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Palatal shelf reorientation in hamster embryos following treatment with 5-fluorouracil

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Summary. A study was undertaken to examine the issue of whether achieving a critical mass of cells and/or palatal shelf volume during vertical development of shelf is essential for reorientation to occur. In control and 5-fluorouracil (5FU)-treated hamster embryos' palatal shelves, at different times during gestation, the numbers of both epithelial and mesenchymal cells were counted and cross-sectional area was measured. DNA synthesis was measured by ^{3}H-thymidine incorporation and was used as an index of growth by cell proliferation. The control data indicated that, unlike development during initial 24 hours, the later period of vertical palatal development was characterized by a steady level of mesenchymal and epithelial cell numbers and palatal shelf area. Following 5FU treatment all the measurements were reduced, and until they reached the equivalent of control values, the palatal shelves did not reorient. The density of mesenchymal cells in the developing palate did not seem to affect cell number. On the basis of the analysis of results of the present study, along with those reported in the literature, it is suggested that, in hamsters, acquisition and maintenance of both a specified number of mesenchymal cells and shelf area, at least 24 hours prior to reorientation, may be critical for ensuing mesenchymal differentiation to enforce palatal shelf reorientation on schedule. 5FU affected these features to delay reorientation of the palatal shelf.

Key words: Palate Morphogenesis, Hamster, Cell Number, 5-fluorouracil, Shelf volume, DNA Synthesis

Introduction

The embryogenesis of mammalian secondary palate occurs through a cascade of dynamic events. These events are: growth of two vertical palatal shelves along sides of the tongue; reorientation of vertical shelves to a horizontal plane above the dorsal surface of the tongue; and finally closure of the horizontal shelves. In order to accomplish these events, coordination amongst various processes of growth and differentiation at the cellular level is essential.

An individual organ or structure shows a characteristic growth and cellular proliferation pattern during its ontogeny. Upon reaching a certain size the organ stops growing (Enesco and Leblond, 1962; Dobbing and Sands, 1973; Cheek, 1975; Summerbell, 1976; Madhavan and Scheiderman, 1977). Since cell number is fundamental to the growth of a structure, it has been suggested that a critical number of cells may be essential for advancement in the morphogenesis of a structure (Mintz, 1971; Wolpert et al., 1979; Bryant and Simpson, 1984).

Recently it was shown that a series of spurts in DNA synthesis, accompanied by both an increase the in the number and density of mesenchymal cells and the volume of shelves, occurs during primordial growth of the vertical shelves (Burdett, Waterfield and Shah, 1988). During the later part of vertical development the palatal shelf appears to be undergoing a maturation process characterized by steady growth in the area of palatal shelf, but not in the cell number (Shah et al., 1989). Hence, a question arises whether a critical number of mesenchymal cells and/or palatal shelf volume are essential for subsequent reorientation of the palatal shelves. The question is of significance because those teratogens which induce cleft palate by affecting the reorientation stage of palate development are administered at a time when the palatal shelves are still growing vertically. In such circumstances it is not clear whether the teratogen would affect, among other growth features, the number of mesenchymal cells in the vertically developing palate, and/or the shelf volume.

Efforts have been made in the past to analyze whether the proliferation of mesenchymal cells is causally related to the reorientation of palatal shelves (Mott et al., 1969;

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Nanda, 1971; Hudson and Shapiro, 1973; Jelinek and Dostal, 1974; Nanda and Romeo, 1975; Cleaton-Jones, 1976; Brinkley and Bookstein, 1986). These studies, however, did not address the issue of whether achieving a critical mass of cells and/or palatal shelf volume during the vertical development of shelf are essential for reorientation to occur.

In the present study we have attempted to analyse these growth related issues in a developing vertical shelf which is preparing to reorient to a horizontal plane. ^{3}H-thymidine uptake, as a measure of DNA synthesis, was used as an index of growth by cell proliferation. In addition, the effect of 5-fluorouracil (5FU) on developing palate was evaluated, because the chemical (1) inhibits DNA synthesis (Cohen et al., 1958; Ellison, 1961; Heidelberger, 1965; Ruddick and Runner, 1974), and (2) when injected into pregnant hamsters produces cleft palate in the fetus by delaying reorientation of the palatal shelves (Shah and Wong, 1980; Shah et al., 1984).

Materials and methods

The maintenance and mating procedures for Golden Syrian hamsters have been described earlier (Shah et al. 1984). On day 11:00 (day 11, hour 0) of gestation, each female was given an intramuscular injection of either 81 mg/kg 5FU suspended in 1 ml saline, or 1 ml saline. Earlier, on the basis of dose-time response study (Shah and Mackay, 1978), and morphological analysis (Shah and Wong, 1980; Shah et al., 1984) it was observed that this regimen of dose and time in hamster induces cleft palate in all the fetuses by delaying the reorientation of palatal shelves by 18-22 hours.

For morphometric analysis, fetuses were obtained from two control and three drug-treated animals at 6-hour intervals between days 11:00 and 12:00 of gestation (Mean number of fetuses per litter 12.8 ± 1.9). In addition, the control fetuses were obtained at 2-hour intervals until day 12:04, (the period when normal hamster palatal shelf reorients), and treated ones at 6-hour intervals until day 13:00 of gestation. The fetal heads were dissected and fixed in 0.1 M phosphate buffered 2.5% glutaraldehyde (pH 7.3) at 0-4° C, post fixed in 1% osmium tetroxide, and dehydrated and embedded in Epon-Araldite as described by Shah and associates (1984). Prior to embedding, each palate was divided into anterior, middle and posterior thirds, and oriented in the Beam-capsule to obtain frontal sections. One micrometer sections were stained with 1% toluidine blue. Only sections from the middle third were used in the analysis because various morphological events of palate development in hamster begin in this area (Shah and Travill, 1976). A 10 × 10 marked glass grid (American Optical Corporation) was inserted into the eye piece of the microscope and superimposed onto the frontal sections at × 250. The total area of the grid was measured to be 0.0927 mm^2. In order to ascertain the area of the palatal shelf in the sections, the boundaries of the shelf were identified by points where the epithelium changed direction (see Fig. 2, Burdett et al., 1988). By counting the number of squares and portions thereof the grid falling over the palatal shelf, the total area was calculated. The number of mesenchymal and basal epithelial cell nuclei within the frontally sectioned palatal shelf were counted. Cell density per mm^2 of the palatal shelf was calculated by dividing the total number of mesenchymal cell nuclei by the shelf area. Nine to 12 palates per litter were used for the cell counting at each gestational age. using a Macintosh computer program (Statfast, 1985) the data were subjected to a two way analysis of variance.

For the measurement of ^{3}H-thymidine uptake, palates of different ages from both control and treated fetuses were obtained. Four to six palates from each litter were incubated in 2ml Delbecco's Modified Eagle Medium supplemented with 10% calf's serum and 0.01 ml of ^{3}H-thymidine (ICN, 57 Ci/mmole) for 3 hours at 37° C in an atmosphere of 5% CO_2 and 95% air. The tissue was then washed with ^{3}H-thymidine-free medium and homogenized in 0.8 ml of 0.5 N NaOH (0-4° C). It was then precipitated with 10% trichloracetic acid (TCA), containing 1% tannic acid (TA), at 0-4° C for 24 hours. The precipitate was centrifuged at 22,000 g for 30 minutes and the supernatant discarded. It was washed three more times in the TCA-TA mixture and centrifuged. Subsequently the precipitate was dissolved in 1 ml 0.1 N NaOH. Eight 0.025 ml aliquotes were taken and placed on Whatman microfiber glass filters and dried. The filter was then immersed in 2.8 ml Amersham aqueous counting scintillant solution and counted on a Philips PW 4700 liquid scintillation counter. Protein concentration was determined by the Biuret method (Gornall et al., 1949). The experiments were repeated three to six times. The data were analyzed by Student t-test to asses the differences in the synthesis of DNA in the developing palates of control and 5FU-treated fetuses at the 5% significance level.

Results

Cross-sectional area of the developing palatal shelf

In control fetuses, the area of the palatal shelf (Fig. 1a) increased gradually from 0.0298 ± 0.0087 mm ^{2} on day 11:06 to 0.0374 ± 0.0088 mm^2 on day 12:00 of gestation. It increased two-fold during the next two hours to 0.0739 ± 0.0116 on day 12:02 of gestation ($P < 0.05$), and then leveled-off. The palatal shelves reoriented from a vertical to a horizontal plane between days 12:00 and 12:04 of gestation.

Following 5FU treatment, the palatal shelf area (Fig. 1a) also increased gradually from 0.0138 ± 0.0038 mm^2 on day 11:06 to 0.0205 ± 0.0035 mm^2 on day 12:00 of gestation. The rate of increase, however, was slow. During the next 12 hours the shelf area increased two-fold, and was 0.0690 ± 0.0259 mm^2 on day 12:12 of gestation ($P < 0.05$). It then leveledoff. Overall, following 5FU administration, the palatal shelf area in the treated fetuses remained significantly lower than in controls until day 12:06 of gestation ($P < 0.05$).

Table 1. Relationship between different parameters of shelf growth and stage of palate development in control and 5FU-treated hamster fetuses.

Stage of Palate Development	Mean shelf Area mm^2 ± SD	Mean Number of Mesenchymal Cells ± SD	Mean Density of Mesenchymal Cells ± SD	Mean Number of Epithelial Cells ± SD
Vertical				
Control	0.0300 ± 0.0066	347.0 ± 67.6	11813,7 ± 2042.2	83.3 ± 8.0
Treated	0.0277 ± 0.0164	249.9 ± 85.4	10820.0 ± 4418.7	62.9 ± 15.0
Reorientation				
Control	0.0536 ± 0.0160	312.0 ± 103.3	5435.0 ± 817.4	84.8 ± 12.6
Treated	0.0635 ± 0.0355	327. ± 109.0	5732.6 ± 1332.8	28.8 ± 26.0
Horizontal				
Control	0.0674 ± 0.0162	272.5 ± 65.3	4098.9 ± 591.1	93.5 ± 14.5
Treated	0.0776 ± 0.0263	331.5 ± 61.6	4518.3 ± 973.0	85.7 ± 17.7

* $p < 0.05$ from the previous stage

Subsequently, however, on day 12:12 of gestation the shelf area in the treated fetuses reached the level comparable to that seen in control fetuses on day 12:00 of gestation. The 5FU-treated palatal shelves reoriented from a vertical to a horizontal plane between days 12:18 and 13:00 of gestation, i.e., approximately 18-22 hours later than seen in controls.

Since the calculation of mean shelf area, in controls between days 12:00 and 12:04 of gestation and in treated between days 12:00 and 13:00 of gestation represent averages of vertical, reorienting and horizontal shelves, the data were rearranged according to the stage of palatal development. One may deduce, from Table 1 that the mean shelf areas in control and 5FU-treated fetuses at comparable stage of palatal development were similar. In controls, the area of vertical shelf increased significantly during reorientation ($P < 0.05$), and was approximately two-fold when the shelves were horizontal. A similar trend was also observed following 5FU treatment.

Mesenchymal cells of the developing palatal shelf

The data summarized in Fig. 1b show that in controls the number of mesenchymal cells remains unchanged between days 11:06 and 12:04 of gestation. Following 5FU treatment, the overall number of mesenchymal cells during this period, with the exception of day 11:18 of gestation, was comparable to that of the controls. On day 11:18 of gestation it was significantly lower in 5FU-treated than in control palates.

Table 1 shows the data on the number of mesenchymal cells according to stage of palatal development. One may infer from the Table that the number of mesenchymal cells remained steady as the shelves underwent reorientation from a vertical to a horizontal plane. A similar deduction may also be made for palates following 5FU treatment.

The density of mesenchymal cells in the developing palatal shelf (number of cells/mm^2 area) is analyzed in Fig. 1c. In controls, there was a significant decrease in the density of mesenchymal cells from 12905.4 ± 791.3 on day 11:06 to 9588 ± 1182.5 on day 11:12 of gestation ($P < 0.05$). The density increased to 13346.4 ± 1271.2 on day 11:18 of gestation, but dropped rapidly thereafter ($P < 0.05$) and was 3749.9 ± 41.42 on day 12:04 of gestation.

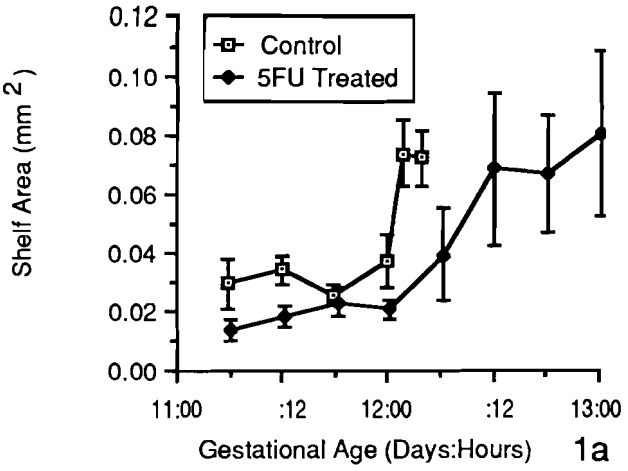
Following 5FU treatment, the density of mesenchymal cells dropped by approximately 50% from 16926.0 ± 1946.1 on day 11:06 to 8773.6 ± 1467.6 on day 11:18 of gestation. It remained steady until day 12:06, and then dropped further by approximately 30% to 5533.4 ± 719.4 on day 12:12 of gestation. Subsequently the density of mesenchymal cells in 5FU-treated palates remained unchanged. Except on day 12:00 of gestation the density of mesenchymal cells in the control and 5FU-treated palates were significantly different ($P < 0.05$).

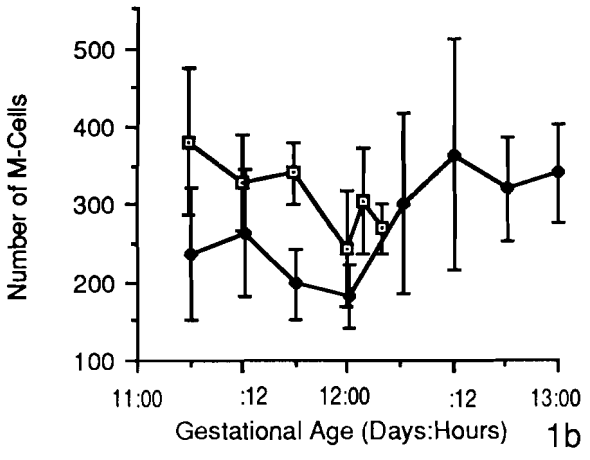
The data on the density of mesenchymal cells in relation to the stage of palatal development are arranged in Table 1. One may deduce that there was no difference in the density between the control and treated fetal palates at comparable stages of development. In both control and treated groups the density of mesenchymal cells dropped by approximately 50% while the shelves were undergoing reorientation.

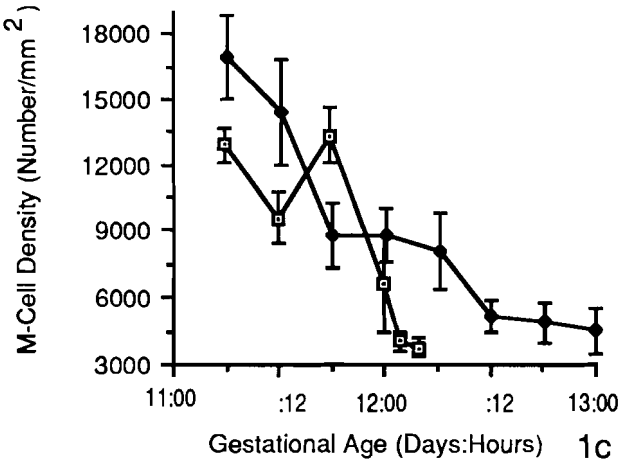
Epithelial cells of the developing palate

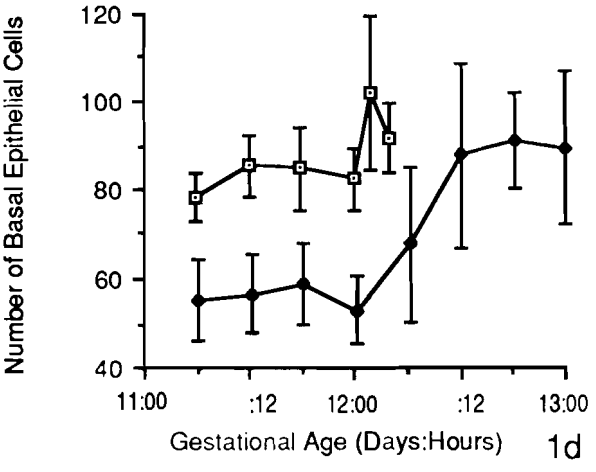
In control fetuses, the number of basal epithelial cells covering the developing palate remained unchanged between days 11:06 and 12:04 of gestation (Fig. 1d). Following 5FU treatment, the number of epithelial cells remained steady between days 11:06 and 12:00 of gestation. Subsequently it increased significantly from 53.0 ± 7.8 on day 12:00 to 87.6 ± 20.9 on day 12:12 of gestation ($p < 0.05$), and then leveled-off. The number of basal epithelial cells, however, remained significantly lower than in controls between days 11:06 and 12:06 of gestation ($P < 0.05$).

The data on the number of epithelial cells in relation to stage of palatal development are presented in Table 1.









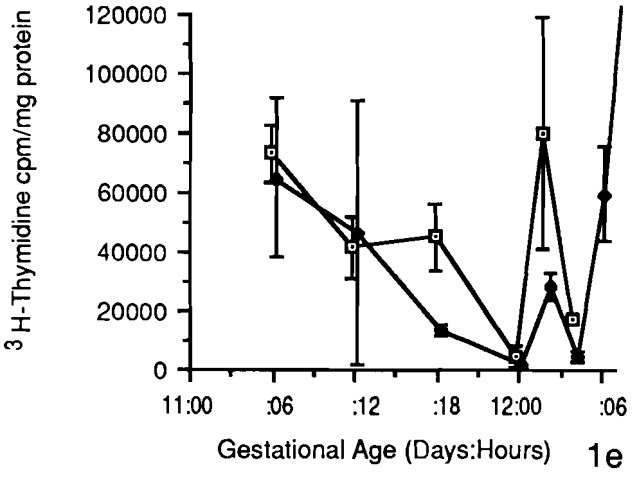


Fig. 1. The data were obtained from the secondary palate in control and 5FU-treated hamster embryos at different times during gestation. **a.** Profiles of cross-sectional areas. **b.** Number of mesenchymal (M) cells. **c.** Density of mesenchymal (M) cells. **d.** Number of basal epithelial cells. **e.** Profiles of DNA synthesis.

One may deduce from the Table that the number of epithelial cells at a comparable stage of palate development was similar in both the control and treated groups. Furthermore, the number of epithelial cells remains steady as the shelves reoriented from a vertical to a horizontal plane.

DNA synthesis in the developing palate

The data on ^{3}H-thymidine uptake are summarized in Fig. 1e. One may infer from the figure that in control fetuses the DNA synthesis decreased significantly between days 11:06 and 12:00 of gestation ($P < 0.05$). It then increased approximately four-fold on day 12:02, followed by a 50% drop on day 12:04 of gestation ($P < 0.05$).

Following 5FU treatment the overall trend in the rate of DNA synthesis was comparable to that of the control. On days 11:18 and 12:02 of gestation, however, the rate was significantly lower in the treated than in the controls ($P < 0.05$).

Discussion

Since the number of cells in a three dimensionally growing structure changes during its ontogeny, a question has been repeatedly raised in the developmental biology literature whether a minimum or specified number of cells is critical for the progression of the morphogenesis of an organ or structure (Waddington, 1957; Weiss, 1968; Wolpert, 1969; Mintz, 1971; Bonner, 1974; Holtzer, 1978; Green, 1980). In mammals, the secondary palate (a mesenchymal structure covered by an epithelium) develops first as a vertical projection which, with advancement in morphogenesis, becomes horizontal. Hence, the issue, whether acquisition of a specified number of mesenchymal cells in the vertically developing shelves is critical for their subsequent reorientation to a horizontal plane, is important. In addition to the inherent interest in determining the number of mesenchymal cells essential for palatal morphogenesis, the information may be useful in analysing the effect of genetically and environmentally induced perturbations of the developing secondary palate. Wolpert and associates (1979) have suggested that the simplest way to interpret such information is that if a structure, at any stage during its development, has too few cells, that part of the structure would not develop or differentiate further. Hence, studies on continually advancing morphogenesis of a structure like the secondary palate, under both normal and abnormal circumstances, might be defined in quantitative terms instead of relying on such discrete developmental indices as embryonic weight, crown-rump lenght, numerical rating systems, etc., which show large inter-and intra-species variations, and hence are difficult to interpret (Dostal and Jelinek, 1972; Shah, 1979).

The control data obtained in the present study, along with those gathered earlier (Burdett et al., 1988; Shah et al., 1989), suggests that three aspects of mesenchymal cell growth appear to be critical in regulating vertical palatal development. These are initiation of growth, rate of growth, and termination of growth. Although the number of stem cells required to initiate the palatal bud in mammals is not known, it was shown in earlier study (Burdett et al., 1988) that during initial palatal development, between days 10:00 and 11:00 of gestation in hamster, two spurts in DNA synthesis occur (presumably corresponding to the number of cell cycles), which leads to an increase in both the number and density of mesenchymal cells. This event is followed by reduction, both in DNA synthesis and mesenchymal cell density, and concurrent stabilization of the mesenchymal cell number between days 11:00 and 12:00 of gestation (Shah et al., 1989). The control quantitative data obtained in the present study on the rate and termination of mesenchymal cell growth during palatal development are in confirmation of these results, and also corroborate those reported for rats and mice by Nanda and Romeo (1975, 1978) and Brinkley and Bookstein (1986). Similar reductions in cell growth and mesenchymal density have been noted during limb development (Hornbruch and Wolpert, 1970; Ede, 1971). Thus, when the data from our previous studies, and the present study, on hamster are considered together it appears that, during palate morphogenesis, the mesenchymal cell number reach a plateau by day 11:00 of gestation, and the numbers do not increase further until after the closure of the palate. Such a timely specified number of cells in a vertically developing palatal shelf may be considered critical because: (1) the number did not increase during reorientation, even though the shelf area was increasing (Table 1); (2) there was no increase in DNA synthesis during the ensuing 24 hours to increase cell numbers, which presumably could have been a mechanism accounting for shelf reorientation; (3) when the cell number did not reach plateau levels following treatment with 6-mercaptopurine, shelf reorientation was prevented (Burdett et al., 1988) (4) when the cell number had been significantly reduced within 18 hours following 5FU administration (Fig. 1b), the shelves did not reorient until the number reached the "specified" level between days 12:06 and 12:12 of gestation, and (5) when the cell number remained unaffected after hydrocortisone treatment on day 11:00 of gestation, the shelf reorientation occurred on schedule (Shah et al., 1989). This analysis would suggest that, in hamster, the putative critical number of mesenchymal cells for palatal shelf reorientation may be reached approximately 24 hours prior to the event. Holtzer (1968) suggested that the acquisition of the critical number of cells in a developing organ generally precedes differentiation. Also, determination of such a specified number may depend on the number of stem cells (Holtzer, 1978), which could be determined "genetitically", as has been suggested in general for other developing organs and structures (Mintz, 1971; Bryant and Simpson, 1984).

It should be noted that the reduction in mesenchymal cell number on day 11:18 of gestation in 5FU-treated palates (Fig. 1b), which was accompanied by a simulta-

neous reduction in the rate of DNA synthesis (Fig. 1e), was due to the loss of damaged mesenchymal cells (Shah et al., 1984). Later when an increase in DNA synthesis (Fig. 1e), accompanied by restoration of at least the ultrastructural appearance (health) of the mesenchymal cells (Shah et al., 1984) occurred on day 12:06 of gestation, the mesenchymal cell number was restored to the level comparable to that seen in controls on day 12:02 of gestation (Fig. 1b). In the drug-treated palates, however, the expansion of the shelf area (reflecting extracellular matrix synthesis), as discussed below, was not restored until day 12:12 of gestation (Fig. 1a). This analysis would suggest that, in the circumstances of the present experiment, 5FU insulted the vertical shelf development not by affecting the DNA synthesis in the mesenchymal cells, but, as was shown earlier (Shah et al., 1984), by damaging the mesenchymal cells and affecting their subsequent differentiation. Indeed 5FU have been shown to affect the differentiation of various post-mitotic cells by interfering with RNA and protein synthesis (Heidelberger, 1965). This, however, does not detract the proposition that an acquisition of a minimum number of mesenchymal cells may be critical for palatal reorientation. Indeed, one may further deduce from the analysis that not only the acquisition but also the subsequent maintenance of critical number of mesenchymal cells during the antepenultimate (vertical) phase of palatal development may be essential for palatal shelf reorientation.

The results of the present study also suggest that there does not appear to be a relationship between the mesenchymal cell number and its density, which could be involveld in regulating the number of cells in the vertically developing palatal shelf, as suggested, for example, for the developing limb (Summerbell, 1977). In addition, the data does not support the proposition made for the developing limb, that the number of mesenchymal cells in a elongating structure may be regulated by the outgrowth of the covering epithelium (Hornbruch and Wolpert, 1970; Ede, 1971; Gould et al., 1972). In the present study, it was observed that following 5FU treatment, when the epithelial cell number was reduced, the number of mesenchymal cells remained fairly constant.

The area (volume or mass) of a structure or an organ expands considerably during its embryonic development. In this regard, the morphogenesis of mammalian seconday palate is unique, because, unlike other organs or structures, the bilaterally symmetrical vertical palatal processes, while expanding in area, change the direction of their development from a vertical to a horizontal plane to meet in the midline. Hence, information on the rate of increase in the area of the developing palatal shelves may be of intrinsic significance to advancing morphogenesis. Since the data obtained in the present study, along with our earlier observations (Burdett et al., 1988; Shah et al., 1989), show that the shelf area increases several fold during the vertical morphogenesis of the palatal processes, the issue is raised whether a minimum critical volume of the vertical shelves is essential for them to undergo reorientation. In addition to the intrinsic usefulness of such information in analysing advancing morphogenesis of the developing secondary palate, further significance of the issue may reside in the frequently recorded observation that in those circumstances where cleft palate developed due to a delay in the reorientation of palatal shelves, the shelves were small (Kochhar and Johnson, 1965; Mott et al., 1969; Andrew and Zimmerman, 1971; Jelinek and Dostal, 1974; Tassinari et al., 1981; Reddy et al., 1986). In these instances it is not known if the proposed minimum critical volume was acquired by the developing palatal shelves.

In an earlier study, it was shown that the area of the vertically developing shelf increased approximately tenfold during the initial 24 hours of hamster palate development, i.e., betwen days 10:00 and 11:00 of gestation (Burdett et al., 1988). In the ensuing 24 hours the shelf area continued to expand, albeit at a slower rate (Shah et al., 1989). Just prior to reorientation, however, there was a spurt in the rate of expansion of the palatal shelf area. Following 5FU administration the expansion of the shelf area was inhibited, and it did not reach the equivalent of control values, observed on day 11:00 of gestation, until after day 12:00 of gestation, i.e., approximately 24 hours late, and the reorientation was correspondingly delayed. On the basis of these observations one could suggest that, in hamster embryos the palatal shelf area accomplished by day 11:00 of gestation, i.e., approximately 24 hours prior to scheduled reorientation time, may be considered critical for subsequent advancement in palate morphogenesis. The acquisition of such an area (space) appears to be the result of controlled growth, and its regulation would be a matter of rate and duration of growth, as has been suggested earlier for many other embryonic structures by Snow and associates (1981). It must be emphasized that reaching a specific area during vertical shelf development may not always assure shelf reorientation. For example, it has been shown that after 6-mercaptopurine treatment of hamsters on day 9:00 of gestation, the vertical shelf area continued to increase well past the critical phase, but the shelf reorientation never occurred, because the drug inhibited the growth of the mesenchymal cells and affected their subsequent cytodifferentiation (Burdett and Shah, 1988; Burdett et al., 1988). On the other hand, following hydrocortisone treatment of hamsters on day 11:00 of gestation, both the mesenchymal cell number and shelf area remained unaffected, and shelf reorientation occurred on schedule (Shah and Travill, 1976; Shah et al., 1989). Obviously the synchronization of complex growth processes (i.e., cell number, shelf area, cell cycle and DNA synthesis, extracellular matrix synthesis, cell arrangements, etc.) in the vertically developing palatal process is crucial for its subsequent reorientation. The events leading to reorientation of the palatal shelf are currently believed to be achieved through critically regulated alterations in the mesenchymal cell behaviour, and synthesis of extracellular matrix (Lessard et al., 1974; Babiarz et al., 1975; Krawczky and Gillon, 1976; Fergurson, 1978a; Shah, 1979; Jacobson and Shah, 1982;

Zimmerman and Wee, 1984; Brinkley and Bookstein, 1986), and are accompanied by a further expansion of the shelf area. Hence, on the basis of these observations, along with those reported in the present study, one may suggest that acquisition of a critical area during the vertical development of palatal shelves may facilitate the differentiation of mesenchymal cells which would later enforce the shelf's reorientation. This possibility is further strengthened by the observation that during the 24 hour period prior to shelf reorientation, even though the shelf area continued to expand, the mesenchymal cell number remained unaltered, and the cell density and DNA synthesis were reduced; and thus the suggestion is consistent with the general concept that, during organogenesis, acquisition of cell number (growth) precedes cell differentiation (Holtzer, 1968; Mintz, 1971). It may be noted that the observations of the present study do not rule out the possibility that synthesis of extracellular matrix may be solely responsible for the expansion of shelf area after day 11:00 of gestation. Also, as a result of prior mesenchymal cell damage (Shah et al., 1984), it is possible that 5FU may affect the synthesis of extracellular matrix, hence the expansion of shelf area, and eventually delay the palatal shelf reorientation, as have been claimed for other teratogens (Jacobs, 1964; Fergurson, 1978b; Jacobson and Shah, 1982; Brinkley and Morris-Wiman, 1984). Indeed, this would reinforce the proposition made in the earlier paragraph that a critical number of healthy mesenchymal cells may be essential for subsequent optimum production of extracellular matrices involved in the palatal shelf reorientation.

In summary, in the present study an attempt was made to determine the significance of various features of growth during vertical development of the palatal shelves, and their subsequent reorientation. During the 24-hour period preceding shelf reorientation, there were no major alterations in the mesenchymal cell number and shelf area, while DNA synthesis dropped. On the basis of analysis of data obtained from the present study, and along with observations reported in the literature, it was suggested that, in hamster, acquisition and maintenance of both a specified number of mesenchymal cells and shelf area, at least 24 hours prior to reorientation, may be critical for ensuing mesenchymal cell differentiation to enforce palatal shelf reorientation on schedule. Failure to achieve them in synchrony could prevent or delay the shelf reorientation. 5FU treatment affected these features to delay reorientation of the palatal shelf.

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