \)

Different from control values:

- \( P < 0.05 \)
conclusion was confirmed by performing immunofluorescence studies using antisera to two basement membrane antigens, type IV collagen and heparan sulphate proteoglycan. Both antisera showed immunoreactivity with a structure apposed between epithelium and mesenchyme, as well as remaining attached to areas of mesenchyme after epithelial removal (Fig. 7A,B). A similar pattern of immunoreactivity was also observed with antisera to the basement membrane glycoprotein laminin (result not shown).

The effects of wounding on reorientation in vitro

Wounds were made on the oral surface running the length of the hard palate and covering approximately 9% of the oral surface, or greater than twice the area of

Fig. 4. Coronal section through palate shelves following 0 h (A) or 6 h (B,C,D,E) of culture (×60). Palate shelves were cultured following treatment with PBS with Ca\(^{2+}\) and Mg\(^{2+}\) (B), PBS without Ca\(^{2+}\) and Mg\(^{2+}\) containing 10 mM-EDTA alone (C), followed by scraping the presumptive nasal surface (D), or scraping the presumptive oral surface (E). Bar equals 100 \(\mu\)m.
Fig. 5. The epithelial surface of palate shelves cultured for 6 h (×150). Oral epithelial surface of a control palate shelf following treatment in PBS with Ca$^{2+}$ and Mg$^{2+}$ (A). Oral epithelial surface of a palate shelf treated in PBS without Ca$^{2+}$ and Mg$^{2+}$ containing 10 mM-EDTA (B). Nasal epithelial surface of a palate shelf treated in PBS without Ca$^{2+}$ and Mg$^{2+}$ containing 10 mM-EDTA followed by scraping of the nasal surface (C). Oral epithelial surface of a palate shelf treated in PBS without Ca$^{2+}$ and Mg$^{2+}$ containing 10 mM-EDTA following by scraping of the oral surface (D). Bar equals 100 μm.

wounding observed after EDTA and oral surface disruption (Fig. 8A). The wounds protruded deep into the underlying mesenchyme and the surface of the wound appeared devoid of epithelium (Fig. 8B). Following 6 h of culture, wound healing could be observed in the palate shelves (Fig. 8C). The mesenchyme in the area of the wound was condensed and contracted with some areas of the mesenchyme still remaining separated. The epithelium at this time had not yet covered the wound. The palate shelves also showed substantial movement toward

Table 3. Morphometric analysis of the oral surface following EDTA and mechanical treatment

<table>
<thead>
<tr>
<th>Area measured</th>
<th>Mean area of micrographs (mm$^2$) ± s.e.</th>
<th>% of total oral surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total oral surface</td>
<td>3323.3 ± 89.1</td>
<td>100</td>
</tr>
<tr>
<td>Area of epithelial removal</td>
<td>1363.8 ± 67.8</td>
<td>41</td>
</tr>
<tr>
<td>Area of basement membrane disruption</td>
<td>130.6 ± 20.7</td>
<td>4</td>
</tr>
</tbody>
</table>

Analysis was performed on scanning electron micrographs of 30 palates immediately following treatment with EDTA for 10 min and mechanical scraping of the oral surface. Micrographs were magnified ×85.
the horizontal position. Measurement of PSI showed that wounding could inhibit reorientation only to a small degree and this inhibition was significantly less than that observed following treatment with EDTA and oral epithelial removal (Table 4).

DISCUSSION

The process of palate shelf reorientation may be directed by forces intrinsic to the palate shelves (Walker & Fraser, 1956). Consistent with this idea is the ability of the palate shelves to reorient in culture in the absence of the tongue, brain and circulation (Brinkley, 1975, 1978). Experiments presented in this paper as well as observations made previously (Brinkley & Vickerman, 1979) suggest that this intrinsic force is particularly associated with the anterior two thirds or presumptive hard palate. The hard palate could reorient whether intact or separated from the posterior third or presumptive soft palate, while the soft palate failed to reorient (Table 1). This result was also observed even after an extended culture period, although some minimal movement was apparent (Fig. 3). This result might imply that soft palate reorientation requires extrinsic factors provided by the hard palate. This process could be accomplished by an anterior to posterior 'zippering' effect of adhesion and fusion along the medial edge (Brinkley & Vickerman, 1979). Alternatively, hard palate reorientation may provide a pulling force for directing the soft palate to a horizontal position. This latter interpretation is supported by observations that the soft palate provides resistance to reorientation of the hard palate. When the two segments were separated, hard palate
reorientation was accelerated while the soft palate failed to reorient. The reorientation of the isolated soft palate may require factors that cannot be expressed in this culture system. The soft palate has been described as remodelling around the tongue. This remodelling may require the presence of the tongue for full expression. However, the importance of the tongue and these other factors appear to be minimal in this culture system since the intact shelf showed substantial reorientation and fusion of the soft palate (Fig. 3).

The major emphasis of this paper was to examine the role of the epithelium in generating the intrinsic force for reorientation. The results observed suggest that epithelium on the oral aspect of the hard palate is a major factor in generating this intrinsic force. Removal of the oral epithelium significantly inhibited the pattern of reorientation in vitro. In contrast, disruption of the epithelium on the presumptive nasal surface had no effect on the degree of movement. The treatment used to remove the epithelium, EDTA and scraping, removed primarily epithelium leaving the majority of the basement membrane intact. This observation was

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**Fig. 7.** Immunofluorescence photographs of palate shelves treated in PBS without Ca\(^{2+}\) and Mg\(^{2+}\) containing 10 mM-EDTA and scraping of the oral surface (×500). Palate shelf was fixed immediately following treatment for histology (A) or immunofluorescence (B,C,D). Immunofluorescence was carried out using rabbit antiserum to type IV collagen (B), antiserum to heparan sulphate proteoglycan (C), or with a control rabbit serum (D). e, epithelium; m, mesenchyme. Bar equals 10 μm.
Fig. 8. Palate shelves following wounding of the oral surfaces. The scanning electron micrograph taken immediately following wounding shows the wound (wd) running the length of the hard palate, mag. ×35. Light micrograph of coronal section through the palate immediately following wounding shows the wound (wd) running deep into the mesenchyme, mag. ×60 (B). Coronal section through wounded palate shelf following 6h of culture. Arrows indicate area of wound healing, mag. ×60 (C). Bar equals 100 μm.

Table 4. The effect of wounding the oral surface of the palate on reorientation of palate shelves in vitro

<table>
<thead>
<tr>
<th>Palate segment measured</th>
<th>Treatment</th>
<th>PSI–SE (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time in culture (h)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Anterior</td>
<td>Control</td>
<td>2·4 ± 0·09 (28)</td>
</tr>
<tr>
<td></td>
<td>Wounded</td>
<td>–</td>
</tr>
<tr>
<td>Mid</td>
<td>Control</td>
<td>1·9 ± 0·05 (28)</td>
</tr>
<tr>
<td></td>
<td>Wounded</td>
<td>–</td>
</tr>
<tr>
<td>Posterior</td>
<td>Control</td>
<td>1·1 ± 0·07 (28)</td>
</tr>
<tr>
<td></td>
<td>Wounded</td>
<td>–</td>
</tr>
</tbody>
</table>

Palate shelves were wounded on the presumptive oral surface using finely sharpened forceps. Following this treatment the palate shelves were separated into hard and soft palate and cultured in DMEM plus 10% FBS.

Different from control values:

^a P < 0·05
^b P < 0·02
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supported by both morphological (Hall & McSween, 1984) and immunohistochemical criteria (Table 3, Fig. 7). However this treatment also created some wounding. It is possible that contraction of such wounds during wound healing may have created a force to overcome the force necessary for reorientation and was responsible for the observed inhibition. This interpretation appears unlikely for two reasons. First, the degree of wounding observed after treatment with EDTA and scraping was minimal, covering only on the average 4% of the oral surface. Secondly, when wounds were created covering two times this area only a small degree inhibition of palate reorientation was observed. It could not be determined whether this small degree of inhibition was due to wound healing or the removal of the epithelium over the wound. These results are consistent with the removal of the oral epithelium and not wounding as being responsible for the observed inhibition of palate shelf reorientation.

Brinkley & Vickerman (1979) have previously suggested that reorientation of the hard palate may actually occur by expression of a pre-existing infrastructure. The oral epithelium or an interaction between the oral epithelium and the mesenchyme may help define this infrastructure. Alternative mechanisms for controlling the process of reorientation include the expression of an active contractile system within the mesenchyme (Babiarz et al. 1975) or the accumulation of a hyaluronic acid (HA)-rich extracellular matrix (Larsson, 1960; Pratt et al. 1973; Furguson, 1977; Brinkley, 1980). It appears most likely that HA plays a role in the reorientation of the mid palate. Enhanced degradation of glycosaminoglycans including HA by chlorcyclizine was able to inhibit reorientation in this area while reorientation of the anterior palate was unaffected (Brinkley, 1982). This is in contrast to removal of the oral epithelium which inhibited reorientation in the anterior palate more effectively (Table 2). It is possible that the intrinsic force for reorientation is generated not by simply one element but by the interaction of a number of elements including the oral epithelium, mesenchyme and a HA-rich extracellular matrix.

Our results suggest that the oral epithelium is particularly important in generating the force for reorientation. How this is accomplished is unclear. The control of growth and differentiation of this epithelium may be responsible for generating a particular structure which is necessary for expression of this force. Luke (1984) has observed an unequal cell division within palate epithelium. The oral epithelium appears to be dividing more rapidly than the nasal epithelium. He has suggested that this differential in cell division and development of rugae on the oral surface may be responsible for palate shelf reorientation. The rugae may be particularly important since they are exclusively associated with the hard palate and their development correlate with reorientation in a number of mammals (Waterman & Meller, 1974; Meller et al. 1980). The formation of rugae on the oral surface may stiffen the structure of the oral epithelium forcing the palate shelf toward the horizontal position. Interference with the development of the epithelium and rugae may then lead to the induction of cleft palate.
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