Study of the Normal Development of Rat Palatal Rugae and the Role of Collagen Fibers in Rugae Development

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Abstract—Sprague-Dawley rat fetuses ranging from 15- to 19-day of gestational age, were studied with their rugae morphogenesis at the light microscopic level. The distribution of collagen fibers in the palatal mesenchyme was analyzed. In addition, an attempt to evaluate the role of collagen fibers in rugae development was made. The specimens were H-E stained and collagen fiber stained according to the Gordon and Sweet method. To evaluate the role of collagen fibers in rugae formation, β-aminopropionitrile (BAPN), a well-known lathyrogen, was orally administered to the pregnant rats. Concurrently, an organ culture of the fetal palate was taken with and without BAPN in the culture media. Rugae in the palate had maturated anteroposteriorly in general. Individual ruga development was accompanied by local epithelial cell proliferation, changes in the position of the basement membrane, and crowding and rearrangement of the mesenchymal cells. Collagen fibers were also accumulated beneath the basement membrane of the rugae with changes of their arrangement pattern from reticular to concentric form. The palatal processes of a 15-day-old fetuses cultured with BAPN showed some mesenchymal invasion by proliferating epithelial cells of rugae. In contrast, the cultured palate of the 17-day-old fetus with BAPN showed an exaggerated protruding of rugae above the epithelial surface.

Following the above procedures, some descriptions of rugae development in rat fetuses were obtained. With these results, it was suggested that mesenchymal cells and collagen fibers may play important roles in rugae formation, and the contrasting results of the organ culture experiment with 15- and 17-day-old fetuses might mean that collagen fibers have the possibility of not inducing but counterbalancing normal development of rugae in rat fetuses.

Key Words: Rat, Fetus, Rugae palatinae, Basement membrane, Mesenchymal cells, Collagen fibers, Organ culture, β-aminopropionitrile (BAPN)

INTRODUCTION

The mammalian palatogenesis, as a typical model of embryonic development and differentiation, has been studied extensively by many authors in several laboratory animals (Humphrey 1969; Walker and Ross 1972; Chaudhry and Shah 1973; Greene and Pratt 1976; Greene et al. 1983; Ferguson 1988; Shah and Ferguson 1988). Most of the studies were focused on the outgrowth of palatal shelves, and their elevation and fusion. Alongside such events,
another morphogenetic change occurs concomitantly on the oral surface of the palatal mucosa and that is the formation of rugae. Though it has received little attention, Luke (1988) has already noticed, rugae formation - the formation of a series of elevated ridges from a flat surface - is thought to represent one of the prototypes of embryonic morphogenesis.

The rugae present on the oral palatal mucosa of mammalians are thought to facilitate intraoral transport of food in several animals (Weijs 1975; Franks et al. 1984). Though rudimentary to the idea of biological functions, rugae in men have been studied in terms of an identification method in forensic medicine (English et al. 1988), racial differentiation in anthropology (Thomas et al. 1987), and as a possible landmark for determining changed teeth positions (Van der Linden 1978; Simmons et al. 1987). Some authors have reported the presence of rugae on the lateral surface of palatal shelves at the time of shelf elevation (Waterman et al. 1973; Waterman and Meller 1974; Ferguson 1978; Meller et al. 1980), and rugae themselves have been referred to as one of the causes of shelf elevation (Bulleit and Zimmerman 1985). In those studies, information about rugae development was fragmentary because the studies were not concerned with rugae development in itself. Luke (1984, 1988) reported more information about rugae morphogenesis. In his work, he observed that a fetal mouse palate already had the same number of rugae as an adult mouse just prior to shelf elevation and that there was rich innervation around them. An aggregation of mesenchymal cells beneath rugae both before and after shelf elevation was observed by Sakamoto et al. (1989). Previous investigations about rugae were not so abundant. Furthermore, studies in fetal tissues were more scanty and unsatisfactory because only a few investigators have been interested in rugae formation. The first aim of this study is to obtain more details about morphological changes during rugae formation.

The amount of collagen in the extracellular matrix of the palatine shelf has been known to increase during palate closure (Pratt and King 1971). Collagen fibers have been referred to as a candidate for the intrinsic factor in shelf elevation (Hassell and Orkin 1976). Besides, they are known to play several important role in the development of various fetal tissues (Paranko 1987; Chen and Little 1987; Fukuta et al. 1988; Nakanishi et al. 1988). It may be possible that collagen fibers would play some roles in rugae formation. For such reasons, in search of factors concerning rugae development, we first thought of collagen fibers and investigated their putative role using well-known lathyrogen, β-amino-propionitrile (Wilk et al. 1972), in vivo and in vitro.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 200-250 gm were used in this study. A virgin female was placed overnight with males. Pregnancy was ascertained by the presence of a vaginal plug the next morning, and the day was designated as day 0 of pregnancy. Dams were sacrificed at 15- to 19-day of pregnancy by cervical dislocation. The fetuses were removed from the uterus and the body weights were recorded. In each litter, three fetuses were randomly selected and fixed by perfusion via the umbilical vein. Fetal heads were dissected and fixed in Bouin's solution for another 48 hours. After removing the tongue and mandible, they were dehydrated and embedded in paraplast. Horizontal sections including palatal shelves with 15-day fetal heads, those with vertical palatal shelves, and parasagittal sections with others, those with elevated palatal shelves, were made. Sections were stained with hematoxilin and eosin for histological observation, and Gordon and Sweet's silver impregnation (Sheehan and Hrapchak 1980) for collagen fibers. To investigate the putative role of collagen fibers in rugae development, a well-known lathyrogen, β-amino-propionitrile (abbreviated as BAPN), was orally administrated at a single dosage of 600 mg/kg (1 gm/kg of BAPN fumarate) (Wilk et al. 1972) to pregnant rats on day 16, 17, or 18 of pregnancy. They were all sacrificed on day 19 and the obtained fetuses were carefully examined for gross malformations and treated for histological observations.

For in vitro studies, organ culture was carried out. The palatal shelves or fused palates of 15-
16- or 17-day fetuses were dissected aseptically, and placed on a Millipore filter (0.45 μm porosity) with the nasal side down. The filter was again placed on a stainless steel grid in a culture dish (Falcon model 3037). Cultures were maintained at 37°C in a humidified incubator with 5% CO₂ in air. Used culture media was MEM (minimum essential medium) with Earle's salt (Sigma) supplemented with 10% fetal bovine serum (Hyclone), and antibiotics were not added. The media were changed every 24 hours. Incubation times were 96, 72, 48 hours in 15-, 16-, 17-day palate, respectively. BAPN was used at a concentration of 36 μg/ml. At the end of incubation, the explants with underlying filter were removed and fixed in Bouin's solution and routinely processed.

RESULTS

1. Normal Development of Rugae

On day 15 of gestation, the palatal shelves were in vertical position (Table 1). The future rugae appeared as linear epithelial ridges somewhat parallel to the coronal plane on the future oral surface, i.e., the lateral surface of a shelf. Their number was 2-3 in each shelf. Histologically the lateral surface was composed of a two-cell layer with basal cuboidal and upper flat cells. The future rugae assumed localized epithelial thickenings composed of a 3- to 4-cell layer (Fig. 1). In that area the epithelial cells were more or less larger and the basement membrane was more eosinophilic and thicker than in other areas. Condensation of mesenchymal cells was observed under the thickened epithelium. In collagen-fiber stained sections, fibers appeared as darkly stained threads throughout the mesenchyme. Collagen fibers just beneath the basement membrane showed a perpendicular arrangement in contact with the basement membrane (Fig. 2).

On day 16, palatal shelves were either elevated with their medial edge epithelia in contact with each other or not elevated. About 6-7 rugae with elevated shelves and 5-6 with non-elevated shelves were observed. The rugae showed somewhat differing appearances according to their location on the palate. The most posteriorly located ruga was composed of a 2-cell layer with elongated basal cells (Fig. 3). The basement membrane was flat or seemed to be slightly depressed. The epithelium was thickened to a 3- to 4-cell layer in the next anterior ruga and the basement membrane was slightly introduced into the mesenchyme. Mesenchymal cells, which had smaller nuclei than surrounding cells, were condensed around the ruga. The more anteriorly located rugae showed further development with more a depressed basement membrane and more aggregated mesenchymal cells (Fig. 4). The center of the deeply depressed basement membrane was lifted again toward the surface in the most anterior ruga (Fig. 5). Collagen fibers were condensed beneath the rugae and they showed a reticular arrangement (Fig. 6).

On day 17, both shelves were almost completely fused and 9 rugae were observed. The basement membranes of the anterior 2-3 rugae were lifted towards to the surface forming an arch (Fig. 7). In the core of the arch, mesenchymal cells were aggregated. In this group, collagen fibers were often found in a concentric arrangement with a flat or elevated basement membrane (Fig. 8). Compared with that of the 16-day palate, the fibers were stained thicker and were in general more dense. Some rugae showed flat basement membranes with increased mesenchymal cell density. Posterior rugae still showed a depressed basement membrane. On day 18, the anterior 3 rugae appeared as a mesenchymal ridge covered with a two-cell layer epithelium except at its apex, where the epithelium was composed of a 3- to 4-cell layer (Fig. 9). Mesenchymal cells were aggregated and arranged concentrically especi-

| Table 1. The state of palatogenesis and the number of rugae at each fetal age group |
|---------------------------------|------------------|-----------------|
| Fetal age                      | Developmental state of palate | No. of rugae |
| 15-day                         | Vertical palatine processes   | 2-3            |
| 16-day                         | Vertical palatine processes or elevated palatine processes | 5-6           |
| 17-day                         | Almost complete fusion of both processes | 9             |
| 18-day                         | Similar to 17-day fetus       | 9              |
| 19-day                         | Fusion of primary and secondary palate | 9             |
ally at the top of the ridge. Long collagen fibers, now arranged anteroposteriorly, were attached at both sides in a ruga (Fig. 10). In inter-rugal areas, the fibers were short and perpendicular to the basement membrane. Some of the posterior rugae were with concentrically arranged collagen fibers. On day 19, the anterior rugae assumed a similar appearance to the 18-day fetus. However the rugal epithelium was without apical thickenings as was shown in the apex of the 18-day rugae. It was composed of more than 3- to 4-cell layers with an eosinophilic free surface (Fig. 11). Collagen fibers and mesenchymal cells also showed a concentric or anteroposterior arrangement. Collagen fibers were more abundant than in 18-day rugae. Almost all posterior rugae showed concentrically arranged collagen fibers. The developmental states of the most anterior rugae in each age group are summarized in Table 2.

2. BAPN Treatment in vivo

The number of living fetuses gathered in a BAPN-treated rat varied ranging from 0 to 7. The average number was 3.8 per mother, less than that of the control group which was 9.8 in average. Their body weight, 2.02 ± 0.17 gm, was smaller than that of the control group (2.21 ± 0.28 gm) but not statistically significant (p > 0.05). They showed no obvious gross malformations and collagen fiber staining was similar with that of the control group. But, collagen fibers in dead fetuses were scanty in the palate mesenchyme.

3. In Vitro Experiment

In 15-day-old palatal processes cultured for 96 hours, 4-6 rugae were observed (Table 3). The

Table 2. Some representative events accompanying rugae formation and the state of anteriorly located rugae at each fetal age observed in H.E. stained and collagen fiber stained sections

<table>
<thead>
<tr>
<th>Representative events</th>
<th>15-day</th>
<th>16-day</th>
<th>17-day</th>
<th>18-day</th>
<th>19-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position of basement membrane</td>
<td>Flat or slightly depressed</td>
<td>Depressed</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>Arrangement of mesenchymal cells</td>
<td>Random</td>
<td>Random</td>
<td>Random or somewhat concentric</td>
<td>Concentric</td>
<td>Concentric</td>
</tr>
<tr>
<td>Involvement of mesenchyme in rugae</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Arrangement of condensed collagen fiber</td>
<td>Minimal condensation</td>
<td>Reticular</td>
<td>Reticular or concentric</td>
<td>Concentric</td>
<td>Concentric</td>
</tr>
</tbody>
</table>

Table 3. Summary of the findings observed in cultured palatine tissues with or without β-aminopropionitrile (BAPN) in the culture media

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of rugae</th>
<th>Position of basement membrane</th>
<th>Arrangement of aggregated mesenchy. cells</th>
<th>Arrangement of condensed collagen fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-day control</td>
<td>4-6</td>
<td>Flat or slightly depressed</td>
<td>Random</td>
<td>Vertical or reticular</td>
</tr>
<tr>
<td>15-day BAPN</td>
<td>5-6</td>
<td>Marked depression in some rugae</td>
<td>Random</td>
<td>Vertical or reticular</td>
</tr>
<tr>
<td>16-day control</td>
<td>6-7</td>
<td>Elevated or flat</td>
<td>Concentric or random</td>
<td>Reticular</td>
</tr>
<tr>
<td>16-day BAPN</td>
<td></td>
<td>Similar to 16-day control</td>
<td>Concentric or random</td>
<td>Reticular</td>
</tr>
<tr>
<td>17-day control</td>
<td>8</td>
<td>Elevated or flat</td>
<td>Concentric or random</td>
<td>Reticular or concentric</td>
</tr>
<tr>
<td>17-day BAPN</td>
<td>8</td>
<td>Markedly elevated</td>
<td>Concentric or random</td>
<td>Reticular or concentric</td>
</tr>
</tbody>
</table>

* In palatal tissues cultured in the presence of BAPN, regardless of their gestational age group, collagen fibers are short and arranged randomly and reticularly throughout the mesenchyme without obvious condensation below the rugae.
basement membrane was flat or slightly depressed. Rarely, an elevated basement membrane was also seen. The epithelium was several cell layers thick with an eosinophilic free margin. Mesenchymal cells beneath the rugae were irregularly aggregated, but not so densely as in vivo. Collagen fibers were more or less condensed beneath rugae and in perpendicular position to the basement membrane (Fig. 12). They often showed a reticular arrangement in some anterior rugae. In palates cultured with BAPN, collagen fibers were shorter and thicker than in control and dispersed evenly throughout the mesenchyme without any distinct localizations beneath the rugae (Fig. 13). Though general morphology was similar to the control, some rugae showed a deeply depressed basement membrane to an extent that had never been seen in any of the other groups (arrows in Fig. 13). In 16-day-old palates cultured for 72 hours, the number of observed rugae was 6-7. The anterior 3-4 rugae showed an elevated or flat basement membrane with concentrically arranged mesenchymal cells. Some of posterior rugae showed slightly depressed basement membrane and the mesenchymal cells were randomly arranged. Collagen fibers were in reticular arrangement beneath rugae but in perpendicular position between rugae. In palates cultured with BAPN, collagen fiber patterns were similar to those of 15-day palates cultured with BAPN. The thickness of the palate was thinner than the control. In 17-day-old palates cultured for 48 hours, about 8 rugae could be identified. Almost all basement membranes of rugae were elevated or flat. Condensed mesenchymal cells and collagen fibers, as a whole, showed a reticular arrangement, but were concentrical in some anterior rugae (Fig 14). Palates cultured with BAPN, collagen fiber patterns were similar to those of 15-day palates cultured with BAPN. The thickness of the palate was thinner than the control. In 17-day-old palates cultured for 48 hours, about 8 rugae could be identified. Almost all basement membranes of rugae were elevated or flat. Condensed mesenchymal cells and collagen fibers, as a whole, showed a reticular arrangement, but were concentrical in some anterior rugae (Fig 14). Palates cultured with BAPN, collagen fiber patterns were similar to those of 15-day palates cultured with BAPN (Fig. 16).

DISCUSSION

In this experiment, the palatogenesis progressed as previously described by many authors (Coleman 1965; Pratt and Greene 1976; Diewert 1978; Ferguson 1978). That is, vertical shelves on day 15 of gestation, shelf elevation and contact of both medial edge epithelium on day 16, nearly closed palate on day 17. On day 16, there were some individual variations showing both elevated or non-elevated shelves in the same litter and between litters (data not presented). Variations in a given litter may be because of such factors as differences in the time of fertilization and implantation, and in the blood supplies due to different positions in the uterus (Ferguson 1978). A somewhat long housing time for mating, overnight (about 12 hours), might result in variations between litters. In fact, though several reports agreed that shelf elevation occurred early on day 16, they showed some differences as to the precise time of shelf elevation - 16 days in Long-Evans rats (Coleman 1965), late on day 15 in Sprague-Dawley rats (Pratt and Greene 1976), 16 days 8 hours in Sprague-Dawley rats (Diewert 1978), 16.3-16.5 days in Wistar rats (Ferguson 1978). For this study, in the case of the 16-day group, elevated shelves were selected for observation.

Rugae morphogenesis has been known to already occur before shelf elevation (Waterman et al. 1973; Waterman and Meller 1974; Bullet and Zimmerman 1985). In this experiment, the presumptive rugae were also observed before elevation on days 15 and 16. However in contrast to Luke (1984), who reported that the same number of rugae as in the adult mouse (8-9 in number) were observed just prior to shelf elevation, only 2-3 and 5-6 rugae were observed in the vertical shelf of 15- and 16-day fetuses, respectively. This discrepancy seems, at a glance, to be reasonable because of some differences between Luke's and our experiments. Luke defined a ruga as an epithelial thickening in histological sections, and we identified rugae under a dissecting microscope. Luke observed in mice instead in rats. However Sakamoto (1989), who also observed in mice,
found only 4-5 recognizable rugae just prior to shelf elevation. Furthermore, our results showed 6-7 rugae even in histological sections of elevated 16-day palates. Luke had not stated in his paper that a localized epithelial thickening had always to be regarded as a future ruga. Therefore, it cannot be ruled out that his results might be overestimated. One of our results that cultured 15-day palate revealed only 4-5 rugae may be further supporting evidence that not all rugae were represented before shelf elevation. A total of 9 rugae, as in adult rats, appeared by day 17 of pregnancy. On day 18, the anterior 3 rugae appeared to include mesenchymal ridges and this coincides with the fact that, in adult rats, they are larger than the others (Weijs 1975).

Several accompanying morphological changes were observed at the sites corresponding to rugae during their maturation. Such events, which have been reported by some authors (Luke 1984; Thomas 1984; Sakamoto et al. 1989), as an aggregation of mesenchymal cells and a changing of their arrangements from random to concentric, were observed. Besides, the basement membranes of rugae changed their positions from flat to introduced into the mesenchyme, and elevated again toward the surface. Collagen fibers were also accumulated beneath rugae, and their arrangement changed from random or reticular to concentric. All the changes were progressed in an anteroposterior sequence as was previously noted by Luke (1984). That is, more developed rugae were located more anteriorly. However that was not so critical, but only a trend. It is well known that mesenchymal tissue or cells are involved in the differentiation and development of embryonic epithelial tissue (Toole and Underhill 1983). In palatogenesis, for the complete differentiation of the palatal epithelium, interaction between mesenchyme and epithelium is also necessary (Tyler and Koch 1977). Brinkley (1986) described the localized difference of mesenchymal cell density during palatogenesis and supposed that the mesenchymal cells might influence the palatal morphogenesis. The present study showed that mesenchymal cells were gathered and changed their patterns of arrangement during rugae formation. This implies that mesenchymal cells may be directly or indirectly involved in rugae formation. There are some examples that mesenchymal cells influence developmental processes by synthesizing chemical substances. In palatogenesis, they have been reported to play some roles by producing prostaglandins (Chepenik and Greene 1981; Alam et al. 1982). Another example was presented by Edelman (1989), who found that N-CAM (neutral type cell-adhesion molecules) secreted by mesenchymal cells was prerequisite for the development of a feather in chick embryos. One of the roles of the mesenchyme is to regulate the formation of the basement membrane and stabilize it (Spooner and Faubin 1980). Though it is generally accepted that the basement membrane is made by epithelium, the mesenchyme has also been observed to contribute to the formation of the basement membrane (Kuhl et al. 1984; Kimata et al. 1985; Simon-Assman et al. 1988). The basement membrane in structures which undergo rapid morphological change, such as growing fetal maxillary processes, was reported to show compositional differences, which were supposed to be related to morphogenesis (Xu 1991). Our results showed that the rugal basement membrane appeared to change its position variously according to the developmental states. Considering our results and others’ reports, the possibility cannot be excluded that the change of the basement membrane might be supported by mesenchymal tissue. It is well known that the mesenchymal cells secrete collagen fibers. Mesenchymal cells, in this study, assumed a very similar condensation and arrangement pattern to those of collagen fibers at similar times. Therefore it might be possible that they are only for the production of collagen fibers, regardless of the role of the fibers. Of course, the present study cannot differentiate how the mesenchymal cells are involved in rugae morphogenesis. Further studies should be done to determine what role, if any, they play in rugae morphogenesis. Collagen fibers, showing increasing density and changes of arrangemental pattern, may influence rugae development. Collagen fibers influence morphogenesis in several organs such as the testis and ovary (Paranko 1987), lung (Chen and Little 1987), and the submandibular gland (Fukuda
et al. 1988; Nakanishi et al. 1988). Luke (1988) supposed the involvement of collagen fibers in rugae formation. But, their influence remains unknown. To investigate the putative role of collagen fibers, BAPN was administered to pregnant rats and the fetuses were examined for gross malformations. BAPN, a well-known teratogen, is known to inhibit intramolecular cross-linking of collagen fiber (Hall 1972; Steffek and Handrickx 1972). When orally administered to pregnant rats, the maximum concentration in the fetus is reached after 6 hours, maintained for 3 hours more and abruptly decreased thereafter to negligible amounts by 24 hours (Wilk et al. 1972). A single oral dose on day 15 of pregnancy provoked cleft palate by altering the development of Meckel's cartilage (Diewert 1981). We administered a single oral dose on day 16 or 17. Many resorptions were observed and some fetal palates showed decreased stainability for collagen fibers. However, in living fetuses, no obvious gross malformations were detected. Steffek et al. (1972) and Barrow and Steffek (1974) reported similar results which differed only in some minor details.

Because in vivo study with BAPN revealed little information about the putative role of collagen fibers, in vitro experiment, which has been used by many investigators in the studies of palatogenesis (Smiley and Koch 1975; Tyler and Koch 1975) for a more controlled environment, was carried out. In 15-day palatal shelves cultured for 4 days, rugae appeared as a thickened epithelium with the basement membrane but not as much as in 19-day fetuses. In 16-day palates cultured for 3 days and 17-day palates cultured for 2 days, the basement membranes were somewhat elevated. It is thought that the behaviour of the basement membrane of cultured rugae was restricted by the gestational age of the fetus at the time of sacrifice. Not only the behaviour of basement membrane, but the number of rugae at the end of the culture process showed differences according to the gestational age of the used fetuses- 4-6, 6-7 and 8 rugae in cultured 15-, 16- and 17-day fetuses, respectively. These results may suggest that rugae still not induced at the time of sacrifice are not developed, and therefore, it may be possible that the inducing factor for rugae is extrinsic rather than intrinsic. Such a suggestion may be supported by the experiment of Capon (1983), who insisted that innervation was an inducing factor in rugae formation. While the observed numbers of rugae on days 15 and 16 were 2-3 and 5-6, the cultured palates of 15- and 16-day fetuses showed 4-6 and 6-7 rugae, respectively. The discrepancy may be due to the interval between induction and beginning of real development of rugae. In other words, the potential for rugae formation might be given before as in palatogenesis, in which the potential for cell death of medial edge epithelium appeared 36 hours prior to actual cell death, and during this time, no morphological change was detected (Hudson and Shapiro 1973).

It was noticed that BAPN in culture media did not change the stainability for collagen fibers, but changed their distribution and arrangement. Because BAPN does not inhibit secretion of fibers (Pratt and King 1972) the unchanged stainability is thought to be accurate. The most outstanding features in cultured tissue with BAPN were that in cultured 15-day fetuses, some proliferated epithelial cells were deeply introduced into the mesenchyme and that in cultured 17-day fetuses rugae showed exaggerated elevation over the surface. In other words, both age groups showed opposite developmental directions of rugae epithelium to each other, probably due to BAPN, whether by direct or indirect influence. This implies that the effects of BAPN are different at different ages. In turn, the deteriorated arrangement of collagen fibers in both groups suggests that collagen fibers may contribute in different ways at different ages. When the basement membrane is introducing into the mesenchyme, collagen fibers seem to exert an elevating force, and when it is being lifted, they seem to exert a pulling down effect. It must be remembered that the arrangement of collagen fibers changed during rugae formation in collaboration with the changes of position of the basement membrane (summerized in Table 2).

Considering the above results, it may be suggested that collagen fibers seem to stabilize or counterbalance the change in the basement membrane during rugae morphogenesis.
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LEGENDS FOR FIGURES

Fig. 1. Ruga on day 15. Epithelium is thickened and the basement membrane is prominent (arrow). Mesenchymal cells are crowded in the rugal region compared to the interrugal region. H-E. x 400

Fig. 2. Lateral surface of 15-day shelf. Short collagen fibers are dispersed throughout the mesenchyme and are perpendicular to the basement membrane. Collagen fiber stain. x 500

Fig. 3. Posterior ruga on day 16. Epithelium is composed of a 2-cell layer but the cells of the basal layer are highly elongated. The basement membrane is relatively flat. H-E. x 400

Fig. 4. Anterior ruga on day 16. The basement membrane is depressed into the mesenchyme. Mesenchymal cell density is increased in the rugal region. H-E. x 400

Fig. 5. Second ruga on day 16. The basement membrane introduced into the mesenchyme is lifted again toward the surface from the center of the ruga. H-E. x 400

Fig. 6. Anterior ruga on day 16. Collagen fibers show reticular arrangement. Collagen fiber stain. x 400

Fig. 7. Third ruga on day 17. Basement membrane is elevated, and mesenchymal cells are aggregated in the mesenchymal core of the ruga. H-E. x 400

Fig. 8. Ruga on day 17 with elevated basement membrane. Collagen fibers show a somewhat concentric arrangement. Collagen fiber stain. x 400

Fig. 9. Second ruga on day 18. Mesenchymal ridge is formed being a part of the ruga. Cells are aggregated and arranged concentrically in the mesenchymal core. Thickened epithelium of the ruga is located on the summit of the ridge. H-E. x 200

Fig. 10. Second ruga on day 18. In the mesenchymal core, collagen fibers show an obviously concentric arrangement. In the interrugal area, fibers are short and perpendicular to the basement membrane and appear less densely. Collagen fiber stain. x 400

Fig. 11. Third ruga on day 19. Epithelium on the summit of the mesenchymal ridge is not so thick compared with the neighboring epithelium. H-E. x 200

Fig. 12. Ruga in 15-day-old palatal process cultured for 96 hours. Long collagen fibers are attached to the basement membrane perpendicularly and reach deep into the mesenchyme. Collagen fiber stain. x 400

Fig. 13. Rugae in 15-day-old palatal process cultured with BAPN. Epithelial proliferations, thought to be rugae, invade into the mesenchyme (arrows). Collagen fibers are short and arranged randomly and reticularly throughout the mesenchyme. On the left side, another ruga is shown. Collagen fiber stain. x 400

Fig. 14. Ruga in 17-day-old palate cultured for 48 hours. The basement membrane is elevated with concentrically aggregated mesenchymal cells. H-E. x 400

Fig. 15. Ruga in 17-day-old palate cultured for 48 hours with BAPN. Exaggerated bulging toward the surface is seen. H-E. x 400

Fig. 16. Ruga in 17-day-old palate cultured for 48 hours with BAPN. Collagen fibers are similar in appearance with that of Fig. 13. Collagen fiber stain. x 400
시급 태자에서 구개주름의 발생과 이에 미치는
아교성유의 역할에 관한 연구

충북대학교 의과대학 해부학교실

황영일·이은영

서울대학교 의과대학 해부학교실

이왕재·이광호·장가용

Sprague-Dawley계 시급의 구개주름 발생에 따른 형태학적인 변화와 아교성유의 태양에
따른 분포를 조사하여 보고 아교성유가 구개주름 발생에 미치는 영향을 추정하고자 본
실험을 시행하였다. 태양 15일에서 19일 사이의 태자 구개주름 구역을 취하여 H-E
염색 및 아교성유염색을 시행하여 관찰하였고 아교성유가 구개주름 발생에 미치는 영향을
알아보기 위하여 B-aminopropionitrile (BAPN)을 임신한 시급에 경구 투여하는 한편 태자의
구개주름 및 구개로 BAPN 존재하에 조직패혈하였다.

관찰결과 각각의 구개주름의 발생은 상과의 부분적인 증식, 기저막의 위치 변화, 중간
염색포의 밀집과 시간에 따른 페열의 변화 등이 동반되었으며 이러한 변화는 대략 약 1주에서
되로의 방향으로 진행되었다. 아교성유는 구개주름 바로 밑의 중간염색조직에서 밀집되어
관찰되었는데 초기에는 그물모양의 구조를 이루다가 점차 동심원모양인 배열을 이루어
나갔다. BAPN을 사용한 조직패혈 실험에서 태양 15일 배양군에서는 구개의 후방에 위치하는
일부 구개주름에서 상과의 홍조의 중간염색조직으로 갈수록 들어가는 양상을 나타낸
반면에 태양 17일 배양군에서는 전체의 구개주름의 표면으로 과장되게 용기하는 모습을
보여주었다.

위의 결과로 미루어 볼 때 구개주름 발생에서 중간염색포와 아교성유는 매우 중요한
역할을 한 것으로 생각된다. 또한 아교성유의 경우에는 BAPN을 사용한 태양 15일과 태양
17일의 조직패혈에서 구개주름의 변화양상이 서로 반대되는 방향으로 나타나는 것은 아마도
아교성유가 구개주름에서 발생을 유발시키는 기능을 하기보다는 그 형성을 조절하는 기능을
수행할 가능성이 많을 것임을 나타내는 것으로 사료된다.