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# The Mechanism of Palatal Shelf Elevation and the Pathogenesis of Cleft Palate

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**Summary.** Both normal Wistar rat fetuses and those with cleft palate induced by 5-Fluoro-2-Desoxyuridine were studied with a view to elucidating the mechanism of palatal shelf elevation and the pathogenesis of cleft palate. It was postulated that normal shelf elevation is brought about rapidly by an intrinsic turgor shelf force generated by binding of water to mucopolysaccharides. Interference with mucopolysaccharide synthesis would seem to be an important factor in the pathogenesis of some types of cleft palate.

**Key words:** Palatal shelf elevation – Cleft Palate – Mucopolysaccharides.

## Introduction

During fetal life the secondary palate develops initially as two bilateral extensions from the main maxillary processes – the palatal shelves. At first, these shelves grow vertically, passing downward on the lateral sides of the tongue, (Fig. 1) but during the eighth week of human development (and about day 16 in the rat), they elevate to a horizontal position above the dorsum of the tongue, fusing with each other and with the nasal septum to form the definitive secondary palate (Fig. 2). This elevation of the palatal shelves is a critical event in normal palatogenesis. However, there is no general agreement about either the mechanism(s) of shelf elevation or its precise spatial and temporal parameters.

The need to understand more fully the details of shelf elevation is underlined by the fact that delay (or failure) of this event probably accounts for 90% of human cases of isolated cleft palate (i.e. without associated cleft lip or cleft primary palate). Since it has been established (Fogh Andersen, 1968; Ross and Johnston, 1972) that in man some 80% of cases of isolated cleft palate are caused by environmental factors, knowledge of the process of normal shelf elevation and of ways in which cleft palate can be induced experimentally is clearly a necessary prerequisite for understanding the aetiology of, and developing rational preventative or restorative treatment for, this distressing abnor-

mality. The investigation of any developmental process or deformity in man is usually severely limited by lack of material and of relevant information about the material, with the result that animal models are essential. In this connection it is widely accepted that the early development of the human face is essentially similar to that of the rat: for this, and other reasons, (placental permeability, size, genetic stability, length of gestation and economy of husbandry) the rat is a favourite model for teratogenic research (Poswillo, 1968b). Therefore, a comprehensive study of palatogenesis in the Wistar rat was made, combining macroscopic, microscopic, ultrastructural and experimental observations on the entire palate of both normal fetuses and those with experimentally produced cleft palate.

## Review of the Literature

Many theories concerning shelf elevation can be found in the literature. These can be divided broadly 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, e.g. (1) descent of the tongue resulting from a marked growth spurt of the mandible relative to the maxilla around the time of shelf elevation (Zeiler et al., 1964; Sicher, 1966; Burdi and Silvey, 1969; Diewert, 1974); (2) depression of the tongue produced by the downward growth of the nasal septum and primary palate (Fraser, 1969, 1971); (3) descent of the tongue as a result of its intrinsic myoneural activity (Wragg et al., 1972a; Wragg et al., 1972b); (4) lowering of the tongue as part of a fetal mouth opening reflex (Humphrey, 1969); (5) lifting of the head off the chest (enabling the mandible and tongue to drop) as a result of either spontaneous contraction of neck muscles (Walker, 1969, 1971) or growth in length of the cervical spine (Ross and Lindsay, 1965) and (6) changes in the angulation of the anterior relative to the posterior cranial base producing a palatal shelf elevating force (Verrusio, 1970). The second group of theories attribute an active role to the shelves themselves rather than to extrinsic structures, e.g. (1) differential growth of the palatal shelves (Wood and Kraus, 1962; Sicher, 1966); (2) hydration and polymerization of intercellular substances producing an elastic elevating force (Lazzaro, 1940; Larsson, 1962; Walker, 1961); (3) the shortening of elastic fibres (Walker and Fraser, 1956); (4) the contraction of newly synthesized collagen (Hassell and Orkin, 1976); (5) the contraction of actomyosin or microfilaments (Lessard et al., 1974; Krawczyk et al., 1975); (6) the contraction of skeletal muscle (Babiarz et al., 1975); (7) increased vascularity producing an erectile force (Gregg and Avery, 1971); (8) remodelling of the shelf with resorption in a vertical direction and new growth horizontally (Polzl, 1904; Pons Tortella, 1937; Coleman, 1965); (9) shrinkage of one side of the shelf by rugae formation (Pourtois, 1972), and (10) differential growth and traction of the shelf epithelium (Pourtois, 1972). This list is not exhaustive: these and other theories have been discussed in detail previously (Ferguson, 1976, 1977a).

Although there have been some attempts (e.g. by Zeiler et al., 1964) to determine when shelf elevation occurs in the rat, the precise timing of this event 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 and Fraser, 1956; Zeiler et al., 1964; Larsson, 1962; Pourtois, 1972; Babiarz et al., 1975) while others maintain that they start at the front (Coleman, 1965; Wragg et al., 1972b).

## Materials and Methods

*A. Normal Fetuses*

Twenty highly inbred (12th generation offspring of a brother/sister mating programme) pregnant female Wistar rats (average wt 300 g) were used in this study. A total of 217 fetuses were obtained

between days 13 and 19, any litters containing less than 8 or more than 12 fetuses being discarded. Because the fetuses of a given litter differ somewhat in their maturity, the developmental ages of the fetuses were calculated from the smear age of the litter adjusted for individual variations in crown-rump lengths (Ferguson, 1976, 1977a). Living fetuses, both inside their amniotic sacs and after removal from the latter, were stimulated by gently touching, in turn, the face, trunk, and limbs, and the nature and duration of any responses noted. Of the fetuses removed from each litter two were placed in normal saline for immediate experimentation, two were used for electron microscopy and the remainder prepared either for macroscopic or light microscopic examination. Both fresh and Bouin fixed fetuses were examined macroscopically and dissected to display the interior of the mouth. 7 μm serial paraffin sections were cut in the coronal, sagittal and horizontal planes and stained with either a combination of 1% aqueous alcian blue (pH = 7.0), Harris' iron haematoxylin & alcoholic eosin, or by Mallory's or Masson's method. In addition, either alcian blue at different pH's, Hale's colloidal iron, acridine orange fluorescence, or hyaluronidase digestion followed by alcian blue staining, were used to detect mucopolysaccharides in the developing palate, the cartilages of the facial region serving as controls. The appearances of these sections were compared with those of corresponding fresh unfixed fetuses sliced at 1 mm intervals in the coronal, sagittal and horizontal planes. The two fetuses selected from each litter for electron microscopy 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 four hours in Karnovsky, postfixed in 1% osmium tetroxide for 45 min, 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.

*B. Abnormal Fetuses*

Twenty comparable pregnant female Wistar rats were used. In order to elucidate the primary lesion responsible for delay in shelf elevation, fetuses had to be studied from the earliest stages of palatogenesis (when it would not be possible to know if the fetuses would have developed cleft palate later) and this necessitated finding a teratogen which would eventually induce cleft palate in 100% of the fetuses in any litter. In preliminary trials, it was found that a single intraperitoneal injection of 100 mg of 5-Fluoro-2-Desoxyuridine (F.U.D.R. – dissolved in injectible water) per kilogram maternal body weight into an unanaesthetised rat at day 12.5 induced cleft palate in 100% of the fetuses recovered at day 17. Using this schedule, 70 living fetuses and 29 dead and resorbing fetuses were recovered between days 14 and 19 from nine rats injected with F.U.D.R.

As controls, a series of unanaesthetised rats were each given a single intraperitoneal injection of the same volume of injectible water at the same stage of gestation. A total of 109 fetuses and two resorption sites were recovered from 11 control rats between days 14 and 19. The resorption rate in the F.U.D.R.-treated animals was 30% as compared with 2% in the control rats. Techniques identical to those described above for normal fetuses were used to study these experimental fetuses.

## Results

### *1. Observations on Normal Fetuses*

The palatal shelves first appear at day 14 as small vertically orientated extensions of the main maxillary processes. They are situated lateral to the tongue and are continuous anterolaterally with the posterior margin of the primary palate (which develops some two days earlier). Initially, the margins of the vertical shelves are straight and parallel to one another, but from day 15.5 to day 16.3 they develop a progressively more sinusoidal outline with a marked 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

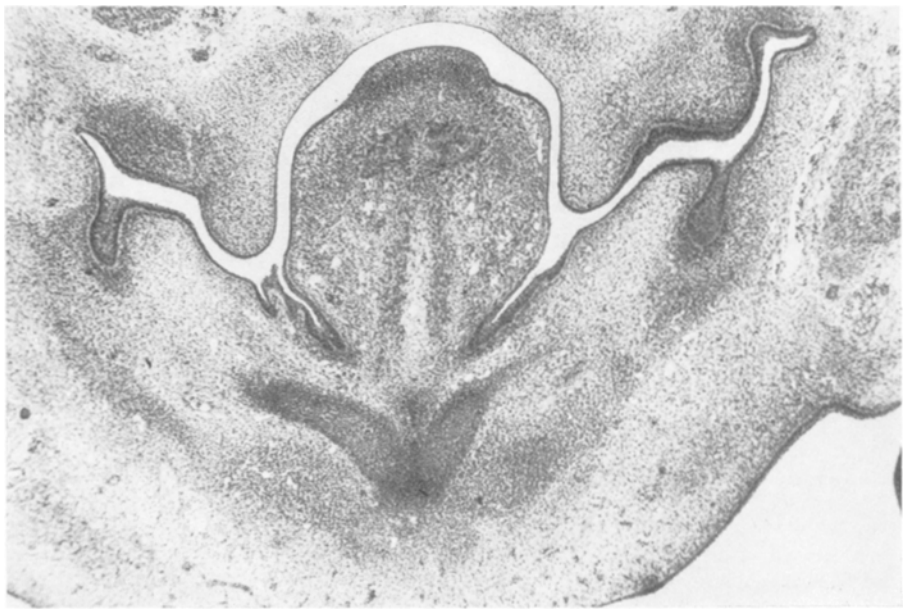


**Fig. 1.** Coronal section of the vertical palatal shelves in a day 15 normal fetus. H & E and Alcian Blue, ×43

the tongue. At day 15 the palatal shelves consist of the "original palatal shelves" (anterior four-fifths) which are long and vertically orientated, but an additional element has formed (the future soft palate) which is stubby and projects horizontally above the dorsum of the tongue in the region of its root (and so does not have to elevate). In the transition zone between the vertical anterior fourfifths and horizontal posterior one-fifth of a shelf, the appearances are such that at first sight it might be erroneously inferred that the shelf is changing from a vertical to a horizontal disposition by differential accretion and resorption.

Elevation of the palatal shelves from a vertical position lateral to the tongue (Fig. 1) to a horizontal one above its dorsum (Fig. 2) occurs between days 16.3 and 16.5. The initially elevated shelves do not immediately make contact with one another. They are closest anteriorly in the region of their maximum convexities (Fig. 3) where the gap between them is rapidly bridged by marginal growth. Contact, followed by epithelial fusion, spreads rapidly in a posterior direction, the future hard palate region being fused within five hours of elevation (Fig. 4). The future soft palate does not fuse till day 17.5 (Fig. 4). Anteriorly, the palatal shelves fuse with a small backgrowth from the posterior border of the primary palate, leaving bilateral defects—the anterior palatine foramina—which persist into adult life (Fig. 4).

At day 14, the mesenchymal cells of the palatal shelves are densely packed and the matrix between them stains faintly for mucopolysaccharides. However, from day 14 to day 16.3 the mesenchymal core of the shelves show a progressive accumulation of mucopolysaccharides and a decrease in cell density associated with a highly oedematous matrix occupying wide intercellular spaces. These changes are most marked in the anterior convexities of the shelves, and least

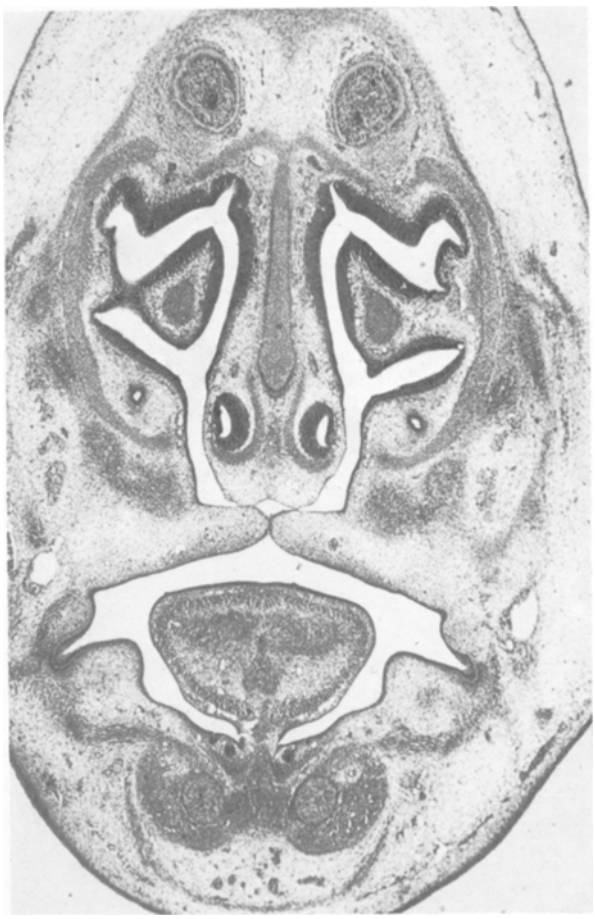


**Fig. 2.** Coronal section showing the earlier stages of fusion between the anterior palatal shelves and nasal septum in a day 16.4 normal fetus. H & E and Alcian Blue, ×27

marked in their posterior one-fifths which are horizontal from the beginning. Incubation of sections with hyaluronidase abolishes subsequent mucopolysaccharide staining. Mitotic figures are evenly distributed throughout the mesenchyme and there is no evidence of any differential shelf growth. Small capillaries are present, but not conspicuous, and there appeared to be no change in shelf vasculature at the time of shelf elevation. Ultrastructurally, the capillaries show no evidence of increased permeability. The shelves do not stain for collagen in ordinary histological preparations, while ultrastructurally there is no evidence of any collagen, (except for an occasional primitive fibril beneath the basement membrane) elastic or muscle fibres in the mesenchymal core of the shelves around the time of shelf elevation (Fig. 5). Indeed, the swollen matrix of the shelf core is without visible structure (Fig. 5). The mesenchymal cells possess free ribosomes, mitochondria, RER cisternae and a clearly defined Golgi complex with secretory vesicles (Fig. 5), but do not contain presumptive contractile elements. The cells are stellate, with long contacting processes giving the cellular component of the mesenchyme a spongy appearance (Fig. 5).

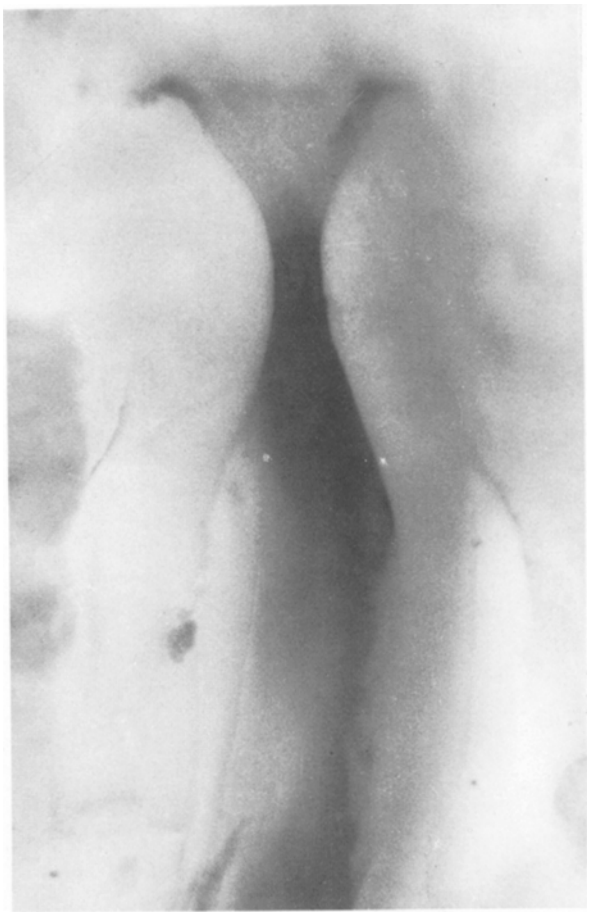


**Fig. 3.** Macroscopic appearance of the palatal shelves immediately following shelf elevation in a day 16.3 normal fetus, ×40

The bases of the vertical palatal shelves are progressively undercut by epithelial invagination with groove formation, evidently providing a fulcrum for shelf elevation. These epithelial ingrowths disappear as the shelves elevate.

The elevated palate did not fuse with the nasal septum along its entire length. Indeed only the anterior one-fifth of the palatal shelves fuse with the septum (Figs. 2 and 6B), i.e. with the part of the septum housing Jacobson's organs (Figs. 2 and 6B). Behind Jacobson's organs, the septum is undercut, rapidly looses height and has a free lower edge. This free septum develops lateral flanges which fuse with corresponding bulges on the lateral nasal walls (Fig. 7). In this way, two sphenoethmoidal recesses are formed above the fused flanges, while a single common nasal passage is formed above the palate (Fig. 7). This common nasal passage is roofed anteriorly by the septal flanges (Fig. 7) but posteriorly, behind the sphenoethmoidal recesses, it is roofed by the cranial base. In previous work the existence of such a common nasal passage in the rat has either been largely overlooked, called the nasopharynx, or regarded as a developmental error!

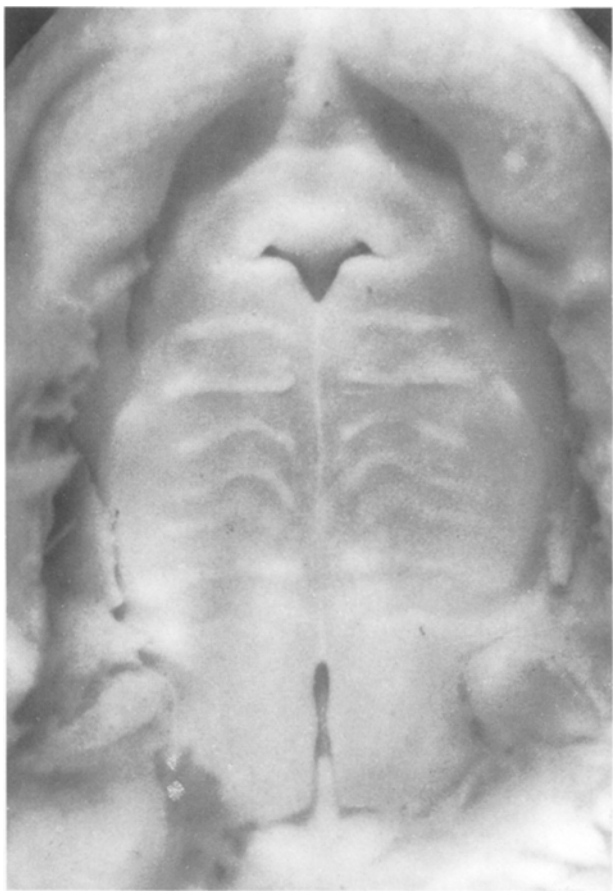


**Fig. 4.** Macroscopic appearance of the palate of a day 16.5 normal fetus. The future hard palate is almost completely fused whereas the shelves of the future soft palate have not yet made contact, ×25

The space needed to create the common nasal passage (simultaneously with shelf elevation), is made available at the expense of the tongue. Prior to shelf elevation, sagittal sections show the tongue to be highly arched and in contact with the cranial floor posteriorly (Fig. 6A). Anteriorly, it is in contact with a bulge made by the primary palate and nasal septum (Fig. 6A). During shelf elevation the tongue is flattened by the elevating shelves and its tip protrudes out of the oral cavity, such protrusion obviously being guided by the sloping bulge of the primary palate and nasal septum (Fig. 6B). Thus space for the common nasal passage is created by tongue protrusion (Fig. 6A and B). Indeed, protrusion of the tongue tip in a fetus is proof positive that shelf elevation has occurred.

Because of the form of the nasal septum, the vomer (which first appears at day 17) is Y-shaped anteriorly where the septum is fused with the palate and U-shaped where the lower edge of the septum is free.

At day 14 the epithelium covering the tips of the palatal shelves is several layers thick. However, by day 16.3 (when the shelves are about to elevate) it has thinned to become only 1–2 cells thick. Ultrastructurally, the surface

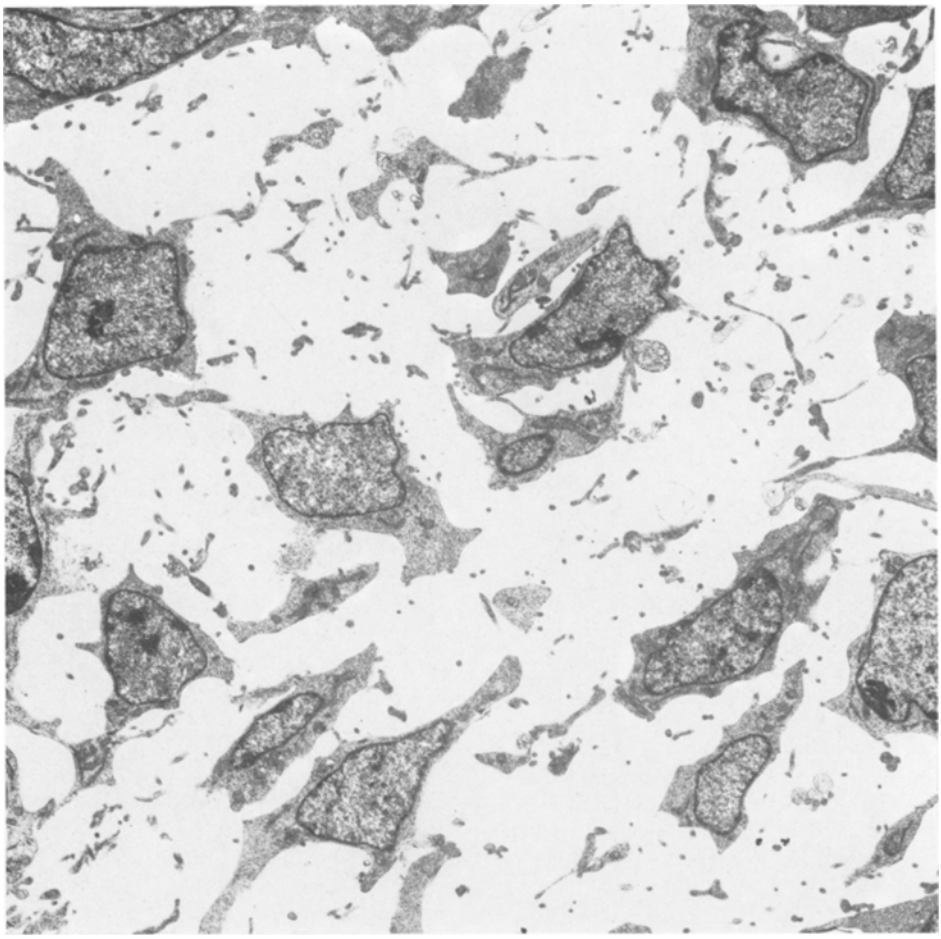


**Fig. 5.** Electron micrograph of the mesenchymal core of a day 16.4 normal palatal shelf. Note the oedematous matrix. Lead & Uranyl, × 5000

of these epithelial cells show small 'microvilli-like' projections, which could well help to 'tack together' the elevated shelves. The epithelial cells, in general, show no evidence of any contractile elements, or unusual mitotic activity.

The epithelial cells of the elevated palatal shelves rapidly fuse with each other by means of numerous desmosomes, to form the epithelial seam. This fusion is very strong and attempts to tease the shelves apart causes tearing of the bodies of the shelves rather than dehiscence at the epithelial seam. No sooner have the epithelial seam cells fused than they show signs of degeneration; numerous lysosomes, autophagic vacuoles and areas of cytoplasmic disruption are evident. Occasional macrophages are present in the mesenchyme near the seam and appear to be engaged in removing cell debris. The seams between each shelf and the nasal septum show similar changes, and the mesenchyme of the ventral end of the anterior nasal septum (which ultimately fuses with the palate) resembles closely that of the palatal shelves, with regard to the

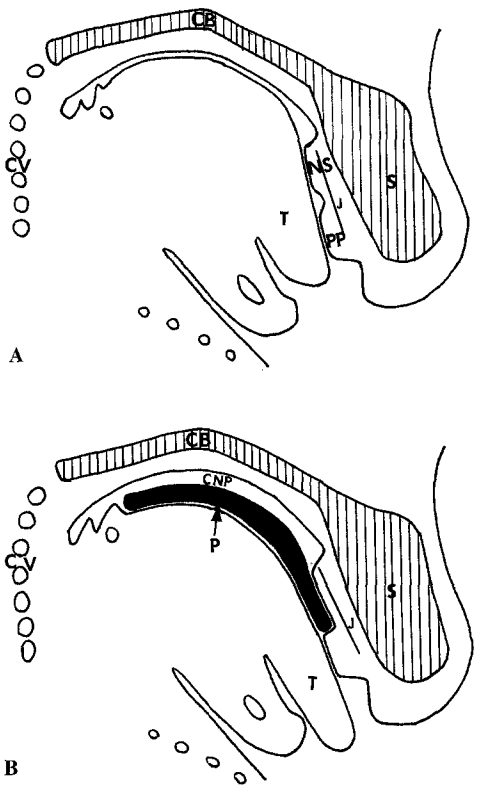



**Fig. 6. A** Tracing of sagittal sections of a day 16.3 normal fetus (before shelf elevation). Note the highly arched tongue and bulge of the primary palate (*P.P.*) and nasal septum (*N.S.*). **B** Tracing of sagittal sections of a day 16.5 normal fetus (after shelf elevation). Note the elevated palate (*P* solid black), common nasal passage (*C.N.P.*) and protruded tongue tip. *CB* cranial base cartilage; *S* nasal septum cartilage; *CV* cervical vertebrae; *T* tongue; *J* Jacobson's organs

intensity of mucopolysaccharide staining and oedema. The epithelial seams have largely disappeared in the hard palate by day 17, and in the soft palate by day 18, so that there is mesenchymal continuity between the two shelves and the septum: epithelial pearls are sometimes present.

The osteogenic blastemata for the maxillary and palatine bones appear at the bases of the shelves about day 16 (i.e. before shelf elevation). The palatal processes of these blastemata rapidly invade the elevated shelves and have almost reached the midline before disintegration of the epithelial seam is completed (Fig. 7). Palatal osteogenesis is well advanced by day 19, at which time the developing bones of each side have made sutural contact (Fig. 8). Muscle first appears in the developing soft palate at day 18.

Both Meckel's cartilages and mandibular ossification are conspicuous prior to shelf elevation. The mandible did not seem to show any growth spurt immediately prior to, or during, the time of shelf elevation; indeed it grew fastest *after* this event, enabling the protruded tongue tip to return to the oral cavity by day 16.8. No reflex myoneural activity of the tongue could be elicited prior to, during, or up to one day after shelf elevation; neither could any mouth opening or head extension reflexes be demonstrated throughout this period.

Prior to shelf elevation the head is flexed with the lower jaw resting on the chest wall. The head remains flexed, both during and after the period

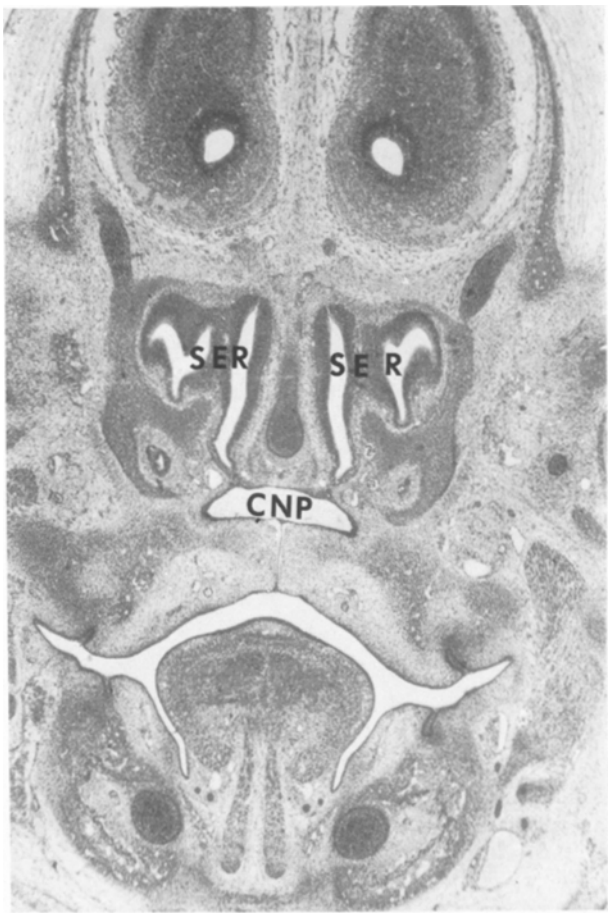



**Fig. 7.** Coronal section of a day 16.4 normal fetus illustrating fusion of the septal flanges with the lateral nasal walls. *S.E.R.* sphenoethmoidal recesses; *C.N.P.* common nasal passage. H & E and Alcian Blue, ×27

of shelf elevation (indeed it is more flexed at day 16.5 than at day 16.3) and the lower jaw is not lifted off the chest wall (Fig. 6A and B). Indeed because of the consistent position of the head on the chest, true descent of the tongue and mandible is impossible. Superimposition of tracings from sections shows that there is no change in the angulation of the anterior cranial base cartilage relative to the posterior cranial base cartilage throughout the period of shelf elevation, neither does the cranial base show any special 'kinks' (Fig. 6A and B). Likewise, there is no dramatic change in disposition of the cervical vertebrae during the period of shelf elevation (Fig. 6A and B). Indeed the only morphological differences detectable between day 16.3 and day 16.5 sections were the elevation of the palatal shelves, disappearance of the shelf epithelial grooves and the nasal septum-primary palate bulge, protrusion of the tongue tip and appearance of the common nasal passage. The appearances of the fetuses recovered from the control injected rats were identical with those of normal rat fetuses.

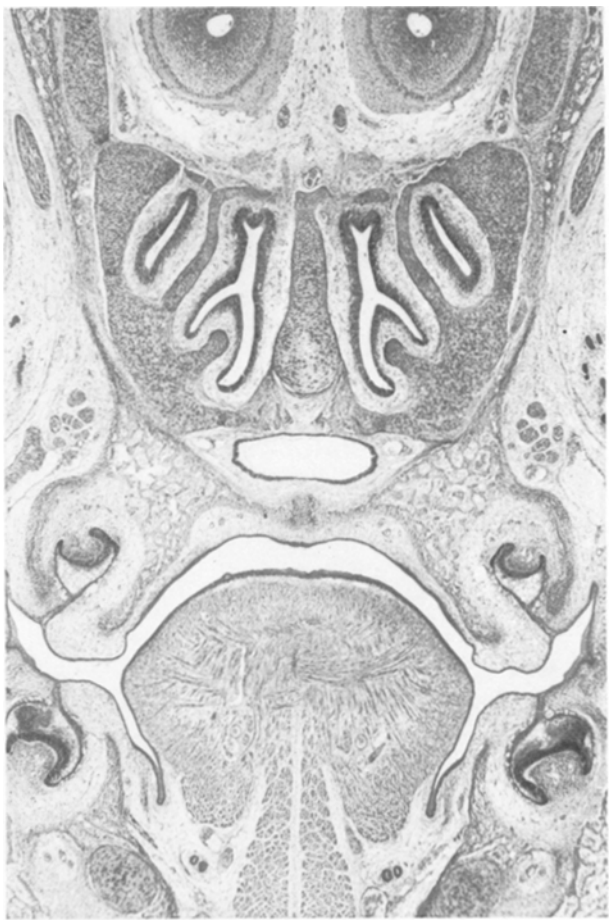


**Fig. 8.** Coronal section of a day 19 normal fetus illustrating the S.E.R., C.N.P. and ossification of the developing maxilla. H & E and Alcian Blue, ×27

*2. Experimental Observations on Fresh Normal Fetuses*

Experimentally, shelf elevation could be induced in freshly removed fetuses (immersed in normal saline to rule out possible surface tension phenomenon) by depression (with a blunt probe) or removal, of the tongue. At day 14 elevation was sluggish, taking about 5 s; at day 15 it took approximately 3 s, while at day 16.3 it occurred in less than one second. This rapid shelf elevation may be termed 'flip up'. Flip-up starts anteriorly, where the shelves show a marked convexity of their margins, and proceeds posteriorly. The shelves elevated most readily when the tongue was depressed at the level of these convexities. The macroscopic appearance of the day 16.3 palate which had been elevated experimentally, was identical to that seen after spontaneous shelf elevation in vivo (Fig. 3). Likewise, if the dorsum of the tongue was depressed in an intact day 16.3 fetus the tip of the flattened tongue protruded from between the lips as after in vivo shelf elevation. The inference is that changes in shape of the tongue are passive and secondary to shelf elevation.

The tongue could not be withdrawn from between the palatal shelves by depression of the mandible (as might occur if there were a mouth opening reflex) unless the latter was depressed so far that tears developed at the corners and in the floor of the mouth. Similarly, it was shown that it would be impossible for the tongue to descend below the palatal shelves and push them up to the horizontal position.

As 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, 1969) an experiment was undertaken in which a horizontal incision was made through the fetal head removing the top three-quarters of the nasal septum, with the brain and calvarium. The remainder of the nasal septum and cranial base were then carefully dissected from the lower part of the cut head, so that one was left looking down on the dorsum of the tongue. Again depression of the latter produced flip up. Indeed, if the mandible and tongue were now excised from beneath the palatal shelves in a day 16.3 fetus, and the shelves displaced (by a probe) to the vertical position, they quickly returned to the horizontal position and they never assumed any intermediate position. This manual displacement and return to the horizontal could be carried out 8–10 times in succession – in fact till the fragile shelf tissue broke. Furthermore, this repeated flip up could be attained in isolated slices of day 16.3 heads containing only the palatal shelves (attached to some lateral oral tissue) and the premaxillary region. Clearly there exists a palatal shelf force intrinsic to the shelves and not generated by external factors. This elevating force was still present in 'dead' palatal shelves of 16.3 day fetuses left unfixed in saline for 24 h, or fixed in Bouin's fluid for 1–2 h. However, after the lower portions of the heads of fresh unfixed 16.3 day fetuses had been incubated with a solution of hyaluronidase (1 mg/ml) for 10 h at 37°C, shelf elevation no longer occurred, although the tissue was rather 'mushy'. These observations, taken in conjunction with the observation that the rising shelf force demonstrated experimentally between days 14 and 16.3 parallels the mucopolysaccharide accumulation and increasing oedema, are consistent with the view that the shelf elevating force is derived from the binding of water to mucopolysaccharide.

### *3. Observations on Fetuses Recovered from F.U.D.R. Treated Rats*

In general, the F.U.D.R. fetuses were smaller than normal and developmentally retarded, exhibiting many signs of depressed mucopolysaccharide synthesis. However, about day 17 the effects of the drug appeared to wear off and the fetuses exhibited considerable catch up in development (Ferguson, 1976, 1977b). The palatal shelves (which did not make their appearance until day 15.8), did not elevate in vivo and at day 18 they became ossified while still in the vertical position. Histologically, the shelves showed adequate growth in length (in some cases if they had elevated they would have overlapped) but they did not stain for mucopolysaccharide, nor did they appear oedematous (Fig. 9). Indeed, the mesenchymal cells remained densely packed and were seen, ultrastructurally,

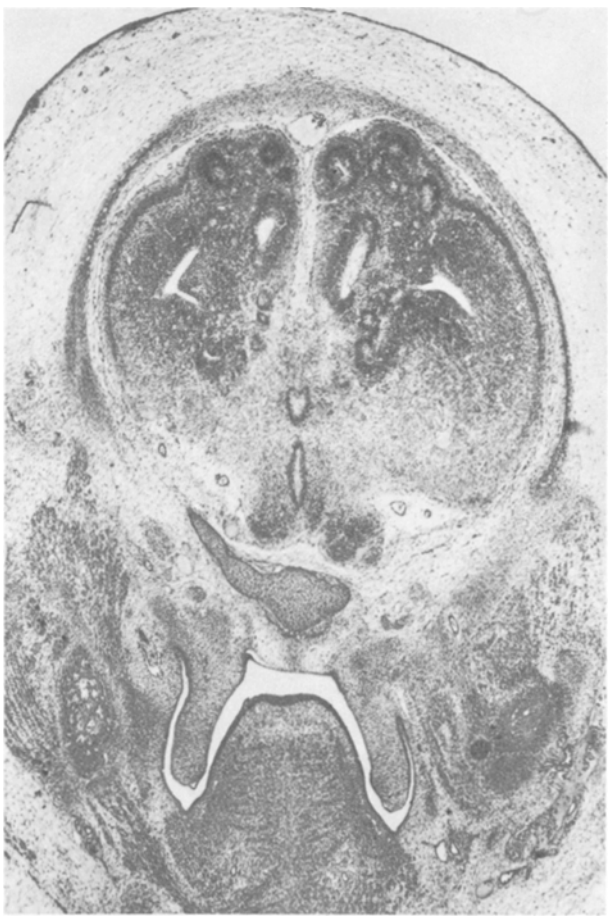
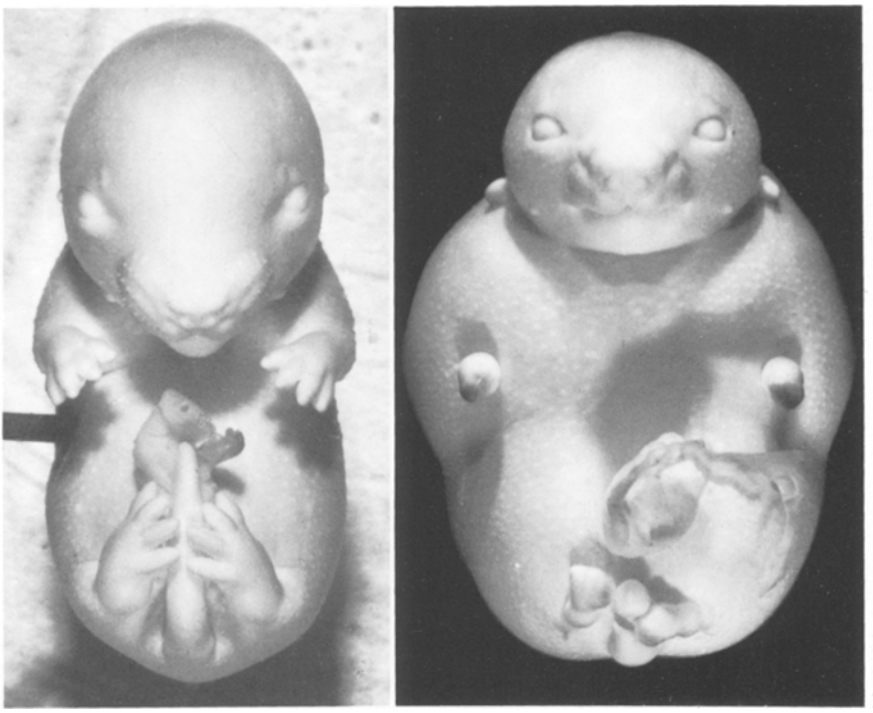


**Fig. 9.** Coronal section of a day 17.5 F.U.D.R. fetus. Note the long vertical palatal shelves and the abnormal brain. H & E and Alcian Blue, ×27

to contain numerous lipid-like deposits. In addition, epithelial undercutting of the shelves did not occur (Fig. 9) and the maxillary and palatine oesteogenic blastemata did not appear till day 17.8. At day 18 some fetuses showed fusion between the tips of the long, vertically orientated, posterior parts of the palatal shelves and the lateral walls of the oral cavity (palato-oral fusion): this involving epithelial fusion, breakdown of the resulting epithelial seam and development of mesenchymal continuity as in normal shelf-shelf fusion. Developing rugae were present on the vertical unelevated shelves. At day 16.5, the ventral end of the nasal septum fused with the the lateral nasal walls, as normally, but since the palatal shelves were unelevated, the common nasal passage and oral cavity remained undivided.

At day 14 there was a massive haemorrhage into the ventricles of the brain and the central canal of the spinal cord, the result of rupture of the internal carotid arteries in the region of the pituitary. From the absence of alcian blue staining in the expected locations of basement membranes it was inferred that blood vessel walls were weak and easily ruptured. Subsequent to this massive



10 11

**Fig. 10.** Macroscopic appearance of a day 17.5 normal fetus ×4

**Fig. 11.** Macroscopic appearance of a day 17.5 F.U.D.R. fetus, compare with Figure 10. Note the bloated appearance, phocomelia and small tail. ×4

neural disorganisation, the fetuses showed some remarkable attempts at neural regeneration (Fig. 9) from surviving neural epithelium.

Compared to the normal (Fig. 10), the day 17 F.U.D.R. fetuses were grossly bloated as a result of a massive subcutaneous infiltration with some unknown material and they showed haemorrhagic, phocomelic limb buds and tail (Fig. 11). This swelling had caused the fetuses to straighten out and both the head and cranial base cartilages were hyperextended (Fig. 11). The vertebral column was represented by a continuous cartilaginous rod, there being no signs of segmentation or disc formation.

Meckel's cartilages were virtually absent (apart from two 100 μm islands at the anterior and posterior ends of the mandible), yet the mandible showed both growth (although it was somewhat retrognathic) and ossification, but the inner ears were malformed.

### *4. Experimental Observations on Fetuses Recovered from F.U.D.R. Treated Rats*

Palatal shelf elevation could *not* be induced by any of the experimental methods outlined in section 2; it was clear that no intrinsic shelf elevating force was present.

## Discussion

On the basis of these observations the following theory 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 produces 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 reaches a threshold level and the elevating force becomes sufficient to overcome the resistance offered by the tongue, so enabling flip up to occur. The tongue is passively depressed, flattened, and its tip protruded out of the oral cavity, so making room for the common nasal passage. Other factors aid the transposition of the palatal shelves. Firstly, the undercutting of the underside of the shelf base by epithelium provides a fulcrum for shelf elevation. Secondly, maxillary and palatine osteogenic blastemata are present just exterior to the shelves and afford a firm base for flip up. The subsequent rapid invasion of the elevated shelves by these blastemata, and the ensuing ossification, soon consolidate the elevated palate.

This theory is in accordance with the fact that the anterior regions of the shelf elevate first both in vivo and experimentally, for these regions show the maximum mucopolysaccharide staining and oedema. In this study, it was estimated by histochemical means that hyaluronic acid constituted over 50% of the mucopolysaccharides present in the palatal shelves, and this estimate agrees with that of Pratt et al. (1973) who found (by biochemical analysis) that hyaluronic acid constituted over 60% of the mucopolysaccharides, the remainder being sulphated glycosaminoglycans. It is clear that $^{35}S$ labelling is of very limited use in following either the normal build up of mucopolysaccharides in the shelves or the difference in mucopolysaccharide content between cleft palate and normal shelves; as this label does not detect hyaluronic acid. While it is obvious that a certain level of head development is a sine qua non for shelf elevation, little evidence could be found to support many previous theories purporting to explain this event. Such theories have been critically analysed previously (Ferguson, 1976, 1977a). The fact which is most difficult to assimilate into many of these theories is the extreme rapidity of shelf elevation. The present theory of shelf elevation postulates a confrontation between shelf elevating force and tongue resistance, and so it is not surprising that depression of the tongue should lead to premature shelf elevation (even at 14 days). It follows that cleft palate is theoretically producible in at least two ways: (1) by decrease in shelf force (as in F.U.D.R. fetuses); (2) by increase in tongue resistance (as seems probable in Pierre Robin-like anomalies produced by amniocentesis and contraction of the fetal membranes – Poswillo, 1968a and b).

The other abnormalities found in the F.U.D.R. fetuses namely: (1) poorly formed or absent cartilages, (2) poor basement membrane synthesis and hence detached epithelia and rupture of blood vessels in the brain, spinal cord, limbs and tail, are also readily explained by defective mucopolysaccharide synthesis. These abnormalities have been discussed in more detail previously (Ferguson, 1976, 1977b). Cleft palate in man is frequently associated with abnormalities of the limbs, brain and vertebral bodies (Ross and Lindsay, 1965; Gorlin

et al., 1971; Gorlin and Cervenka, 1974) and one is tempted to speculate that such defects are likewise induced by defective mucopolysaccharide synthesis. In this connection it is of interest that a number of drugs which are known to induce cleft palate in the rat also depress mucopolysaccharide synthesis (Ferguson, 1976, 1977b) and such drugs have been incriminated (on epidemiological evidence) in the aetiology of human cleft palate (Saxen, 1975). From the evidence presented in this paper the avoidance, at least during the first twelve weeks of pregnancy, of drugs known to depress mucopolysaccharide synthesis is recommended.

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