Cytodifferentiation of the mouse secondary palate in vitro: morphological, biochemical, and histochemical aspects

By VICTOR IDOYAGA-VARGAS,\textsuperscript{1} CARLOS E. NASJLETI\textsuperscript{2} AND JULIO M. AZCURRA\textsuperscript{3}

From the Mental Health Research Institute, University of Michigan and Veterans Administration Hospital, Ann Arbor, Michigan

SUMMARY

Previous investigations had indicated that the epithelial cells at the medial surface of the palate differentiate prior to fusion. An increase in adhesiveness and signs of partial autolysis were reported to be part of the differentiation. It was not determined, however, whether or not the autolytic processes may result in the total lysis of the cells, even if fusion is prevented. This problem was approached in the present investigation by culturing shelves in isolation, thus preventing fusion. The morphological properties of the presumptive fusing cells were then observed during and after the actual time of fusion. Differentiation was assessed in vivo at equivalent developmental ages. In addition, the role of the lysosomes in the process of fusion was investigated. This was done by measuring the activities of two lysosomal enzymes, glucosaminidase and arylsulfatase, before, during, and after fusion in vivo and at the equivalent times in vitro. Glucosaminidase activity was also localized histochemically.

The in vitro and in vivo differentiation were similar. The cells of the mesenchyme produced intramembranous bone and loose mesenchyme. The nasal surface differentiated into a pseudostratified columnar ciliated epithelium, while the oral surface became stratified squamous epithelium. At the medial surface, however, the morphological changes during fusion in vivo were different from those observed at the equivalent time in vitro. Few, if any, of the presumptive fusing cells forming a bi-layered or multilayered epithelium degenerated in the explants, while many cells forming the epithelial seam in vivo underwent complete degeneration. In addition, after fusion, the medial surface of the palatal explants was covered by a stratified squamous and/or pseudostratified columnar ciliated epithelia. In vivo, the mesenchyme merged as a consequence of the total destruction of the seam. The specific activity of glucosaminidase increased progressively, reaching a peak three days after fusion. In all cases no statistically significant differences were found between the in vivo and in vitro values. Arylsulfatase specific activity remained the same both in vivo and in vitro. Histochemically glucosaminidase activity was localized in the cells undergoing degeneration during fusion. It is concluded that fusion is a necessary condition for the completion of the autolysis of the presumptive fusing cells. The cells may alter their developmental fate, forming a stratified squamous and/or pseudostratified columnar epithelia, if fusion is prevented.

\textsuperscript{1} Author’s address: Oak Ridge National Laboratories, Oak Ridge, Tennessee, 38830 U.S.A.
\textsuperscript{2} Author’s address: Veterans Administration Hospital, Ann Arbor, Michigan, 48105 U.S.A.
\textsuperscript{3} Author’s address: Instituto de Anatomia Gral y Embriologia, Facultad de Ciencias Medicas, Buenos Aires, Argentina.
INTRODUCTION

One of the main events during the morphogenesis of the oro-facial region is the closure of the secondary palate. This consists of the process of elevation and fusion of the palatine shelves, both of which have been imputed to be involved in the production of cleft palate (Fraser, 1967). Thus, it becomes important to understand the mechanisms underlying shelf elevation and shelf fusion.

Conspicuous among these mechanisms are the changes in the epithelial cells at the medial surface of the palatine shelves. These cells acquire the potentiality to fuse during their development (Pourtois, 1966), a potentiality which is expressed as an increased adhesiveness (Pourtois, 1968) and signs of partial autolysis of the cells before the actual time of fusion (Mato, Aikawa & Katahira, 1967; Angelici & Pourtois, 1968). It was not determined, however, whether or not the autolytic processes may result in the total lysis of the cells even if fusion is prevented. The dissociation of the processes of fusion and degeneration may help to decide which alternative is valid. If palate closure is prevented and the cells undergo complete degeneration, then epithelial autolysis is independent of fusion. The degeneration of cells during development is accompanied by an increase in lysosomal activity (Scheib-Pfleger & Wattiaux, 1962; Weber, 1963; Salzgeber & Weber, 1966). Thus, an increase in lysosomal activity may be expected if the presumptive fusing cells degenerate. However, if by preventing fusion cells do not degenerate, then palatine closure and epithelial seam formation is a necessary condition for total cell lysis.

In the present investigation an approach was made to the problem of whether the cells degenerate regardless of fusion. Palatine shelves were cultured in isolation and the activity of two lysosomal enzymes was measured. These enzymes were β-2-acetamido-2-deoxy-D-glucoside acetamidoglucohydrolase (Glucosaminidase; E.C.: 3.2.1.30) and arylsulfatesulphohydrolase (Arylsulfatase; E.C.: 3.1.6.1). In previous studies in vitro (Pourtois, 1966; Vargas, 1967), the palatine shelves were explanted with the medial surface in contact to permit analysis of the fusion process. In the present work the shelves were explanted without permitting contact with each other; fusion was thus prevented and conditions established for the observation of the presumptive fusing cells before and during the equivalent time of fusion in vivo. Moreover, by appropriately extending the culture period, the cells of the oral and nasal surfaces differentiated so as to allow a comparison with the cells at the fusing edge. This provided information on the fate of the cells at the medial surface after fusion.

MATERIALS AND METHODS

Organ culture

Pregnant mice of the G.P. white (N.I.H.) Swiss strain were used in this study. The time of pregnancy was determined by the plug method, and the final
Cytodifferentiation of mouse palate in vitro

selection of the embryos was made using the morphological criterion of Walker & Fraser (1956). Anesthetized pregnant mice were killed by cervical dislocation. The embryos were immediately removed and the palatine shelves dissected out in balanced salt solution (B.S.S.) Hanks's medium, as described previously (Vargas, 1967). Isolated palatine shelves were explanted, nasal or oral surface up, on a medium of the following composition:

Solution A:
NCTC-109 (Evans et al. 1956) 22.5 vol.
Fetal bovine serum 5.0 vol.
Sodium bicarbonate, 7.5% 0.25 vol.

The above-mentioned chemicals were obtained from the Microbiological Associates.

Solution B:
Ionagar 2, Colab. Laboratories, Inc. 0.4 g.
Bi-distilled water 22.5 vol.

To obtain the final medium, solution B was autoclaved, allowed to cool and mixed with solution A.

Palatine shelves were dissected from 13½ and 14½ (prior to fusion) days post-conception (P.C.) and were cultured from 1 to 4 days according to the five different schedules described below. The controls consisted of histological serial sections of embryonic heads aged 12½ to 18½ days P.C. In the experimental group, one palatine shelf was fixed in Bouin's immediately after dissection and the homotypic shelf was explanted. The culture schedules were as follows:

Series I. Explants dissected at 13½ days P.C. and cultured for one day; total developmental age was 14½ days.
Series II. Explants dissected at 13½ and 14½ days P.C. and cultured for two days and one day, respectively; total developmental age was 15½ days.
Series III. Explants dissected at 13½ and 14½ days P.C. and cultured for three and two days, respectively; total developmental age was 16½ days.
Series IV. Explants dissected at 13½ and 14½ days P.C. and cultured for four and three days, respectively; total developmental age was 17½ days.
Series V. Explants dissected at 14½ days P.C. and cultured for four days.

Three experiments were carried out for each age group for a total of 192 explants.

Biochemistry

The in vivo experiments utilized embryos of 13½, 14½ and 17½ days P.C. In vitro experiments utilized those of 12½ days P.C. which were cultured for 24, 48 and 120 hours.

The fused palates were dissected out from the maxillary arch and the nasal septum and separated into individual shelves which were pooled and suspended in 0.25 M sucrose. The suspension was homogenized vigorously up to 20 times
by means of a mechanically driven pestle rotating in a glass Potter–Elvejhem homogenizer at about 1300 rev/min.

For the assay of glucosaminidase, the tubes contained between 400 and 550 µg of palate homogenate proteins, 0.15 ml of 4-8 mM p-nitrophenyl-2-acetamido-2-deoxy-β-glucopyranoside (Pierce Chemicals, Rockford, Illinois) and 0.68 ml of phosphate–citrate buffer (Elving, Markowitz & Rosenthal, 1956), pH 4.4. (Proteins were determined according to Lowry, Rosebrough, Farr & Randall, 1951.) In the blanks, the tissue homogenate was replaced by 0.1 ml of 0.25 M sucrose. The tubes were incubated for one hour at 37 °C and the enzymic reaction was stopped by the addition of 1 ml of cold 4% (w/v) trichloroacetic acid. The tubes were centrifuged for 10 min at 3000 rev/min, and 0.7 ml of the supernatant fluid was mixed with 0.7 ml of a 5:1 (v/v) mixture of glycine carbonate, pH 10.5 and 0.5 N NaOH. The optical density was determined at 412 nm.

In the arylsulfatase assay, 0.65 ml of 1 M pyridine buffer, pH 5.0 and 0.15 ml of p-nitrocatechol sulphate dipotassium salt (Sigma Co., St Louis, Mo.) were added to tubes containing 400–500 µg of palate proteins. The final molarity of sucrose in the tubes was 0.25 M and the final volume was 0.93 ml. In the blanks, the tissue homogenate was replaced by 0.1 ml of 0.25 M sucrose. The tubes were incubated for 1 h at 37 °C. The reaction was stopped by the addition of 0.25 ml of cold 8% (w/v) trichloroacetic acid. The tubes were centrifuged and 0.9 ml of the supernatant were transferred to tubes containing 0.9 ml of 5 N NaOH. The optical density was detected at 550 nm.

**Histochemistry**

Heads of embryos ranging from 13½ to 14½ days P.C. were fixed in formol calcium and processed for the histochemical detection of N-acetyl-β-D-glucosaminidase (Hayashi, 1965).

**RESULTS**

Table 1 summarizes the temporal development of the morphological changes.

*Epithelia*

The epithelium covering the oral, nasal and medial surfaces of the palatine shelves was composed, before elevation, of a basal layer of cubic cells and an outer layer of squamous cells. The cells of the basal layers were actively dividing in all surfaces. In the oral surface, the cells differentiated to form a stratified squamous epithelium, while in the nasal surface they became stratified columnar. The columnar epithelium then produced typical cilia. The epithelial cells of the opposed medial surfaces came into contact at about 14½ days. The seam thus formed contained many degenerated cells. The seam subsequently became discontinuous and the mesenchyme of both primordia merged.
Table 1. **Histodifferentiation of the secondary palate**

<table>
<thead>
<tr>
<th>Developmental age (days)</th>
<th>Oral surface</th>
<th>Nasal surface</th>
<th>Medial surface</th>
<th>Connective tissue</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12½</td>
<td>Double layered</td>
<td>Double layered</td>
<td>Double layered</td>
<td>Loose</td>
<td>—</td>
</tr>
<tr>
<td>13½</td>
<td>Stratified cuboidal</td>
<td>Double layered</td>
<td>Double or multi-layered</td>
<td>Loose</td>
<td>—</td>
</tr>
<tr>
<td>14½</td>
<td>Stratified cuboidal</td>
<td>Stratified columnar</td>
<td>Fused</td>
<td>Loose</td>
<td>—</td>
</tr>
<tr>
<td>15½</td>
<td>Stratified cuboidal</td>
<td>Pseudostratified columnar ciliated</td>
<td>Fused</td>
<td>Loose</td>
<td>Intramembranous</td>
</tr>
<tr>
<td>16½ through 18½</td>
<td>Stratified squamous without stratum corneum</td>
<td>Pseudostratified columnar ciliated</td>
<td>Fused</td>
<td>Loose</td>
<td>Intramembranous</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14½; I*</td>
<td>Stratified cuboidal</td>
<td>Stratified columnar</td>
<td>Double or multi-layered</td>
<td>Loose</td>
<td>—</td>
</tr>
<tr>
<td>15½; II*</td>
<td>Stratified squamous</td>
<td>Stratified columnar ciliated</td>
<td>Stratified squamous and/or stratified columnar ciliated</td>
<td>Loose</td>
<td>Intramembranous</td>
</tr>
<tr>
<td>16½; III*</td>
<td>Stratified squamous</td>
<td>Pseudostratified columnar ciliated</td>
<td>Stratified squamous and/or stratified columnar ciliated</td>
<td>Loose</td>
<td>Intramembranous</td>
</tr>
<tr>
<td>17½; IV*; V*</td>
<td>Stratified squamous with stratum corneum (s.c.)</td>
<td>Pseudostratified columnar ciliated</td>
<td>Stratified squamous with s.c. and/or columnar ciliated</td>
<td>Loose</td>
<td>Intramembranous</td>
</tr>
</tbody>
</table>

* Experimental series number.
Mesenchyme

The mesenchyme of the palate, before elevation, showed densely packed cells with relatively scant intercellular substance. During fusion, however, the neighboring cells of the medial area became further separated while the cells of the dorsolateral portion differentiated into osteocytes. The osteocytes subsequently underwent ossification which progressively extended toward the midline. The histogenetic changes in the palate can be followed in Fig. 1 A, C, E, G.

Histogenesis in vitro

The changes observed in the cells of the oral surface were similar to those observed in vivo, though they occurred more rapidly. The cells differentiated into a stratified squamous epithelium, forming a stratum corneum. The cells of the nasal surface differentiated following the same morphological and temporal patterns seen in vivo producing a pseudostratified columnar ciliated epithelium.

The histogenesis of the medial surface was followed throughout the culture periods. In explants of Series I the mesenchyme was covered with epithelium containing two or more cell layers. However, portions of the palatine shelves of Series I and the remaining series were not covered by epithelium. The nuclei of the epithelial cells covering the mesenchyme showed the heterochromatin homogeneously distributed. Some condensation of the heterochromatic material was noticed near the nuclear edge. Other nuclei were seen undergoing mitosis. The cytoplasm of the cells was devoid of vacuoles. Nevertheless, in some cells cytoplasmic granules with intense basophilia were occasionally found. The presence of the granules was not confined to the cells of the medial surface. Karyopycnosis, or signs of cytoplasmic degeneration, was seldom observed in Series I through V.

Figure 1

(A) Cross-section of a 13½ palatine process, showing a loose mesenchyme and a bi-layered epithelium. × 800.
(B) Oral surface of a Series IV explant, belonging to the same experiment from which the specimen in (A) was taken. Notice the presence of a stratified squamous epithelium. × 1250.
(C) Oral surface of a 17½-day-old mouse palate. The epithelium is stratified squamous. × 1250.
(D) Nasal surface of the same explant of (B). A pseudostratified columnar ciliated epithelium can be observed. × 1250.
(E) Nasal surface of the same palate of (C), showing a respiratory epithelium. × 1250.
(F) Intramembranous bone developed in the same explant of (B) and (D). × 1250.
(G) Intramembranous bone present in a region of the same palate of (C) and (E). × 1250.
The double or multilayered epithelium was replaced in Series II by a stratified squamous or a pseudostratified columnar ciliated epithelium. Both joined in many cases at the medial surface. The appearance of the stratum corneum in Series IV was the last change noticed at the unfused edge.

**Mesenchyme**

The mesenchymal cells followed the same morphological patterns of differentiation as *in vivo*. In the dorsolateral areas osteocytic activity was evident in the explants of Series II. The progression toward the midline was achieved as *in vivo*. The rest of the mesenchymal cells proliferated and became progressively separated from each other. Histogenesis *in vitro* is illustrated in Fig. 1B, D, F; Fig. 2A; Fig. 3A.

An analysis of Table 1 obtained the following conclusions by comparing the *in vitro* and *in vivo* results. (a) *In vitro*, the cells of the oral surface differentiated faster; unlike *in vivo*, they formed a stratum corneum. (b) The nasal surface differentiated in a manner similar to that in *in vivo*. (c) At the time when fusion would normally occur, the epithelial cells at the medial surface of the explants did not degenerate. *In vivo*, however, many cells underwent complete autolysis. (d) After fusion, cornified and/or respiratory epithelia covered the mesenchyme at the unfused edge. A similar situation was observed in a shelf that was not fused *in vivo* (Fig. 3B, C). (e) The mesenchyme underwent similar patterns of differentiation *in vivo* and *in vitro*.

**Biochemical observations in vivo and in vitro**

In order to achieve the optimal assay conditions for glucosaminidase, the velocity of hydrolysis as a function of time of incubation and of enzyme concentration was tested. Fig. 4 shows that enzyme activity was linear up to 2 h incubation, and Fig. 5 illustrates the linearity of the reaction as a function of different amounts of palate protein. The velocity of the glucosaminidase reaction appeared to be maximum at pH 4-4 (Fig. 6). An identical pH optimum was obtained by Shuter, Robins & Freeman (1970) for the same enzyme in the central nervous system of the rat.

Fig. 7 illustrates the saturation curve of the enzyme with its substrate; an apparent $K_m$ value of 0.96 mM was calculated from this curve. Idoyaga-Vargas and Sellinger (1971) had reported a $K_m$ value of 0.96 mM for glucosaminidase of...
Figs. 4–7. The kinetics of glucosaminidase activity is illustrated. (See explanation in the text.)

Glial cells isolated from the cerebral cortex of the rat. Table 2 shows the results obtained with glucosaminidase and arylsulfatase as a function of age. The specific activity of glucosaminidase increased progressively up to a value of 8.68 in vivo and 12.67 in vitro. The difference between the ages was under all conditions statistically significant, with P-values ranging between 0.01 and 0.005. The age dependency of arylsulfatase specific activity values was slightly different. However, these differences were not statistically significant except between the in vitro 48- and 120-h values.

Examination of the ratios of the specific activity values of glucosaminidase
Table 2. Specific activities of glucosaminidase and arylsulfatase in palatal total homogenate as function of age

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>In vivo</th>
<th></th>
<th>In vitro</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13(\frac{1}{2}) days</td>
<td>14(\frac{1}{2}) days</td>
<td>17(\frac{1}{2}) days</td>
<td></td>
</tr>
<tr>
<td>Glucosaminidase</td>
<td>2.71 ± 0.33</td>
<td>3.86 ± 0.26</td>
<td>6.87 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>1.73 ± 0.06</td>
<td>2.10 ± 0.24</td>
<td>2.32 ± 0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>120 h</td>
<td></td>
</tr>
<tr>
<td>Glucosaminidase</td>
<td>3.69 ± 0.84</td>
<td>6.26 ± 1.07</td>
<td>12.67 ± 2.10</td>
<td></td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>2.93 ± 0.63</td>
<td>3.35 ± 0.85</td>
<td>5.56 ± 0.74</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as total units of enzyme activity per mg of palate protein/h. Each value is given with the corresponding standard errors of means.

Fig. 8. Glucosaminidase specific activity from palatal total homogenates, in vivo and in vitro, plotted as a function of total developmental age. The specific activity is expressed as micromoles of \(p\)-nitrophenol liberated/mg protein/h.

and arylsulfatase at the different age points revealed an upward trend with increasing age, suggesting a preferential increase of glucosaminidase activity with age.

The relationship between the activity of glucosaminidase and the process of fusion is depicted in Fig. 8. There was an increase in the specific activity of the hydrolase both in vivo and in vitro during fusion. The highest specific activity was noted, however, three days after closure of the palate.
Histochemical observation in vivo

When 13½-day-old embryo heads were sectioned for the histochemical detection of glucosaminidase activity in the region of the secondary palate, virtually no activity was observed. However, at 14½ days a reaction was noticed in the area of fusion. Fig. 9A illustrates the localization of the enzyme activity in the triangular epithelial area formed as a consequence of fusion. The cells that are being expelled from the seam also appear to show enzyme activity (Fig. 9B). However, the fusion zone was not the only area within the palate where activity could be detected, since the lateral portions, presumably containing macrophages, also stained (Fig. 9C).

The above histochemical evidence strongly suggests that glucosaminidase activity and hence lysosomes are closely related to cellular degeneration occurring during fusion of the secondary palate.

DISCUSSION

Differentiation of embryonic tissues into stratified squamous epithelium (Soriano, Saxen, Vainio & Toivonen, 1964), respiratory epithelium (Aydelotte, 1963), and bone without a previous cartilage template (Fell & Robinson, 1930), were all demonstrated to occur in vitro. In the present investigation, cells of the explanted palatine shelves differentiated, forming cornified and respiratory epithelia, as well as intramembranous bone. The attention, however, was focused on the differentiation of the epithelial cells at the medial surface. Pourtois (1968) has described a stratification of the outer layer of the epithelium occurring in the marginal region of the palatine shelves. Analogous transformations were observed in some of our explants. This change may be attributed to an increased adhesiveness of the peridermal cells at the medial surface. Moreover, epithelio-mesenchymal recombinations supported the conclusion that the differential adhesiveness is induced by the underlying mesenchyme (Pourtois, 1969). However, the adhesivity of the peridermal cells may be a general property shared by other types of epithelia at a specific time of their cytomorphosis. This has been indicated by the in vitro fusion of epithelial surfaces of different embryonic origins (Vargas, 1968; Pourtois, 1969; Goss, Bodner & Avery, 1970; Bodner, Goss & Avery, 1970).

Greulich (1964) reported that the basal cells of the epidermis give rise to two daughter cells. These may retain the characteristics of basal cells for an indeterminate period of time after cell division. For each cell division, however, another basal cell migrates and differentiates, to be subsequently eliminated after degeneration. In this way the steady state of the epithelium is maintained. If this applies to the palatine epithelium, one may be able to find cells degenerating before fusion on the nasal, oral and medial surfaces. Such degenerative cells have been reported in these three surfaces (Sweeney & Shapiro, 1970). Thus, in order
Cytodifferentiation of mouse palate in vitro

to critically test the assumption that complete epithelial autolysis is a necessary consequence of differentiation before palate closure, it must be ascertained whether there is an increase in cell degeneration at the medial surface of the palate before fusion.

The literature reveals studies of cells undergoing degeneration before fusion (Smiley, 1970; Sweeney & Shapiro, 1970), as well as evidence of lysosomal activity in the palatine epithelium (Waterhouse & Squier, 1966; Andersen & Matthiessen, 1966; Mato, Aikawa & Katahira, 1966, 1967; Angelici and Pourtois, 1968; Farbman, 1969; Hayward, 1969). However, a quantitative demonstration of an increased number of degenerating cells at the fusing edge has not been made. Other embryonic systems in which cell degeneration is accompanied by increased lysosomal activity have been described (Weber, 1963). Therefore, lysosomal activity may serve in certain circumstances as an indication of cell degeneration. If cell degeneration is occurring at a higher rate before than during fusion, then a simultaneous increase in lysosomal activity should be expected. However, in the present work, the specific activity of the lysosomal enzyme, glucosaminidase, was lower before than during fusion and was highest after fusion. The finding of a higher specific activity during fusion than prior to it suggests the degeneration of a higher number of cells during the fusion process per se. That the enzyme is involved in cell degeneration is indicated by its histochemical localization in the degenerating cells at the zone of fusion during the closure of the palate. Some activity, however, was also evident in the epithelial cells throughout the surfaces and in the mesenchymal cells. The increase in glucosaminidase activity may not indicate only degeneration at the medial surface during fusion. The enzyme may be involved in other hydrolytic processes taking place in the palate. For increase in glucosaminidase specific activity in palate explants, during the equivalent time of fusion in vivo, is not accompanied by morphological signs of cell degeneration at the medial surface. The occurrence of these processes, that are apparently independent of fusion, may account in part for the increase in specific activity of the enzyme in the explants during the actual closure. In addition, both in vivo and in vitro, the specific activity of glucosaminidase reached the highest value when morphological differentiation was achieved. The cells of the oral mucosa lose organelles such as mitochondria.

**Figure 9**

(A) Cross-section of the fusing area from a 14½-day-old mouse embryo. Observe the localization of glucosaminidase activity in the midline. The reaction product is present in the triangular area and in a group of cells below it. They represent the remnants of the epithelial seam formed during fusion. × 800.

(B) Cross-section of the fused area from a 14½-day-old mouse embryo. In the midline cells containing the reaction product are being expelled from the palate. × 800.

(C) Mesenchymal cells of the dorsolateral portions of the palate from a 14½-day-old embryo show localization of glucosaminidase activity. × 800.
during keratinization (Rogers, 1964). It is possible that the lysis of organelles is mediated by lysosomes. Thus, glucosaminidase, a lysosomal enzyme located in palatal oral epithelium, may be involved in the lysis of organelles. The increase in glucosaminidase activity observed when differentiation of the oral mucosa occurs would then partly reflect the higher lysosomal activity in the cells undergoing keratinization.

During fusion a morphological comparison was made between the presumptive and actual fusing areas. This was done under three different conditions, in vitro, in vivo and both. In the first, isolated rat palatine shelves, cultured up to the equivalent time of fusion in vivo, showed no evidence of spontaneous epithelial breakdown. However, when cultured with the medial surface or a Millipore filter in contact the epithelium was destroyed (Shapiro, personal communication). The second revealed similar results when electron microscopical examination was made of the same respective areas in vivo (Mato et al. 1966, 1967; Farbman, 1968, 1969; De Angelis & Nalbandian, 1968; Hayward, 1969; Smiley, 1970). Finally in the third, the epithelial cells of the seam in vivo were compared with the presumptive fusing cells in vitro. This comparison, made in the present studies, showed that while many cells undergo degeneration in vivo, few if any presented clear signs of degeneration in vitro. These morphological observations are compatible with the assumption that fusion is a necessary condition for degeneration to occur. Although partial autolysis may indicate differentiation of the cells at the medial surface (Angelici & Pourtois, 1968), fusion appears indispensable for their complete lysis. Consequently, if fusion is prevented, the fate of the cells can be altered. The presumptive fusing cells may develop different morphological characteristics or become part of the respiratory and/or Malpighian-like epithelia. The joining of both epithelial types without transition supports the latter alternative. If this is the case, it may not be an isolated developmental event. For an analogous situation develops during the morphogenesis of the wing bud in the chick embryo. Cells of the posterior necrotic zone are genically determined to die. A ‘death clock’ is set at stage 17. However, the cells can be diverted to a new developmental fate if they are grafted before stage 22 to the dorsal side of the wing bud. There they may develop cartilage or form extra feathers (Saunders, 1966).

In conclusion, the morphological, biochemical and histochemical findings of the present investigation are consistent with the concept that fusion is a necessary condition for the complete autolysis of prospective fusing cells. The cells may alter their developmental fate to form Malpighian- or respiratory-like epithelia if fusion is prevented.

The authors wish to thank Dr O. Z. Sellinger for reading the manuscript and making useful comments, and Mrs P. Petiet for her technical assistance. The critical discussions of Dr R. Narbaitz in the early part of this work are also gratefully acknowledged. This work was supported in part by Grant MH-07417, and one of us (V. I.-V.) was a postdoctoral trainee of the U.S. Public Health Service.
REFERENCES


SWEENEY, L. R. & SHAPIRO, B. L. (1970). Histogenesis of Swiss white mouse secondary palate from the nine and one half days to fifteen and one half days *in utero*. I. Epithelial–mesenchymal relationships: light and electron microscopy. *J. Morph.* 130, 435–450.


*Manuscript received 23 June 1971*