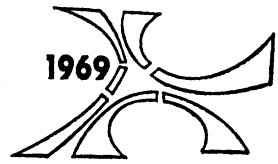
Basic Research in Cleft Palate—An Appraisal and Some Suggestions



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It is my purpose in this paper to concentrate upon the status of research which ostensibly is directed toward discovering the etiological factors and the mechanisms underlying palate malformation in man. I shall then attempt, by a brief survey of certain epidemiological information and new knowledge about human embryology, to restate old problems and to offer for your consideration some new hypotheses that might merit investigation. Let me state in advance that I am convinced that much of the research to date, while of importance to general biology, is not directed toward, nor does it have the potentiality of attaining, an understanding of certain unique features of cleft palate development in man.

Status of Epidemiological Investigations

It is of primary importance, in the analysis of disease or malformations in man, to digest the information that is obtained from epidemiological surveys. In the case of cleft palate the following summary of data might be made.

a) Clefts are reported to be most frequent among Mongoloid populations, slightly less among Caucasoids, and considerably less among Negroids (*37*, *43*, *75*). It must be kept in mind, however, that in most populations surveys have been hampered by the poor quality of birth records and other vital statistics. Furthermore, many populations, particularly those in Asia, Africa, and South and Central America, have not been surveyed at all. A recent report by the World Health Organization does not support the contention of others that in Mongoloids the frequency of clefts is higher (*89*). We agree with Greene and associates (*38*) when they wrote:

> The whole problem of the relation of race to the occurrence of facial clefts . . . is provocative and needs further investigation. Additional studies are needed of racial mixtures, as well as of the occurrence of clefts in defined racial or ethnic groups living in the United States and in groups living in the countries of their ancestral origin, such as Japan, China, Philippines, and parts of Africa, Latin America and Europe.

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b) Clefts that do not occur in syndromes are not reported to occur in significantly higher frequencies in isolates, that is, in small populations where inbreeding is the rule rather than the exception. In gene-mediated malformations or syndromes, inbreeding often causes unusually high prevalence of such conditions, such as in congenital dislocation of the hip among Apache Indians (*60*), Laplanders (*70*), Canadian Indians (*83*), and Italian villagers (*78*). Albinism among the San Blas Indians of Central America is extraordinarily frequent (*42*). Cooley's anemia, another recessive condition, is found among certain Italian, Greek, Syrian, Armenian, and Iranian isolates (*76*). High incidences of hereditary diseases occur in triracial isolates in Maryland and South Carolina (*103*, *104*). In the case of cleft palate, assortative mating does not appear to increase its frequency above that of the general population.

c) Family studies have demonstrated familial incidence of about 40 % for cleft lip (with or without cleft palate) and about 20 % for cleft palate alone (*26*). There is little clear-cut evidence from pedigrees that supports unequivocally any one genetic mode of inheritance, but generally it is thought that cleft palate is genetically distinct from cleft lip (with or without cleft palate) in those cases where the type of familial occurrence permits a genetic hypothesis (*26*, *28*, *105*).

d) In monozygotic twins there is about 36 % concordance for cleft lip and/or palate (*24*, *71*, *80*). If clefts were exclusively gene-mediated the expected concordance of this defect in monozygotic twins would be 100 %.

e) Clefts of lip and/or palate in human fetuses do not occur alone but are apparently always accompanied by multiple defects throughout the body (*51*, *58*). In cleft palate patients there is evidence that almost the same condition prevails (*86*). These associated defects are not syndromic but occur in a haphazard, totally unpredictable way. This has also been noted by Green and associates (*38*).

f) Similarly, numerous tooth buds found in cleft human fetuses show clear-cut abnormalities (*45*), and in cleft children, 65 % show multiple crown abnormalities of the primary and permanent dentitions (*45*, *56*).

g) There is no evidence to indicate that seasonal influences, parental age, birth rank distribution, and parental occupation (*26*, *34*, *105*) are factors in producing clefts. Greene and associates (*38*), however, claim disparity between parental ages is a factor in clefts.

In a relatively short period the views of Fogh-Andersen have undergone a notable change, and, in view of his stature in the field, should be recorded here. In 1961 he stated (*25*):

> . . . the main etiological factor of congenital cleft lip and palate in man is that of heredity . . . . It is shown that two different cleft malformations exist with no genetic connection and with a different manner of inheritance, viz. 1) harelip with or without associated cleft palate, occurring most often in males, and mostly inherited as a recessive character (so-called "conditioned dominance"), and 2) cleft palate alone, most frequently found in females, only in-

herited in a smaller number of cases and then as a dominant character with failing manifestation.

Attention is drawn to the possibility that the natal frequency of cleft lip and palate will increase in the future on account of the growing number of affected individuals kept alive and satisfactorily repaired—and thus having good chances of bringing offspring into the world.

In 1964 Fogh-Andersen (*26*) took a more equivocal stand with the following statements.

Most likely the majority of congenital deformities are due to a combination of exogenous factors and a gene-pattern, predisposing more or less to congenital malformations in general or to single, specific malformations.

Among cases of cleft palate alone a familial occurrence was demonstrable in only 19%, indicating a presumable greater influence of exogenous factors in this type of cleft.

It is as yet impossible to pronounce with certainty on the nature of exogenous factors that might be active here. . . . The recently proved bigger incidence of malformations among children of diabetic mothers in connection with the increasing number of diabetic women who nowadays bear children is at least one factor which might cause an increasing incidence of cleft deformities.

With the thalidomide catastrophy in mind and with our knowledge of the enormous abuse of tablets of nearly all kinds which has taken place during the last few decades in most civilized countries—particularly sedatives, hypnotics and tranquillizers—we cannot exclude the possibility that certain drugs or other substances taken by the pregnant woman during the first months of pregnancy might interfere with embryonic development—no matter whether such interference is intensified by a hereditary predisposition to malformations or not.

From the epidemiological studies we can make the following summation.

a) Clefts occur universally in man, with apparently a significantly lower frequency in those Negroid populations studied.

b) While there may be a predisposing genetic pattern underlying most clefts, there are very few cases where the evidence supports a simple genetic theory of inheritance.

c) The evidence from twins clearly indicates the predominant role of environmental factors.

d) Clefts most frequently are associated with other malformations throughout the body but not in any predictable pattern, as in the case of hereditary syndromes, such as, club foot, ulceration of the soles of the feet, Apert's syndrome, Cockayne's syndrome, ectopia cordis, cleft lip-palate-popliteal pterygium-digital and genital anomalies, cleft palate-flattened facies and multiple congenital dislocations, mandibulofacial dysostosis, cleidocranial dysostosis, craniofacial dysostosis, cebocephalia, Greig's syndrome, Klippel-Feil syndrome, Marfan's syndrome, and orodigitofacial syndrome (*35*, *36*).

Teratogenic Factors in Laboratory Animals

For the great majority of clefts, which apparently are not part of any hereditary syndrome, there has been no environmental factor (or factors)

which can be identified as a causative agent. As Greene (*37*) concludes:

> Though there is strong evidence that both genetic and environmental forces are involved in cleft formation, the genetic mechanism has not been described adequately nor have specific environmental agents been identified. Theories based on environment alone or on genetics alone cannot fully explain the data in the literature.

Granted that heredity plays an important role as a predisposing factor, thus accounting perhaps for the racial differences in incidence noted previously, there must be exogenous agents which are ubiquitous, since all populations in the world show a frequency of from 1 cleft in 500 to 1 cleft in 1200 live births. The search for these agents must be pressed with urgency.

Laboratory research designed to establish the normal and abnormal developmental pathways of palate formation has concentrated heavily upon experiments with animals—primarily mice and rats. At least two strains of mice (A/Jax and Strong a) have been developed which consistently produce 8 to 12% spontaneous clefts. The type of cleft is generally complete cleft of lip and palate, with occasionally an isolated cleft palate. Clefts have been observed in most mammals, including dogs, cats, cattle, sheep, pigs, lions, jaguars, and yaks. Recently we reported on the first cleft palate found in a subhuman primate—the marmoset (*55*), and now we are preparing a report on a spontaneous cleft palate in a rhesus monkey (*59*).

Strangely enough there are no published data which present representative histological sections through the head of a rodent with a spontaneous cleft. On the other hand there is an abundant literature on induced clefts. There are many teratologic agents which can successfully induce clefts in rodents. These include: riboflavin deficiency, folic acid deficiency, vitamin B12 deficiency, deficiency of other nutrients, hypervitaminosis A, trypan blue, ionizing radiation, hormones, amniocentesis, salicylates, chlorcyclizine, 6-aminonicotinamide, and pteroylglutamic acid. From the accumulated data and conclusions put forth by these investigations on rodents, one is led to the belief that there are potentially a great many kinds of teratogens which, if administered at the proper dosages and critical periods, will produce clefts with remarkable consistency in various rodents. However, it is difficult indeed to imagine that these same agents play any role in the formation of clefts or other craniofacial abnormalities in man. Cortisone, the most commonly employed teratogen in rodents (*30*, *31*, *32*, *33*, *46*, *65*, *96*, *101*) must be administered in very high doses in order to produce clefts in mice. These doses are much higher than what one would expect to be present in human circulation. Although cases of cleft palate in offspring of women treated with very high doses of cortisone have been reported (*41*), this possible association must be further investigated. At any rate, treatment with such massive doses of cortisone is rare and certainly cannot be considered seriously as an important factor in human cleft palate throughout the world.

A highly potent synthetic steroid used as a substitute for cortisone has been reported to act in very low doses equivalent to those used for treatment of asthma or chronic rhinitis (*98*). Other anti-allergic drugs, such as meclizine, also produce cleft lip and cleft palate in the rat (*47*). Nevertheless, even if it were demonstrated that these different types of drugs are capable of producing, and actually do produce, cleft palate in man, it would explain only the few cases in which those agents have been used by the mother at critical periods of pregnancy. The same argument could be applied to other teratogens, such as vitamin A (*100*), salicylic acid (*67*), or X-irradiation (*13*), all of which produce cleft palate in rodents when used in high doses but which can only rarely be associated with human cases of clefts. Nevertheless, the studies of these teratogenic agents in rodents have been very valuable since they have supplied information about the mechanisms by which palatal development can be interrupted, and, having been interrupted, the paths that abnormal development seems to follow. Thus, in the case of cortisone, it has been found that cleft formation involves extensive biochemical alterations of palatal metabolism as demonstrated by biochemical and histochemical techniques. Mechanisms of production of cleft palate may be common to man as well as lower mammals, and information derived from experiments with diverse teratological agents may contribute to our understanding of these mechansims, despite the fact that the teratogens used cannot be seriously considered as factors in the production of human clefts. Kalter (*46*) warns that the argument

> . . . that the induced defects represent phenocopies is in most cases only poorly supported, either by proof that the genetic and induced phenotype were closely alike or that they shared a common pathway of development, even in part.

While none of the researchers in experimental cleft investigations would even hint that one can transfer results obtained on rodents to man, there is nevertheless apparent a tacit assumption to this effect on the part of many. For example, the report by World Health Organization (*89*) states with great assurance:

> Posterior cleft palate alone is inevitably midline and results from failure of fusion of the maxillary palatal processes determining failure of separation of the mouth from the nasal cavity.

Anatomy of Cleft Palate in Human Fetuses

It may well be that the mechanism of palate formation in rodents and other mammals is the same as in man, but this must be demonstrated. As one who has examined normal and cleft palate development primarily in man and other primates, I would like to suggest that there is considerable evidence which places man apart from rodents in this area of embryology. This evidence principally bears upon the argument that in rodents cleft palate results from the failure of the shelves to become horizontal, or, having assumed a horizontal position, to come into contact with each other.

There is a variety of explanations to support either suggested process. It seems very probable that cleft palate formation in rodents, as induced by most teratogenic agents, is a pre-fusion phenomenon, whatever the mechanism. It is my contention, however, that this does not hold true for the great majority of cleft palates in man. Examination of some 80 serially sectioned heads of fetuses with clefts of the palate only has led me to the conviction that such clefts result from deterioration of tissue subsequent to palate fusion with eventual rupture of the palate. Since palatal fusion is completed by 54+ days, and since the horizontal processes of both maxillary and palatine bones meet in the midline at about 10 weeks, I suggest that the majority of cleft palates in man occurs between 7½ and 10 weeks *in utero*. Some of my reasons for this hypothesis are based upon the following arguments.

a) Anatomically normal palate development in man is different in certain important respects. A report of the 1967 Workshop on Cleft Lip and Palate (*29*) stated:

> 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.

b) Cleft palate in human fetuses shows a number of striking dissimilarities from that in rodents.

c) There are apparently discrete structural markers in the palate which argue against failure of the shelves to make contact.

d) There is some experimental evidence to suggest that certain universally occurring teratogenic agents could be active in producing clefts in man.

Let us examine first some features of palate embryology which are unique for man. The shelves first make contact at about 47 days (*57*) and a week later (54+ days) have completely fused along the midline (Figure 1). Apparently the same timing holds for the rhesus monkey (*88*). In man the fusion process leaves numbers of epithelial rests in the midline (Figure 2). Several of these fail to break down and eventually (by the 9th or 10th week) enlarge, become highly keratinized, and form pebble-like pearls (Figure 3). These occur in all human fetuses thus far examined. Burke (*12*) noted the common occurrence of "cysts" but was mistaken about the chronology of palate closure (54+ days). He stated:

> Cystic formation was observed in the hard palates of 31 of the 32 fetuses. The only fetus lacking cysts was estimated to be approximately 3 months of age. This would place palatal development at the stage shortly after closure of the paired palatal shelves of the developing maxillas, thus precluding the possibility of cystic development at such an early period of growth.

They persist beyond birth, often to the second year of life. They are not pathological, as has often been reported, but are normal consequences of palate fusion in man (*57*). With one exception they are always located in

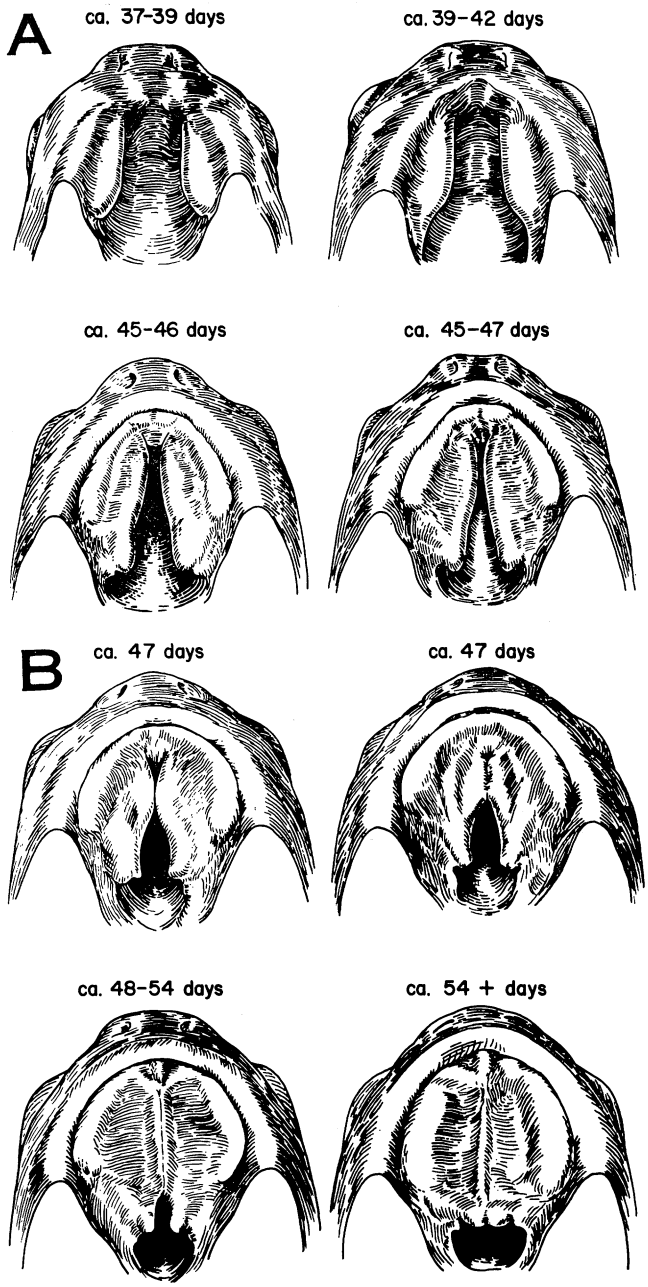

FIGURE 1. A, development of the human palate (37–47 days). B, closure of the human palate (47–54+ days). Reprinted from Kraus, B. S., H. Kitamura, R. A. Latham. *Atlas of Developmental Anatomy of the Face: With Special Reference to Normal and Cleft Lip and Palate.* New York: Harper & Row, Hoeber Medical Division, 1966.

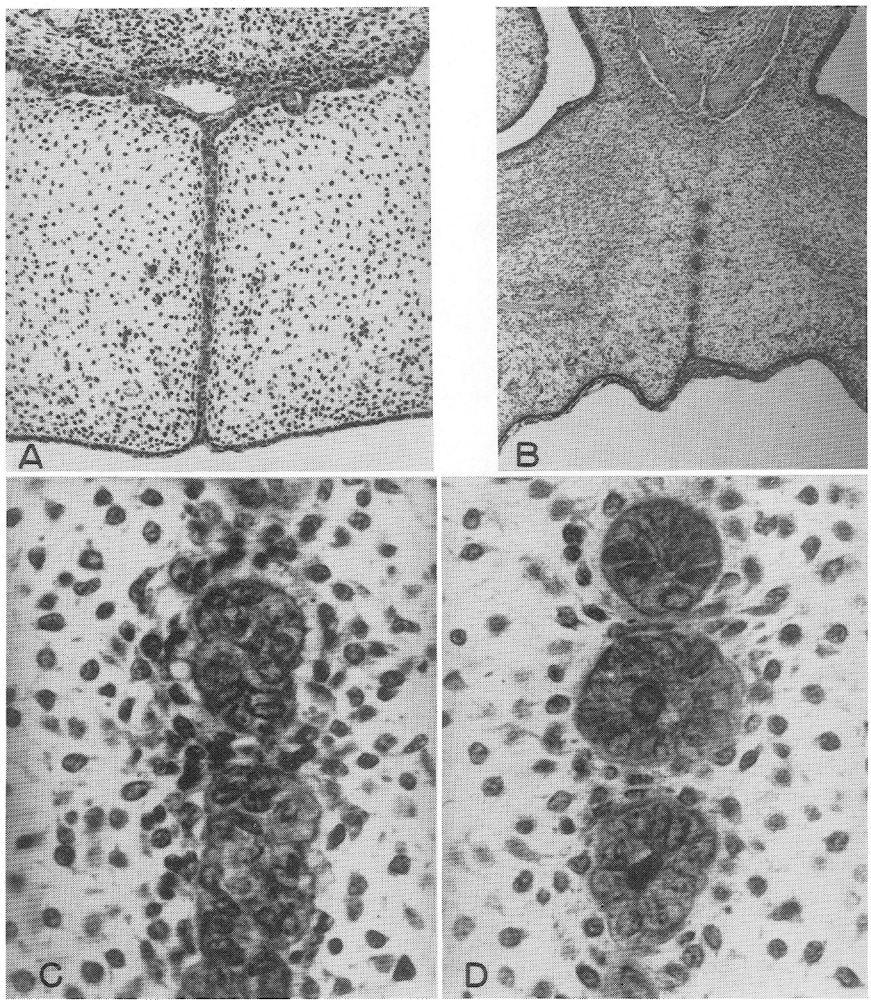

FIGURE 2. Frontal sections through the palates of human embryos and fetuses to show the mode of development of epithelial rests. A, a 47-day human embryo with beginning breakdown of the epithelial lamina. B, a 9-week human fetus showing a number of isolated epithelial remnants in the midline. C, a 9-week human fetus showing details of the breakdown of the epithelial lamina and the early formation of the epithelial remnants. D, a 10-week human fetus, showing three well-developed epithelial rests in the midline.

the midline of the hard palate. The exception is in the plane defined by a line passing through the two canine tooth buds. In this plane are found, in the embryonic period, the traces of the nasopalatine ducts. The ducts disappear, in precisely the same manner in which the epithelial layers of the contacting palatal shelves break down, leaving epithelial rests which soon keratinize to become pearls. Thus in this one plane there often occur bilaterally placed epithelial pearls in addition to the midline ones (Figure 4).

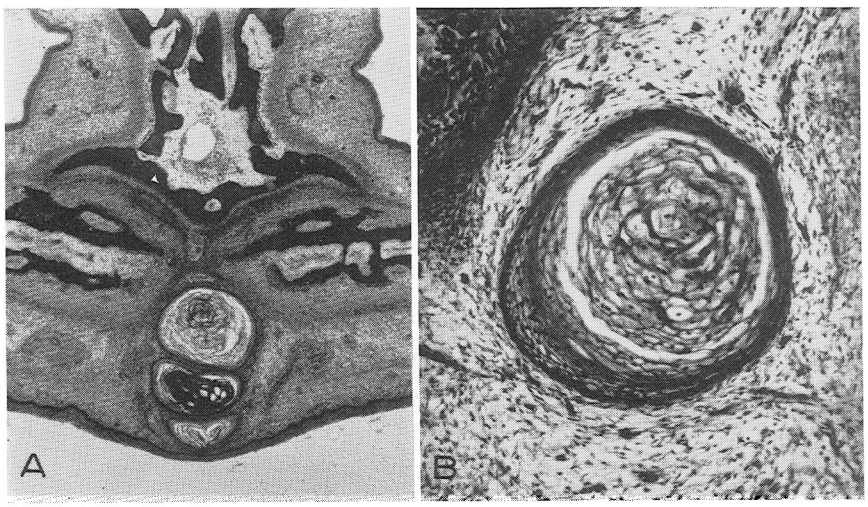

FIGURE 3. Well-developed, keratinized epithelial pearls. A, a 17-week human fetus with three pearls in the midline. B, a 13-week human fetus, enlarged to show details of epithelial pearl development.

At the 1968 Miami Beach meeting of the American Cleft Palate Association, I reported on the evolutionary significance of epithelial pearls (*53*). They have not been found in rodents. In fact, even epithelial rests are of rare occurrence. When they do occur they disappear rapidly thereafter (*1*). In the marmoset, a South American monkey, and in rhesus and presbytis, both African monkeys, a single epithelial rest will remain in the midline for at least two or three months. In presbytis, a single partially keratinized pearl has been observed. In the anthropoid apes, a large epithelial pearl has been recorded in the midline of the palate of a gibbon fetus, and a pearl-like structure has been found in an orangutan fetus. In a gorilla fetus, no sign of a pearl was observed. Apparently, in the evolution of the palate leading toward man, the persistence, number, and keratinizing tendencies of epithelial rests increases radically.

Now it must be obvious that the "universal" occurrence of pearls in the human fetus must be a reflection of evolutionary changes in the biochemistry and hence underlying genetic constitution of the mesenchyme and epithelium of the palatal shelves. There is therefore a significant difference between rodent and man in this developmental process and one cannot ignore it by attempting to interpret the human in the light of the rodent condition.

Spontaneous clefts in mice are primarily of the lip and palate. Rarely an isolated cleft palate will occur. Unfortunately we know of no instance in which the head of a mouse with spontaneous cleft of palate alone has been serially sectioned. However, in our laboratory we have serial frontal sections of over 80 human fetal heads with isolated cleft palate. The fetuses ranged in age from 8 weeks to term. All the clefts began in the hard or soft

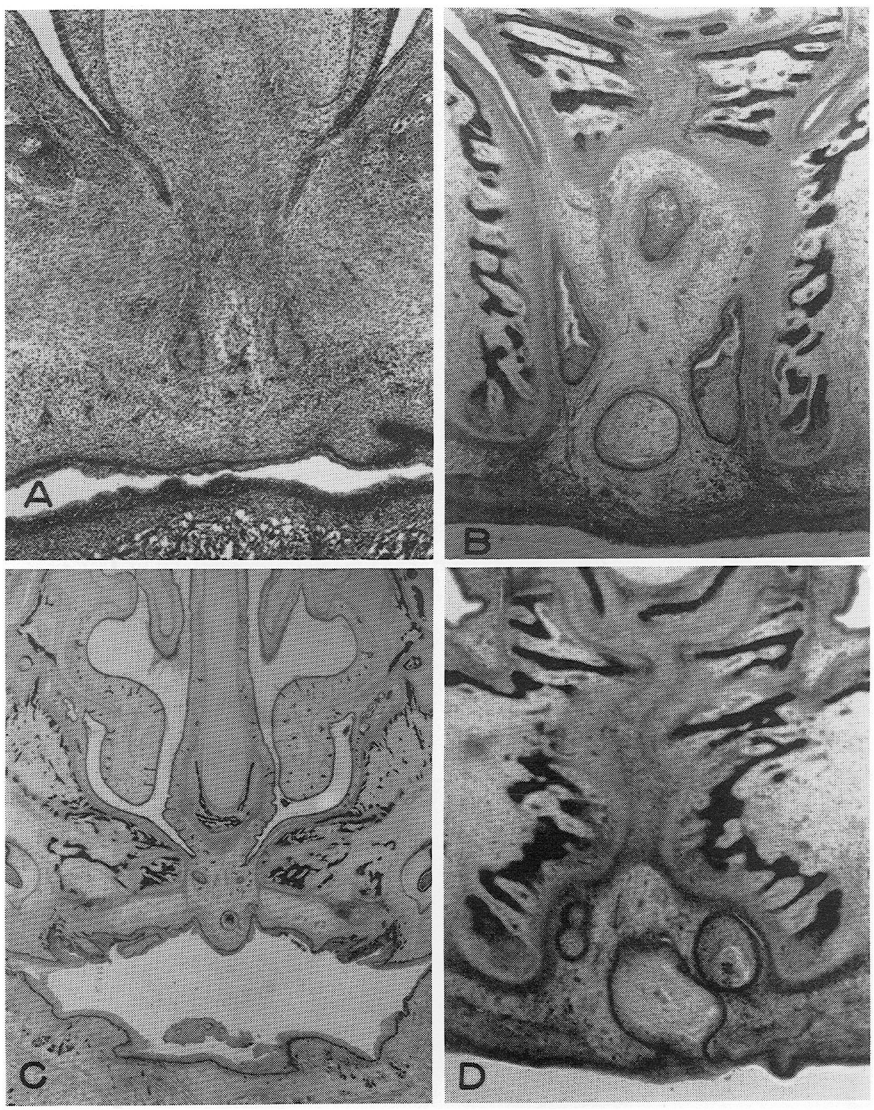

FIGURE 4. The development of bilateral epithelial pearls as a consequence of breakdown of the nasopalatine ducts. A, breakdown of the nasopalatine ducts and beginning formation of bilateral epithelial rests in a 63-day human fetus. B, epithelial pearls in the mid-palatal area. The bilateral pearls are of nasopalatine duct origin; the two midline pearls are the product of fusion of the palatal shelves in a 20-week human fetus. C, a single midline pearl and two nasopalatine duct pearls in a 17-week human fetus. D, the same in another 17-week fetus showing the beginning extrusion of the midline pearl via the oral mucosa.

palate, in some cases as far anteriorwards as the incisive foramen (*87*). An examination of these sections, particularly in the area *anterior to cleft itself*, reveals a number of very interesting phenomena. It will be remembered that the current theories about *induced* cleft palate formation in

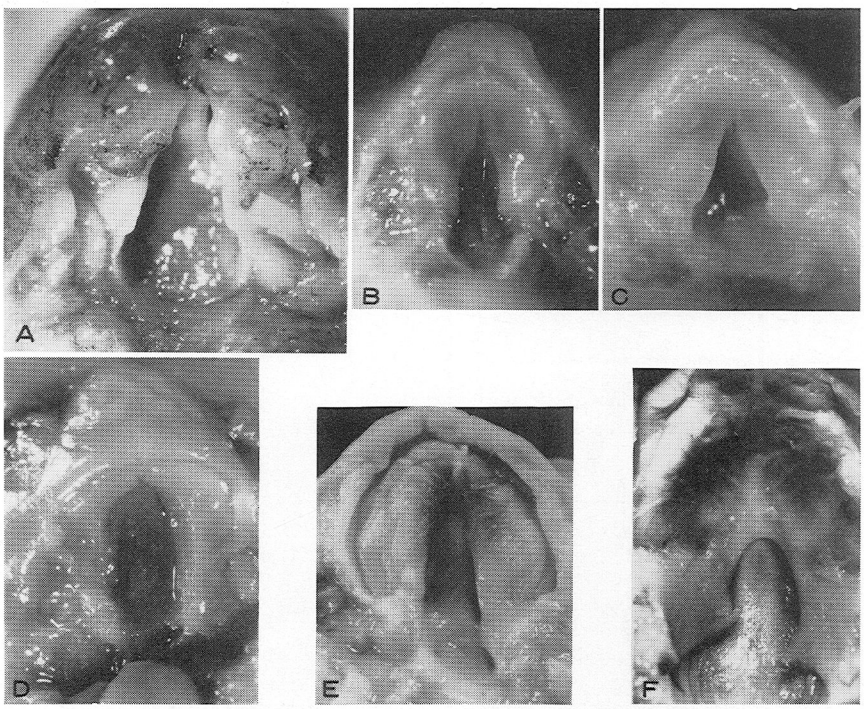

FIGURE 5. Types of cleft palate in aborted human embryos and fetuses. A, specimen X436, a 43-day embryo. B, specimen W269, a 47-day embryo. C, specimen X3079, a 9-week fetus. D, specimen X3651, a 9-week fetus. E, specimen Q3, a 14-week fetus. F, specimen X193, a 19-week fetus.

rodents hold that the shelves, for one reason or another, fail to make contact or to fuse. We know of no published illustrations of histological sections of mice or rats anterior to the induced cleft itself. However, sections through the cleft area make it appear quite probable that: a) the shelves have indeed failed to "horizontalize" or to make contact, and b) the cleft therefore is bilateral throughout its length (*3*, *22*, *23*, *32*, *33*, *63*, *64*, *66*, *69*, *96*, *99*). What is more, published illustrations of induced palatal clefts in mice and rats invariably show the same phenomenon: the cleft extends from the primary palate posteriorly throughout the entire length of the hard and soft palate (*15*). This is not the case in human cleft palate, where the cleft may involve only portions of the palate. Furthermore in its most anterior aspect the patent cleft is usually unilateral, becoming bilateral as it extends posteriorly (Figure 5). Recently, however, Dr. LeJour-Jeanty has induced all varieties of clefts in rats using hadacidin, a derivative of penicillin mold. She has demonstrated that most of the clefts resulted from post-fusion rupture, often with subsequent repair.[1] Her sections illustrate much the same phenomena that occur in human clefts.

[1] Personal communication, April 25, 1969.

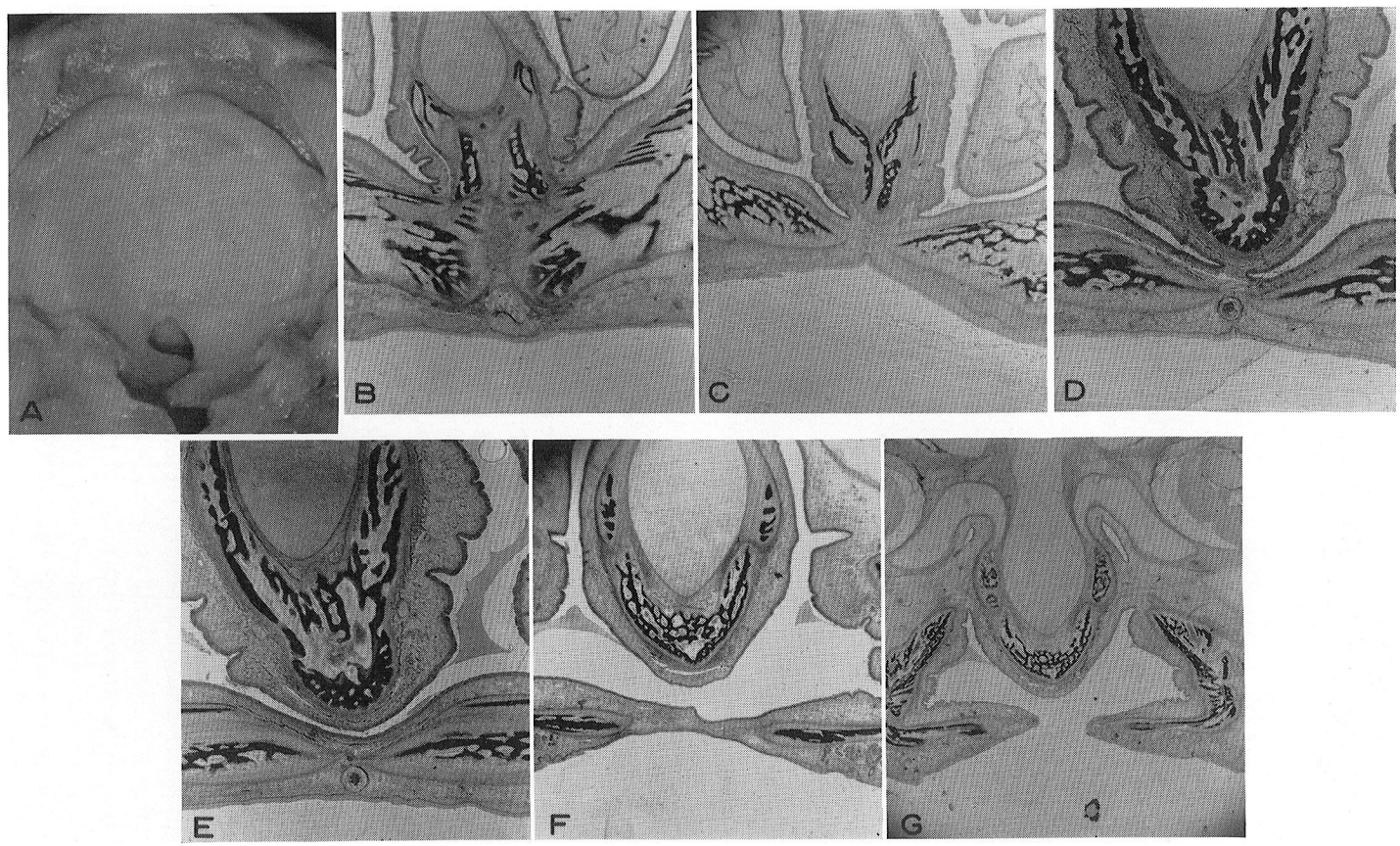

FIGURE 6. Selected frontal sections through the palate of specimen X2326, a 15-week human fetus. A, a palatal view. B–G, sections of the hard palate progressing posteriorwards to show progressive degeneration of the palate and the beginnings of the cleft (see text).

Let us examine several human fetuses with isolated cleft palate. In each case we shall begin with several sections located anterior to the patent cleft itself, then show the most anterior point of the cleft, and finally a section midway through the cleft. Let me preface this by stating that the phenomena which occur in the ones illustrated are, in general, present in all 80 specimens thus far examined, with no exceptions.

X2326 is a 15-week human fetus with a cleft of the soft palate (Figure 6A). The first section (Figure 6B), cut on a plane between the canine and second primary incisor, shows an abnormally large separation between the cartilaginous septum and the palate, with marked craniad displacement of the medial bony walls of the palatal processes. There is also a bending of the septum towards the right. Farther back, at the level of the canine, there is asymmetry of the inferior conchae and a thinning of both palatal shelves where they meet the septum in the midline (Figure 6C). In Figure 6D, there is undercutting of the inferior border of the septum. The corpus of the vomer bone is hypertrophied and the medial portions of the two horizontal processes of the maxillary bone are thin and do not meet at the midline. In Figure 6E, the palate is free of the septum and a pearl is found in the midline. Next (Figure 6F), the medial portion of the palate becomes very thin, the septum straightens out, and the maxillary bones are farther apart than was the case more anterior to this point. The shelves give an appearance that suggests "stretching" which probably is the result of the continued growth in breadth of the face and mouth at this time. The tissue is clearly necrotic. Finally, there is a separation of the two halves of the palate (Figure 6G). The specimen is abnormal in several respects. The palatine bone extends farther posteriorly than it should, particularly the horizontal processes, and the cartilaginous septum is quite bulbous. Have the shelves failed to contact and to fuse? If so, this would have taken place at the 8th week and should not have involved the palate and other facial structures anterior to the cleft.

W92 is a full-term human fetus with almost complete cleft of the hard and soft palate (Figure 7A). It appears to be unilateral at its anterior point. The palate is abnormally high and narrow. The frontal section just anterior to the cleft shows two strands of tissue apparently connecting the septum to the nasal surface of the palate (Figure 7B). However, the space in between was occupied by a large epithelial pearl. The section goes through the second primary molar, as do the following ones. The palatine bones do not meet at the same level; one dips below the other in the midline. The septum is bent slightly to the right. The next section (Figure 7C) shows a thinning of the left palatine shelf. This continues as we progress posteriorwards. A close-up shows the degeneration of tissue and sharp bending of the vomer bone. The cleft first appears to the left of the midline. Then the right side breaks free of the vomer, resulting in a midline cleft; that is, both shelves are separated from each other and from the septum (Figure 7D). Another close-up (Figure 7E) shows two large pearls, one above the

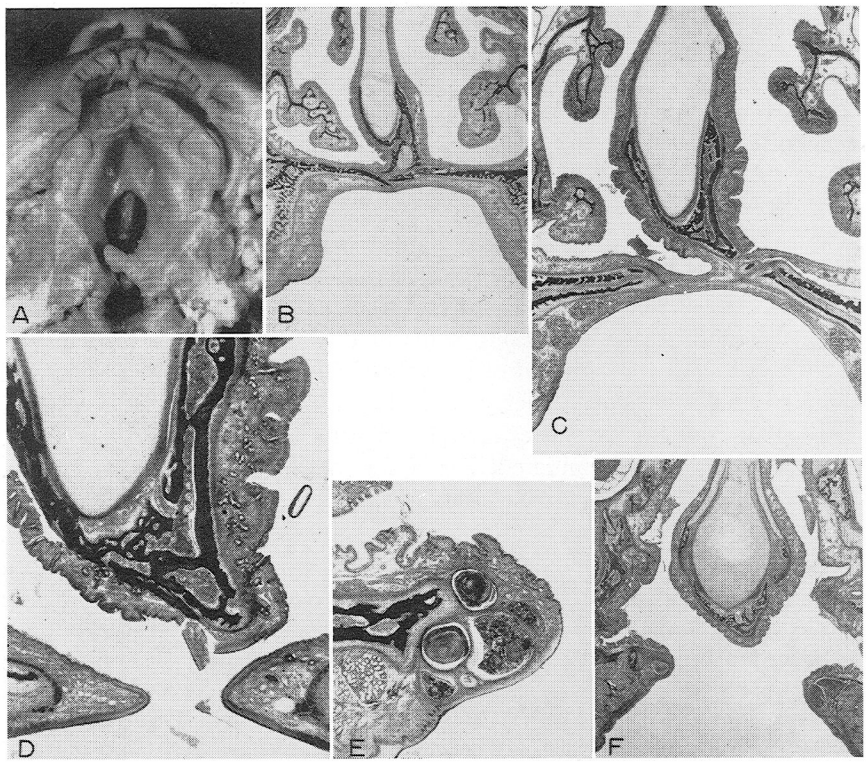

FIGURE 7. Frontal sections of the hard and soft palate of specimen W-92, a full-term human fetus. A, palatal view. B-F, successive sections through the hard palate to show degeneration of the shelves anterior to the cleft with a close-up (E) of midline pearls on the left detached shelf.

other and medial to the bone on the left side. In the final section, at the juncture of hard and soft palate, a single pearl can be seen on the left shelf (Figure 7F). In the noncleft portion of the face there are multiple abnormalities, as there were throughout the body (*57*).

The next specimen, X158, is that of a 12-week human fetus with a cleft of the hard and soft palate (Figure 8A). The distortion is obviously an artifact, but the internal structures show true malformations. The first section (Figure 8B) is through the anterior portion of the canine and shows a midline pearl and a pearl to the left which is a remnant of nasopalatine duct fusion. The septum is bent to the left. The preosteoid tissue of the right maxilla (shown on the left of the figure) has become detached from the bone, which is poorly developed. The former midline, site of palatal fusion, has been shifted to the left. In the next section (Figure 8C) another duct pearl makes its appearance to the right of the midline pearl. The next section is postcanine and shows a marked attenuation of midline palatal tissue (Figure 8D). The maxillary bones are far apart although they should

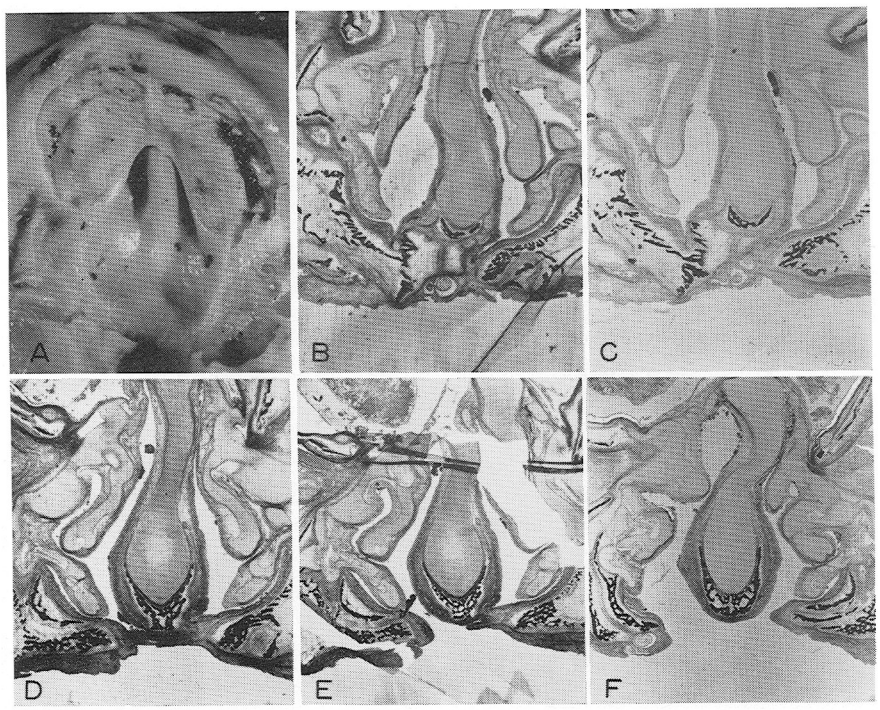

FIGURE 8. Frontal sections of the hard palate of specimen X158, a 12-week human fetus. A, a palatal view. B–F, successive sections of the hard palate showing bilateral nasopalatine duct pearls (B and C) and gradual deterioration of the palate to the point of clefting.

be in direct contact at this point. Figure 8E shows the most anterior border of the cleft, which is on the left side. The right palatal shelf is still fused, though tenuously, to the septum. The next section, at the junction of maxillary and palatine bones, shows both shelves separated from the septum, which has recovered its vertical orientation in its lower half (Figure 8F).

Specimen 129 is a 12-week American Indian fetus (Figure 9A). It has a cleft of the soft and part of the hard palate. In the succeeding series of sections a normal 12-week fetus is represented on the left side of each figure at the same approximate plane. Even in the region of the lip, abnormalities of structure are apparent in the bending of the septum, the configuration of the cartilaginous nasal capsule, and the enlarged nasal cavity on the left side (Figure 9B). In the plane of the central incisors (Figure 9C) a portion of the left maxillary bone is absent and a hemorrhagic area is in its place. The paranasal cartilages are hypertrophic and situated unusually far forward. The nasal capsules fail to penetrate the inferior conchae. In the next section, in the area of the canine, we can observe the right and left duct pearls (Figure 9D). Next, in the postcanine region (Figure 9E),

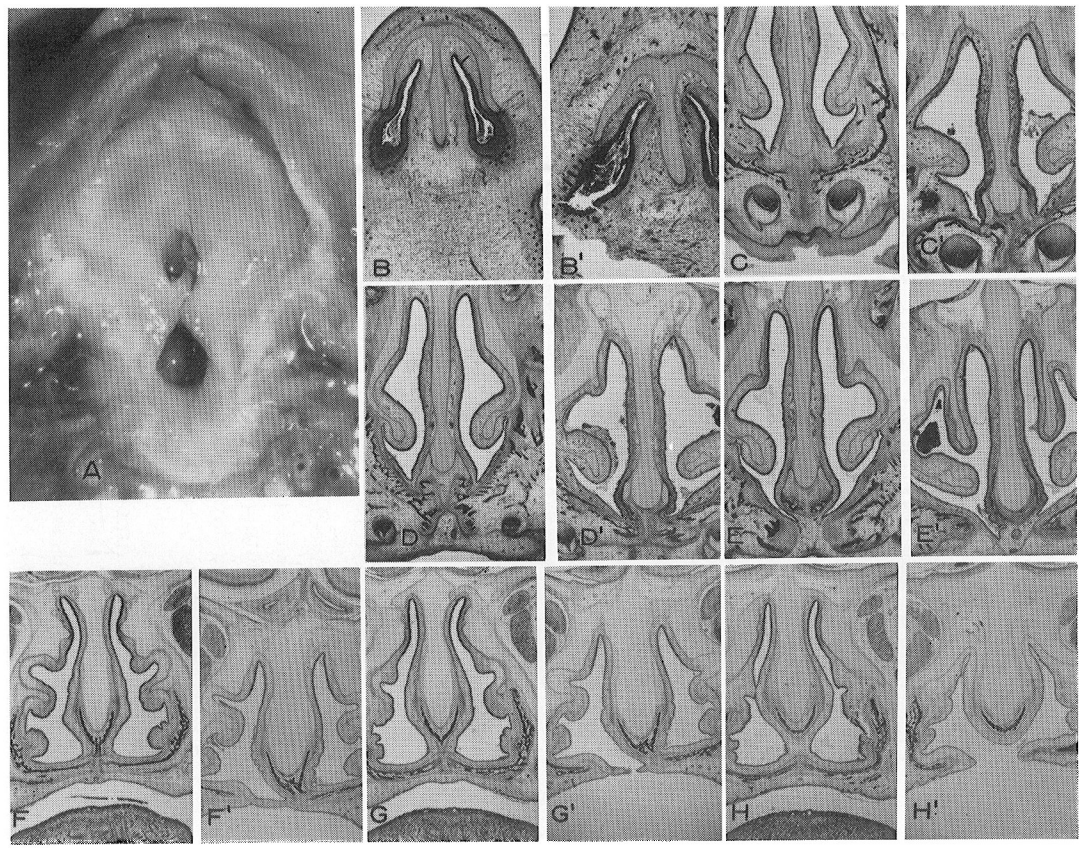

FIGURE 9. Successive frontal sections through the lips, primar, and hard palate of specimen 129, a 12-week American Indian fetus. To the left is shown a comparably section in each case of a noncleft 12-week human fetus. The cleft specimen is indicated as B^1, C^1, etc (see text).

we see a deterioration of the tissue of the left shelf, giving the septum an "undercut" appearance. Two pearls are found in the midline, which has shifted slightly to the right as the septum again bends in that direction. In the next section (Figure 9F), the left shelf has become extremely thin, as if stretched, and the horizontal process of the maxillary bone is very meager. Next we see the breakdown of the left shelf, not at its original junction with the septum, but within its own tissue (Figure 9G). This occurs at the level of the second primary molar. The corpus of the vomer bone is now strongly bent toward the intact palatal shelf on the right. The connection between the septum and the right shelf then becomes very tenuous and breaks down, thus producing a bilateral cleft (Figure 9H). On the free portion of the right shelf, where the midline was once located, we see two pearls, one above the other.

It would, therefore, seem that the theory which holds that in cleft palate the palatal shelves have either failed to elevate or, having elevated, have failed to make contact and fuse, is not applicable to man, however true it may be for induced clefts in rodents. The progressive degeneration and breakdown of the palate that lies anterior to the patent cleft, plus the occurrence of epithelial pearls on one or the other of the unattached shelves, make it clear that simple failure of the shelves to elevate or fuse is not the cause of any of the clefts we have seen in the human fetus. This alone could not explain the many other defects found in these fetuses, not only in the facial area adjacent to the cleft, but throughout the rest of the body as well. Study of human cleft palate fetuses leads us to postulate that fusion had taken place in the majority of cases and that subsequent degeneration of tissue due to exogenous factors had led to rupture of the fused shelves as a result of continuing growth in breadth of the head (*50*). Politzer (*77*) had postulated post-fusion rupture of the palate as a cause of cleft palate in 1937, and our laboratory has advanced the same hypothesis subsequently. Without denying that certain genetic constitutions, or gene-patterns, predispose to such malformations, it is nevertheless important to realize that certain exogenous factors must be present to initiate reactions which lead to these abnormalities. What is more, these factors must be ubiquitous since cleft palates are of universal occurrence, regardless of race, culture, or climate. We have pointed out that the various teratogens which successfully induce cleft palates in rodents are not ubiquitous. Therefore it is essential to search for teratogens which are universally present and which can be demonstrated to be able to induce clefts and associated malformations similar to those found in man.

Let us first consider the experimental animal of choice. I have suggested earlier (*54*), and still maintain, that the logical laboratory animal should be the rhesus monkey. Since our discovery of a cleft palate in the rhesus, another cleft has been found in this species by Swindler.[2]

[2] Swindler, D., personal communication, March 12, 1969.

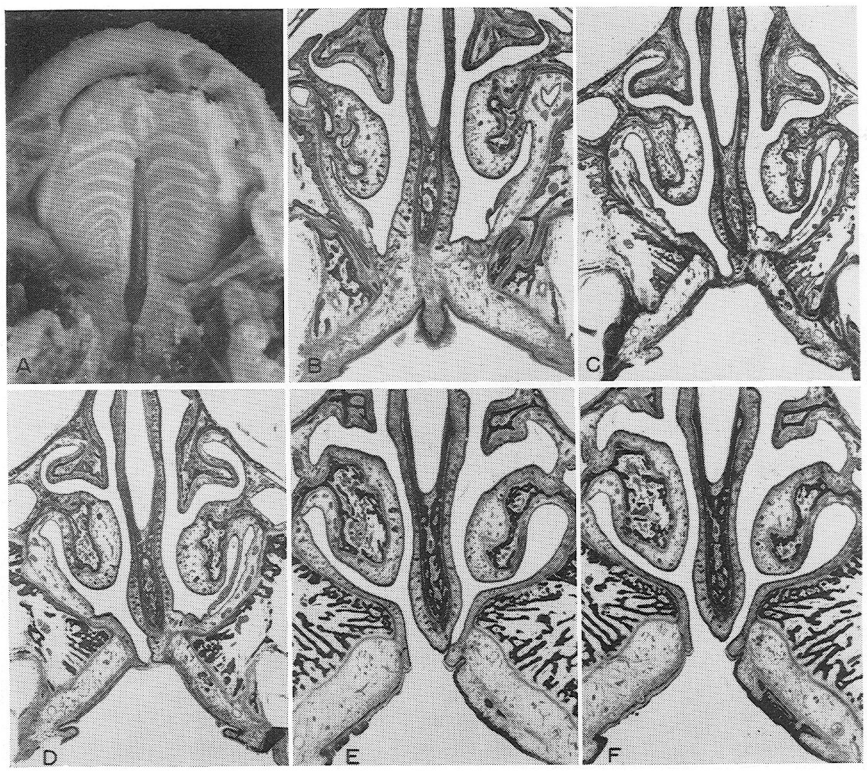

FIGURE 10. Palatal view (A) and frontal sections through the palate of the first spontaneous cleft of a rhesus monkey to be reported. The specimen is that of a full-term fetus (168 gestational days).

In both the marmoset and the rhesus, serial sections show precisely the same sort of phenomena we have described for man (Figures 10A and 10B). The anatomy, the chronology of palate development, the phylogenetic relationship, and the availability of the rhesus, all favor selection of this animal for experimentation to discover teratogens which will produce phenocopies of the spontaneous cleft and associated malformations in man.

Two types of environmental agents which seem to satisfy all the conditions mentioned are viruses and antibodies, and it would appear that experiments designed ultimately for testing with the rhesus monkey should employ these teratogens first on rodents. The prevalence of viruses in our environment and the increasing prevalence of immune diseases in man, plus the fact that it is now well known that both viruses and anti-organ sera can produce malformations in both rodents and man, make it almost mandatory that these agents be tested for the production of cleft palate.

Viruses

Since Gregg (*39*) observed that the rubella virus is capable of altering organogenesis in the human embryo, an increasing interest in the relation-

ship between viruses and congenital malformations has been developing. In man, rubella virus is the best known viral teratogen; earlier reports described an increased incidence of spontaneous abortions, stillbirths and defects of the heart, eyes (glaucoma, cataract, microphthalmia), ears, brain, and teeth, in infants born to mothers who had suffered rubella during the first trimester. Selzer (*84*) has described the isolation of rubella virus from one human embryo and the placental tissues of four women who had clinical rubella some time prior to study. Inclusion bodies were found both in placental and fetal tissues.

The virus producing cytomegalic inclusion disease is also known to produce malformations in the human fetus (*102*). In this case, viral inclusions have also been recovered from placenta and affected fetuses. There have been isolated reports indicating that other viruses may be responsible for the production of malformations in the human embryos (*93*). Thus, Blattner and Heys (*5*) have recorded a few well-documented cases in which maternal measles was followed by the birth of children with mental retardation, heart disease, cleft lip, or other malformations. Similarly they have indicated that abortions and congenital anomalies occur in the offspring of mothers affected during pregnancy with mumps, influenza, and coxsackie virus. Nevertheless, in all these cases the relationship between viruses and malformations has not been confirmed by adequate epidemiologic studies.

Substantiating evidence is supplied by experimental production of anomalies by the injection of viruses in animals. Thus, the production of malformations by attenuated vaccines of hog cholera (*106*) and ovine blue tongue viruses (*81*) has been proven.

Especially interesting experiments have been conducted using a group of rat viruses which are capable of producing malformations in hamster embryos and fetuses. The two most interesting viruses of this group are known as the rat virus (R. V.) and the H-1 virus.

Toolan (*94*) had observed that newborn hamsters developed a characteristic malformation of their heads when injected with extracts of a number of human tumors previously transplanted into rats. This malformation, which was called "mongolism", consisted in a flattened foreface, protruding eyes and tongues, absent or abnormal teeth, and osteolytic lesions in bones. Viral injections made directly into the fetuses during the last seven days of pregnancy showed the same effect. Toolan also demonstrated that normal rat serum was capable of neutralizing the teratogenic action of the extracts. In a later work (*95*), the H-1 virus was identified in the cell free fractions of the tumor extracts, demonstrating that the observed malformations were produced by this virus. Interestingly enough, it was observed that mongoloid hamsters developed antibodies against the virus and, if bred, were able to transmit these antibodies through the placenta. As a result of this, the offspring of mongoloid hamsters never were affected by the postnatal injection of viruses.

The rat virus (R. V.) was found by Kilham and Olivieri (*49*) in tissue cultures in which tumor-bearing rat material was inoculated. This virus

does not produce apparent disease when inoculated into rats, which are its natural host, nor into mice. However, when injected into new-born hamsters it produces a typical malformation (*48*) previously described by Toolan (*94*) under the name "mongolism". The infected hamsters transmitted the infection to their littermates.

While both viruses, R. V. and H-1, produce the same type of malformation when injected into newborn animals, only the H-1 virus is capable of producing malformations during the first half of pregnancy. Thus, Ferm and Kilham (*20*, *21*) found that when H-1 virus is injected intravenously into the pregnant hamster from the sixth to the twelfth day of pregnancy it produces a very high embryonic mortality. Lower doses injected on the sixth or seventh day produce, in addition to many embryonic deaths and resorptions, a high proportion of malformations in the surviving embryos. Facial clefts are included among the numerous malformations encountered. It is important to mention that no pathological symptom was developed by the injected mothers. Three main types of studies are needed in order to determine if viruses are involved in the production of cleft palate in man.

a) Basic knowledge must be obtained in laboratory animals relative to the mechanisms by which viruses can produce clefts.

b) Viruses or viral antibodies should be isolated from human newborn or aborted fetuses with clefts, or from their mothers. Epidemiological studies should show that there is a significant difference between the frequency with which a given virus is found in cleft fetuses and newborn and the frequency with which the same type of virus is found in the general population of newborn.

c) Reproduction of clefts in experimental animals by the same viruses which have produced them in man. For obvious reasons these experiments should be made in animals as closely related to man as possible (subhuman primates).

Antibodies

The first anti-organ antisera to be used for inducing malformations was reported by Guyer and Smith (*40*). These investigators induced a variety of malformations in the rabbit by injecting anti-lens antisera into the rabbit during pregnancy. In 1958, similar results were obtained by Miller (*73*); the percentage of malformation obtained was low. Miller further established that the failure of previous investigators to repeat the results by Guyer and Smith was due to the fact that the teratogenic agents were injected into the mother too late in pregnancy.

Since these original studies a number of other investigators, using specific anti-organ antisera, have obtained similar results with species other than rabbits (*18*, *27*, *61*, *62*, *74*).

In 1940, Seegal and Loeb (*82*) studied the effect of anti-placenta antisera on the fetus of the pregnant rat. These authors noted a high percentage of intrauterine deaths and resorptions. Using kidney antisera, Brent (*9*) was

able to produce a high percentage of malformations in rats, the incidence of malformations appearing to be related to dose. This was confirmed by David (*16*). A subsequent study by Brent (*7*) further demonstrated that a specific dosage of immune sera at a specified time during pregnancy yielded a spectrum of malformations including 4.7% to 6.6% cleft palate when injected at the 8th and 9th days of pregnancy. Brent (*8*) has since obtained similar results using anti-placenta antiserum.

The role of the immune processes in the production of diseases in man, particularly as related to the pregnant mother and her offspring, has received increasing attention since the early 1940's. Levine and associates (*68*) demonstrated that antibodies against blood cells containing the RH factor could be produced by mothers not initially possessing this factor; furthermore, these antibodies could cross the placental barrier and produce lesions in the fetus.

At present there is a variety of disease entities in man which are related to immunological mechanisms. Anti-nuclear antibodies have been found in patients with *lupus erythematosus* (*72*); antibodies against kidney in patients with glomerulonephritis (*52*); against thyroid tissue in patients with Hashimoto's disease (*79*), primary myxedema (*17*), and thyrotoxicosis (*11*); against muscle in myasthenia gravis (*91*); against intestinal mucosae in ulcerative colitis (*10*); against heart tissue in rheumatic fever (*97*); against the stomach in pernicious anemia (*44, 92*); against adrenal glands in primary adrenal atrophy (*2*); and against nervous tissue in multiple sclerosis (*14*).

In some cases it has been demonstrated that antibodies of this type can be transported from the mother to the fetus. Thus, the passage of antibodies through the placenta has been demonstrated in cases of *lupus erythematosus* (*4*), thyrotoxicosis (*85*), myasthenia gravis (*90*), cretinism (*6*), et cetera.

We can summarize the data mentioned above by pointing out that evidence has been obtained demonstrating the following facts:

a) That anti-organ antibodies obtained experimentally are capable of producing malformations in the offspring if injected into the pregnant female.

b) That adult human organisms produce in many instances anti-organ antibodies of different types.

c) That anti-organ antibodies present in pregnant women may cross the placenta and reach the fetus.

Consideration of these three facts suggests the possibility that anti-organ antibodies may be involved in the production of some human congenital malformations whose etiology thus far remains unknown.

Conclusion

A survey of epidemiological data, of experimental research in cleft palate on rodents, and of the anatomy and histology of cleft palate in human fetuses, has led us to suggest that a different orientation in research is neces-

sary if we are to progress toward an understanding of the mechanisms and etiology of cleft palate in man. Although the genetic constitution without doubt is an important factor role as a predisposing host in many cases and to varying degrees, environmental agencies are clearly indicated as playing key teratogenic roles in clefting of the palate. Since none of the teratogens used on rodents satisfy the requirements of a ubiquitous environmental agent operating on all races and populations of man, we propose the use of viruses and anti-organ antibodies to produce cleft palate and associated malformations—not in the rodent, but in the rhesus monkey. The latter is recommended as the experimental animal of choice, since in its normal and cleft anatomy it is strikingly similar to man, whereas the rodent is not. Furthermore, the evidence is in favor of clefting in man and in rhesus to occur after fusion has taken place and before bony invasion of the palatal shelves is complete, in other words, between 7½ and 10 weeks *in utero*. Degeneration of palatal tissue, induced by teratogenic action, plus continued rapid increase in breadth of the face, cause rupturing of the palate with consequent clefting, to various extents, of the palate.

Both viruses and anti-organ antibodies are known to cause congenital malformations in man. As Ebert (*19*) points out: "Antibodies and cell-mediated reactions are more than experimental tools of the embryologist; they may be teratogenic agents in man".

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