Section I. Epidemiology, Etiology, and Pathogenesis of Cleft Lip and Palate

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Cleft lip and palate have probably been the target of more research on developmental causes and mechanisms than have most other congenital malformations. A blend of experimental, epidemiological, and genetic studies continue to generate a variety of hypotheses on the underlying causes of these orofacial cleft types. Yet, actual primary cause-and-effect relations still remain elusive to most investigators. Recent reviews (*Teratology*, Vol. 6, 1972; *The Cleft Palate Journal*, Vol. 10, 1973) continue to emphasize the need for more data on the morphogenic phases of palatal closure coupled with similar events in the consolidation of early facial primordia leading to the lip and surrounding facial areas.

Although detailed observations are available on a variety of events ranging from palatal shelf "horizontalization" to the nature of contractile elements within the shelf substance during the critical phases of closure, we still need to reconcile other factors (e.g., *in vivo* vs. *in vitro* experimental differences, intra- and interspecies polymorphisms) before specific cause-and-effect mechanisms can be isolated, for understanding either normal palatogenesis or the variety of clinical anomalads whose patterns include clefts of the lip and palate. While these earlier reviews of the etiologies and pathogenesis of facial clefts have not brought out clearcut causal answers, they have made it clear that an understanding of the early beginnings of most orofacial clefts, including those of the lip and palate, will call for a two-fold attack emphasizing both population data and early (prenatal) morphogenic aspects of these facial regions as seen in both human and related sub-human animal models.

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A. Epidemiological Advances

One of the most catalytic hypotheses in this area of facial clefting (Kalter, 1954; Fraser, et al., 1957) proposes that a majority of spontaneously occurring malformations, including clefts of the lip and palate, result from an interplay between many genetic and environmental factors rather than a single factor (genetic and/or environmental). Only the effect of genetic factors have so far been shown in humans.

While the identification of a specific gene or chromosome responsible for palate development in humans is still a goal to most investigators interested in the genetics of orofacial development, most advances have capitalized upon contrived or natural defects. The association between cleft lip and cleft palate and D1 trisomy has at least set forth an association factor between clefting and abnormal chromosome arrangements. Specifically, Loevy and coworkers (1975) have shown that cleft lip and palate in regular and translocation trisomy 13 are similar in incidence and severity but that the incidence and severity of these defects are lower in cases of mosaic and partial trisomy 13. Recent analyses of two major racial groups, caucasoid and oriental, showed no positive relationship between the various demographic factors and the risk of CP and CL (P) (Ching and Chung, 1974). This corroborates the earlier finding on the association between paternal age and the incidence of the same lip and palate clefts (Perry, et al., 1972). Clefts of the lip and palate have also been examined (Hay and Barbano, 1972), along

with a variety of other congenital malformations, with regard to maternal age and birth order; but no convincing evidence has been shown for birth-order effects independent of parental age. More specifically, these authors found a higher incidence among older mothers of all clefts except the isolated cleft lip. However, it was also shown that, when the isolated CL was associated with other malformations, a marked relation to parental age emerged.

The study of interracial crosses in Hawaii as related to orofacial clefting (Chung, et al., 1974) has also shown in a large sample of ascertained data, for example, that the caucasian incidence in oral clefts among Hawaiians is about the median value among other large caucasian samples keying on the same defects of the lip and palate (Leck, 1972). Work still continues on the epidemiologic associations between cleft incidences and such parameters as birth incidence, sex types, location and severity of clefting, maternal age and birth order, season of birth, and geographical distribution. Chi (1974) reports in his New South Wales hospital study, for example, that the incidence of cleft lip and palate births was 1.21 per thousand live births and that no significant differences were observed between the sexes for the isolated cleft palate. Chi also shows that unilateral clefts of the lip were more prevalent on the left side which corroborates the similar finding by Zilberman (1973). In addition to not finding a relationship between season and CP and CL (P), Chi has clearly shown that there is a higher relationship between the isolated cleft palate and other body malformations than exists between CL (P) and associated defects. In terms of the often more-difficult to identify submucous cleft of the palate, it has been reported (Stewart, et al., 1972) that the submucous cleft has a 1:1200 incidence among children in large urban cities.

Continuing now with a consideration of the environmental component of the multifactorial model which has been used to explain the conditions (but not the definitive mechanisms) leading to the CL and CL (P), it still appears that few hard data have been reported since mid-1972 concerning the environmental factors in the etiology of human oral clefts. In population studies, several associations have been suggested but none of these have been firmly confirmed. For example, associations have been made between orofacial clefts and maternal consumption of therapeutic drugs which produced nausea (Richards, 1972) and those anti-convulsant drugs administered to epileptic mothers (South, 1972). Furthermore, short-term increases in the incidence of cleft palate as well as various seasonal trends in the incidences of clefting have also been reported (Oakley and Flynt, 1973) which provides some evidence for the impact of the environment on clefting.

In a recent epidemiologic and experimental study of 599 cases of oral clefts on the etiologic variables in cleft lip and palate (Saxen, 1975a) the concept of the classical multifactorial model in the production of mammalian oral clefts was generally supported. This detailed investigation not only corborated the earlier pioneer finding between the etiologies of CP and CL (P) but also confirmed that only CP incidences were related to geographical variations in the Finnish sample (Saxen and Lahti, 1974). Similarly, Saxen also demonstrated that threatened abortion was associated only with CL (P), and the association of therapeutic drugs throughout the pregnancy (1975b) as well as exposure to the influenza viruses seemed more closely associated with CL (P) than with cleft palate alone. These cumulative studies by Saxen have done much to show that genetic factors may be more involved with CP in controlling susceptibilities, whereas environmental agents appear to be the more important variables in identifying the levels of response in CL (P) production.

Once having been afflicted with cleft palate, it seems reasonable to ask how cleft palate subjects compare intrafamilially in terms of educational, occupational, and marital status. As compared with their parents, nearest-age siblings, and married nearest-age siblings, it was found (McWilliams and Paradise, 1973) that there were no overall differences in educational achievement between cleft palate individ-

uals and their non-cleft siblings. This study also showed, however, that clefted individuals dropped from secondary schools at a higher rate than their non-cleft sibs and that a significantly larger number of cleft palate patients remained single.

B. Experimental Studies on Cleft Mechanisms and Causes

1. Morphogenic studies on normal mechanisms operable in human and other mammalian animals

Although controversy still exists on the mechanism or mechanisms which bring the palatal shelves into a horizontal position prior to their fusion, it appears that more significant inroads have been made in understanding what happens during the contact and fusion phases of palatal closure. Major emphasis has been placed on palatal development with the expectation that, while the details of palate and lip consolidation may well differ, the underlying developmental principles and mechanisms may be common to both anatomic regions.

It is important to ask first what is happening at surfaces of the palatal shelves before and during fusion, since failure of complete approximation and fusion of the shelves has been implicated as a cause of cleft palate. Pourtois (1970) showed that adhesiveness of the rat palatal shelves *in vitro* depends upon increased levels of a glycoprotein surface coating of the shelves resulting in shelf-to-shelf binding. In a following study, Mato and co-workers (1972a) have shown in human tissues that the plasma membrane of human superficial epithelial cells increased in density with a "specific coating" observed immediately before contact of the shelves. Later reports also confirm that there is a carbohydrate-rich coating present along the medial or leading edges of the shelves prior to fusion in the rat (Pratt, et al., 1973) and rabbit (DePaola, et al., 1975). This build-up of cohesive substances has also been demonstrated in organ culture in which high levels of glycoproteins (DePaola, 1974) and mucopolysaccharides (Pratt, et al., 1973) are shown in palates prior to fusion. It has subsequently been demonstrated in culture studies of rabbit palatal fusion that there is an increase in glycoprotein synthesis in the rabbit palate *in vitro* at a time commensurate with the onset of palatal shelf contact and adherence (DePaola, et al., 1975b).

Not all the attention has been focused on palatal shelf surface phenomena in terms of adhesive surface coatings. A histochemical and electron microscopic study of comparably-aged rodent and human fetuses showed increased lysosomal activity and derangement of epithelial cells along the leading edges of the palatal shelves prior to contact and prior to fusion between shelves and between shelves and nasal septum (Mato, et al., 1972b). These marked changes in surface epithelium prior to any contact between shelves has furthered the concept that presumptive epithelial fusion areas of the palate are "programmed" for a series of changes which precede contact of the shelves. This has been corroborated in a series of *in vitro* studies (Smiley and Koch, 1972) which demonstrated epithelial breakdown and increased lysosomal activity in single and isolated palatal shelves. This concept of epithelial "programming" prior to shelf contact and fusion has elicited still other *in vitro* studies which show that the triggering of epithelial breakdown prior to shelf fusion is dependent upon a definite surface contact between palatal primordia (Goss and Avery, 1975).

These studies also recognized that any differences may well be reconciled by basic differences in animal types, animal strains, and perhaps most importantly, basic differences in the experimental techniques used (e.g. *in vitro* and *in vivo* techniques). For example, Smiley and Koch (1975) have clearly shown that it is possible to vary the biological responses of palatal processes *in vitro* by varying the nutrient and physical substrate of the tissue, and even by altering the position of the palatal processes with regard to a gas-fluid interface. In other words, the conditions in which the palatal tissues, or any tissue or cells, are grown must be carefully defined, i.e., not all *in vitro* studies are alike. In one of the few *in vitro* studies of palatal development using human tissues, it has been shown

that the same techniques employed in animal models can be used for man, especially as needed for *in vitro* stimulation of shelf fusion, epithelial pearl formation, and the sequelae of post-fusion ruptures of the palate (Goss, 1975).

Without regard to whether palatal epithelial changes are intrinsically determined or not but recognizing that they do occur, several studies to explain palatal fusion using evidence from the scanning electron microscope (SEM) have been reported (Shapiro and Hutchinson, 1972; Waterman, 1972). These studies have laid the ground work for the clearcut SEM demonstration in A/Jax mice that epithelial changes are localized to presumptive contact and fusion areas of the palatal shelves and are characterized by (1) a loss of distinct cellular boundaries, (2) the appearance of intercellular gaps, and (3) a progressive accumulation of a filamentous material on the shelf surfaces which are destined to fuse (Waterman, et al., 1973). Although these data must be used guardedly in explaining cleft production, the data do imply that failure of these epithelial changes to occur, for whatever reason, can lead to clefts of the palate and, perhaps, of the lip.

Another area of recent interest is concerned with the spatial arrangement of structures in the oral cavity at the time of palatal closure. The often made observation that palatal clefts may be produced by a limitation of oral cavity space which impedes the downward displacement of the tongue (Ross and Lindsay, 1965; Poswillo, 1966; Nanda, 1970) prompted a study of coronal head sections of mouse embryos that showed that tongue lowering and the movement upward of palatal shelves into the space previously occupied by the tongue were events secondary to the medial movements of the oral cavity walls which initiates shelf horizontalization (Wragg, et al., 1972). In a similar study of oral cavity space based on clefts produced in cortisone-treated mouse fetuses, it was shown that, if shelf movement upward is delayed, the intershelf cleft is not increased by continued growth in width of the head (Green and Kochar, 1973). These investigators concluded that cortisone, can cause a delay in shelf movement and may also interfere with events that occur subsequent to shelf horizontalization leading to shelf fusion. In yet another look at the capacity of the tongue as an active component in shelf closure, Wragg and coworkers (1972) have shown that the fetal rat myoneural apparatus is indeed functional at the time of palatal closure so that it would not be necessary for jaw drop or intrinsic shelf force to remove the tongue from between the separated palatal shelves.

2. Teratological studies on the mechanisms of cleft palate formation

While the missions of the many current efforts to explain normal and abnormal dentofacial development are targetted toward man, the obvious limitation on use of the human embryo to explain human embryogenesis has driven investigators toward appropriate animal types whose early dentofacial, e.g., palate, development is thought to be in compliance with the multifactorial model and its genetic-environment interplay. Knowing that the similarities in morphogenesis between various mammalian types are closest during comparable early embryonic times, investigators then have some justification for identifying mechanisms of dentofacial development in mammalian embryos and then projecting these findings to the human situation. Question still remains on the actual similarities in development between the mammals. In any event, studies in mammalian palatal development since mid-1972 continue to have a teratologic flavor in which normal palatal fusion is disrupted using teratogens such as lithium (Loevy, 1973), tranquilizers (Walker and Patterson, 1974) and cortisone in mice (Hackman and Brown, 1972; Spain, et al., 1975) and hamsters (Shah and Travill, 1976).

While the preceding are *in vivo* approaches to palatal teratogenesis, *in vitro* studies combined with the use of cortisone (Saxen, 1973) and salicylates (Saxen, 1975) have shown again that certain teratogens have the capacity to delay closure time without, however, pinpointing whether

the teratogen action is during or after shelf horizontalization. The underlying rationale for the variety of teratologic approaches to understanding palatal development is that if a suspected mechanism of normal development can be interfered with under controlled experimental conditions, then inferences can be made with regard to the importance of that interfered-with system in normal morphogenesis.

Knowing that normal palate formation in man and related animal types is contingent initially upon such events as actual shelf formation and the acquisition of proper size, recent emphasis continues to be on the events of shelf horizontalization, intershelf contact, and coelescence. Specifically, what changes are occurring within the shelves during their progressive horizontalization? Based on a previous finding that the total amount of collagen in the rat embryo, and specifically the secondary palate, increases exponentially during the time of palatal closure (Pratt and King, 1971), it has been shown that the lathyrogenic compound B-aminopropionitril (BAPN) will induce cleft palate in the rat and mouse when administered at the critical times of palatal closure. Specifically, horizontalization and closure of the palate are associated with increases in cross-linked collagen within the shelves whereas a dramatic decrease in cross-linking is found in BAPN induced cleft palates (Pratt and King, 1972; Steffek et al., 1972). Still unanswered is the actual mechanism by which the lathyrogen BAPN inhibits cross-linking in the embryonic palatal cleft. Does the reduced cross-linked collagen affect horizontalization and/or fusion of the shelves? Mato and Uchiyama (1972) stress the importance of ultrastructural changes of the leading-edge epithelium at the stage of shelf contact. It has been subsequently shown (Mato, et al., 1975) that BAPN retards or completely suppresses the "programmed cell death" of abutting shelf epithelium which must precede intershelf bridging by mesenchymal cells. As an extension of this 1975 study, it was also shown that cross-linked collagen fibers did not develop adjacent to the basal lamina. This led to the conclusion that BAPN decreased the "connection capacity" between embryonic mesenchyme and epithelium resulting in palatal clefting. Although no definite conclusions can be made regarding the pathogenesis of BAPN-induced cleft palate, it does appear that the regressive changes in the leading-edge palatal epithelium prior to fusion do not occur, even if the shelves are in contact. It appears that a teratogen, like BAPN, can produce a cleft, but the actual primary mechanisms of action remain clouded.

Another recent attempt to demonstrate the relationship between induced cleft palate following teratogen administration has supported a key teratologic principle that there is a synergistic effect resulting in higher incidences of mouse CP when both pyridoxine-deprived diets are coupled with cortisone (Miller, 1972). It is this synergistic action of teratogens, along with teratogen latency, which is of prime importance to the contemporary teratologist, regardless of whether or not the palate is the specific experimental target system.

Teratogens have been used to demonstrate key events and structures involved in palatogenesis and to show alterations in structures contiguous with the palate. For example, what is the role of the embryonic chondrocranium, including the cranial base and nasal capsular cartilages, in the formation of the palate? While there is mixed evidence as to whether deviations in the normal morphology of the chondrocranium is primary to palatal clefting or a mere association in time, it has been shown that, at least for the relationship between mouse and nasal septum development and normal palate formation, there is no difference in chondrocranial morphology in a comparison of cortisone-induced CP mice and normals (Sakizlioglu, et al., 1974).

The cranial base portion of the chondrocranium has not gone unnoticed with regard to its possible role in normal development of the palate. Using earlier reported hypotheses that cranial base shape or behavior has a direct bearing on palate closure or lack thereof (Harris, 1967; Verrusio, 1970), it has been suggested that in-

creased activity of labelled cells in the presphenoid region of the mouse cranial base, at times immediately prior to and during palate closure, coincided with a straightening of a "kinked" presphenoid segment. This report (Long, et al., 1973) hypothesized that teratogens which produced clefts in the A/J mouse also inhibited base straightening that is essential for the upward movement and contact of the palatal shelves. Other factors besides induced and spontaneous alterations in the craniofacial skeleton have also received attention. Diewert (1976) demonstrated a positive correlation between alterations in palatal blood vessels and palatal clefts induced in rat fetuses with such teratogens as cortisone, vitamin A, and 6-amino-nicotinamide. Yet, the author emphasizes what to many is quite apparent, i.e., morphologic associations in time may not necessarily be cause-and-effect relationships.

Obviously, the use of teratogens in identifying mechanisms is important, but the assignment of the cause-and-effect relationship must be made with extreme caution. This is underscored by recent reports that even the rather customary and benign air shipment of mice to an investigative laboratory can enhance the clefting incidence in CP teratologic studies (Meskin and Shapiro, 1971; Brown, Johnston and Niswander, 1972).

Conclusions

It is clear that identifiable cause-and-effect relationships in the onset of cleft lip and cleft palate still remain a goal for most investigators. Advances, both epidemiologic and experimental, have indeed been made in associating altered events during critical periods of palatogenesis with clefts. Yet, primary mechanisms and primary loci of deviant growth still remain refractory even with contemporary technology ranging from electron microscopy, immunoassay methods, and radioautography. While the common goal is the better understanding of human malformations from both a developmental and treatment perspective, it is obvious that the thrust of the many investigative efforts on mechanisms pivots around appropriate animal models and investigative protocols, e.g., teratogens, environmental parameters, which are not far removed from the human.

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