A short review of the normal embryonic development of the facial primordia is necessary before reviewing the factors that may interfere with this development leading to clefts of the lip and the palate.

In the developing embryo, migration of cell masses, fusion of facial processes and the differentiation of tissues are three important events that lead to an adult appearance. There is a certain pattern of development, but cells also respond to environmental signals. Since both factors are present and interact, it is difficult to ascertain the exact role of each of them.

The facial primordia (a series of small buds of tissue that forms around the primitive mouth) are made up mainly of neural crest cells that originate from the cranial crest (rev by Ferguson, 1988). Neural crest cells migrate to the primitive oral cavity where, in association with ectodermal cells, they form the maxillary processes. Palatal shelves from these processes are evident at embryonic day 45 in humans. An intrinsic force, mainly produced by the accumulation and hydration of hyaluronic acid-1, is progressively generated within the palatal shelves and reaches a threshold level which exceeds the force of resistance factors (e.g. tongue). Synthesis and hydration of hyaluronic acid by palatal mesenchyme is stimulated by epidermal growth factor and transforming growth factor beta. The erectile shelf elevating force is partly directed by bundles of type I collagen which run down the center of the vertical shelf from its base to its tip. Moreover, the epithelial covering and associated basement membrane of the palatal shelf exhibit differential traction, which serve to constrain and direct the swelling osmotic force. Al-
so the palatal mesenchymal cells are themselves contractile and secrete various neurotransmitters that effect both mesenchymal cell contractility and glycosaminoglycan dehydration and therefore play a role in palate morphogenesis [1].

At this precise developmental stage, the shelves rapidly elevate to a horizontal position above the dorsum of the tongue. Self elevation probably occurs within minutes or hours. The medial edge epithelia of the approximating palatal shelves fuse with each other, developing cell adhesion molecules and desmosomes to form a midline epithelial seam. The epithelial seam starts to thin by expansion in palatal height and epithelial cell migration onto the oral and nasal aspects of the palate [1] and then degenerates, establishing mesenchyme continuity across the intact horizontal palate. Medial edge epithelial cells cease DNA synthesis 24–36 hrs prior to shelf contact and this is referred to as programmed cell death (PCD). The basement membrane on each side of the epithelial seam remains intact even when it has completely thinned. Epithelial-mesenchymal recombination experiments have demonstrated that epithelial differentiation is specified by the mesenchyme and that medial edge epithelial cell death is rather a “murder” by the underlying mesenchyme than an intrinsic epithelial suicide (rev by Ferguson, 1988). The ways in which mesenchyme could signal epithelial differentiation is either through extracellular matrix molecules (i.e. collagen molecules), through soluble factors (i.e. growth factors), direct cell-to-cell contact, or a combinations of all the above. The actual period of fusion of the mesenchymal shelves may be just a matter of minutes, but complications in events leading up to and during fusion will result in a palatal clefting of varying severity.

 Also, seam disruption occurs by migration of a large number of epithelial seam cells (perhaps 50%) into the palatal mesenchyme (rev by Ferguson, 1988). These fragments very quickly become indistinguishable from other palatal mesenchyme cells. The epithelia on the nasal aspect of the palate differentiate into pseudo-stratified ciliated columnar cells while on the oral aspect of the palate they differentiate into stratified squamous non-keratinized cells. Osteogenic blastemata for the palatal processes of the maxillary and palatine bones differentiate in the mesenchyme of the hard palate while several myogenic blastemata develop in the soft palate.

During the period of shelf elevation, there is almost no growth in head width but constant growth in head height. This establishes a conductive orofacial environment that permits the expanding palatal shelves to occupy a position above the dorsum of the tongue.

In human embryos, palatal shelves elevate simultaneously on day 43 (22–24 mm CRL), and the palate is closed by day 55 (33–37 mm CRL). Mesenchymal fusion is complete by day 60 (45–46 mm CRL).

**Pathogenesis of CL and CP**

In studying different types of orofacial malformations, animal specimens have been proved to be especially helpful because they permit observation of embryological and fetal stages that lead to malformations found at birth.

The majority of congenital craniofacial malformations occur during the first 5–12 weeks of fetal development [2]. The embryonic period (from 3–9 weeks) is the most sensitive period during which teratogens can be particularly damaging. This is especially true for midline morphologic disorders such as cleft lip and palate. They are considered to be a polygenic multifactorial problem in which genetic susceptibility is influenced by multiple and probably cumulative environmental factors, interacting altogether to shift the complex process of morphogenesis of the primary and secondary palates, toward a threshold of abnormality at which clefting may occur (multifactorial/threshold model). Both the genetic and the environmental factors have not yet been established.

Cell death is a normal phenomenon seen in the developing embryo (PCD). It is also a common feature seen in embryos after exposure to a variety of teratogens that induce craniofacial malformations. There are three distinct types of PCD (rev by Sulik, 1988). Type 1 is characterized by cellular condensation, fragmentation, phagocytosis and finally lysosomal degradation. Type 2 is characterized primarily by the appearance of large lysosomes which initiate cellular degradation. Type 3 occurs without the involvement of lysosomes and without apparent phagocytosis.

The sites of cell death vary depending upon the teratogen (or genetic insult) and the exposure time (i.e. developmental stage of the embryo). There seems to be a selective sensitivity of cells; tissues with high proliferative activity are more likely to show cell death than tissues that proliferate more slowly. Other factors may also be involved, i.e. the state of cellular differentiation, differential drug distribution or other specific cellular characteristics. Both the disappearance and expansion of areas of PCD may have a role in teratogenesis (rev by Sulik, 1988).

Pathogenesis is probably caused by one of the following mechanisms:

1) Anatomic obstruction, i.e. the tongue obstruction hypothesis – only when associated with
mandibular underdevelopment [4]. In cases where the chin is compressed against the sternum, the tongue may interpose in the space between the ascending shelves. The resultant palatal deficiency is U-shaped not V-shaped by disturbance in growth potential not tissues that may have been affected by disturbances of ecto-mesenchyme or other phenomena at the cellular level [5].

2) Interference with cell differentiation or migration, either through hormonal defect, biochemical defect, or extrinsic biochemical interference. Numerous studies have substantiated the association between teratogens and clefting. Such teratogens may be individually operative in a subgroup of individuals that is genetically and biologically susceptible. Conversely, several different teratogens may act together on a single mechanism controlled by only a few genes. At present our knowledge of the teratogens that are associated with clefting is very limited. Only a few substances such as retinoic acid (used in the treatment of acne and viral infections has not clearly been established. One possible reason is viral infection, which may have a seasonal variation in the incidence of CLP. An association between clefts of the lip and palate formation lasts over 15 days in humans. Therefore in many syndromes, cleft lip and palate may be accompanied by anomalies of different parts of the body. Many developing systems can be disturbed simultaneously by teratogenic influences which operate over a long period of morphodifferentiation. But despite the fact that there are over 150 recognized disorders in which CL, CP or both may represent one feature, it is widely believed that the majority of affected individuals are otherwise structurally normal (rev by

an embryo to the disturbance of lip formation. Where the size of the facial processes is reduced and they are not in tight apposition, there is an increased probability of cleft lip. Experimental support of the previous is found in the work of Trasler, 1968 and Brown, Hetzel, Harne & Long, 1985, reviewed by Poswillo, 1988, where the spontaneous development of cleft lip and palate in A strain mice is attributable to the pointed facial processes that prevent wide areas of contact. On the other hand, in the C57 black strain of mouse, the larger facial processes facilitate wider contact of the processes and therefore clefting doesn't develop.

While anatomical variation is one potential predisposing factor in the development of cleft lip and palate, other factors also exist. It is well established that at the time of consolidation of the facial processes, there is a concurrent program of spontaneous cell death (PCD). It is involved in the removal of the epithelial debris from the developing nasal placode. When this PCD is more extensive than necessary and repair of mesenchyme is disturbed, a weakness develops in the forming lip and alveolus. The continued action of growth traction forces may further disrupt the association of the facial processes with the lip margins being pulled apart. Resultant clefts of the lip may vary from a simple groove in the muscle to a complete cleft that includes the nasal floor [7].

In regards to submucous cleft palate and bifid uvula, both can be considered microforms of isolated palatal clefting. Probably, the disturbances in the local mesenchyme occurred at the time of the ossification of the palatal bridge and merging of the margins of the soft palate. These phenomena occur between 7–10 weeks of human development [8].

An association between clefts of the lip and cleft palate has been observed. Animal studies suggest that “following the failure of lip closure there is an overgrowth of the probial tissues which then divert the tongue into the nasal cavity. The mesenchymal obstruction of the tongue can delay the movement of one or both palatal shelves, so that opportunities for palatal fusion are lost” (rev by Poswillo, 1988).

Lip and palate formation lasts over 15 days in humans. Therefore in many syndromes, cleft lip and palate may be accompanied by anomalies of different parts of the body. Many developing systems can be disturbed simultaneously by teratogenic influences which operate over a long period of morphodifferentiation. But despite the fact that there are over 150 recognized disorders in which CL, CP or both may represent one feature, it is widely believed that the majority of affected individuals are otherwise structurally normal (rev by
Genetic Factors

Polygenic inheritance refers to conditions determined by a large number of genes. Each of them has a small effect, but they act additively (i.e. hair color) [12].

Multifactorial inheritance refers to conditions determined by a combination of factors each with a minor but additive effect (i.e. blood pressure) [11] and has been developed to describe the observed non-Mendelian recurrences of common birth defects. It includes both polygenic origin and undefined environmental factors that will increase the variation in incidence depending on race and geographic region. The multifactorial inheritance pattern is more difficult to analyze than any other type of inheritance but it is thought to account for much of the normal variation in families, as well as for many common disorders, e.g. congenital malformations.

The normal rate of development can be thought of as a continuous distribution in which, if it is disturbed, a serious malformation may occur. This has been described as the multifactorial/threshold model and several human congenital malformations reveal family patterns that fit this model. Cl with or without CP and CP alone are included in this category.

Cl with or without CP shows both geographical and racial variations which means that it could be explained by the multifactorial/threshold model. In contrast CP alone shows little variation in different racial groups. This may mean that isolated CP will not fit the purely multifactorial model.

To date, there have been three pedigrees reported in which CP is clearly inherited as a single-gene X-linked disorder [13–15]. One of these pedigrees is described to a large Icelandic family (293 individuals) that shows Mendelian inheritance of X-linked secondary cleft palate and ankyloglossia [15]. Family analysis has shown that the frequency of CP among all the members of this family was much higher among the male than among the female CP probands. There was no incidence of male to male transmission in this large family. The X-linked mode of inheritance of CP is indicated by the family distribution. Also the large number of members of this family together with the availability of many X-chromosome probes has made it possible to localize the defect subchromosomally (using RFLP-restriction fragment length polymorphism studies for linkage) to the q13-q21.1 region of the X chromosome [16]. Finer mapping and the use of cell lines from patients with deletions of the X chromosome have further localized the defect to Xq21.31-q21.33 [17].

In the case of Cl with or without CP, Melnick et al, 1980 [18] reviewed worldwide CL/P recurrence risk data and found that both a multifactorial/threshold model and a monogenic with random environment component model fitted poorly.

Farrall and Holder, 1992 [19] refer to the work of several investigators. According to their report: Marazita, 1984, in his analysis of a subset of Danish CL/P families, found no support for a MF/T model but suggested the possibility of a major gene. Also Marazita et al, 1986, have reported an analysis of ten English multigenerational CL/P families collected by Carter, 1982. They were able to reject an MF/T model and demonstrated that a major locus acting on a multifactorial background (mixed-model) gave a reasonable fit. Chung, 1986, analyzed a series of Danish and Japanese CL/P families and concluded that the best fitting model predicted a recessive major gene acting on a multi-
factorial background (mixed-model). Chung et al, 1989, analyzed Hawaiian families from several racial groups and found that the data was consistent with a major-gene/multifactorial model (mixed model). Ardinger, 1988, has provided additional evidence for an association between the locus for transforming growth factor alpha (TGFα) and the CL/P locus. TGFα is believed to be the embryonic form of epidermal growth factor, which is believed to regulate the proliferation and differentiation of palatal epithelial cells both in vitro and in vivo. Hecht et al, 1991, analyzed mid-western U.S. Caucasian families and showed consistency with a major-locus model. He found that the dominant or codominant models with decreased penetrance fitted the best. Both the MF/T model and the mixed model with a dominant major gene effect were found to provide an explanation for the familiar clustering pattern. Marazita et al, 1992, analyzed almost 2000 Shanghai families and found that the best fitting model was that of an autosomal recessive major locus.

Farrall and Holder [19], in their own analysis, have shown that “the extensive published recurrence risk data, which have been interpreted to be consistent with an MF/T pattern of inheritance, are equally compatible with an oligogenic model with perhaps as few as four genes.”

In conclusion, the extensive recurrence risk data, which has been widely interpreted as providing evidence of a polygenic multifactorial trait, are now thought to be consistent with a model with a major-gene effect contributing to about 1/3 of CL/P and acting on a multifactorial background. For CL/P, the observed decline in risk with decreasing relatedness to the proband is incompatible with any generalized single-major-locus (gSML) model of inheritance and is suggestive of multilocus inheritance.

**Teratogenes**

Palatal shelf elevation and fusion depends on fetal neuromuscular activity, growth of the cranial base and mandible, production of extracellular matrix and contractile elements in the palatal shelves, shelf adhesion, PCD of the midline epithelial seam and fusion of the ectomesenchyme between one shelf and another. All these phenomena must act in perfect harmony over a short period of time in order to produce normal palatogenesis. Factors that interfere with any of these events could lead to a cleft.

**Vitamin A**

By introducing human teratogenic agents (e.g. excess vitamin A) into the maternal diet of a strain mice, it has been observed that 100% of offspring are born with the expected deformity [7]. Renewed interest in retinoic teratogenicity has followed the introduction of 13-cis-retinoic acid as an effective treatment for severe cystic acne. Inadvertent use of 13-cis-retinoic acid during the first trimester of human pregnancy has been reported to result in a spectrum of malformations termed retinoic acid embryopathy (RAE) [20] and includes microtia or anotia, micrognathia and in some cases CP. The induction of CP following administration of excess vitamin A to pregnant laboratory animals is well documented [21]. Most of the early animal studies involved exposure to forms of vitamin A that are stored in the maternal liver and that, therefore, have a relatively long half-life; they also involved multiple administrations of the drug or examined the developmental end-point only, thereby excluding study of the developmental changes that lead to CP.

A study by Kochhar and Johnson, 1965, reviewed by Sulik, 1989, describes palatal clefts for which the shelves were very small or entirely absent; these resulted from insufficient maxillary prominence mesenchyme. These investigators also found that size reduction of the palatal shelves occurred only posteriorly in most cases.

The use of all-trans-retinoic acid, which is of short half-life, has shown that incidence of cleft palate peaks at more than one developmental stage in both hamsters and mice (Kochhar, 1973, rev by Sulik, 1989).

The changing incidence and severity of secondary palatal malformations that may be induced within a narrow window of time (over a 16 hour period) appear to be related to a corresponding change in the pattern of PCD in the first visceral arch. It has been shown that 13-cis-retinoic acid increases the amount of cell death in regions of PCD in C57Bl/6J mice, a strain which is particularly prone to spontaneous craniofacial malformations [3, 22]. The distribution of excessive cell death in regions of PCD provides a basis for understanding the composition of syndromes in which malformation appears to be unrelated by tissue type or location [22].

What has been described as vitamin A cell necrosis is consistent with type-2 cell death (rev by Sulik, 1988). On the other hand, it has been noted that not all lysosomal membranes of all cells lyse. Only those membranes that are at a particular stage of differentiation or which have been perturbed in some other way lyse. Membrane destabi-
lization by the retinoids may interfere with many cellular functions. For example, blebbing of the neural crest cell membrane was noticed following retinoic exposure. This may interfere with the migratory ability of these cells. Recovery follows removal of the retinoic acid in vitro. In vivo, recovery from a brief interference with cell migration might also be expected but sufficient recovery probably does not follow the excessive cell death of progenitor cells.

Treatment of female C57Bl/6J with 13-cis-retinoic acid at the early stage of pregnancy (8d14hr to 9d0hr) has a more severe effect on the secondary palate [22]. 12 hours after the 8d14hr treatment time, embryos have 13 to 20 somites. Extensive expansion of cell death at this time would be expected to have a major effect on almost the entire secondary palatal shelf complex, thereby resulting in severe hypoplasia and clefting. Minor effects would be expected to involve only the posterior portion of the maxillary prominences, thereby resulting in deficiency in the posterior aspect of the secondary palate.

12 hours after the 9d6hr treatment time (late treatment), embryos have approximately 30 to 34 somites. Expansion of cell death in embryos at this stage of development results primarily in foreshortening of the secondary palate, which occurs at the expense of its posterior portion. Major effects on the entire palatal shelves would not be expected at this treatment time. Later treatment times are mostly associated with induction of limb malformations [23].

Phenytoin

Also, “under the influence of teratogenic doses of phenytoin, the lateral nasal process fails to expand to the size necessary for tight tissue contact with the medial nasal process” (rev by Poswilllo, 1988). Abnormal differentiation of the cellular processes of the ectomesenchymal cells is probably associated with this condition, where the lip and primary palate form.

Ethanol

Ethanol (alcohol) is an important human teratogen. It is estimated to severely affect 1.1/1000 live births and have lesser effects in 3–4/1000 children born. Its abuse during pregnancy results in fetal alcohol syndrome (FAS) which involves a wide variety of malformations in many organs. Abnormalities that are not diagnostic of FAS, but are associated with maternal ethanol abuse are termed fetal alcohol effects (FAE) (rev by Sulik, 1988). Treatment of C57Bl/6J female pregnant mice with ethanol when the embryos have approximately 7–10 somites results in a pattern of malformation that is consistent with the DiGeorge sequence (midline clefts in the nose and cleft palate are features of this sequence. The DiGeorge sequence has been described in the offspring of alcoholic mothers.

Among the cellular effects of ethanol are increased peroxidase activity, interference with cytoskeletal components, diminished DNA synthesis and suppressed rates of cell division, direct effects on membranes resulting in excessive fluidity (reviewed by Sulik, 1988). Recent investigations illustrate excessive cell death within 12hr following maternal treatment. The rates of cell death are similar to the normal rates of cell death seen in PCD, but the areas of cell death are expanded. The reason for this excessive cell death is not yet clear. One possible explanation is that exposure to ethanol results in lipid peroxidase/formation that leads to rupture of lysosomal membranes and release of hydrolytic enzymes (type-2 cell death).

Hyperthermia

Hyperthermia has teratogenic effects and the facial malformations induced include, among others, cleft lip and/or cleft palate. CNS is particularly sensitive to hyperthermia. Facial abnormalities have been associated with human maternal hyperthermia at 4–7 weeks (rev by Sulik, 1988). The type and extent of damage depend on the duration of temperature elevation and the extent of elevation. Also, low sustained temperature elevations appear to be as damaging as repeated spikes of higher elevation. Elevations of 1.5–2.5 degrees Celsius above normal body temperatures represent the threshold for teratogenesis in humans. Such elevations can result from excessive exercise, the use of hot baths and saunas and febrile episodes.

Again in the case of heat-induced teratogenesis, cell death is considered to play a major role, with the mitotic cells being the most susceptible. The pathogenesis of heat-induced malformations in areas other than the CNS have not been studied yet. It has been suggested though that hyperthermia could result in intra and extracellular leakage of lysosomal enzymes which could lead to type-2 cell death.

Ionizing Radiation

Ionizing radiation acts as a direct insult to the embryo. The malformations induced are similar to those noted following exposure to ethanol, retinoic acid or hyperthermia. The cellular mechanisms of radiation-induced teratogenesis are not com-
Cleft lip and palate are incompletely understood. They vary from sublethal injuries affecting differentiation and cellular interactions, to effects on rates of proliferation and cell death (rev by Sulik, 1988). Response of the cells to the radiation is dependant on cell cycle. Also, in some instances cell death is linked to chromosomal damage. Some studies have shown that irradiation results in altered permeability of intracellular structures and enzyme release, i.e. rupture of lysosomal membranes, and suggest that this results from lipid peroxide formation.

**Hypoxia**

Of particular interest is hypoxia-induced cleft lip. Hypoxia in the human embryo may result from cigarette smoking, reduced atmospheric oxygen levels and also placental insufficiency. Previous studies have shown size reduction and abnormal apposition of the facial prominences as possible pathogenetic mechanisms. The presence of cellular debris resulting from cell death in the deepest aspects of the invaginating nasal placodes, as well as overall growth retardation of the facial prominences to contact and fuse (rev by Sulik, 1988). There are also direct effects suggested of oxygen deficiency on the cells which lead to glycolysis followed by acidification of intercapillary spaces and subsequent necrosis resulting from intra and extracellular leakage of lysosomal enzymes. It is interesting to note that chemicals that interfere with oxidative enzymes such as phenytoin induce cleft lip in mice.

**Antimetabolites**

Methotrexate and aminopterin are two uncommon antimetabolites that can induce craniofacial dysplasia and cleft palate in humans. Their action is inhibitory of DNA synthesis through competitive folic acid antagonism. The pathogenesis of methotrexate involves fluid imbalance, resulting perhaps from interference with osmoregulatory cells in extraembryonic capillary beds, which is partially responsible for the malformation.

**Metabolic Disorders**

An interesting finding associated with lip clefting was that of mitochondrial myopathy of cleft muscles [24]. Facial muscle specimens from the cleft site were characterized by disorganized fibers, going in many different directions. The number of fibers appeared to be decreased and there is more connective tissue between the muscle fibers. The fiber diameters were also found to be much smaller. NADH stain and electron microscopy revealed a large accumulation of mitochondria at the central portion of the fiber, giving a star shaped appearance to the fiber. The mitochondria are more variable in shape than normal, and the cristae are more densely packed than expected.

These abnormalities in mitochondrial size, location, cristae and number suggest a form of metabolic defect that could underlie cleft lip deformities. The suggested explanation is that a defect in energy production could result in insufficient cellular migration and proliferation and thus be the pathophysiological basis for cleft lip. As mentioned, in addition to the mitochondria, the cleft lip muscles were found to be abnormal. However, no signs of group denervation or reinnervation were found and the motor end plate structure appeared normal. These findings argue against denervation or abnormal innervation as a cause of the abnormalities. Since the innervation was normal, the muscle atrophy was attributed to an inability of these muscles to function properly, which was furthermore attributed to the mitochondrial energy production abnormality or to the lack of normal fiber orientation. If the causative factor is the fiber orientation, one would expect this to improve following adequate surgical reconstruction of the muscle at the time of lip repair. On the other hand, changes secondary to cellular energy problems would not be expected to improve following surgery.

In general, a common target for some teratogens (i.e. cells in regions of PCD that represent a developmental weak point) provides reason to expect interactive effects. Repeated exposure of teratogens in subthreshold doses of more than one agent could result in potentiation. Potentiation indeed occurs after repeated exposures to vitamin A and hyperthermia.

**Conclusions**

At present, our knowledge of the teratogens that are associated with clefts is not very extensive. Some of these substances (such as retinoic acid) have been confirmed to have direct effects on facial morphogenesis but many more await identification.

Metabolic disorders, inherited or not, may play a role in the pathogenesis of clefting.

Our knowledge of cell biology is increasing rapidly and may eventually lead to the understanding and possibly prevention of clefts of the lip and palate. This can particularly apply in cases with monogenic etiology and in chromosomal disorders.
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Received: 23.03.2012
Accepted: 16.04.2012

Praca wpłynęła do Redakcji: 23.03.2012 r.