

Meetings

Cleft Lip and Cleft Palate

Clefts of the primary and secondary palate are among the most important congenital malformations, judging by the burden of expense and unhappiness they impose on society. Yet evidence of the embryological mechanisms by which they occur is scattered, scanty, and conflicting. In view of this fact, a small group of workers, directly involved in research on palate formation and its failure, met in Denver 22–23 May 1967 to evaluate the current status of knowledge in the field. Two days were spent in comparing data, slides and opinions, and although no intellectual breakthroughs were achieved, or indeed anticipated, the resulting consolidation of knowledge may stimulate and orient further efforts in this area. In the following account, references will not be given to published work, but unpublished observations will be acknowledged by including the participant's name in parentheses.

The primary palate (lip and gum) is formed from the maxillary and nasal processes at an early stage of development when many important organogenetic processes are rapidly occurring. There are, as one might expect, differences among species in morphology of the early face. For instance, in the rat and mouse the central part of the upper lip is formed by the maxillary processes, which meet each other in the midline. In man it is formed by the medial nasal processes. Rat and mouse differ in the relative size and position of medial and lateral nasal processes. Yet the mouse, rat, and human embryos are remarkably similar in size when the face is forming, and basically the process of face formation appears to be the same in the mammalian species studied.

Evidence from amphibia and chick shows that the nasal placodes are induced in the presumptive face epithelium primarily by the parts of the forebrain that will become the olfactory lobes. The mesenchyme of the swellings, or processes, that form the early face are derived from neural crest cells

that migrate from the neural crest, above and below the optic cup, and by their proliferation raise the overlying ectoderm to form the processes. Extirpation of the neural crest on one side of the forebrain region frequently results in clefts of the primary palate (or beak, comparable to the mammalian lip) on the same side. Thus a cleft lip could be determined by interference with neural crest cell migration at an embryonic stage well before there was any sign of a face. What factors determine the routes of migration of the neural crest cells thus becomes a matter of some relevance. They appear to follow natural cleavage planes between mesoderm and ectoderm or endoderm (M. C. Johnston, University of Toronto). Much more study of this important process is required.

The appearance of a cleft lip requires a breakdown of tissue between the medial nasal, lateral nasal, and maxillary processes in the area where they meet (the isthmus). Maintenance of an intact isthmus depends on fusion between the epithelia just in front of it, at the posterior end of the nasal pit (that is, between the lateral surface of the medial nasal and the medial surface of lateral nasal and maxillary processes). Internally the fused epithelia form the nasal fin. In the mouse, at least, spontaneous lateral cleft lip results from partial or complete failure of fusion between these epithelia, with subsequent breakdown of the isthmus. Differences in shape of the embryonic face may alter susceptibility to cleft lip by changing the relation of medial nasal to lateral nasal and maxillary processes, thus making it more or less easy to maintain epithelial fusion (D. G. Trasler, McGill University). This concept is supported by the results of treatment during pregnancy with salicylate. In a strain of mice in which the medial nasal processes are set close together, and do not diverge widely towards the maxillaries, the treatment causes lateral cleft lip. However, in a strain with widely spaced, sharply di-

verging medial nasal processes a median cleft is produced.

Similarly, in the rat, maternal treatment with methyl salicylate, trypan blue, or 9-methyl pteroylglutamic acid, causes cleft lip associated with a failure of the normal extension laterally of the medial nasal process, and with a short maxillary process. It was not clear whether the latter was caused directly by the treatment, or was secondary to the defect in the medial nasal process. The same teratogens can also produce median clefts which apparently result from insufficient approximation of the medial processes (I. Monie, University of California Medical Center, San Francisco).

Although the conference was directed to the question of pathogenesis rather than etiology, some discussion of causal factors was inevitable. Cleft lip in both mouse and man shows a strong familial tendency, indicating that genetic factors are involved. However, there is no simple mode of inheritance, at least for the usual types of defect. Anomalies of tooth morphology in children with cleft lip, indicating early developmental interference, were proposed as evidence of a teratogen causing abnormalities not limited to the primary palate—possibly a virus (B. Kraus, University of Pittsburgh). Conceivably the dental anomalies could result from abnormal stresses resulting from the cleft in the primary palate. The question remains open.

In summary, formation of the primary palate and lip is a complicated process, involving the delicate integration of many processes, and can be disrupted in many ways. Interference with the origin, migration, or proliferation of neural crest cells could prevent successful formation of the facial processes. Alterations in the points of induction of the nasal pits, or in the paths of migration of the neural crest cells, could bring about changes in face shape that would make the embryo more, or less, able to achieve and maintain fusion of the processes. There could be interference directly with fusion of the processes, or formation of the nasal fin. Both genetic and environmental factors could impinge at various points of the system. More information is needed about the anatomical and biochemical events involved in formation of the face, both normal and abnormal. What determines the migration of the neural crest cells, the sites of the nasal placodes, and the topographical relations

of the facial processes? It may be that an approach to prevention may come from learning how to modify these prerequisites to successful lip formation rather than from studies directed at the stage when the defect is actually appearing.

The secondary palate closes well after the primary palate at a stage when most organogenetic processes are over. The palatal shelves, which will eventually form the roof of the mouth, first appear as downgrowths from the inferior surface of the maxilla. The shelves are posterior to the primary palate and on either side of the tongue, as if compressing it. There are differences among species in the dimensions and shape of tongue, jaws, and shelves, but the extent to which they reflect differences in the mechanism of palate closure is not known.

In order to close, the shelves must change their position from the vertical to the horizontal plane, which requires displacement of the intervening tongue. The process involves a complicated interaction of the shelf movement, tongue resistance, mandible growth, and head growth. All these factors need to be coordinated with one another and probably with many other factors to achieve successful closure.

At the beginning of closure the shelves are lying parallel to one another, with the tongue between them. Palatal movement begins posteriorly and proceeds anteriorly. A finger-like projection appears to extend backward at the posterior end of the shelf, and it is this projection that first moves in above the tongue. Possibly this is aided by changes in the contours of the posterior pharyngeal wall which may displace the back of the tongue downward sufficiently to allow the inward movement of the shelf projections (F. C. Fraser, McGill University). If the tongue is experimentally removed in the living embryo just before closure normally begins, the shelves rapidly move upward and medially toward the horizontal, even against gravity. Such experiments have sometimes been confounded by the effects of surface tension, but movement does occur when the shelves are completely immersed in fluid (B. E. Walker, University of Texas Medical Branch, Galveston; D. G. Trasler). Thus there is a force (shelf force) building up within the shelves which causes them to move against the resistance of the intervening tongue.

At the beginning of closure, the tip

of the tongue is lying behind the primary palate. It then moves forward, sliding down the sloping posterior surface of the primary palate and coming to lie under its inferior surface. There is evidence (mouse) that shelf movement begins before tongue displacement. It may be that the primary palate acts as an inclined plane, displacing the whole tongue downward as it moves forward, thus creating a space above the tongue into which the shelves can move (F. C. Fraser). Forward and downward movement of the tongue may be aided by forward growth of the mandible (I. Monie).

Reorientation of the shelf from vertical to horizontal proceeds postero-anteriorly until the whole shelf becomes horizontal. There was no consensus on whether this is brought about by a change in shape, with the medial wall of the shelf bulging into the space above the tongue and concurrent retraction of the ventral edge, or a 90-degree sweep of the shelf in "barn-door fashion." In the rat, at least, it may be the former at the back and the latter at the front. In some embryos fixed during closure, one shelf is horizontal and the other vertical with the tongue displaced toward the side of the horizontal shelf. Perhaps in the struggle of the shelves toward each other against the resisting tongue, it is easier for one shelf to move medially than for both to do so simultaneously. We do not know what proportion of embryos go through this stage but there is evidence that some do not (D. G. Trasler). After both shelves become horizontal, they become progressively more flattened. Their free edges approach one another to meet, at a point about one-third of the length of the shelf from the front. Epithelial fusion then spreads anteriorly and posteriorly, followed by breakdown of the apposed epithelia to give mesenchymal continuity. Formation of the soft palate occurs by merging, rather than fusion (man).

Little is known of the nature of the force that causes the shelves to move. Possibilities include mitotic growth changing the stresses on encircling epithelia, increase in volume of the mesenchyme or intercellular material, changes in vasculature, or a change in the physical properties of some supporting framework that gives the shelf its shape. There does not appear to be collagen in the shelves previous to closure, but there is rapid synthesis of acid muco-

polysaccharide. The most convincing model was a mechanical one based on a report that at the beginning of palate closure the cranial base is flexed in the area of the craniopharyngeal canal. As palate closure progresses there is a gradual reduction in flexure of the cranial base until, when the shelves are horizontal, the cranial base is straight. This can be represented by a piece of parafilm cut in the outline of a sagittal section of the vertical shelf, with a piece of string attached to the convex border to represent the cranial base. When the string is pulled, and the convexity of the base reduced to a straight line, the distal part of the model shelf will rotate to a plane perpendicular to the proximal part, much as the embryonic shelf appears to do (A. C. Verrusio, National Institute of Neurological Diseases and Blindness). The shelf force might therefore result from a straightening out of the shelf's attached edge, by longitudinal growth of the floor of the skull, or decrease in the angulation of the cranial base, or both. This mechanism would require a flexible framework in the shelf, that might possibly be formed by acid mucopolysaccharide.

The tongue, too, may play a role in shelf closure. Several questions need further investigation: Is the tongue displaced by the moving shelves, possibly aided by changes in contours of the pharyngeal wall, or by forward growth of the mandible and the inclined plane of the primary palate, or by sudden changes in degree of flexion of the head as it is in the rabbit (B. E. Walker), or by a combination of several of these? After the tongue comes to lie beneath the shelves, it may aid flattening of the shelves and hasten the meeting of their free edges (D. G. Trasler). However, in the rat, fusion does eventually occur in shelves explanted with their maxillary base, in the absence of the tongue (R. Gerstner, New York University). In the rabbit the tongue appears to be an important factor in shelf movement. The shelves do not move to the horizontal rapidly when the tongue is pulled out of the way experimentally, as they do in the mouse. There is evidence that shelf closure may depend on displacement of the tongue from between the shelves by sudden hyperextension of the neck and pressure on the shelves from below by the displaced tongue (B. E. Walker).

The anatomical differences between

species in the embryology of palate formation are small, but not negligible. If these observations are to have any relevance to man we need to know which, if any, of the anatomical differences reflect differences in the mechanism of closure.

Theoretically cleft palate could occur as the result of: delay in shelf movement, due to decreased shelf force, or increased tongue resistance, or decreased tongue pressure, or other obstruction to the shelf; an abnormal shelf, which may be too narrow, or pathologically incapable of movement; failure of shelf fusion; abnormal head width; or postclosure reopening. Many of these possibilities have been demonstrated, either through the use of teratogens or study of mutant genes.

Decrease in shelf force has been demonstrated *in vivo* following treatment with cortisone, hypervitaminosis A, and x-ray (B. E. Walker). Cortisone added to the medium *in vitro* delays movement of explanted shelves towards each other, when the palatal region is explanted at a stage before movement begins (R. Gerstner). The modes of action of such teratogens, and others that may act on the shelf force, are still unclear. Several teratogens (for example, cortisone, salicylates) decrease synthesis of acidic mucopolysaccharide, which is normally occurring rapidly in the shelves preceding closure [K.-S. Larsson, Karolinska Institute, Stockholm (published observation)]. A variety of antimetabolic agents (for example, x-ray, colchicine, 6-aminonicotinamide) may cause cleft palates. Changes in fluid content have been reported, but it is not clear whether these and other alterations in shelf metabolism are the cause or the result of interference with shelf movement. According to the hypothesis that lengthening of the shelf base is the basis for the shelf force, antimetabolic agents may act by delaying growth of the cranial base, and so might agents that interfere with cartilage growth, such as cortisone and 6-aminonicotinamide. There is need for further detailed study of the morphology, vasculature, and biochemistry of normal and teratogen-treated shelves before and at the normal time of closure.

Increased resistance of the tongue to shelf movement can occur in many ways. Overconstriction of the embryo, as from oligohydramnios (for example, following amniocentesis in the mouse) may result in the tongue being crammed

between the shelves. However, reduction in amniotic fluid volume appears not to be the precipitating cause of cleft palate following treatment with cortisone or 6-aminonicotinamide. Failure of forward movement of the tongue, due to micrognathia, may be the cause of the cleft palate in the Pierre Robin syndrome, though other explanations are possible. The cleft palate that frequently occurs with cleft lip appears to result from obstruction to forward movement of the tongue by the unduly large median process present in embryos with cleft lip. Theoretically, an unduly large tongue could increase tongue resistance and delay movement. However, no examples of this were cited. Paradoxically an unusually small tongue may get cupped between the shelves so firmly that it is difficult to dislodge, as in the recessive "shorthair" mutation in the mouse. A small tongue could also delay the shelf flattening and approach to the midline that occurs after the shelves have moved above the tongue.

If displacement of the tongue depends on hyperextension of the head (for example, in the rabbit), cleft palate could result from interference with this mechanism (B. E. Walker) by cervical, vertebral deformities as in cleft palate induced by chlorcyclizine (A. J. Steffek, National Institute of Dental Research) or in the Klippel-Feil syndrome; by oligohydramnios; or by lack of muscle tone as in the "fetal muscle degeneration" mutation of the mouse.

Whether active movement of the tongue may aid the shelves to wriggle their way in above it is an open question. The tongue responds to artificial stimulus at the time of shelf closure, but it is not known whether the myoneural mechanism for movement is functional at this time. Obstruction to shelf movement by structures other than the tongue, such as an anterior encephalocoele, is another possible cause of cleft palate which is observed rarely in the mouse (D. G. Trasler) and man.

Changes in the interrelation of shelf and tongue may lead to more-or-less delay in shelf movement to the horizontal plane. If growth of the head continues there will be a particular stage in development beyond which, if the shelves come up, they will no longer be able to meet, and a cleft palate will result. The variability in stage of shelf movement can be thought of as a continuously distributed varia-

ble, and the point beyond which they cannot meet as a threshold separating normality from abnormality. In this sense cleft palate is a threshold character.

A number of examples are known of shelves that are too abnormal to permit closure. In the urogenital mutant of the mouse the shelves are too narrow. The "phocomelic" mouse mutant has shelves with abnormal cartilaginous bars which appear to interfere with movement. Hypervitaminosis A results in hypoplastic palate shelves with abnormal cartilage and bone formation in the rat. Chlorcyclizine and related compounds result in a fusion of the tongue with the vertically oriented palatal shelves. However, in this case the glossopalatine fusion is probably not the cause of the cleft. Palatal clefts have also been induced by chlorcyclizine in a nonrodent species, the ferret (A. J. Steffek).

Although failure of fusion of the horizontal shelves is clearly not the mechanism by which some cleft palates occur, it is certainly a possibility for others. In culture, studies of fusion (that is, adherence of the apposed epithelia to form a septum, which is subsequently ruptured so that the mesenchyme of the two shelves is continuous) show that cell adhesiveness increases at the free edges of the shelves immediately before shelf elevation. Experiments involving dissociation and recombination of cell layers indicate that this "zone of adhesiveness" appears in the epithelium as the result of an induction by the underlying mesenchyme (M. Pourtois, University of Pittsburgh). The mesenchyme may also influence the rupture of the epithelial wall. Vitamin A in excess in the medium causes the mesenchyme to separate from the epithelium. Under these conditions the shelves could still achieve epithelial fusion but not the subsequent breakdown of the epithelia. Prevention of epithelial breakdown was also observed in the presence of cortisone, which had a cytotoxic effect on the mesenchyme (M. Pourtois and V. Vargas, University of Pittsburgh). In the mouse, palates have been observed at term in which the shelves are in contact but not fused. These may result from abnormal head growth leading to the shelves meeting at abnormal angles. Thus, the epithelia in contact are not competent to fuse (D. G. Trasler).

Postclosure reopening, possibly as a result of viral infection (B. Kraus), is

another possible origin of cleft palates. The existence of epithelial pearls in the line of fusion of the human palate (though not in lower mammals) and their presence in the shelves of a human fetus with cleft palate could be interpreted as evidence of midline breakdown of the fused palate. However, further evidence is needed.

Finally, the shelves may move to the horizontal at the right time, but fail to meet because the head is too wide. No proven case of this situation was cited. Possible examples may be the cleft palate following treatment with folic acid antagonists (D. G. Trasler) or associated with hypertelorism in man.

In summary, palate closure is a complicated process involving synchronized interactions of shelf, tongue, jaw, and head. Major interference at various points of the system either by specific environmental agents or by mutant genes can bring about failure to close. Study of these examples as well as further study of the normal embryology of closure may lead to further understanding of the process. It is likely that many "spontaneous" clefts in man may result from interaction of a large number of minor genetic and environmental factors which together cause delay in movement of the shelves to a point at which, when they do come up, they are too far apart to meet. Efforts to reduce the frequency of cleft palate in man should be directed not only to a better understanding of the process of closure and to the identification of specific environmental teratogens, but also to learning how to promote early shelf closure, thus reducing the probability that the embryo will reach the threshold of abnormality (F. C. Fraser). Much remains to be done.

This workshop was convened by the Oral-Facial Growth and Development Program, Extramural Programs, National Institute of Dental Research (NIDR), Bethesda.

In attendance were: F. C. Fraser, McGill University, Montreal (chairman); R. Gerstner, New York University; M. C. Johnson, University of Toronto; B. Kraus, University of Pittsburgh (co-chairman); K.-S. Larsson, Karolinska Institute, Stockholm; I. Monie, University of California Medical Center, San Francisco; M. Pourtois and V. Vargas, University of Pittsburgh; A. J. Steffek, NIDR; D. G. Trasler, McGill University; A. C. Verusio, National Institute of Neurological

Diseases and Blindness; and B. E. Walker, University of Texas Medical Branch, Galveston. K. Hisaoka and R. C. Greulich (NIDR) attended as observers.

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Mammalian Oviduct: International Symposium

Progress has been made within the last decade in the biology of the oviduct. Such progress resulted from modern techniques and instrumentation in microanatomy, neurophysiology, genetics, endocrinology, biochemistry, pharmacology, and biophysics. In order to familiarize investigators in biological and health sciences with the application of techniques of these disciplines and the results to date, an international conference on the Mammalian Oviduct was held at Washington State University, Pullman, 31 July-4 August 1967. Participants were from England, the Netherlands, Puerto Rico, Scotland, Sweden, and the United States.

Oviductal organogenesis in many mammalian species is strikingly similar. During late fetal development, however, structural differences among species begin to become apparent. In *in vitro* studies, Dorothy Price and her co-workers showed that the fetal oviduct of the guinea pig fails to respond to testicular and adrenogenic hormones, and early morphogenesis does not depend upon specific hormonal stimulation.

The development of Müllerian ducts during fetal life is not dependent on maternal, placental, or fetal hormones for maintenance and early development. The oviductal segment of the primitive Müllerian duct can show much hormone-independent growth, coiling, and epithelial differentiation. The stage of development of the oviduct of the neonate at birth suggests prenatal hormone influence during late fetal life. The fetal ovaries and adrenals, which become metabolically active, may stimulate the fetal oviduct.

Improved methods of fixation for light and electron microscopic studies involve perfusion techniques for rapid fixation in 2.5 percent solution of glutaraldehyde in Sørensen phosphate buffer pH 7.4. *In situ* preservation is essen-

tial to establish the width of oviductal lumen or to evaluate the anatomical relationship between the oviductal epithelium and the developing embryo. Certain cyclical changes in the structure of the oviduct may or may not relate to function, but in some instances, such changes promise insight into some basic biologic processes. The elaborate lymphatic system of the oviduct seems to respond to steroid hormones. There is an increasing awareness that the uterus, and perhaps even the oviduct, plays an important role in the control of ovarian function. Both luteolytic and luteotrophic effects of uteri and of uterine contents (IUD or embryos) have been recorded. These effects are frequently local. Sometimes they do not seem to be mediated systemically. The possible local effect may be resolved by more thorough studies of the oviducto-uterine vascular, lymphatic, and nervous systems.

Histochemical techniques have been used to study the distribution of metabolites and enzymes, and the metabolic patterns of different regions of the oviduct. Man has more glycogen in the inner circular muscle than in the outer longitudinal layer of the oviduct. The amount of glycogen shows cyclical changes, being low postmenstrually, rather high postovulatory, and particularly high during pregnancy and in the early puerperium. These variations are very similar to those in the human myometrium. Succinic dehydrogenase, DPN-diaphorase, amylophosphorylase, and other enzymes have been demonstrated in oviductal muscle but evidence of a correlation to the sexual cycle is lacking. Histochemical techniques have been used to study the nervous regulation of the oviductal musculature.

In the rhesus monkey, ciliogenesis occurs in the oviduct during the normal menstrual cycle, particularly in the fimbriae. Deciliation of the fimbriae in the luteal phase depends on the presence of a functional corpus luteum. The ampulla shows no such dramatic deciliation. R. M. Brenner showed that after ovariectomy, the ampulla loses most of its cilia. Thus, the real difference between the fimbriae and ampulla is one of degree of responsiveness to changes in blood levels of estrogen and progesterone. Ovariectomy in the rabbit does not cause deciliation of the oviduct. Comparative studies on the uptake of micromolecules during ciliogenesis may use elec-