Etiology and Pathogenesis of Congenital Cleft Lip and Cleft Palate, an NIDR State of the Art Report *

A. BURDI,[1] M. FEINGOLD,[2] K. S. LARSSON,[3] I. LECK,[4]
E. F. ZIMMERMAN [5] AND F. C. FRASER [6] (Chairman)
[1] Department of Anatomy, University of Michigan Medical School,
Ann Arbor; [2] Department of Pediatrics, Tufts University School of
Medicine, Boston; [3] Teratology Laboratory, Karolinska Institute,
Stockholm; [4] Regional Cancer Epidemiology Unit, Christie
Hospital, Manchester, England; [5] Division of Fetal
Pharmacology, Children's Hospital Research
Foundation, Cincinnati, Ohio, and
[6] Department of Biology, McGill
University, Montreal

This report was prepared by a subcommittee on "Etiology and Pathogenesis" of a group commissioned by the American Speech and Hearing Association, under contract NIH 71-643 to the National Institute of Dental Research, to prepare a report on "Clinical Research in Cleft Lip and Palate–the State of the Art." The collaboration of Dr. C. S. Chung, Dr. M. C. Johnston, and Dr. J. D. Niswander of the National Institute of Dental Research is gratefully acknowledged. Other journals will publish reports from the following subcommittees:

Speech and Psychosocial Aspects
Pediatric and Otologic Aspects
Surgical Aspects
Anatomical and Physiological Aspects
Orofacial Growth and Dental Aspects

A. EXPERIMENTAL STUDIES ON MECHANISMS AND CAUSES

1. Cleft palate

Cleft palate has probably been the target of more research on causes and mechanisms than has any other congenital malformation. Perhaps this is because palate closure is one of the last important morphogenetic events, occurring at a stage when the embryo is relatively large and correspondingly easier to observe. Nevertheless, there is still much to be learned. The following discussion will review representative problems and tactical approaches.

a. The observational approach

There are two possible approaches to the study of palate closure and its failure. One is to observe the normal process by all possible methods from macroscopic to ultrastructural and to infer, from the chronology of change, that certain events were the cause of certain subsequent events. For instance, the palate shelves grow down from the walls of the oronasal cavity lateral to the tongue, and then move to a "horizontal" position above the tongue. Examination of a series of embryos may show that the tongue drops before the shelves move and appears to press on the under surface of the shelves during their transition to the horizontal (Walker, '68). On the other hand, if the shelf is seen to have moved towards the horizontal and the tongue has not dropped out of the way (Walker and Fraser, '56) it seems likely that the shelf plays an active role in the process. The truth is probably somewhere in between. The first mechanism appears predominant in the rabbit and the second in the mouse. More, careful observations of human embryos at the time of palate closure may help to show what it is in man.

We still do not know what provides the force in the shelves that causes them to reorient themselves to the horizontal. Certain possibilities can be ruled out. Movement is not likely to depend on elastic fibres in the shelf, since there are none there when the shelves move. Other possibilities can be suggested. If, e.g., active

microfilaments or microtubules were demonstrated in places where contraction would result in shelf movement one would be tempted to suppose that they provided the necessary shelf force. But for these and many other aspects of palate closure it may not be possible to prove the causal interrelation by simple observation. If mucopolysaccharide synthesis, or increased epithelial mitotic activity, or vascular engorgement, precedes shelf movement, it does not necessarily follow that they contribute to shelf movement.

The study of spontaneous clefts in human embryos suffers from the fact that all observations are post hoc, and one can only make inferences about what went on at the time the defect occurred. Thus observations on lack of mesenchymal "penetration" in cleft lip embryos do not tell us whether the mesenchyme failure caused the cleft or vice versa (Stark, '54).

Similar difficulties apply to the question of whether active tongue movement is necessary for palate closure. Humphrey ('69) and others have shown that the human fetus moves its mouth at the time of palate shelf movement but is this necessary for normal closure? The experimental evidence is conflicting (Walker, '69; Jacobs, '71) and we do not know if failure of tongue movement is a mechanism for the failure of palate closure in man. In spite of these difficulties, however, there is much to be learned from careful study of human embryos, both normal and abnormal.

b. *The experimental approach*

The study of teratogenically induced defects can circumvent some of the problems of trying to infer causes from post hoc observations. If treatment with a teratogen produces the defect in virtually all exposed embryos, then one can look for relevant changes in the fixed embryo before the overt defect appears, knowing that that particular embryo would have developed the defect if it had continued to grow. The discovery that cortisone administered early in pregnancy produces cleft palates in 100% of the embryonic mice in one strain so treated (Fraser and Fainstat, '51) allowed study of effects on the palate before its failure to close, and

contributed to the understanding of the underlying mechanisms (Fraser, '69).

The approach is to interfere with closure in some specific way, on the assumption that if process X is blocked, and the cleft palate follows, process X is necessary for palate closure. Early work along these lines was at a rather primitive level. With respect to cortisone-induced cleft palate it could be shown that the treatment results in a delay in movement of the shelves to the horizontal to the point where, when they do get there, the head has grown so much that they are too far apart to meet (Walker and Fraser, '57). Strains in which normal closure was relatively late had the highest frequencies of induced cleft palate. This led to the concept of palate closure as a *continuous distribution* — the stage at which the shelves move —, and a *threshold* — the latest stage at which they can still reach each other to fuse and form the palate (Fraser et al., '57). Progress was aided by comparisons between genetically different inbred strains — an approach that is still relatively unexploited and should be encouraged.

Analysis of various teratogens on various genotypes has suggested a number of factors which may contribute to shelf closure (Fraser, '69). The shelves appear to move to the horizontal against the resistance of the intervening tongue, which may or may not help by moving out of the way or pushing on the shelf. The stage at which the shelves move may be influenced by an intrinsic shelf force, the mechanical effect of changes in the cranial base, width of the head, width of the shelf, length of the head, forward movement of the jaw (and hence the tongue), size and mobility of the tongue, epithelial competence to fuse, pharyngeal musculature, and neck extension. A mutant gene or teratogen could interfere with palate closure by acting at any one of these points. Alternatively, and perhaps more commonly in man, a cleft palate may result from minor changes, individually indistinguishable, at several points in the system, all interacting to delay shelf movement beyond the threshold. This is what is meant by a multifactorial, threshold system.

But, to return to the example, how does

cortisone cause the delay in shelf movement? Cortisone does many things. It inhibits sulfomucopolysaccharide synthesis (Larsson, '62), decreases amniotic fluid (Fraser et al., '67), increases water retention (Jacobs, '66), inhibits RNA synthesis (Zimmerman et al., '70), inhibits mitosis (Bullough, '62), stabilizes lysosomal membranes (Weissman and Thomas, '63), causes myopathy (Walker, '71), and probably does several other things. Which of the things that cortisone changes is process X, the one that causes the cleft palate? Or may it even be several effects conspiring together to delay shelf movement beyond the threshold? The same question can be asked about many other teratogens that cause cleft palate. Isotope and other molecular techniques are being applied to the problem, and they should be encouraged, but more attention must be paid to the limitations of the methods and to the interpretation of results. For instance, measurements on palatal shelves a day after they have failed to close may be misleading, since the tissues may be obviously abnormal by then.

The tactic here is to see which of the effects of a teratogen correlates best with the end point — the frequency of induced cleft palate. For instance, if different genotypes vary in their susceptibility to a teratogen one could give the teratogen to several different strains, and measure the effect on process X. If the effect on X correlated well with the frequency of cleft palate, this would support the hypothesis that process X was the one through which the teratogen caused cleft palate. A poor correlation would argue against X being the key process. But not conclusively — one would have to consider the possibility that the poor correlation results from strain differences in maternal metabolism, placental transfer, or protein binding of the teratogen, or differences in normal embryonic developmental patterns leading to differences in cleft palate frequency.

And let us not forget that even if we prove conclusively how a teratogen interferes with palate closure we have only learned something about the cleft palate caused by that teratogen, which may not be what happens in a "spontaneous" cleft. Maternal emotional stress does not seem to be associated with cleft lip or cleft palate in man (Fraser and Warburton, '64), in spite of claims to the contrary, and it is very difficult to produce cleft palates in mice by maternal treatment with ACTH (Heiburg et al., '59), suggesting that the maternal adrenal output of glucocorticoid is just barely able to reach the teratogenic level. Studies of teratogenically induced clefts have their value but not, so far, in demonstrating the causes of spontaneous clefts in man.

On the other hand, the demonstration of maternal effects on embryonic susceptibility to teratogenically induced and to spontaneous cleft (Fraser, '69) could provide information relevant to prevention in man. Experiments with reciprocal crosses and, more recently, blastocyst transfers and ovary transplants are providing this kind of information and should be pursued.

The use of teratogens as experimental tools is beginning to contribute to a better understanding of developmental mechanisms and their failures. For instance, a number of teratogens inhibit mucopolysaccharide synthesis in the palate shelves before closure, particularly glucocorticoids and salicylates (Larsson, '62; Larsson and Bostrom, '65). But triamcinolone, at a dose that causes a high frequency of cleft palate, does not inhibit acid mucopolysaccharide synthesis nearly as much as cortisol at a dose causing similar frequencies of cleft palate (Andrew and Zimmerman, '71). This suggests that inhibition of acid mucopolysaccharide synthesis is not the cause of the cleft palate. But it does not prove it — the lack of correspondence between teratogenic action and inhibition of acid mucopolysaccharide synthesis by different corticosteroids may result from differences of absorption, or change in sulfate pool (Herbai, '71), or in a synergistic effect on amniotic fluid, for instance. Triamcinolone also inhibits RNA synthesis (Zimmerman et al., '70), and this may result in impairment of many cell functions. The effect on DNA synthesis has also been studied. The question is still unresolved, but the point is that the powerful tools of modern molecular biology are just beginning to be applied to such problems. There is good hope that further extensive and critical studies will clarify many of these questions.

Other teratogens under study have simi-

lar problems and similar promise. Hypervitaminosis A increases mucopolysaccharide synthesis (Kochhar et al., '68) — making the shelves too stiff? — decreases mitosis (Kochhar, '68) — making the shelves too small? —, and possibly changes the capacity of the shelves to fuse. X-irradiation and cytotoxic agents may act by destroying populations of rapidly dividing cells (Scott et al., '71). Chlorcyclizine, in the rat and mouse, causes edema (Posner, '71) which may provide mechanical interference to shelf movement. Amniotic-sac puncture constricts the embryo which may cause cleft palate by mechanical interference (Trasler et al., '56). Lathyrogens inhibit crosslinking of collagen which could interfere with the shelf force (Pratt and King, '72).

We hope that the preceding discussion has made it clear that the day has passed when one can justify experiments that expose embryos to presumptive teratogens "just to see what will happen." Emphasis should now be on critical studies of the mechanisms by which teratogens bring about their effects.

Organ culture has now reached the point where it is useful in studying organogenesis and this approach should be exploited more fully. It is interesting to note from studies on organ culture that preparatory changes occur in the epithelium of the palate shelves before fusion (Morgan, '69), and that fusion will not occur if the epithelia come into contact either too early or too late (Pourtois, '68). Again, we do not know of any teratogens that act this way, though hypervitaminosis A might be a candidate. The technique has also been used to demonstrate the migration of neural crest cells into the facial processes in rat embryos (Johnston and Krasnes, unpublished), as previously demonstrated in the chick (Johnston, '66).

It should be emphasized again that the palate is closing in a rapidly growing organism and that many factors interact to achieve the final result. It is difficult to disentangle them and evaluate their relative importance. For instance, if the shelf force depends on straightening of the cranial base (Verrusio, '70), and if a teratogen acts by inhibiting this process, it is no use looking for the site of action

in the shelves. The growth of the cranial base cartilage has been studied by direct measurement (Harris, '67; Hart et al., '69) and by autoradiography (Long et al., '72), and maternal treatment with 6-aminonicotinamide has been shown to inhibit the straightening of the cranial base (ibid). The effects of other teratogens on cranial base growth should be studied. Species differences may also present difficulties. If closure of the human palate were like that of the rabbit rather than that of the mouse one would need to be cautious about extrapolating conclusions from mouse to man. Nevertheless, work on experimental animals has contributed a great deal of understanding, and has promise of continuing to do so. This approach must be extended to primates, as a test of their relevance to man.

2. Cleft lip, with or without cleft palate

The causes of clefts of the lip (or more precisely of the primary palate, i.e., premaxillary segment) are less well understood. Formation of the primary palate occurs much earlier in development than secondary palate closure. There is considerable evidence from three apparently independent studies (Lejour-Jeanty, '65; Trasler, '68; and Andersen and Mathiessen, '68), conducted on rodent, rabbit, and human embryos, that the primary palate forms by an "invagination-fusion" mechanism. In the first place the epithelium of the olfactory pit "tunnels" backward to reach and fuse with the epithelium in the roof of the stomadeum thereby cutting off an isthmus of mesenchyme. Enlargement of the isthmus by surrounding mesenchyme "consolidates" the primary palate. No mesenchyme penetration of an epithelial wall is involved. This new concept agrees with work on lower vertebrates (e.g., Bertmar, '65). Mesenchyme migrates from the neural crest and proliferates rapidly to form the facial swellings or processes (Johnston, '66), the position of which may depend on inductive stimuli from the developing eyes and ears. There is some evidence to suggest that the size and shape of the facial swellings are related to the embryo's susceptibility to cleft lip (Trasler, '68), and that a critical degree

of apposition between the medial and lateral nasal and maxillary swellings may be a developmental threshold for cleft lip. The same tactical considerations apply here as for cleft palate.

It is more difficult to cause cleft lip experimentally. Almost all teratogens that are effective in inducing clefts of the primary palate [CL(P)] seem to have their greatest effects on rapidly dividing cell populations: radiation (Warkany and Schraffenburger, '47), anticancer drugs ·such as folic acid antagonists (Asling, '62) and hadicidin (Chaube and Murphy, '62; Lejour-Jeanty, '66, '70), mitotic inhibitors such as vinblastine (DeMeyer, '64a) and vincoblastine (DeMeyer, '64b), and, possibly, aspirin (Trasler, '65). Lejour-Jeanty ('66) has studied the pathogenesis of hadicidin-induced cleft lip noting fairly selective cell death in the lateral nasal processes and forebrain. These studies favor the idea of there having to be a critical amount of mesenchyme in the "consolidating" facial processes for normal primary palate formation.

Again, there are maternal effects that may point the way to preventive measures.

3. Conclusions

Observations on normal palate closure and experimental analysis of mechanisms by the use of teratogens and genetic differences have contributed greatly to our understanding of the process and how it may fail, but there is much to learn. Knowledge of lip formation and its failure is much less advanced than that of palate closure, but similar concepts and strategies apply.

The methods of modern molecular biology are just beginning to be applied to the problem. Although analysis is difficult, being complicated by the fact that the process is proceeding in utero, in an amnion, in an embryo in which many things are happening at once, progress is being made. There is need for further research along the following lines.

a. *Continuing study of the normal process in human embryos*

Understanding the normal increases the probability of understanding the abnormal, and developing preventive measures.

Conclusions based on experimental material must be confirmed in man by study of therapeutically or spontaneously aborted embryos. Much valuable material is being wasted, either through being discarded or being stored in inaccessible places, or being inadequately processed. There is a need for an *organized system for collecting embryos* in a state suitable for analysis (e.g., karyotyping, biochemistry, gross and microscopic anatomy), with proper antecedent histories, and a system for making them available to interested scientists. Individuals at strategically located centers could be trained to collect such embryos, process them, and study or distribute them for study. A system for collecting abortuses on a national scale exists in Japan, but the results would be unrepresentative of a North American population. A workshop on technical procedures and logistical problems should be organized.

It is now technologically timely to go beyond subjective descriptions of the fate of human facial and palatal primordia. Increased numbers of aborted human embryos will provide data on the volumetric and spatial changes in embryos with clinical and family histories, and with observations on near relatives. For instance, there is evidence that the palate closes later in female than in male embryos (Burdi and Silvey, '69), which agrees with the higher incidence of cleft palate in females, predicted by the multifactorial, threshold model. Are there also sex differences that would explain the excess of cleft lip in males, or asymmetries that would account for the excess of left-sided clefts of the lip?

b. *Further study of how teratogens cause cleft palate*

This will (i) improve understanding of the mechanisms by which cleft palate can occur; (ii) elucidate possible synergisms between factors — e.g., possible interactions between a susceptible genotype, a transient maternal zinc deficiency, and oligohydramnios resulting from pernicious vomiting; (iii) warn of possible teratogenic hazards in man, such as drugs, pesticides, herbicides, food additives, or chelating agents. Demonstration of terato-

genicity in experimental animals does not necessarily mean teratogenicity in man however, and failure to demonstrate teratogenicity of an agent in animals does not mean it will be harmless to the human embryo (Fraser, '64). Experimental testing is a guide, not a safeguard; (iv) allow a search for possible preventive measures — e.g., ways of making the shelves move earlier (without having disruptive effects elsewhere), would increase the resistance of the embryo to all cleft palate-producing factors.

c. *Utilization of present methods of organ culture and development of better ones, to aid in the objectives above*

B. GENETICAL STUDIES IN MAN

Perhaps the greatest drawback to genetical and epidemiologic research on clefts of the lip and of the palate has been the unfortunate tendency to lump them together. There is good embryological and genetical evidence that isolated cleft palate is developmentally and genetically different from cleft lip (Fraser, '70), which may or may not have an associated cleft of the palate. Conclusions based on data in which the two types were combined have led to considerable confusion.

1. *Cleft lip* ± *cleft palate*

There are now several extensive bodies of data on families of probands with cleft lip, with or without cleft palate, providing frequencies of recurrence in various categories of relatives. These are, in general, reliable and are useful for genetic counseling (Fraser, '71).

In an unselected series of children with cleft lip or cleft palate a small proportion would be caused by major mutant genes (these children often have associated defects which constitute a syndrome), a small proportion by chromosomal aberrations (these also are likely to result in syndromes), a few by nonrecurrent environmental factors, and the majority by the interaction of many factors, individually indistinguishable — the multifactorial group.

For cleft lip and palate a number of features are compatible with a multifac-

torial, threshold model (Fraser, '70). These include (a) the increase in risk to sibs after two sibs are affected over that after one is affected; (b) the fact that the probands of the sex with the lower frequency (females) have the higher recurrence risk in their first degree relatives; (c) the increase in recurrence risk with increasing severity; (d) the sharp fall-off in frequency from first to second degree relatives and much smaller decrease from second to third degree; (e) the fact that the frequency in sibs is about the square root of the frequency in the population. If this is the correct model the increased fertility of treated patients will cause only a slow rise in frequency — if treatment were to increase fertility to normal there would be roughly a 5% rise in frequency per generation. Furthermore, the existence of an environmental component to the etiology raises the possibility of reduction in frequency by environmental means.

On the other hand, the familial distribution is also compatible with other models, such as a major recessive gene with reduced penetrance and an admixture of sporadic cases (Chung and Morton, unpublished). However, the relation of recurrence risk to sex of proband and severity of defect would require a number of assumptions about the nature of the sporadic cases that make this hypothesis less attractive than the multifactorial one. Sophisticated methods of analysis (Chung and Morton, unpublished) would make it possible to discriminate the models by data from offspring of affected parents with an affected child. But such families are rare; sufficient data can be obtained only by extensive cooperative studies.

It would be useful to identify measurable indications of genetic susceptibility. Data from teratological studies on mice suggested that shape of the embryonic face might be such an indication (Trasler, '68). If so, first degree relatives of affected patients should have face shapes that differed, statistically, from the average. Preliminary studies support the hypothesis both on the basis of surface topography (Fraser and Pashayan, '70), and cephalograms (Niswander and Johnston, unpublished). Further data are needed to substantiate these findings and reveal which changes are the most discriminant. Ceph-

alometry is the most accurate method of measurement, but if the measurements are to be useful as guides for counseling, it would be more practical to use surface features, which can be measured quickly and without special equipment.

The most efficient indication of genetic predisposition would be unaffected monozygotic twins of children with cleft lip, since they would presumably be near the threshold. A small series of twins does show the expected features (Johnston, unpublished), but many more monozygotic discordant pairs are needed.

Another indication of genetic susceptibility would be the occurrence of "microforms," or minor degrees of the major defect. There are many uncritical studies and unjustified claims in this area (Fraser, '71). To be a microform the anomaly must occur more frequently in the near relatives of persons with the major defect than in the general population. This appears to be true for cleft uvula and submucous cleft palate in the families of patients with cleft palate. It does not seem to be true for nostril asymmetry or missing lateral incisors in relation to cleft lip. Further critical studies would be useful.

2. *Cleft palate*

Because isolated cleft palate is less frequent than cleft lip (with or without cleft palate), there are many fewer genetic data available. Cases of cleft palate probably fall into the same four categories — major mutant genes, chromosomal, environmental, and multifactorial — but the proportion of multifactorial cases may be smaller than for cleft lip. There is a need for the collection of more information on frequencies in relatives, on twins, and on the physical characteristics of unaffected near relatives.

3. *Syndromes*

Cleft lip and/or palate may be part of a syndrome of defects, in which case its cause and mechanism will be related to that of the syndrome. Over 100 syndromes are known (Gorlin et al., '71) that involve clefts of the lip or palate, of which about a third are caused by major mutant genes

and a few by recognized chromosomal aberrations, and many have no recognized cause. An appreciable portion of the latter may be chromosomal aberrations too subtle to be recognized by standard methods. The development of new methods of chromosome staining (Caspersson et al., '70), which allow detection of small deletions, duplications, translocations, and inversions, may reveal the causes of many syndromes at present placed in the "idiopathic" category. Greatly increased activity is occurring in this area.

"Syndromology," the recognition of characteristic associations of defects in the same individual, is becoming such an active and sophisticated field that it might almost be called a subspecialty (Smith, '70). The underlying assumption is that a syndrome has a specific etiology, even if it is unknown. The identification of syndromes is of value because:

a. it is a necessary prerequisite for the identification of their causes;

b. it results in removal of syndromes from series of cases in the "multifactorial" group, thus reducing heterogeneity and improving the reliability of the data;

c. it improves prognosis, both of the patient's future and of the risk of recurrence in the family;

d. it may be helpful in revealing developmental relations and causal mechanisms. For instance the array of defects that occurs in the holoprosencephaly syndrome (DeMeyer et al., '64) indicates a developmental relation between the forebrain and face, as demonstrated experimentally in amphibia (Jacobson, '66), and supports experimental evidence regarding the role of neural crest cell migration in facial morphogenesis (Johnston, '66). "Comparative syndromology" may also help to reveal developmental mechanisms. For instance a majority of syndromes in which micrognathia is a feature also include cleft palate as a feature, whereas many syndromes involving cleft palate do not include micrognathia. This provides indirect evidence that the micrognathia of Pierre Robin syndrome is a contributory cause to the cleft palate (Fraser, unpublished).

Syndromology is a field that still presents some difficulties, however. For one thing there is a great need for normative

standards. Many syndromes are characterized by various subjectively determined features such as low-set ears, wide-spaced eyes, low-set thumbs, or micrognathia. It is necessary to develop criteria for such features that are both objective and easily determined at the bedside. We need to be able to assess objectively whether an ear is low-set, or a mandible small, without radiologic cephalometry, for example.

Furthermore, not all features of a syndrome occur in every case, and some features occur in some normal individuals. There is a need for data on the frequencies of features such as confluent eyebrows and hypertelorism in the general population, and for extensive data on the frequencies with which the features of a syndrome occur in cases of the syndrome.

Keeping track of the growing wealth of information on syndromes is also a problem. Many syndromes are rare, so that no one investigator sees more than a few of any one kind. It is a tedious and sometimes fruitless task to search the literature for other reports of a rare combination of defects that an investigator has just seen for the first time. Moreover, many syndromes are recognized by a "gestalt" visual impression, difficult to describe other than by a picture (Gellis and Feingold, '68). There is a need for a central computerized repository of information which would list all reported cases of unusual combinations of defects, grouped into categories. It should be so organized that a pediatrician, for instance, who had a patient with features, A, B, D, M, and X, could extract from the registry all syndromes that included these features, as well as individual case reports that might represent still-unrecognized syndromes. Ideally the system would also produce a series of pictures of children with the syndrome into which the case might fit, to provide the gestalt image.

More than one computerized syndrome-identification service exists, based on the frequencies of various anomalies in the general population and in syndromes. Though these are not yet very discriminatory, perhaps because the information stored is inadequate, the approach looks promising and should be encouraged.

C. EPIDEMIOLOGY

Epidemiological studies of malforma-

tions have been of four main kinds: (1) *descriptive* studies comparing their incidence in the various groups into which children can be classified on the basis of routine data; (2) *correlative* studies in which groups differing in exposure to factors that could be causal are examined for parallel variations in incidence, or vice versa; (3) *analytic* studies in which evidence of differences in exposure to such factors is sought by comparing individuals with and without defects; and (4) *experimental* studies in which action is taken to reduce the chances of exposure to a suspected cause and the cases in which this action has been taken are compared with others. Because of serious inadequacies of reporting, birth certificate data are virtually useless for epidemiological studies (Meskin and Pruzansky, '67). Ascertainment continues to be a significant problem in the use of epidemiological data.

Only descriptive studies appear so far to have yielded much positive information about the common defects; and in the case of oral clefts even the results of descriptive studies, apart from those concerned with sex and ethnic group, have been somewhat inconclusive by comparison with recent family studies.

1. Description studies of cleft lip (with or without cleft palate)

Studies of incidence in different places and ethnic groups suggest that the main sources of variation are genetic. In 25 recent series, each of which included over 10,000 births, was relatively homogeneous ethnically, and had reasonably complete ascertainment (Leck, '72), the statistical variance between populations of different "races" — Mongoloids (high incidence), Negroids (low), Caucasoids (moderate), and Latin Americans (between Caucasoids and Mongoloids) — was eight times as high as the variance between populations of the same "race" in different places. The ethnic differences persist even in places such as Hawaii and Birmingham, England, where members of two primary races live. The figures for American Indians (Tretsven, '63; Miller, '64; Niswander and Adams, '67; Lowry and Renwick, '69) are all higher than for Caucasoids and some are above the Mongoloid range

also. Clearly, then, incidence varies with ethnic group. Differences in face shape may account for at least some of these variations. Those of mixed ethnic group seem to be intermediate in incidence between their races of origin with no evidence of maternal effects (Morton et al., '67; Ching, '70).

There is little to suggest that incidence varies with place or time. Two British studies (Pleydell, '60; Knox and Braithwaite, '63) reported higher rates in towns than in the surrounding country, at any rate for cleft lip alone, but genetic differences could not be ruled out.

Leck ('72) showed no secular or seasonal trend in Birmingham. On the other hand, an upward secular trend was reported from Denmark (Fogh-Andersen, '67), and cleft lip without cleft palate was significantly commoner in some years than others in Northeast England (Knox and Braithwaite, '63). Some previous studies of season suggested that incidence was particularly high in spring (Edwards, '61; Fujino et al., '63; Slater et al., '64; Hay and Wehrung, '70) although others did not (Charlton, '66; Stark et al., '70).

Another aspect of the relation of incidence to space and time is that these two variables may interact — in other words that mothers who produce affected children at about the same time may tend to live closer together than average, as they might if an infection was concerned in etiology. Studies both in Northeast England (Knox, '63b) and in parts of the US (Stark et al., '70) suggest that some but not all series show clustering of this kind. The evidence is strongest for cleft lip without cleft palate.

Males are affected by cleft lip more often than females. In many series, although not all (Leck, '72), the sex difference was greater for cleft lip and palate than for cleft lip alone. However, the fact that on US birth certificates underreporting of cleft lip alone is more serious in males than females (Hay, '67b) raises doubts about these differences. Where the palate is involved the severity of its involvement tends to be greater in females (Meskin et al., '68).

There was no significant variation in incidence by social class, maternal age, or parity in the most recent Birmingham series (Leck, '71a). There is evidence in

other studies that incidence rises at maternal ages over about 35 (e.g., MacMahon and McKeown, '53). According to US birth certificate data, the completeness of which does not seem to vary with maternal age (Hay, '67b), the excess at high maternal ages is almost confined to children with multiple defects, among whom it is extremely marked (Hay, '67a) — perhaps because chromosomal aberrations are involved in these groups. The incidence of cases with no other defects in the same data did increase with paternal age, but only very slightly. Other studies of parental age have given inconsistent results.

Although the incidence of cleft lip without cleft palate seems from some of these results to be rather labile the overall impression for cleft lip with or without cleft palate is that, for cases not associated with other defects, there are no recognizable environmental influences, since there must be a few of these that would not be correlated with place, time, parental age, parity, or social class. Because of its insensitivity to these influences, in striking contrast to the behavior of neural tube defects, we may have to accept that in cleft lip the nongenetic element in causation — which seems from family studies and the above data on ethnic groups to be a relatviely small element — is at present largely undefinable "intrauterine noise."

2. Descriptive studies of cleft palate (without cleft lip)

Variation in incidence of cleft palate not associated with cleft lip is rather different. Its variance between populations of different primary races is not significantly greater than between those of the same race, although the latter variance is of the same order as for cleft lip (Leck, '72). Although there is no more evidence of variation in time or by social class for cleft palate alone than for cleft lip its incidence is significantly increased in the children of relatively old women of high parity in Birmingham, England. Similarly the incidence of cleft palate with no other defect showed a more marked increase with parental (primarily paternal) age than did cases of other clefts with no other defect in Hay's ('67b) US birth certificate series, contrary to the case for clefts combined with other defects.

Nonrandom environmental influences appear, therefore, rather more likely to affect the incidence of cleft palate occurring as a single defect than is the case for cleft lip, and ethnic and family studies suggest that the influence of the genotype on incidence is correspondingly less — although even in cleft palate, twin studies and the sex difference (a higher rate in females than males) would appear to indicate that this influence is considerable. Perhaps Fogh-Andersen ('42) was near to the truth when he suggested that in this condition, unlike cleft lip, the familial cases may differ in etiology from the sporadic ones.

It follows that although little of value seems likely to emerge from further descriptive studies of the epidemiology of either defect as a whole, there would be a case for comparing the epidemiology of familial and nonfamilial cases of cleft palate alone. Hay's ('67a) finding of a striking increase in maternal age for clefts combined with other defects suggests that further descriptive epidemiological studies of the commoner combinations might also be worthwhile, especially as they seem likely to include a higher proportion involving a specific causal gene or teratogen than do the clefts without other malformations.

As with twin studies the problem in studying the epidemiology of familial cases or of combinations of defects is to obtain adequate data, which may involve the unbiased surveillance of millions of births. The closest practicable approach to such a system for the United States might be to revive the National Cleft Lip and Palate Intelligence Service, under which copies of all birth certificates recording malformations and 1% of other birth certificates used to be sent by 29 states to the PHS Dental Health Center in San Francisco (Greene et al., '65). This would need to be augmented by parallel data on stillbirths, and by follow-up of samples of each group of children to estimate how biased the data are by incomplete and inaccurate ascertainment.

3. Correlative, analytic, and experimental studies of clefts

In the field of clefts these three approaches have been largely unrewarding — as they generally are when the hypotheses under test have no basis in descriptive studies. Even the positive results that have been reported are difficult to interpret, because one never knows how many unpublished negative results have been obtained for each published positive one.

The correlative approach, comparing incidence in groups differing in their exposure to factors that could be causal, was used to study the relative frequency of clefts and other defects in 12 cohorts of children born 26–40 weeks after influenza epidemics (Leck et al., '69; Leck, '71). Four of the 12 cohorts were born after the first A2 influenza epidemics in their communities, and these showed no increase in the incidence of cleft lip. The other eight were born after epidemics due to recurrences of familiar viral strains; and in each of these cohorts the incidence of cleft lip alone, although not of cleft lip with cleft palate, was more than 18% higher than expected; three of these eight increases were significant at the 5% level (with 1 tail). However, since the increases were not confined to children that were at a particular stage of their embryonic development when the epidemics occurred it is difficult to believe that the association was causal. It is perhaps more likely that the *survival* of embryos affected by these defects, or exposed to conditions predisposing to them, may be differentially affected by epidemics. There is some precedence for this, in mice, where embryos with spontaneously occurring cleft lip appeared relatively resistant to 6-aminonicotinamide (Goldstein et al., '65).

The main need exposed by this finding may be for more study of the pattern of clefts among aborted human embryos, which would appear from the high frequency of clefts reported by Nishimura ('70) to account for far more cases than survive to a viable age. Knox's ('63b) studies of the sex ratios of uncles, aunts, and cousins of children with clefts suggest that the abortion rates among such relatives may also be abnormal; he found, e.g., a deficiency of the kinds of relatives that might have had one of the same X chromosomes as the children with cleft lip alone.

4. Conclusions

a. There is no evidence from popula-

tion studies of incidence that major environmental factors influence the frequency of cleft lip (with or without cleft palate, but without other defects), and further epidemiological studies directed to the detection of such factors are not likely to be fruitful, unless they are designed to detect far more subtle influences than previous studies could. Clinical and family investigations directed to the question of etiological heterogeneity could be useful.

b. For isolated cleft palate, on the other hand, and for clefts combined with other defects, there is a little evidence for nonrandom environmental influences. Further studies, particularly looking for differences between familial and nonfamilial cases of cleft palate, and between clefts with and without other major defects, might be informative. For such enquiries, as for twin studies, data on a much larger population would be needed than are now being assembled at any one place in the United States.

c. More information is needed on the *distribution of clefts in human embryos* (e.g., those aborted deliberately), which is likely to reflect any variations in risk more closely than the pattern at birth (to which many affected individuals do not survive long enough to contribute).

d. There is a need for data on prenatal events associated with cleft lip. Retrospective studies are likely to be biased, and prospective studies expensive and tedious. A compromise approach would be to study the physiology and metabolism of mothers of affected children — looking, for instance, at folate levels, vitamin A metabolism, presence of antiinsulin factor, toxoplasma antibodies, glucose tolerance, and corticoid levels. Such mothers could also be followed through subsequent pregnancies, 5% of which could be expected to result in affected children. This would probably require a collaborative study.

Analytic studies, comparing other characteristics of individuals with and without defects, have suggested association between clefts and (i) nausea and vomiting in pregnancy (Drillien et al., '66; Richards, '69); (ii) miscellaneous drugs, including antiemetics (Richards, '69); (iii) maternal bleeding (Drillien et al., '66; Fraser, '70); (iv) toxemia (Fraser, '70); (v) increased antagonism to insulin (Vallance-

Owen et al., '67), and (vi) toxoplasma antibodies (Erdelyi, '57). Folic acid deficiency and abnormalities of serum proteins have both been reported in abnormally high proportions of mothers of malformed children generally (Langman, '61; Hibbard and Smithells, '65). Several preliminary reports suggest that antiepileptic drugs may occasionally cause cleft lip and palate (Pashayan et al., '71) and this is a question that should be thoroughly investigated.

Finally, there has been one *experimental study* in which vitamin supplements were given during subsequent pregnancies to some but not all of a group of mothers of children with clefts. Although the difference in subsequent incidence of clefts between the two groups was in favor of the treated group, it was not statistically significant (Peer et al., '63).

If these findings are not fortuitous some (such as the association with maternal bleeding) may be due to the malformation producing the other findings, rather than the reverse; and others (such as the positive nutritional and biochemical findings) may indicate genetically determined abnormalities that predispose to clefts, rather than environmental factors. In either case further study of the possible relation of nutrition and biochemistry to clefts (especially during pregnancies of women that have already had affected children) could well be rewarding, since if it were found that, e.g., a genetically determined low level of tissue folate could cause clefts it might be possible to override this effect by massive doses of this vitamin in high-risk cases.

Recommendations (not in order of priority)

1. The techniques of molecular biology are beginning to be applied to mammalian teratology. Careful, critical work in this field should be encouraged with a view to a better understanding of normal and abnormal developmental mechanisms, environmental predisposing factors, and possible preventive measure.

2. Systems should be developed, on a regional or national basis, for preserving and making available for morphologic, genetic, and biochemical study embryos obtained from spontaneous and therapeu-

tic abortions. A workshop should be organized to develop such a system.

3. Further epidemiological studies should be done only to test specific hypotheses. Studies based on information from birth certificates will be untrustworthy until there is a marked improvement in reporting.

4. Twins, and particularly discordant monozygotic twins, should be exploited more fully both to identify predisposing features and to evaluate the effect of the malformation on the total phenotype. This would require collaboration, since no one center sees enough twins, and perhaps a data bank.

5. Prospective studies should be organized, on a collaborative basis, on the prenatal biology of mothers of affected children during subsequent pregnancies, in an attempt to identify predisposing environmental factors.

LITERATURE CITED

Andersen, H., and M. Mathiessen 1967 Histochemistry of the early development of the human central face and nasal cavity with a special reference to the movement and fusion of the palatine processes. Acta Anat., 68: 473–508.

Andrew, F. D., and E. F. Zimmerman 1971 Glucocorticoid induction of cleft palate in mice; no correlation with inhibition of mucopolysaccharide synthesis. Teratology, 4: 31–37.

Asling, C. W. 1961 Congenital defects of the face and palate following maternal deficiency of pteroylglutamic acid. In: Congenital Anomalies of the Face and Associated Structures. S. Pruzansky, ed. Thomas, Springfield, pp. 173–187.

Bertmar, G. 1965 The olfactory organ and upper lips in dipnoi, an embryological study. Acta Zool., 46: 1–40.

Bullough, W. S. 1962 Growth control in mammalian skin. Nature, 193: 520–523.

Burdi, A. R., and R. G. Silvey 1969 Sexual differences in closure of the human palatal shelves. Cleft Pal. J., 6: 1–7.

Caspersson, T., L. Zech, C. Johansson and E. G. Modest 1970 Identification of human chromosomes by DNA-binding agents. Chromosoma, 30: 215–227.

Charlton, P. J. 1966 Seasonal variation in incidence of some congenital malformations in two Australian samples. Med. J. Aust., 2: 833–835.

Chaube, S., and M. L. Murphy 1963 Teratogenic effect of hadacidin (a new growth inhibitory chemical) on the rat fetus. J. Exp. Zool., 152: 67–73.

Ching, G. H. S. 1970 A genetic and epidemiological study of cleft lip and cleft palate in Hawaii. Ph.D. Dissertation, University of Hawaii.

DeMeyer, W. 1964a Cleft lip and jaw induced in fetal rats by vincristine. Arch. Anat., 48: 181–186.

———— 1964b Vinblastine-induced malformation of face and nervous system in two rat strains. Neurology, 14: 806–808.

DeMeyer, W., W. Zeman and C. G. Palmer 1964 The face predicts the brain: diagnostic significance of median facial anomalies for holoprosencephaly (arhinencephaly). Pediatrics, 34: 256–263.

Drillien, C. M., T. T. S. Ingram and E. M. Wilkinson 1966 The Causes and Natural History of Cleft Lip and Palate. Livingstone, Edinburgh.

Edwards, J. H. 1961 Seasonal incidence of congenital disease in Birmingham. Ann. Hum. Genet., 25: 89–93.

Erdelyi, R. 1957 The influence of toxoplasmosis on the incidence of congenital facial malformation. Plast. Reconstr. Surg., 20: 306–310.

Fogh-Andersen, P. 1942 Inheritance of Harelip and Cleft Palate. Busck, Copenhagen.

Fraser, F. C. 1964 Experimental teratogenesis in relation to congenital malformations in man. In: Proceedings Second International Congress Congenital Malformations. International Medical Congress, New York.

———— 1969 Gene-environment interactions in the production of cleft palate. In: Methods for Teratological Studies in Experimental Animals and Man. H. Nishimura and J. R. Miller, eds. Igaku Shoin, Tokyo, pp. 34–49.

———— 1970 The genetics of cleft lip and cleft palate. Am. J. Hum. Genet., 22: 336–352.

———— 1971 Etiology of cleft lip and palate. In: Cleft Lip and Palate. W. C. Grabb, S. W. Rosenstein and K. R. Bzoch, eds. Little, Brown, Boston, pp. 54–65.

Fraser, F. C., D. Chew and A. C. Verrusio 1967 Oligohydramnios and cortisone-induced cleft palate in the mouse. Nature, 214: 417–418.

Fraser, F. C., and T. D. Fainstat 1951 The production of congenital defects in the offspring of pregnant mice treated with cortisone. Pediatrics, 8: 527–533.

Fraser, F. C., and H. Pashayan 1970 Relation of face shape to susceptibility to congenital cleft lip. J. Med. Genet., 7: 112–117.

Fraser, F. C., B. E. Walker and D. G. Trasler 1957 Experimental production of congenital cleft palate; genetic and environmental factors. Pediatrics, 19: 782–787.

Fraser, F. C., and D. Warburton 1964 No association of emotional stress or vitamin supplement during pregnancy to cleft lip or palate in man. Plast. Reconstr. Surg., 33: 395–399.

Fujino, H., K. Tanaka and Y. Sanui 1963 Genetic study of cleft-lips and cleft-palates based upon 2828 Japanese cases. Kyushu J. Med. Sci., 14: 317–331.

Gellis, S. S., and M. Feingold, eds. 1968 Atlas of Mental Retardation Syndromes. US Government Printing Office, Washington, D.C.

Goldstein, M. B., F. C. Fraser and K. Roth 1965 Resistance of A/Jax mouse embryos with spontaneous congenital cleft lip to the lethal effect of 6-aminonicotinamide. J. Med. Genet., 2: 128–130.

Gorlin, R. J., J. Cervenka and S. Pruzansky 1971 Facial clefting and its syndromes. Birth Defects Orig. Art. Ser., 7: 3–49.

Greene, J. C., J. R. Vermillion, S. F. Hay and S. Kerschboum 1964 Epidemiological study of cleft lip and cleft palate in four states. J. Amer. Dent. Ass., 68: 387–404.

Harris, J. W. S. 1967 Experimental studies on the closure and cleft formation in the secondary palate. Scientific Basis of Med., Ann. Rev., pp. 356–370.

Hart, J. C., G. R. Smiley and A. D. Dixon 1969 Sagittal growth of the craniofacial complex in normal embryonic mice. Arch. Oral Biol., 14: 995–997.

Hay, S. 1967a Incidence of clefts and parental age. Cleft Pal. J., 4: 205–213.

——— 1967b Variables in under-reporting of clefts on birth certificates. Publ. Hlth. Rep., 82: 985–993.

Hay, S., and D. A. Wehrung 1970 A study of seasonal incidence of congenital malformations in the United States. Br. J. Prev. Soc. Med., 24: 24–32.

Heiberg, K., H. Kalter and F. C. Fraser 1959 Production of cleft palates in the offspring of mice treated with ACTH during pregnancy. Biol. Neonat., 1: 33–37.

Herbai, G. 1971 Effect of adrenalectomy, corticosteroids and some other antiinflammatory agents, salazopyrine, thyroxine and vitamin A on the exchangeable sulphate pool and on sulphate incorporation in vivo into costal cartilage in the mouse. Acta Pharmacol. Toxic., 29: 164–176.

Hibbard, E. D., and R. W. Smithells 1965 Folic acid metabolism and human embryopathy. Lancet, 1: 1254.

Humphrey, T. 1969 The relation between human fetal mouth opening reflexes and closure of the palate. Am. J. Anat., 125: 317–344.

Jacobs, R. M. 1966 Effects of cortisone acetate upon hydration of embryonic palate in two inbred strains of mice. Anat. Rec., 156: 1–4.

——— 1971 Failure of muscle relaxants to produce cleft palate in mice. Teratology, 4: 25–30.

Jacobson, A. G. 1966 Inductive processes in development. Science, 152: 25–34.

Johnston, M. C. 1966 A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. Anat. Rec., 156: 143–155.

Knox, G. 1963a The family characteristics of children with clefts of lip and palate. Acta Genet., 13: 299–315.

——— 1963b Detection of low intensity epidemicity: application to cleft lip and palate. Br. J. Prev. Soc. Med., 17: 121–127.

Knox, G., and F. Braithwaite 1963 Cleft lips and palates in Northumberland and Durham. Arch. Dis. Child., 38: 66–70.

Kochhar, D. M. 1968 Studies of vitamin A-induced teratogenesis: effects on embryonic mesenchyme and epithelium, and on incorporation of H^3-thymidine. Teratology, 1: 299–310.

Kochhar, D. M., K. S. Larsson and H. Boström 1968 Embryonic uptake of S^{35}-sulfate: change in level following treatment with some teratogenic agents. Biol. Neonat., 12: 41–53.

Langman, J. 1961 The influence of teratogenic agents on serum proteins. In: Congenital Anomalies of the Face and Associated Structures. S. Pruzansky, ed. Thomas, Springfield, pp. 149–161.

Larsson, K. S. 1962 Closure of the secondary palate and its relation to sulpho-mucopolysaccharides. Acta Odont. Scand., 20: suppl. 31: 1–35.

Larsson, K. S., and H. Boström 1965 Teratogenic action of salicylates related to the inhibition of mucopolysaccharide synthesis. Acta Paediat. Scand., 54: 43–48.

Leck, I. 1971 Further tests of the hypothesis that influenza in pregnancy causes malformations. HSMHA Hlth. Rep., 86: 265–269.

——— 1972 The etiology of human malformations; insights from epidemiology. Teratology, 5: 303–314.

Leck, I., S. Hay, J. J. Witte and J. C. Greene 1969 Malformations recorded on birth certificates following A_2 influenza epidemics. Publ. Hlth. Rep., 84: 971–979.

Lejour-Jeanty, M. 1965 Etude morphologique et cytochimique de développement du palais primaire chez le rat (complète des observations chez le lapin et l'homme). Arch. Biol., 76: 97–168.

——— 1966 Becs-de-lièvre provoquées chez le rat par un dérivé de la pénicilline, l'hadacidin. J. Embryol. Exp. Morph., 15: 193–211.

Lejour, M. 1970 Cleft lip induced in the rat. Cleft Pal. J., 7: 169–186.

Long, S. Y., K. S. Larsson and S. Lohmander 1972 The uptake of ^{3}H-thymidine in the cranial base of cleft palate mice. Teratology, 5: 261 (abstr.).

Lowry, R. B., and D. H. G. Renwick 1869 Incidence of cleft lip and palate in British Columbia Indians. J. Med. Genet., 6: 67–69.

MacMahon, B., and T. McKeown 1953 The incidence of harelip and cleft palate related to birth rank and maternal age. Am. J. Hum. Genet., 5: 176–183.

Meskin, L. H., S. Pruzansky and W. H. Gullen 1968 An epidemiologic investigation of factors related to the extent of facial effects. I. Sex of patients. Cleft. Pal. J., 5: 23–29.

Meskin, L. H., and S. Pruzansky 1967 Validity of the birth certificate in the epidemiologic assessment of facial clefts. J. Dent. Res., 46: 1456–1459.

Miller, J. R. 1964 The use of registries and vital statistics in the study of congenital malformations. In: Proceedings 2nd International Conference on Congenital Malformations. M. Fishbein, ed. International Medical Congress, New York, pp. 334–340.

Morgan, P. R. 1969 Recent studies on the fusion of the secondary palate. Lond. Hosp. Gaz., 72: 5–19.

Morton, N. E., C. S. Chung and M. P. Mi 1967 Genetics of Interracial Crosses in Hawaii. Karger, Basel.

Nishimura, H. 1970 Incidence of malformation in abortions. In: Proceedings Third Intertional Conference on Congenital Malformations. F. C. Fraser and V. A. McKusick, eds. Excerpta Medica, Amsterdam, pp. 275–283.

Niswander, J. D., and M. S. Adams 1967 Oral clefts in the American Indian. Publ. Hlth. Rep., 82: 807–812.

Pashayan, H., D. Pruzansky and S. Pruzansky 1971 Are anticonvulsants teratogenic? Lancet, 2: 702–703.

Patten, B. M. 1971 Embryology of the palate and maxillofacial region. In: Cleft Lip and Palate. W. C. Grabb, S. W. Rosenstein and K. R. Bzoch, eds. Little, Brown, Boston, pp. 21–53.

Peer, L. A., H. W. Gordon and W. G. Bernhard 1963 Experimental production of congenital deformities and their possible prevention in man. J. Int. Coll. Surg., 39: 23–25.

Pleydell, M. J. 1960 Anencephaly and other congenital abnormalities. An epidemiological study in Northamptonshire. Br. Med. J., 1: 309–314.

Posner, H. S. 1971 Two treatments alter the incidence of cleft palate obtained in rats after chlorcyclizine administration. Teratology, 4: 238–239 (abst.).

Pourtois, M. 1968 La fusion des crêtes palatines et son altération par quelques agents tératogènes. Arch. Biol., 79: 1–75.

Pratt, R. M., and C. T. G. King 1972 Inhibition of collagen crosslinking associated with amino-propionitrile-induced cleft palate in the rat. Dev. Biol., 27: 322–328.

Richards, I. D. G. 1969 Congenital malformations and environmental influences in pregnancy. Br. J. Prev. Soc. Med., 23: 218–225.

Scott, W. J., E. J. Ritter and J. G. Wilson 1971 DNA synthesis inhibition and cell death associated with hydroxyurea teratogenesis in rat embryos. Dev. Biol., 26: 306–315.

Slater, B. C. S., G. I. Watson and J. C. McDonald 1964 Seasonal variation in congenital abnormalities; preliminary report of a survey conducted by the Research Committee of Council of the College of General Practitioners. Br. J. Prev. Soc. Med., 18: 1–7.

Smith, D. W. 1970 Recognizable Patterns of Human Malformation. Saunders, Philadelphia.

Stark, C. R., J. D. Niswander, V. T. Bardanouve and B. Iba 1970 Temporal-spatial clustering of oral-cleft births in Michigan and Montana. Cleft Pal. J., 7: 826–845.

Stark, R. B. 1954 The pathogenesis of harelip and cleft palate. Plast. Reconst. Surg., 13: 20–29.

Trasler, D. G. 1965 Aspirin-induced cleft lip and other malformations in mice. Lancet, 1: 606–607.

———— 1968 Pathogenesis of cleft lip and its relation to embryonic face shape in A/J and C57BL mice. Teratology, 1: 33–49.

Trasler, D. G., B. E. Walker and F. C. Fraser 1956 Congenital malformations produced by amniotic-sac puncture. Science, 124: 439.

Tretsven, V. E. 1963 Incidence of cleft lip and palate in Montana Indians. J. Speech Hearing Disord., 28: 52–57.

Vallance-Owen, J., F. Braithwaite, J. S. P. Wilson, J. R. G. Edwards and D. G. Maurice 1967 Cleft lip and palate deformities and insulin antagonism. Lancet, 2: 912–914.

Verrusio, A. C. 1970 A mechanism for closure of the secondary palate. Teratology, 3: 17–20.

Walker, B. E. 1968 Variations of palatine shelf morphology in rabbit embryos. Anat. Rec., 160: 445–446.

———— 1969 Correlation of embryonic movement with palate closure in mice. Teratology 2: 191–198.

———— 1971 Induction of cleft palate in rats with anti-inflammatory drugs. Teratology, 4: 39–43.

Walker, B. E., and F. C. Fraser 1956 Closure of the secondary palate in three strains of mice. J. Embryol. Exp. Morph., 4: 176–189.

———— 1957 The embryology of cortisone-induced cleft palate. J. Embryol. Exp. Morph. 5: 201–209.

Warkany, J., and E. Schraffenberger 1947 Congenital malformations induced in rats by roentgen rays. Am. J. Roentgen., 57: 455–463.

Weissman, G., and L. Thomas 1963 Studies on lysosomes. II. The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. J. Clin. Inv., 42: 661–669.

Zimmerman, E. F., F. Andrew and H. Kalter 1970 Glucocorticoid inhibition of RNA synthesis responsible for cleft palate in mice: a model. Proc. Nat. Acad. Sci. USA, 67: 779–785.