J. Anat. (1978), **125**, 3, pp. 555–577
With 5 figures
Printed in Great Britain

Palatal shelf elevation in the Wistar rat fetus

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(*Accepted* 3 *March* 1977)

INTRODUCTION

The elevation of the palatal shelves from a vertical position lateral to the tongue to a horizontal position above the tongue is a critical event in palatogenesis. However, there is no general agreement about either the mechanism(s) of shelf elevation or its precise spatial and temporal parameters. Many theories concerning shelf elevation are present in the literature and these are summarized in Table 1. The theories can be broadly divided into two groups. In the first group the shelves are thought of as playing an entirely passive role, being elevated as a result of some extrinsic activity, whilst in the second group an active role is attributed to the shelves themselves rather than to extrinsic structures.

Although there have been some attempts (e.g. by Zeiler, Weinstein & Gibson, 1964) to determine when the palatal shelves become horizontal in the rat, the precise timing of shelf elevation in this animal remains uncertain. In addition, there is confusion regarding the sequence of events in shelf elevation: some authors assert that the shelves start to elevate at the back (Walker & Fraser, 1956; Zeiler *et al.* 1964; Larsson, 1960, 1962, 1974; Pourtois, 1972; Stark, 1973; Babiarz, Allenspach & Zimmerman, 1975) while others maintain that they start at the front (Coleman, 1965; Andersen & Matthiessen, 1967; Burdi & Faist, 1967; Wragg, Diewert & Klein, 1972).

It was decided to make a comprehensive study of palatogenesis in the rat, combining macroscopic, microscopic, ultrastructural and experimental observations on the entire palate, because so far no one seems to have done this. Important data obtained from teratological experiments, in which cleft palate was induced pharmacologically, will be the subject of a future communication (Ferguson, 1978).

MATERIALS AND METHODS

Twenty pregnant female Wistar rats (average weight 300 g) were used in this study. The onset of pregnancy was determined by vaginal smearing, the day of finding sperm in the smear being called day 0 (the precise time of fertilization is, of course, unknown, but is taken conventionally to be at midnight at the start of day 0). All the female and male Wistar rats used were highly inbred, being the twelfth generation offspring of a brother/sister mating programme. A total of 217 fetuses was obtained between day 13 and day 19, any litters containing less than 8 or more than 12 fetuses being discarded. Living fetuses, both inside their amniotic sacs and after removal from the latter, were stimulated by gently touching in turn the face, trunk

Table 1. *Some theories concerning the mechanism of palatal shelf elevation*

Group 1: Extrinsic factors

Theory	References
1. Descent of the tongue resulting from a marked growth spurt of the mandible relative to the maxilla around the time of shelf elevation	Asling *et al.* 1960; Zeiler *et al.* 1964; Coleman, 1965; Sicher, 1966; Poswillo, 1968; Burdi & Silvey, 1969; Diewert, 1974
2. Depression of the tongue produced by the downward growth of the nasal septum and primary palate	Zeiler *et al.* 1964; Fraser, 1967, 1969, 1971; Abramovich, 1972
3. Descent of the tongue as a result of its intrinsic myoneural activity	Wragg, Smith & Borden, 1969, 1972; Wragg, Diewert & Klein, 1972
4. Tongue pushing the shelves up	Walker, 1971; Arey, 1974
5. Lowering of the tongue as part of a fetal mouth opening reflex	Humphrey, 1968, 1969, 1971
6. Lifting of the head off the chest (enabling the mandible and tongue to drop) as a result of:	
(*a*) spontaneous contraction of the neck muscles	Walker, 1969, 1971, 1974
(*b*) growth in length of the cervical vertebrae	Ross & Lindsay, 1965
(*c*) differential growth spurt in head height	Joondeph & Wragg, 1971
7. Changes in the angulation of the anterior relative to the posterior cranial base producing a palatal shelf elevating force	Verrusio, 1970; Larsson, 1974

Group 2: Factors intrinsic to the shelves

Theory	References
1. Differential growth of one side of the palatal shelf	Wood & Kraus, 1962; Sicher, 1966; Andersen & Matthiessen, 1967; Poswillo, 1968; Furstman, Sol Bernick & Mahan, 1971
2. Hydration and polymerization of intercellular substances producing an elastic elevating force	Lazzaro, 1940; Larsson, 1960, 1962, 1974; Walker, 1961
3. The shortening of elastic fibres	Walker & Fraser, 1956
4. The contraction of newly synthesized collagen	Hassell & Orkin, 1976
5. The contraction of actomyosin or microfilaments in smooth muscle and other cells	Lessard, Wee & Zimmerman, 1974; Krawczyk, Gillon & Szabo, 1975
6. The contraction of skeletal muscle	Babiarz *et al.* 1975
7. Increased vascularity producing an erectile force	Gregg & Avery, 1971
8. Remodelling of the shelf with resorption in a vertical direction and new growth horizontally	Polzl, 1904; Pons-Tortella, 1937; Coleman, 1965; Johnston, Hassell & Brown, 1975
9. Shrinkage of one side of the shelf caused by the appearance of rugae	Pourtois, 1972
10. Traction caused by developing bone blastemata	Pourtois, 1972
11. Differential growth of the shelf epithelium	Pourtois, 1972
12. Differential epithelial adhesiveness and traction	Pourtois, 1972
13. Expanding functional matrices	Moss, 1971
14. Surface tension	Atnip, 1963

and limbs with either a blunt piece of wire or a hair, and the nature and duration of any responses noted. Of the fetuses removed from each litter, usually two were placed in normal saline for immediate experimentation, two were used for electron microscopy, and the remainder were prepared either for macroscopic or light microscopic examination. The fetuses destined for macroscopic or microscopic examination were weighed (under normal saline) to the nearest half milligram and then fixed for 1 week in either Bouin's fluid or 10 % formol saline, after which time they were reweighed. Using a pair of vernier calipers the crown–rump length, the maximum head width, the maximum head height, the average length of the upper limbs, the average length of the lower limbs, and the distance between the upper and lower limbs were measured to the nearest half millimetre in each fetus. Both fresh

and Bouin-fixed fetuses were examined macroscopically, using a zoom dissecting microscope. One or two fetuses from each litter were dissected with sharp needles to display the interior of the mouth. Three others were sliced serially at 1 mm intervals in the coronal, sagittal and horizontal planes respectively, and the appearance of these 1 mm slices compared with corresponding 7 μm paraffin sections from other fetuses of the same litter stained with either a combination of 1 % aqueous alcian blue (pH = 7·0), Harris' iron haematoxylin and alcoholic eosin, or by Mallory's or Masson's method. In addition, either alcian blue at different pHs (Cook, 1972), Hale's colloidal iron, hyaluronidase digestion/alcian blue staining (Cook, 1972) or acridine orange fluorescence were used to detect mucopolysaccharides in the developing palate, the cartilages of the facial region serving as controls. The two fetuses selected for electron microscopy from each litter were quickly removed and, under a pool of Karnovsky fixative (pH = 8·0), the palatal region was excised by making two horizontal incisions, one through the mandible and tongue, and the other through the cranial base. The palatal tissue block was fixed for 4 hours in Karnovsky, post-fixed in 1 % osmium tetroxide for 45 minutes, dehydrated in acetone and propylene oxide, and embedded in Durcupan. Ultrathin sections were cut, stained with lead citrate and uranyl acetate, and viewed in an A.E.I. electron microscope.

Fetal age determination

The putative (or 'smear') age of a fetus, timed from midnight on the evening before finding sperm in the smear until the time of killing the mother, could well be as much as 4 or 5 hours in error with respect to the true 'conceptual' age, which is very difficult to determine. In addition, it is well known that the fetuses in a given litter differ in their degree of maturation because of differences in their times of fertilization and implantation, and in the effects of local factors such as differing blood supplies. In a developmental study it would clearly be advantageous to use a 'developmental age' rather than an age solely determined from the time of finding sperm in the vaginal smear. With these considerations in mind a statistical analysis was undertaken to determine the utility and accuracy of certain morphological parameters as indicators of 'developmental age'.

Data from 217 fetuses were subjected to analysis using an I.C.L. 1906S computer: the programs were adapted from the appropriate sections of the X.D.S. 3 or S.P.S.S. statistical package handbooks. The original data, computer sheets and calculations are available for reference in the Anatomy Department, Queen's University, Belfast. The coefficients of correlation (of the measurements) of each of the following morphological parameters with the smear ages of the fetuses (in hours) were as follows: fresh weight, 0·9268; weight after fixation, 0·9029; CR length, 0·9661; maximum head width, 0·9370; maximum head height, 0·8760; average length of upper limb, 0·9406; average length of lower limb, 0·9183; distance between limbs, 0·9394. The nearer to unity the correlation coefficient, the more accurately can developmental age be predicted from the morphological criterion in question. It is clear that the highest correlation coefficient was attained with CR length. The Coefficient of Determination (square of the correlation coefficient) for CR/smear age was 0·933 (= $0{\cdot}9661^2$) which means that 93·3 % of any age increase is associated with a comparable increase in CR length. Regression analysis ($P < 0{\cdot}0001$) of age on CR length gave the equation $P = 292{\cdot}4748 + 6{\cdot}7841 . L$, where P = predicted developmental age of the fetus in hours, L = crown–rump length in millimetres,

292·4748 is the regression constant and 6·7841 is the regression coefficient. The standard deviation from regression is 9·6957. It must be emphasized that this equation only holds for fetuses between 13 and 19 days smear age (S age). The predicted developmental age in hours P (referred to from now on as CR age) and the difference between the CR age and the S age were calculated for each fetus. It was found that, around the critical time of shelf elevation, CR age was a better indicator of palatal maturation than S age, as will be seen on reference to Figures 1B–E which show some stages of palatal closure in a litter of fetuses with S age = 16·4 days but of varying CR ages. Using the linear regression line of S age on CR it was shown that palatal shelf elevation in the Wistar rat fetus is a *rapid* event occurring between 16·3 and 16·5 days (CR age): thus all fetuses at 16·3 days (CR age) showed vertical palatal shelves, while all at 16·5 days (CR age) showed horizontal shelves.

In an attempt to predict developmental age even more accurately multiple correlation/regression analysis was performed and the correlation coefficient of smear age (in hours) with all eight morphological criteria was found to be 0·982. In this case the multiple regression equation was:

$$P^1 = 283{\cdot}83 + 30{\cdot}65.\mathrm{WT} - 37{\cdot}73.\mathrm{FW} + 4{\cdot}92.\mathrm{CR} + 3{\cdot}65.\mathrm{HW} - 0{\cdot}16.\mathrm{HH} + 2{\cdot}84.\mathrm{UL} + 1{\cdot}49.\mathrm{LL} + 0{\cdot}54.\mathrm{DL},$$

where P^1 = predicted age in hours, WT = fresh weight, FW = weight after fixation, CR = crown–rump length, HW = maximum head width, HH = maximum head height, UL = average length of upper limb, LL = average length of lower limb, and DL = distance between upper and lower limbs. The standard deviation from regression is 7·26. It will be seen that the predicted developmental age obtained by multiple analysis was only marginally more accurate than that obtained using the single analysis of age on CR length alone, so that multiple analysis was not employed as a predictive measure in the routine dating of fetuses.

RESULTS

Observations on normal fetuses

S age 13·58, CR age 13·88–14·44

At this stage the fetus is markedly flexed and the median nasal, maxillary and mandibular processes are apposed to the cardiac bulge. The fused mandibular processes are retrognathic, their anterior limit being in approximately the same coronal plane as the middle of the eye. The tongue lies well back in the oronasal cavity: it is highly arched and in contact with the roof of the cavity. No palatal shelves are present. No reflex responses could be elicited at this stage.

S age 14·42–14·54, CR age 14·27–15·01

The mandibular arch still rests on the chest wall, but has grown forward to reach a level slightly posterior to the primary palate, the latter being continuous superiorly with the primary nasal septum. The primary septum houses Jacobson's organs and in it there is a mesodermal condensation foreshadowing the differentiation of the septal cartilage. Behind the primary palate the primary nasal septum rapidly loses height and disappears. Palatal shelves are now present. They hang vertically from the maxillary processes on either side of the tongue. Anteriorly they are continuous with the posterolateral margins of the primary palate. The tongue is still a highly arched

structure which fills the posterior two thirds of the oronasal cavity; its tip is just behind the primary palate in approximately the same coronal plane as the opening of the posterior nasal choanae into the oronasal cavity. When mandibular arch and tongue are cut away the edges of the palatal shelves are seen to be straight. Histologically the palatal shelves contain densely packed mesenchymal cells in a matrix which stains faintly with alcian blue. The epithelium covering the lateral aspect of the shelf is thickest at the junction of the shelf with the main maxillary process. More posteriorly, in the region of the root of the tongue, the shelves decrease in height until they are represented merely by bulges, and behind this they disappear. Meckel's cartilages are now recognizable and cartilage is also present in the developing vertebræ, cranial base and nasal septum. It must be emphasized that the space representing the oronasal cavity seen in histological sections is an artefact: in fresh specimens the cavity is a potential one only. The upper lip is now defined and rows of vibrissal anlagen have appeared. No reflexes could be elicited at this age.

S age 15·33–15·85, CR age 15·29–16·14

At 15·3 days (CR age) the lower jaw has grown forward under the back of the primary palate; the gap in the upper lip has narrowed and two tactile vibrissal anlagen (colliculi piliferi angulares) have appeared. The growth forward of the lower jaw and tongue has resulted in the tip of the latter passing under the posterior edge of the primary palate. The tongue is still highly arched and makes contact with the roof of the oronasal cavity except anteriorly where the secondary nasal septum has started to grow downwards. The anterior four fifths of each palatal shelf is relatively long and still hangs vertically by the side of the tongue (Fig. 2A), but the posterior one fifth (i.e. in the region of the root of the tongue) is stubby and projects horizontally above the dorsum of the tongue (Fig. 2B). In the transition zone between the vertical and horizontal parts of a shelf the appearances are such that at first sight it might be inferred (erroneously) that the shelf is being remodelled by differential accretion and resorption. This is discussed later on. The point is emphasized that the posterior one fifth of each palatal shelf (future soft palate) grows horizontally from the beginning and does not have to elevate (Fig. 2B). At 15·3 days (CR age) the palatal shelves are still straight (Fig. 1A), but at 15·5 days (CR age) they have a sinusoidal edge with a convexity in their anterior one third. This means that the anterior one third of each palatal shelf is deeper than the rest and projects further down the side of the tongue. At 15·5 days (CR age) the tip of the tongue is well underneath the primary palate, and at 15·8 days (CR age) it is just behind the upper lip. At 15·8 days (CR age) the convexity of the margins of the anterior one third of the palatal shelves is marked, and here the shelves appear to be constricting the base of the tongue, so that in coronal sections this organ is mushroom-shaped.

Anteriorly, the nasal septum (both primary and secondary parts) has grown in height and the septal cartilage, vomeronasal cartilages and the lateral nasal cartilages are all evident, while Jacobson's organs have increased in size. Posterior to the primary palate the short secondary nasal septum rests on the dorsum of the tongue. Meckel's cartilage has developed still further, the upper and lower incisor and molar tooth germs have appeared and the tongue musculature has begun to differentiate (e.g. the genioglossus anlagen are evident). At the junction between palatal shelf and main maxillary process there is a groove where the epithelial thickening (noted at 14·5 days) has invaginated the shelf mesenchyme (Fig. 2A). Mitotic figures are evenly distributed throughout the shelf mesenchyme and the matrix now stains more

definitely with alcian blue and Hale's colloidal iron. Small blood vessels are evident, but not conspicuous, in the palatal shelves.

On electron microscopy the epithelial cells of the palatal shelves are seen to be stellate and their processes make contact with one another (Fig. 5A). A labyrinth of intercellular spaces is a conspicuous feature (Fig. 5A). The cells possess a relatively large nucleus while their cytoplasm contains many free ribosomes, some mitochondria, occasional RER cisternae (frequently associated with a dilated perinuclear cisterna) and a few glycogen aggregates, but, generally speaking, no unusual features. The epithelium rests on a basement membrane beneath which an occasional collagen fibre is to be seen. The core of the shelf is very loosely arranged, exhibiting a network of stellate mesenchymal cells with contacting processes, so that it resembles a sponge (Fig. 5B). Coursing through the mesenchyme are numerous small capillaries (Fig. 5B). They show no evidence of pores or gaps between their endothelial cells which might indicate unusual permeability. Small unmyelinated nerve fibres are present, but no smooth muscle fibres, collagen or elastic fibres. The matrix in fact appears oedematous and without visible structure (Fig. 5B). The mesenchymal cells have a large nucleo-cytoplasmic ratio, and do not show microtubules or filaments or other presumptive contractile elements. They contain numerous free ribosomes, some mitochondria, some RER cisternae and a clearly defined Golgi complex with secretory vesicles.

No reflexes could be elicited at this stage.

S age 16·33–16·42, CR age 16·14–16·30

The fetus is now less flexed, but the lower jaw still rests on the chest wall. The relationship of the lower to the upper jaw is similar to that on day 15·8 (CR age). The tip of the tongue reaches further beneath the primary palate, but still lies behind the upper lip (Fig. 3A). The palatal shelves are still vertical. They are longer (in the vertical direction), especially anteriorly where their border is now markedly convex. The central region of the *primary* palate exhibits a small posterior projection. The nasal septum generally has grown in height, but behind the primary palate its height diminishes until it disappears into the roof of the oronasal cavity about the middle of the dorsum of the tongue. Anteriorly, Jacobson's organs lie partly in the septum

Fig. 1 (A–F). Macroscopic appearances of the developing palate from day 15·3 to day 16·6 (CR age). Viewed from below, mandible and tongue removed.

(A). Palatal shelves in a 15·3 day (S and CR age) fetus. Note these shelves are vertically orientated and therefore this figure only shows their margins. ×28.

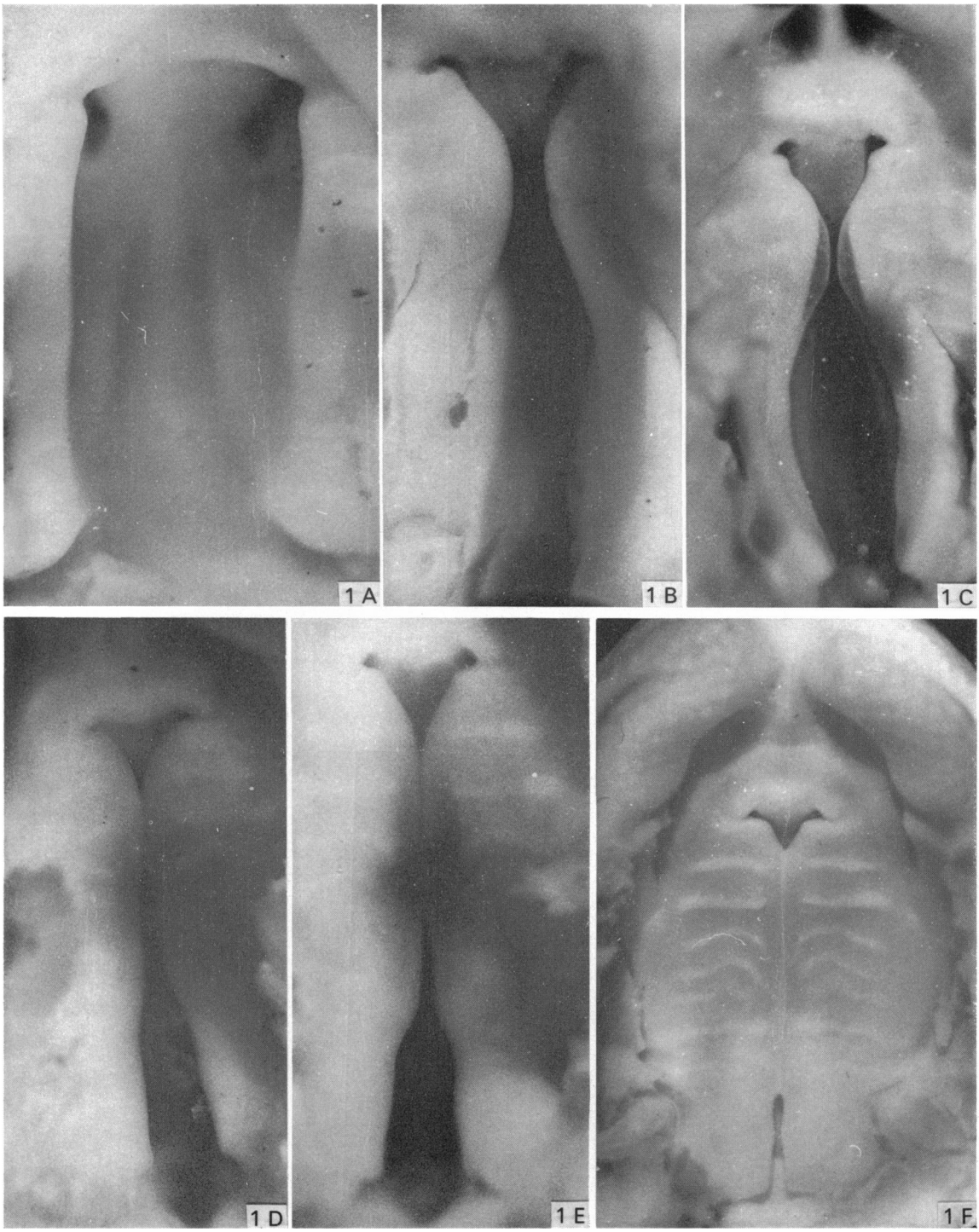
(B). Horizontal palatal shelves in a fetus aged 16·4 days S age, but 16·3 days CR age. Flip up has just occurred and the shelves approximate but do not contact each other in the region of their maximum convexities anteriorly. ×22.

(C). Palatal shelves in a fetus from the same litter as fetus 1B, i.e. S age = 16·4 days but CR age = 16·39 days also. Note the rapid marginal growth of the anterior convexities which still have not contacted each other. ×20.

(D). Palatal shelves in a fetus from the same litter as fetuses 1B and 1C, i.e. S age = 16·4 days and CR age = 16·43 days also. The shelves have just contacted each other anteriorly and fusion is spreading posteriorly as the shelf margins approximate each other. ×20.

(E). Palatal shelves in a fetus from the same litter as fetuses 1B–D, i.e. S age = 16·4 days but CR age = 16·5 days. Note that the posterior margins of the shelves have grown towards each other and fused. ×20.

(F). Palatal shelves in a fetus; S age = 16·5 days, CR age = 16·6 days. Fusion of the future hard palate is largely complete, apart from the anterior defect, whereas the palatal shelves of the future soft palate (which were horizontal from the beginning) have not yet contacted each other. ×20.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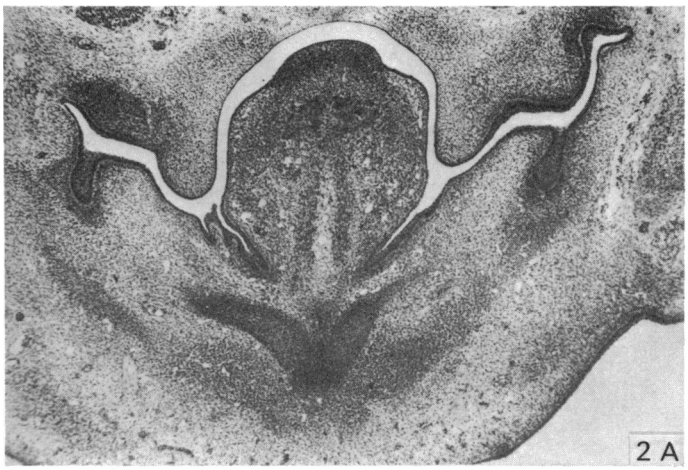
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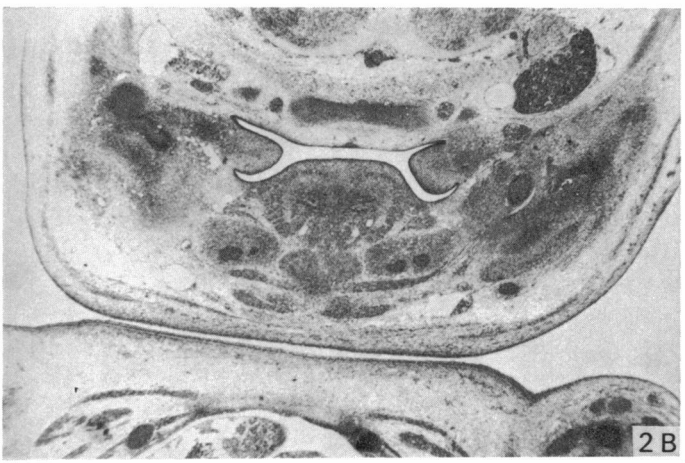
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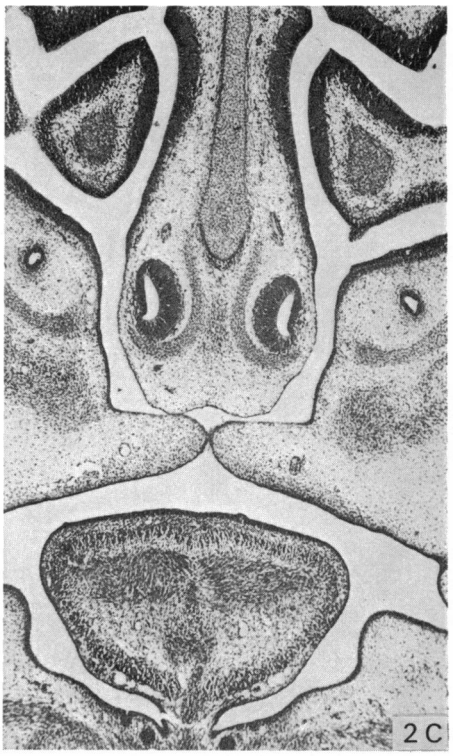
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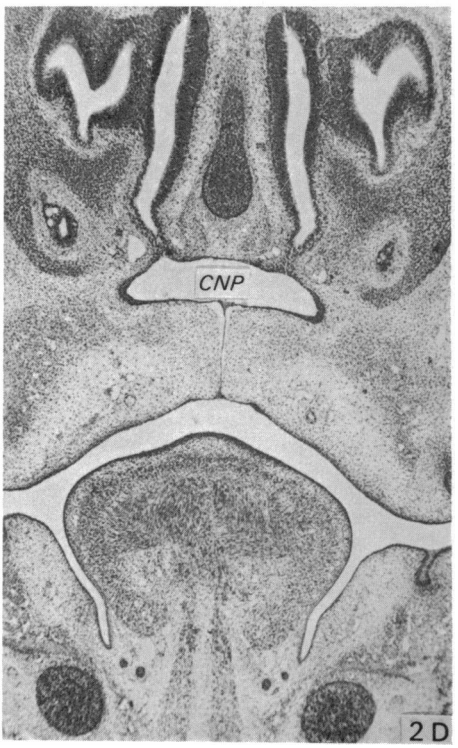


2 D

above the primary palate and partly in the free portion of the septum further back (Fig. 3A). Nasal conchae are now apparent. Both the primary palate (including the posterior projection mentioned) and the free edge of the nasal septum are in contact with the dorsum of the tongue, and sections cut in the sagittal plane show that the bulging into the mouth of the primary palate and nasal septum appears as if directing the tip of the growing tongue out of the oral cavity (Fig. 3A). The vertebral and cranial base cartilages are now well developed: there is no kink in the posterior cranial base cartilage (as Larsson (1974) asserted in an attempt to explain shelf elevation). Intramembranous ossification of the mandible has begun lateral to Meckel's cartilages, and maxillary and palatine osteogenic blastemata are present, but do not extend into the palatal shelves. The palatal shelves still show an epithelial groove on their lateral aspect. The epithelium covering the tip of the shelf has become thinner and is now only 1–2 cells thick. Rugae are present on the lateral aspect of the shelves. The mesenchymal cells in the shelves are even more loosely arranged, with an overall decrease in cell density. No evidence of differential mesenchymal growth was noted and the shelves still do not stain for collagen. However, the matrix of the shelves, particularly anteriorly, now stains quite densely with both alcian blue and Hale's colloidal iron: incubation of sections with hyaluronidase abolishes this staining. The posterior ends of the shelves are still horizontal, and here alcian blue staining is relatively less intense. The ultrastructure of the shelves is quite similar to that described for day 15·8 (CR age) except that, as mentioned in the histological observations, the mesenchymal cells are more loosely arranged and the epithelium at the shelf tip is only 1–2 cells thick.

Slight contralateral reflexes of the 'total pattern' type, involving rotation of the head, rump and limbs away from the stimulus, could now be elicited. However, no movement of the jaws or tongue was obtained.

S age 16·52–16·79, CR age 16·43–16·99

As mentioned previously, shelf elevation occurs some time during this period. Examination of at least 30 fetuses at this stage revealed that the head was still flexed; indeed if anything it was slightly more flexed than at day 16·3 (CR age), and, in particular, the lower jaw still rested on the chest wall (Fig. 3B). At 16·4 days (CR age) the position of the lower jaw relative to the upper jaw was similar to that at 15·8 days (CR age) (Fig. 3B): i.e. there had been no relative growth spurt in the

Fig. 2 (A). Coronal section through the anterior region of the tongue and vertical palatal shelves in a day 15·3 (CR age) fetus. H & E and alcian blue. ×35.

(B). Coronal section through the root of the tongue and the small posterior palatal shelves (future soft palate) which are horizontal from the beginning, in a day 15·3 (CR age) fetus. H & E and alcian blue. ×24.

(C). Coronal section through the anterior region of the elevated palatal shelves in a day 16·4 (CR age) fetus. Note that epithelial fusion is just about to occur both between the shelves themselves and between the shelves and the bifid lower end of the nasal septum. Note also Jacobson's organs, the vomeronasal and nasal septal cartilages. H & E and alcian blue. ×45.

(D). Coronal section through the nasal septum posterior to the ending of Jacobson's organs in a day 16·5 (CR age) fetus. Note the incipient fusion of the flanges of the now shortened nasal septum, with the bulges of the lateral nasal walls (associated at their bases with blood vessels), thus separating two sphenoethmoidal recesses above from the common nasal passage (*CNP*) below. Note also the fused palatal shelves with intact epithelial seam and the osteogenic blastemata of the maxilla. H & E and alcian blue. ×45.

(E). Coronal section of a day 16·5 (CR age fetus) in the region where the sphenoethmoidal recesses end. Note also the common nasal passage, fused palatal shelves, molar tooth germs and ossifying mandible. H & E and alcian blue. ×24.

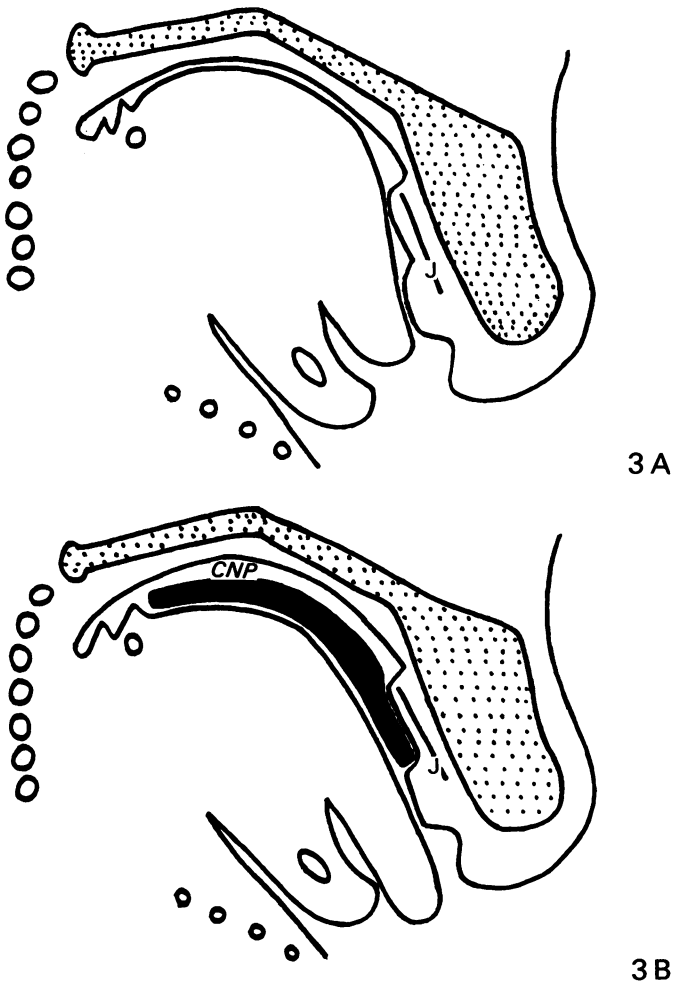


Fig. 3 (A). Diagram drawn from tracings of sagittal sections of 16·3 day (CR age) fetuses. Note the relationship between the arched tongue and cranial floor posteriorly; and the bulge of the primary palate and nasal septum anteriorly, which appear to be directing the tongue tip out of the oral cavity. The angulation of the cranial base and cervical vertebrae and the relationship between the lower jaw and chest wall should also be noted. *J* = Jacobson's organs.

(B). Diagram drawn from tracings of sagittal sections of 16·5 day (CR age) fetuses. Note the elevated palatal shelves (solid black) and the common nasal passage (*CNP*). Space for the common nasal passage (compare Fig. 3A) is provided by protrusion of the tip of the flattened tongue out of the oral cavity, such protrusion obviously being facilitated by the sloping bulge of the primary palate and nasal septum. The role of the nasal septum as a 'stop' for the palate is obvious, and it should also be noted that there has been no change in the angulation of the cranial base or cervical vertebrae, and no change in the relationship of the lower jaw and chest wall.

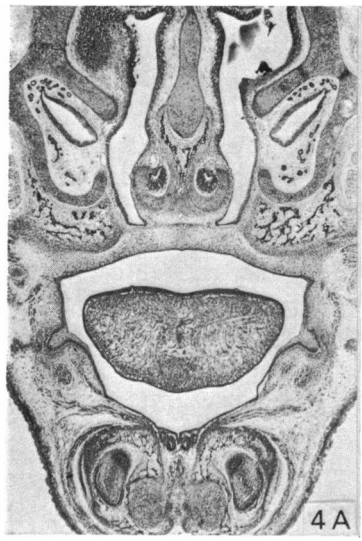
lower jaw prior to shelf elevation (as some authors have asserted). The tongue protruded between the lips in those members of the litter of 16·4 day fetuses (S age) in which the palatal shelves had elevated, but no such protrusion was present in those fetuses in which the shelves were still vertical (Figs. 3A, B). In fetuses with elevated shelves the tongue had changed its shape from a highly arched structure, filling the oronasal cavity, to a broad flat structure (oval in cross section), lying

beneath the shelves (Figs. 2C, 3A and B). Cutting away the tongue and lower jaw in fetuses from litters at 16·4 days (S age) revealed a variety of stages of palatal closure, as illustrated in Figures 1B–E which show the palates of some fetuses from one such litter placed in CR age sequence. Immediately after elevation the shelf edges do not make contact, although they are closest to each other anteriorly in the region of their greatest convexity: posteriorly they are more widely separated (Figs 1B, C). However, within 2 hours of elevation the anterior shelf convexities have contacted and fused with each other, leaving a Y-shaped gap in the palate in front of the region of contact, and a long straight gap behind it (Figs. 1D, 2C). The anterior gap evidently is closed later by a combination of backward growth of the nasal septum and primary palate and forward growth of the palatal shelves. Posteriorly the shelves grow rapidly towards each other (Fig. 1E) and epithelial fusion proceeds from before backwards (Figs. 1B–F), the major part of the future hard palate being fused within 5 hours of shelf elevation (Fig. 1F). The extreme posterior parts of the shelves, which were horizontal from the beginning, remain widely separated (Fig. 1F). At 16·4 days (CR age) the fused shelves are very easily torn apart, but at 16·5 days (CR age) they are firmly adherent and any attempt to tease them apart causes tearing of the bodies of the shelves rather than dehiscence at the epithelial seam marking the site of fusion (Figs. 2C–E). The epithelium on the oral aspect of the shelves near the midline becomes greatly thinned during the process of fusion. By 16·6 days (CR age) relatively rapid forward growth of the lower jaw has resulted in the tip of the tongue returning from between the lips into the oral cavity.

The palatal shelves stain quite heavily with alcian blue and Hale's colloidal iron, and, as before, hyaluronidase incubation inhibits this staining. There has been a further decrease in cell density and the matrix looks highly oedematous (Figs. 2C–E). Oedema and matrix staining intensity are most marked anteriorly where the shelves first meet (Fig. 2C). The cells and matrix of the mesenchyme at the lower end of the nasal septum, where the septum will fuse to the palate, appear very similar to that of the adjacent palatal mesenchyme, and have similar staining properties (Fig. 2C). The posterior ends of the palatal shelves do not stain with alcian blue as heavily as the anterior ends, and they have greater cell density. The shelves show no localized condensations of cells, nor are collagen, elastic or muscle fibres present (Figs. 2C–E). The developing maxillary and palatine osteogenic blastemata begin rapid invasion of the horizontal palatal shelves between 16·4 and 16·5 days (CR age), that is before disintegration of the epithelial seam. Blood vessels are most numerous in relation to the bone blastemata: elsewhere the vasculature appears much as it was prior to shelf elevation (i.e. there is no evidence of vascular erection). The epithelial invaginations and grooves noted earlier have now disappeared.

Ultrastructurally the mesenchyme presents similar features to those described on day 16·3 (CR age). The fusing epithelia (both shelf–shelf and shelf–septum), however, show some interesting features. The cells of each shelf adhere to each other by means of numerous desmosomes. After fusion, the epithelial seam rapidly degenerates: numerous lysosomes, autophagic vacuoles and areas of cytoplasmic disruption are evident in the seam cells. Occasional macrophages are present in the mesenchyme near the seam and appear to be engaged in removing cell debris.

Superimposition of tracings from sagittal sections of 16·3 and 16·5 day (CR age) fetuses revealed that there had been little change in either the structure or angulation of the cranial base cartilages, or in the cervical vertebrae (Figs. 3A, B). The nasal septum/primary palate bulge disappeared concomitant with shelf elevation (Fig. 3B).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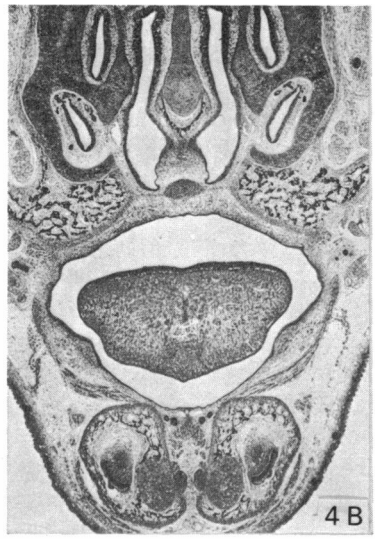
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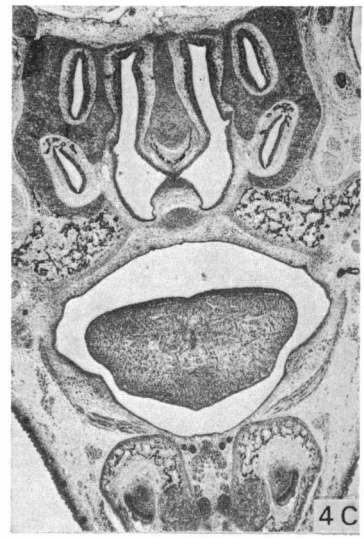
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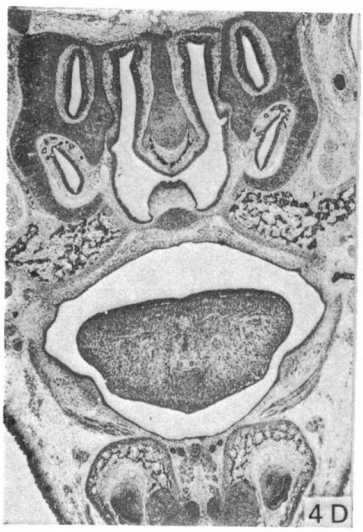
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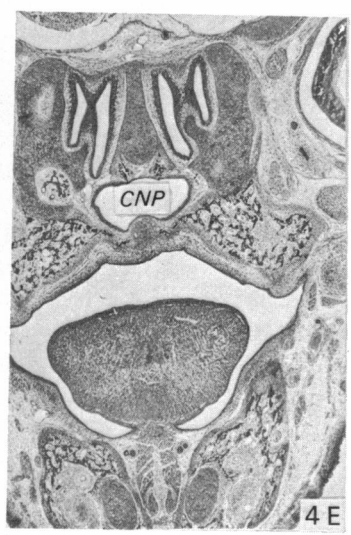
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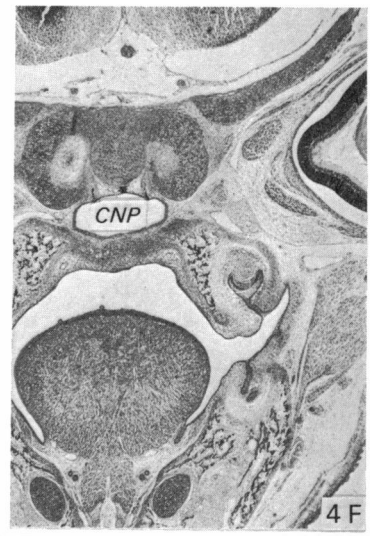
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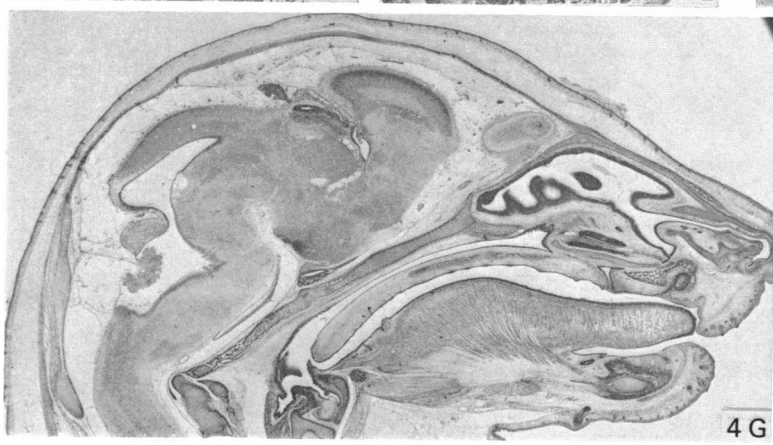
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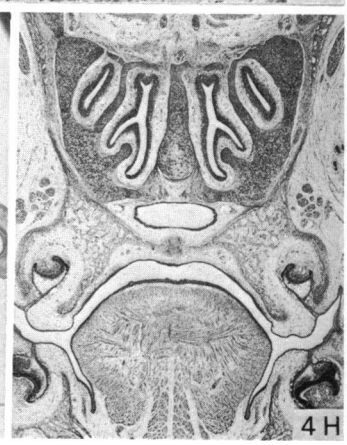
4 H

Anteriorly, the elevated palatal shelves fuse separately with the somewhat bifid lower edge of the nasal septum, leaving a small space between the three structures which is not obliterated until the 18th day (Fig. 2C). However, the palate is not fused to the septum throughout its entire length (Figs. 2C–E, 3B and 4G). Instead only the anterior one fifth of the palatal shelves fuses with the septum: this part of the septum houses Jacobson's organs. Behind the posterior ends of Jacobson's organs the septum rapidly decreases in height and has a free lower edge. The transition from palately-attached septum to free septum is marked by histological appearances which suggest that the lowest part of the septum is here, being detached from the main upper part by a pinching off process involving epithelial ingrowth (as in Figs. 4A–F). A little further back the free lower edge of the nasal septum develops lateral flanges which fuse with corresponding bulges developing on the lateral nasal walls (Fig. 2D). These latter bulges each contain a few large blood vessels. The septal flanges separate paired sphenoethmoidal recesses above from a *common nasal passage* below (Fig. 2D). This common nasal passage, roofed anteriorly by the septal flanges and floored by both hard and soft palates, further back lies directly beneath the cranial floor (where septum and recesses are no longer present) (Figs. 2D, 2E, 4E and 4F). It is clear that the paired sphenoethmoidal recesses and the common nasal passage become continuous with each other, and with the main nasal cavities, in the region where the nasal septum hangs free, i.e. neither attached to palate nor to lateral nasal walls.

Only 'total pattern' contralateral reflexes could be elicited, as at day 16·3, and again no movement of mandible or tongue could be obtained.

S age 17·60–17·85, CR age 16·99–17·84

Although the head is still flexed, the anterior part of the lower jaw is no longer in contact with the chest wall. The mandible is now growing forward faster than the upper jaw, whereas previously their growth rates were similar. The palate is now

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Fig. 4 (A). Coronal section through the anterior region of the palate and nasal septum in a day 18 fetus. The ventral end of the nasal septum is completely fused to the palate. The Y-shaped vomer, Jacobson's organs, and the start of a pinching in of epithelium superior to Jacobson's organs should be noted. H & E and alcian blue. ×18.

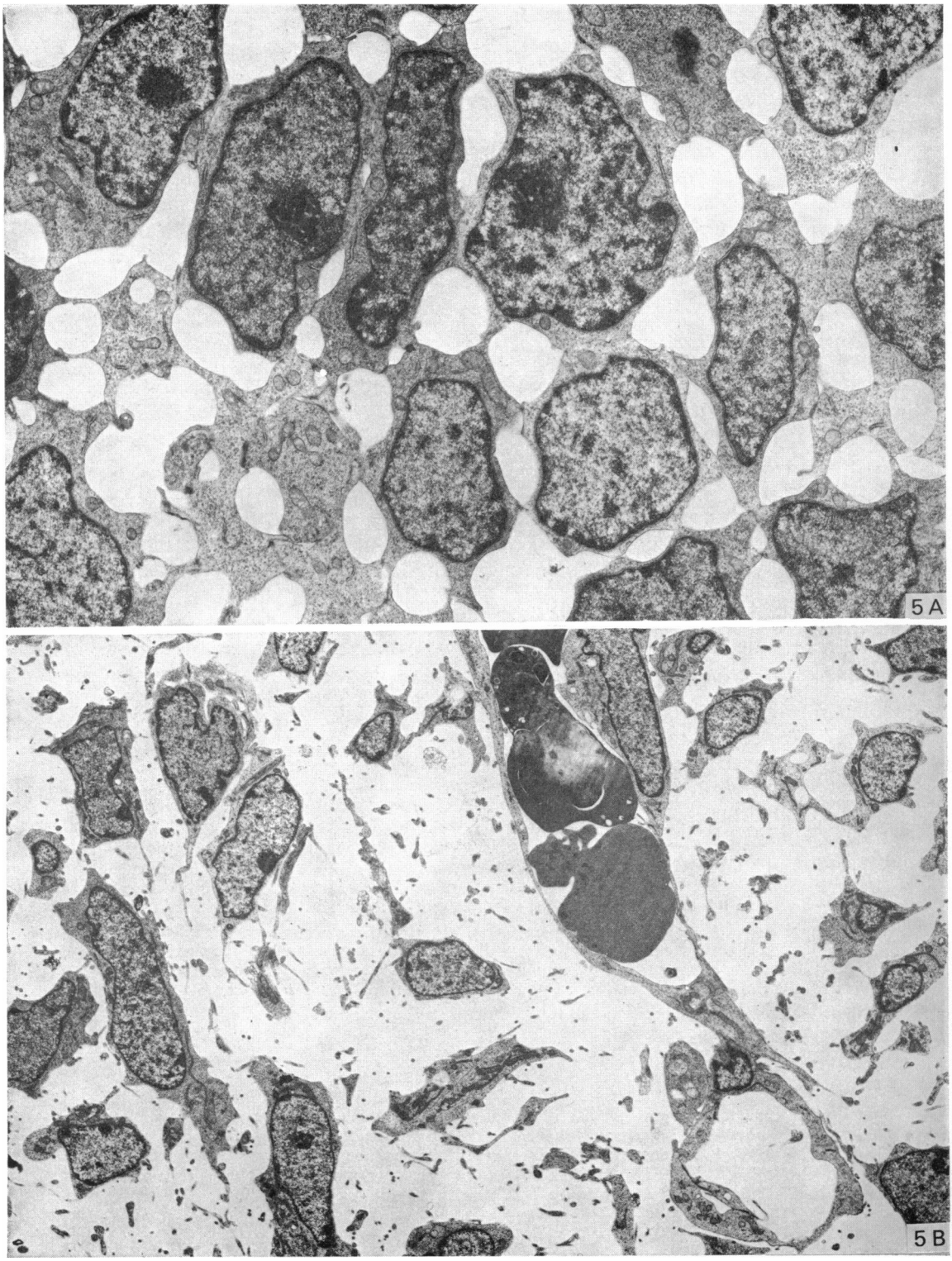
(B)–(D). Coronal sections cut posterior to that in Fig. 4A illustrating the progressive epithelial undercutting which results in a decrease in the height of the nasal septum, detachment of the palate from the main septum, and disappearance of the vomer keel, so that the vomer is now U-shaped. Posterior to Fig. 4D the septum hangs free for a short distance attached neither to palate nor to lateral nasal walls. H & E and alcian blue. ×18.

(E). Coronal section cut posterior to that in Fig. 4D illustrating the flanges of the nasal septum, the sphenoethmoidal recesses, the common nasal passage and the palate, the latter now showing signs of the ossifying maxillary bones and the palatal suture. H & E and alcian blue. ×18.

(F). Coronal section cut posterior to that in Fig. 4E showing the blind ending of the sphenoethmoidal recesses, the common nasal passage and the developing palate. H & E and alcian blue. ×18.

(G). Parasagittal section of the head of a day 19 fetus. Note the nasal cavity, premaxilla, incisor tooth germ and anterior palatine foramen anteriorly. The anterior portion of the secondary palate is fused to the nasal septum in the region of Jacobson's organs, posterior to this the common nasal passage runs above the palate. Before ending blindly the sphenoethmoidal recess shown lies superior to the anterior quarter of the common nasal passage, separated from it by the septal flanges; the cranial base then forms the superior relation of the posterior three quarters of the common nasal passage. H & E and alcian blue. ×7·5.

(H). Coronal section of a day 19 fetus illustrating the sphenoethmoidal recesses, the cartilage in the septal flanges, the common nasal passage, the ossifying palatal processes of the maxilla and the developing palatal suture. H & E and alcian blue. ×14.


5A
5B

fused throughout its entire length. Anteriorly the V-shaped defect has been filled in except for the two anterior palatine foraminae which persist into adult life. The characteristic pattern of rugae is present. The epithelial seams between the palatal shelves, and between the shelves and septum, have largely disappeared, although some epithelial remnants can be seen, particularly posteriorly where fusion takes place later than anteriorly. The seam is still intact in the future soft palate (the last region to fuse). The palatal processes of the maxillary and palatine bone blastemata have grown further into the palate and in some places the blastemata of the two sides have made sutural contact. The blastema for the vomer has appeared, and anteriorly where the septum is fused to the palate it is Y-shaped, the arms of the 'Y' encompassing the ventral end of the septal cartilage and the stalk of the 'Y' extending between Jacobson's organs and the vomeronasal cartilages of each side (as in Fig. 4A). More posteriorly the epithelial undercutting of the septum results in the loss of the stalk of the 'Y', so that the vomer becomes U-shaped (as in Figs. 4B–D). More positive and varied reflexes could be elicited at this age and occasionally a *very slight* downward movement of the lower jaw was observed as part of the response.

S age 18·58–18·83, CR age 18·69–19·53

Continued growth and uncurling of the fetus have resulted in the head and lower jaw lifting off the chest. The lower jaw has continued its rapid growth forward and is now in the adult position relative to the upper jaw. The tip of the tongue lies slightly behind the upper lip. The palate itself has essentially the same form as at 17 days. Previously with two-point fusion between nasal septum and palate there was a semicircular space in the midline lined with epithelium. This space has now been obliterated and the epithelium is disintegrating (Fig. 4A). The lateral flanges of the nasal septum now contain cartilaginous extensions from the lateral nasal cartilages (Fig. 4E). Posteriorly the epithelial seam has disappeared in the soft palate and muscle is beginning to differentiate: e.g. the tensor palati is conspicuous. Many different types of reflexes could be elicited at this age, including mouth opening and tongue protrusion, but the actual amount of movement was slight.

S age 19·39, CR age 18·97–20·95

The head is now almost at right angles to the rest of the body with the mandible well clear of the chest wall, otherwise relationships are much as they were at day 18, including those of the common nasal passage. Here it is worth noting that the common nasal passage is a normal feature of the adult skull and preserves the relationships it displayed in the fetus (Figs. 4A–G). The midline palatal suture is clearly developed (Fig. 4G) and in it there are occasional epithelial pearls. A wide range of positive and negative reflexes could be elicited.

Fig. 5 (A). Ultrastructural appearance of the epithelium covering the lateral aspect of the palatal shelf in a day 15·3 (CR age) fetus. Note the labyrinth of intercellular spaces. Lead and uranyl. × 8000.

(B). Ultrastructural appearance of the mesenchymal core of a day 15·3 (CR age) palatal shelf. Note the oedematous matrix with no visible structure, and the numerous mesenchymal cell processes. The mesenchymal cells show no unusual features. Lead and uranyl. × 3200.

Experimental observations on fresh normal fetuses

In order to elucidate possible mechanisms of shelf elevation, several experiments were performed on freshly removed fetuses between day 13·5 and day 16·5 (CR age). The fetuses were dissected free of their membranes and all experiments were performed with the fetus completely immersed in normal saline, so as to avoid possible surface tension phenomena.

When a blunt probe was inserted into the mouth of a day 14 fetus (CR age), and the dorsum of the tongue depressed, the stubby palatal shelves were seen to move slowly into the horizontal position. After the probe was removed and the jaws allowed to stay closed for about one minute, when the mouth was subsequently reopened it could be seen that the tongue has resumed its natural position in the oronasal cavity, with the shelves orientated vertically once again. Depression of the dorsum of the tongue again resulted in slow elevation of the palatal shelves. When the dorsum of the tongue was depressed in a day 15 fetus (CR age) the shelves moved to the horizontal position much more quickly. Furthermore, when the probe was removed and the mouth allowed to close, subsequent re-opening of the mouth revealed that the shelves had remained in the horizontal position and that the tongue had flattened considerably. Following depression of the dorsum of the tongue in a day 16·3 fetus (CR age) the shelves moved even more rapidly to the horizontal position, and once again remained there. This time the tip of the flattened tongue protruded from between the lips as after spontaneous shelf elevation at day 16·4 (CR age) (Fig. 3B).

In order to investigate whether or not the tongue could be withdrawn from between the palatal shelves by opening the mouth (as might occur in a mouth opening reflex) the probe was placed on the tip of the mandible of a 16·3 day fetus (CR age) and the latter slowly depressed. The tongue was not withdrawn from between the palatal shelves unless the mandible was depressed so far that substantial tears developed at the corners and in the floor of the mouth. It was concluded that the degree of mouth opening required for tongue withdrawal from between the palatal shelves is far in excess of that obtainable by mouth opening reflexes or neck extension. If the mandible was carefully removed, leaving the tongue between the palatal shelves, and the tongue was then removed, it was possible to study shelf elevation from below. At 16·3 days (CR age) the elevated shelves did not make contact with each other, but were separated by a gap which was narrowest anteriorly where the shelves have their maximum convexity. The macroscopic appearance of the 16·3 day (CR age) palate which had been elevated experimentally, was identical to that seen after spontaneous shelf transposition at 16·4 days (CR age) (Fig. 1B). It has been suggested that the palatal shelves may move to the horizontal position because of some extrinsic force such as that produced by changes in the angulation of the cranial base (Verrusio, 1970) or by a sudden lowering of pressure in the nasal part of the oronasal cavity following reflex tongue withdrawal (Humphrey, 1968, 1969, 1971). To test these possibilities, a horizontal incision was made through the fetal head which removed the top three quarters of the nasal septum, brain and skull. The remainder of the nasal septum and cranial base was then carefully dissected out of the lower part of the cut head, so that one was looking down on the dorsum of the tongue. When the latter was gently depressed in day 14 fetuses (CR age) the shelves elevated slowly, rising above the dorsum of the tongue in approximately 5 seconds. In day 15 fetuses (CR age), however, elevation

took approximately 2–3 seconds, while in day 16·3 fetuses (CR age) shelf elevation occurred in less than 1 second. This rapid shelf elevation may be termed 'flip up'. The shelves elevated first in the region of their maximum convexity (i.e. in their anterior one third), and they elevated most readily when the tongue was depressed at a level with the convexities. When the mandible and tongue were now excised from beneath the palatal shelves in a day 16·3 fetus (CR age), and the shelves were pushed by a probe into a vertical position, they still flipped up quickly to the horizontal position when the probe was removed. This manual displacement of the shelves with subsequent return to the horizontal position could be carried out 8–10 times – in fact until the delicate shelf tissue was torn from its mooring to the side of the mouth. The shelves never remained in any intermediate position; they moved immediately from the vertical to the horizontal position. Isolated slices of fetal heads containing only the palatal shelves (attached to some lateral oral tissue) and the premaxillary region were prepared at 16·3 days (CR age). The shelves could be depressed into vertical positions by a probe and when the probe was removed they flipped up again rapidly. Clearly there exists a palatal shelf force, intrinsic to the shelves and *not* caused by external factors such as low nasal pressure, cranial base changes or surface tension. Shelf elevation could still be attained experimentally in 16·3 day fetuses (CR age) which had been left unfixed in isotonic saline for 24 hours, although the speed of the event was decreased. Even palatal shelves of 16·3 day fetuses (CR age) fixed for 1–2 hours in Bouin's fluid flipped to the horizontal position in about 5 seconds. This proves that living cells are not required for actual flip up. However, after the lower portions of the heads (nasal septum and cranial base removed) of fresh unfixed 16·3 day fetuses (CR age) had been incubated with a solution of hyaluronidase (1 mg/1 ml) for 10 hours at 37 °C, flip up could not be obtained experimentally, but it must be confessed that the shelves were 'mushy' at this stage.

DISCUSSION

On the basis of these observations the following argument is advanced to account for shelf elevation. The gradual build up of mucopolysaccharides, predominantly hyaluronic acid, in the palatal shelves from day 14 to day 16·3 is assumed to produce an increasingly powerful elevating force because of the turgor associated with the strong water binding tendencies of these substances. At 16·3 days this turgor evidently reaches its peak and the elevating force becomes sufficient to overcome the resistance offered by the tongue. The shelves then spring up over the tongue and become horizontal. The tongue is somewhat depressed and broadens to occupy the space vacated by the palatal shelves. Besides mucopolysaccharide turgor, other factors would appear to aid the transposition of the palatal shelves. Firstly, the ingrowth of epithelium in a superomedial direction and its subsequent breakdown at the base of the shelf undercut the shelf and at the same time provide a fulcrum about which hingeing can occur. Secondly, maxillary and palatine osteogenic blastemata are present just exterior to the shelves prior to elevation and afford a firm base for palatal 'flip up'. The subsequent rapid invasion of the palatal processes by these blastemata and the ossification which follows stiffen and strengthen the elevated palate.

When the shelves flip up they only make contact with the anterior part of the nasal septum; behind this the palate is unsupported above and a space is cut off

from the oronasal cavity, above the palate, which may be termed 'the common nasal passage' (Figs. 2C–E, 3B and 4A–H). This space was previously occupied by the upper part of the arched tongue (Figs. 3A, B). The space for this passage is found at the expense of the tongue, for which there is now insufficient room in the oral cavity, and in consequence the tip of the tongue protrudes from the mouth (Fig. 3B). This protrusion is evidently facilitated by the sloping bulge of the anterior nasal septum and primary palate (Fig. 3A). It is entirely passive (there is no evidence of neuromuscular involvement) and occurs as the shelves elevate. In fact, when examining a freshly delivered fetus the protrusion of the tongue tip is proof positive that flip up has occurred. From this analysis it would appear that the anterior nasal septum has three roles: firstly, its sloping surface guides the tip of the tongue out of the mouth; secondly, it acts as a stop for the anterior parts of the elevating palatal shelves (which have not yet fused), this in turn preventing the more posterior parts of the shelves from contacting the cranial floor and keeping open space for the common nasal passage (Figs. 3A, 3B and 4G); thirdly, it facilitates palatal shelf fusion anteriorly by maintaining a firm surface across which the shelf edges may grow and meet.

Failure in the past to recognize the existence and significance of the common nasal passage (and, consequently, ignorance of its mode of development), and the fact that the nasal septum only reaches the secondary palate anteriorly, has led to confusing and erroneous accounts of palatogenesis in the rat. Thus Furstman *et al.* (1971) illustrate the common nasal passage but confuse it with the nasopharynx, while De Angelis (1975) labels it the nasal cavity. The lateral septal flanges described in the present paper were misinterpreted by Furstman *et al.* (1971), who described the nasal septum as gradually and completely fusing with the lateral nasal walls and obliterating the posterior parts of the nasal cavities – ignoring the existence of the spheno-ethmoidal recesses. Furstman *et al.* (1971) also described the whole nasal septum as fusing initially with the palate and then the posterior part of the septum 'pulling away'. In fact a small part of the anterior *septum* is undercut from the rest of the septum and remains attached to the palate in the process of forming the common nasal passage (Figs. 4A–D). This undercutting explains the absence of the vomer 'keel' posteriorly. These authors also described an increase of cell density in the palatal processes prior to rotation, and this cell proliferation was supposed to account for shelf elevation. The reverse, however, is the case.

Wragg *et al.* (1970) and Wragg, Diewert & Klein (1972) observed tongue protrusion following shelf elevation, but they did not appreciate its relation to the provision of space for the common nasal passage. Diewert (1974) stated that tongue protrusion was present one day prior to shelf elevation, but this was never the case in the present series. Wragg *et al.* (1970, 1972) and Greene & Kochhar (1973) concluded from an examination of frozen sections that no space whatsoever was present or created in the oronasal cavity prior to, during, or after shelf elevation. However, whilst it is true that some of the spaces seen in routine histological sections are artefacts, the common nasal passage is a real and new space created at the time of shelf elevation. Humphrey (1969), Wragg *et al.* (1972) and Greene & Kochhar (1973) were clearly wrong to conclude that the palatal shelves contacted each other immediately following shelf elevation, and that any gap observed histologically was artefact: a small gap is, in fact, clearly seen in fresh specimens examined macroscopically (Fig. 1B, C).

Many of the theories regarding shelf elevation to be found in the literature were

based on studies which paid little attention to the accurate dating of fetuses. Not only has this led to varying estimates of the time of shelf elevation in the rat (from day 15 to day 17), but it has also masked the rapidity of the event. Any theory of shelf elevation must take account of the fact that such elevation occurs in a matter of seconds. This tends to rule out theories which postulate critical growth changes, e.g. a spurt in mandibular growth or changes in the angulation of the cranial base (such theories also being irreconcilable with the fact that flip up occurs experimentally in the absence of all tissue except the palatal shelves). It also means that day to day comparisons of fetal histology are inadequate: the histology must be studied from hour to hour, at least around the time of flip up. For example, Verrusio (1970) compared the histology at day 15 with that at day 17 in arriving at his conclusion that changes in the angulation of the cranial base were decisive.

The mucopolysaccharide turgor theory propounded here explains why the anterior regions of the shelf flip first, both *in vivo* and experimentally, for these regions stain heaviest for mucopolysaccharide and are the most oedematous. Moreover, the gradual accumulation of mucopolysaccharides (correlatable with the accumulation of the necessary synthesising organelles in the mesenchymal cells) in the palatal shelves from day 14 to day 16·3, noted histologically, correlates with the gradual build-up of shelf force determined experimentally during this period. The observation that incubation of the fetal head with hyaluronidase abolishes the shelf force is confirmatory of the general theory. It is estimated that hyaluronic acid constitutes over 60 % of the total acid mucopolysaccharides present in the rat palatal shelves prior to flip up, the remainder being sulphated glycosaminoglycans (Pratt *et al.* 1973). Hyaluronic acid is extremely hydrophilic, its open coil type molecule being capable of binding considerable quantitites of water (Laurent, 1970) so that the postulate of a turgor force elevating the shelves is compatible with the biochemical facts. In this connexion it is of interst to note that the mucopolysaccharides of the cornea (of the horse) can develop a turgor force of 75–85 g/cm^2 (60 mmHg) *in vitro* (Hedbys, 1961; Maurice, 1969; Maurice & Riley, 1970).

Larsson (1960, 1962, 1974) alleges that histochemical methods are not sensitive enough to detect differences in the mucopolysaccharide content of the developing palatal shelves, or differences between the mucopolysaccharide content of normal and cleft palates. Instead, he strongly advocates the use of ^{35}S labelling. However, the present study clearly demonstrates that the accumulation of mucopolysaccharides in the palatal shelves can be followed histochemically. The present author (Ferguson, 1978) has also demonstrated (histochemically) depressed mucopolysaccharide synthesis in fetuses with cleft palate induced by 5 fluoro-2-desoxyuridine. Also it must be stressed that ^{35}S will not detect hyaluronic acid (which comprises over 60 % of the total mucopolysaccharide content of the palatal shelves).

Nanda (1970) and Andrew & Zimmerman (1971) argued strongly against any role of mucopolysaccharides in palatal development. However, these authors used ^{35}S autoradiography exclusively and their methodology is open to serious criticism. Thus Nanda (1970) studied the incorporation of ^{35}S into rat palatal shelves at day 17, i.e. at a time when the palate is already fused and bone formation is in an advanced state. Larsson (1974) severely criticized the work of Andrew & Zimmerman (1971), referred to above, on the grounds that their ^{35}S techniques were faulty. Nanda (1970) and Andrew & Zimmerman (1971) are obviously very rash in concluding that, "the inhibition of mucopolysaccharide synthesis by glucocorticoids is not responsible for the formation of cleft palates during fetal development. It would appear that

mucopolysaccharide synthesis is not responsible for the internal shelf force whereby palatal shelves become horizontal."

Many other existing theories of shelf elevation are inconsistent with the results of the present study. Not only is there no mandibular growth spurt prior to shelf elevation, but indeed the mandible grows fastest *after* this event. No myoneural activity of the tongue could be induced experimentally: the changes observed in the tongue at the time of flip up are clearly passive, and indeed can be simulated in dead fetuses. It can also be argued that spontaneous tongue movement would positively interfere with palatal shelf fusion, particularly posteriorly where their only support is the quiescent dorsum of the tongue. Jacobs (1970*a*, *b*, 1971) tried to induce cleft palate by giving large doses of neuromuscular inhibiting agents but failed, from which it seems clear that muscular activity is not required for palatal development. *A fortiori* Holt (1975) found no differences between the lingual myoneural apparatus of normal fetuses and those with cleft palate. Mouth opening reflexes leading to tongue displacement (Humphrey, 1968, 1969, 1971) are ruled out, not only because such reflexes cannot be elicited up to the time of shelf elevation, but also because experimental evidence shows their postulated mode of action to be false. There was no evidence of sudden neck extension at the time of flip up – indeed the head and neck were, if anything, more flexed after flip up, confirming the observations of Wragg *et al.* (1970) but refuting those of Diewert (1974). Larsson (1974) described a kinking of the cranial base prior to flip up, but this was not confirmed. In fact, flip up occurred even after the cranial base had been removed. No support was found for views that shortening of elastic or collagen fibres, actomyosin filaments or smooth muscle fibres play a part in shelf elevation, for such elements are not present in the relevant parts of the palate at the time of flip up. Hassell & Orkin (1976) observed ruthenium red staining material in the palatal shelves and thought it might be primitive collagen, but it is more likely to be mucopolysaccharide. Much evidence for the collagen theory of shelf elevation stems from experiments in which cleft palate was induced by β-aminoproprionitrile (Pratt & King, 1972) but it should be noted that this compound can also cause an 18 fold increase in the activity of acid hydrolyase – an enzyme system responsible for the degradation of mucopolysaccharides (Kauffman & Delbalso, 1975). Coleman (1965) concluded that the posterior part of the palate became horizontal by a remodelling of the shelves, with resorption in a vertical direction and new growth medially above the tongue. However, it is possible that he over-emphasized the histological appearances at the transition zone between the anterior vertically orientated shelves and the posterior parts of the shelves (soft palate primordia) which are horizontal from the beginning. Atnip (1963) could not produce experimental shelf elevation when the fetuses were submerged in saline, so he concluded that surface tension accounted for shelf elevation under conditions described by other workers. However, flip up was obtained in the present experiments, all of which were conducted under normal saline.

Some of the previous confusion in the literature (e.g. regarding the role of the tongue and nasal septum in shelf elevation) may have arisen from a failure to appreciate that at this time two separate but related processes are occurring: (1) shelf elevation and (2) creation of the common nasal passage. It is clearly important to distinguish the primary factors involved in each process (as discussed). Whilst it is agreed that a certain overall level of head development is required for shelf elevation, and that this latter event may involve a coordinated interaction between various

structures (Greene & Pratt, 1976), it would appear that the primary force responsible for shelf elevation is an intrinsic one generated by the hydration and polymerization of shelf mucopolysaccharides.

SUMMARY

Palatogenesis in the Wistar rat fetus was studied macroscopically, microscopically, ultrastructurally and experimentally between days 13 and 19. The developmental ages of the fetuses were calculated from the smear age of the litter adjusted for individual variations in crown–rump lengths. Palatal shelf elevation occurs at day $16{\cdot}4 \pm 0{\cdot}1$. Experimentally induced shelf elevation in freshly delivered fetuses was sluggish at day 14, but by day 16·3 it occurred in less than 1 second. Both shelf elevation and shelf fusion begin anteriorly where the shelves show a marked convexity of their margins, and proceed posteriorly. The extreme posterior part of each shelf (future soft palate) is horizontal from the beginning. The matrix of the shelf mesenchyme (especially in the region of the anterior convexities) shows an increasing accumulation of mucopolysaccharides from day 14 to day 16·3 and becomes increasingly oedematous. The shelf attachment to the main maxillary process is progressively undercut by epithelial invagination, producing a fulcrum for shelf elevation. The maxillary and palatine osteogenic blastemata are present at the base of the shelf prior to elevation and rapidly invade the shelves after the event. The elevated palatal shelves fuse with the nasal septum anteriorly, but posteriorly the palate is not attached to the septum. The posterior septum at first has a free lower edge, but then it develops lateral flanges which fuse with corresponding bulges on the lateral nasal walls. In this way two sphenoethmoidal recesses are formed above the fused flanges, while a common nasal passage is formed above the palate, roofed anteriorly by the septal flanges and posteriorly by the cranial base. The space needed to create (simultaneous with shelf elevation) the common nasal passage is made available by flattening of the tongue and protrusion of its tip out of the oral cavity – this protrusion being facilitated by the sloping bulge of the primary palate and nasal septum. Many existing theories of shelf elevation are inconsistent with these observations. It was concluded that shelf elevation occurs very rapidly at a rather precise developmental stage and that turgor (due to binding of water to mucopolysaccharides) is the intrinsic force which elevates the shelves, a force which at 16·4 days reaches a threshold level enabling the shelves to force their way up and over the intervening tongue.

I would like to thank Professor J. J. Pritchard for his valuable help in the preparation of this paper.

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