The Dentition of Monozygotic Human Twin Fetuses with Cleft Lip and Palate: A Case Report

BERTRAM S. KRAUS, Ph.D.
SEISHI W. OKA, D.D.S.
Pittsburgh, Pennsylvania

Among the 250 aborted human fetuses with cleft lip and/or palate in the collection of the senior author was a pair of twins of 20 weeks gestational age showing mirror-imaged cleft lip and palate. The fetuses are Caucasoid and male. The report of the attending physician indicated normal delivery for the first fetus and a breech presentation for the second. The placenta was single and was delivered intact. The pathology report was as follows:

The placenta shows two umbilical cords which are attached to the fetal surface about 10 cm from each other. Intervening between the cords is a thin, transparent membrane-like material. One length of cord is 20 cm and the second length of cord, attached to the placenta, is 30 cm. These cord structures show no true or false knots or any other unusual features. The membranes show no significant vessels to be present on their surfaces and the maternal surface of the placenta is intact. The maternal surface, on measuring, is 17 x 14 cm. One section of the membranes intervening between the cords was taken and one section of the placental elements was taken. Each of the fetuses shows a hare lip and cleft palate deformity which is quite severe. There is mirror imagery in this deformity. In one fetus it is on the right side (X-4022); in the other it is on the left side (X-4023). Each of these fetuses measures 27 cm in length and their weights are 1 lb, 3 oz and 1 lb, 3½ oz. Other than for the hare lip and cleft palate deformity, the development appears to have progressed in a normal fashion. Each fetus shows a 10 cm length of umbilical cord (interpreted by the authors to mean a 10 cm distance between the two cords). Sections were not taken.

Both sections of the placenta reveal that the villi are quite mature and highly vascularized, and contain anuclear red cells. There is minimal intervillous fibrin deposition. The sections through the membrane between the two amniotic sacs reveal that it consists of two amnions placed back to back without any intervening chorion. The features are those of a mature placenta and of uni-ovular twins.

The only previous study of the tooth buds of aborted twin and triplet fetuses was that of Goodman and Kraus (*2*). Autopsy reports were not available for the eight pairs of twins and three sets of triplets utilized in that report, hence an analysis of crown morphology of the developing

Dr. Kraus is Director and Dr. Oka is Research Associate in the Cleft Palate Research Center, University of Pittsburgh.

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primary teeth was undertaken to discover if there was a likelihood of determining zygosity on the basis of degree of morphological concordance. A rating system was devised wherein a value of *four* was assigned if the analogous teeth of twin pairings were different; *two,* if they were judged similar; and *zero,* if they were identical. Although these authors concluded that the dental evaluation revealed four pairs of monozygotic twins, five pairs of dizygotic twins, and two pairs of unknown zygosity, they pointed out the need for a study of fetal twins for whom a zygosity determination had been made on the basis of examination of the membranes.

The discovery of the present pair of fetal twins provides an opportunity to re-examine the question of the validity of dental evaluation in zygosity determination in a case where monozygosity has apparently been established by histological examination of the membranes. However, the presence of a cleft lip and palate, mirror-imaged, in each member of the pair introduces some complicating factors. Recent studies have shown that a significantly high number of morphological abnormalities occur in the developing dental crowns of human fetuses with cleft lip and/or palate (*3*). The present twin fetuses, therefore, offered the opportunity for examining whether such abnormalities, if present, showed concordance and were mirror-imaged or occurred on the same side.

Material and Method

A complete photographic record of the twin fetuses was obtained. Twin X-4023 had a left-sided cleft of the lip; twin X-4022 had a right-sided cleft of the lip (Figure 1).

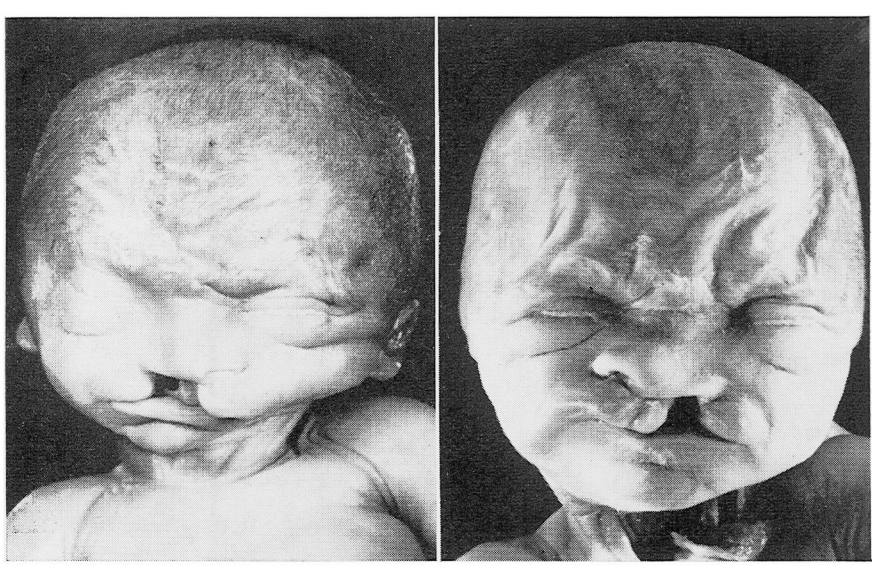

FIGURE 1. Frontal view of 20-week-old monozygotic cleft fetuses: X-4022, left, and X-4023, right.

Fetus X-4022		*Fetus X-4023*	
right	*left*	*right*	*left*
	maxillary		
a	a	a	a
b′	b	b	b′
b″			b″
c	c	c	c
d	d	d	d
e	e	e	e
6	6	6	6
	mandibular		
a–b	a	a	a–b
	b	b	
c	c	c	c
d	d	d	d
e	e	e	e
6	6	6	6

FIGURE 2. Enumeration of recovered teeth from the twin fetuses. Code is: a, primary central incisor; b, primary lateral incisor; c, primary canine; d, primary first molar; e, primary second molar; 6, permanent first molar; b′, primary lateral incisor; and b″, primary supernumerary (probably lateral) incisor.

The tooth buds were extracted, stained in alizarin red S, and stored in 50% glycerin. Each bud was photographed from the lingual and the profile of each was traced for superimposition. Occlusal views of the molars were taken in addition. The teeth which were recovered are indicated in Figure 2.

Tracings were made of the cleft of each twin from photographs taken from a palatal view. The extensive collection of human fetal tooth buds maintained at the Cleft Palate Research Center was used for controls and comparative studies.

Results

Photographs of the clefts are shown in Figure 3A and B, together with tracings made from them. The tracing of X-4023 was reversed to facilitate

FIGURE 3a. Palatal views of the clefts of X-4022, left, and X-4023, right.

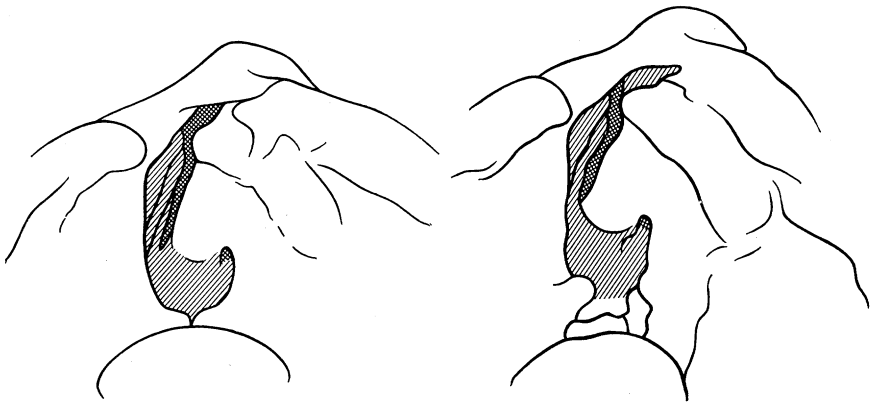

FIGURE 3b. Tracings of the clefts of the twin fetuses. That of X-4023, right, is reversed to facilitate comparison with X-4022, left.

direct comparison of the profiles of the major structures involved (Figure 3B). It is apparent that there is remarkable similarity between the two mirror-imaged lip and palatal deformities except in the region of the uvula.

The developmental stages of the tooth crowns (Table 1) agree with the report of the attending physician that the gestational age was 20 weeks. The mandibular first molars were in Stage X, the second molars were also in Stage X, the maxillary first molars were in Stage X, and the maxillary second molars were in Stage IV (*4*). This places the age of the twin fetuses between 19 and 22 weeks, with a mean gestational age of approximately 20½ weeks. The calcification status of the incisors and canines collectively indicates an age of over 17 weeks. Comparison of the four primary molars in each twin with the diagrams of sequential patterns of calcification found in Kraus and Jordan (*4*, pp. 124–126) narrows the calculation of age to 20 weeks.

METHODOLOGY. The two fetuses were compared, tooth by tooth, in the following manner. Thin paper tracings of the lingual profiles were superimposed. Tracings of teeth from the same side, right or left, were di-

TABLE 1. Age of the fetus based on developmental status of each primary tooth.

primary tooth	*maxillary*		*mandibular*	
	developmental stage	*mean age*	*developmental stage*	*mean age*
central	not available	14+ wks	not available	14+ wks
lateral	"	16+ wks	"	16+ wks
canine	"	17+ wks	"	17+ wks
1st primary	X	22+ wks	X	23 wks
2nd primary	IX	19 wks	X	22 wks
1st permanent	V	22 wks	VI	22 wks

rectly superimposed; those of teeth from opposite sides of the mouth were compared by inverting one of the tracings. If the calcified portions of the crowns superimposed identically, the two teeth were called concordant. If not, they were designated as discordant. There could thus be four possible comparisons: right with right, left with left, right with left, and left with right (Figure 4). A diagram representing the situation for any four teeth with regard to concordance and discordance is shown in Figure 5. The diagram is interpreted to mean that there was concordance (identical superimposition) between the upper right lateral incisor of X-4022 and the upper left lateral incisor of X-4023; and discordance between the two rights, the two lefts, and between the upper left of X-4022 and the upper right of X-4023. The concordance is between the laterals of the cleft side in each fetus. The uncalcified lower portion of the crown was disregarded in this comparison because of the soft tissue which is easily distorted during extraction, staining, and handling.

In addition to the lingual profile tracings, the calcified areas of the

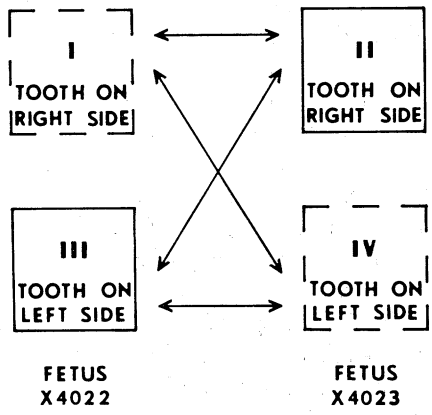

FIGURE 4. The four types of comparisons of the teeth used in this report. The cleft side in each fetus is indicated by broken lines.

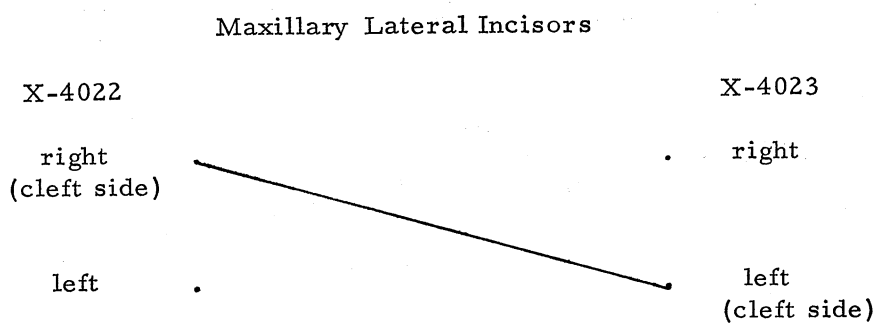

FIGURE 5. Diagram representing any four teeth with respect to concordance and discordance.

molars were traced from the occlusal view and were superimposed in the same way.

Maxillary Central Incisors. The maxillary centrals are within the normal range of variation in their morphology and degree of calcification (Figure 6). On all four incisors the incisal edge, however, is characterized by a central mammelon which is dominant over the mesial and distal mammelons. Although there is little variation among the four incisors in profile (Figure 7), it is obvious that there is mirror imaging. The right incisor of fetus X-4023 superimposes almost exactly on the left of fetus X-4022, and vice versa.

Maxillary Lateral Incisors. The lateral incisors are normal in morphology (Figure 8), but those on the cleft sides (right lateral in the case of X-4022 and left lateral in the case of X-4023) are approximately one-half the size of the laterals on the noncleft side. Concordance occurs only

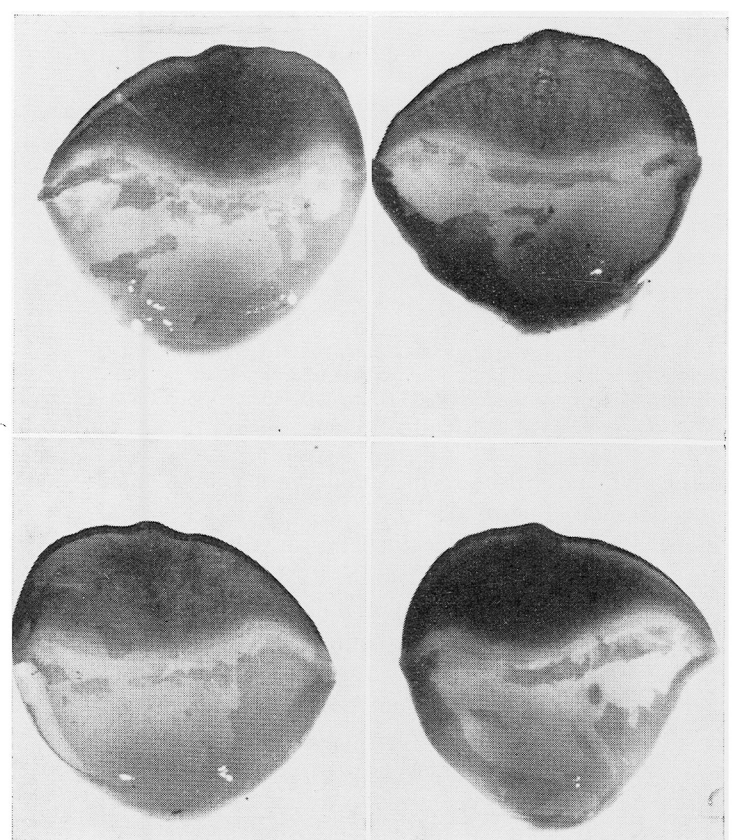

FIGURE 6. Lingual views of the maxillary central incisor buds: maxillary right shown at top, X-4022, left, and X-4023, right; maxillary left shown at bottom, X-4022, left, and X-4023, right.

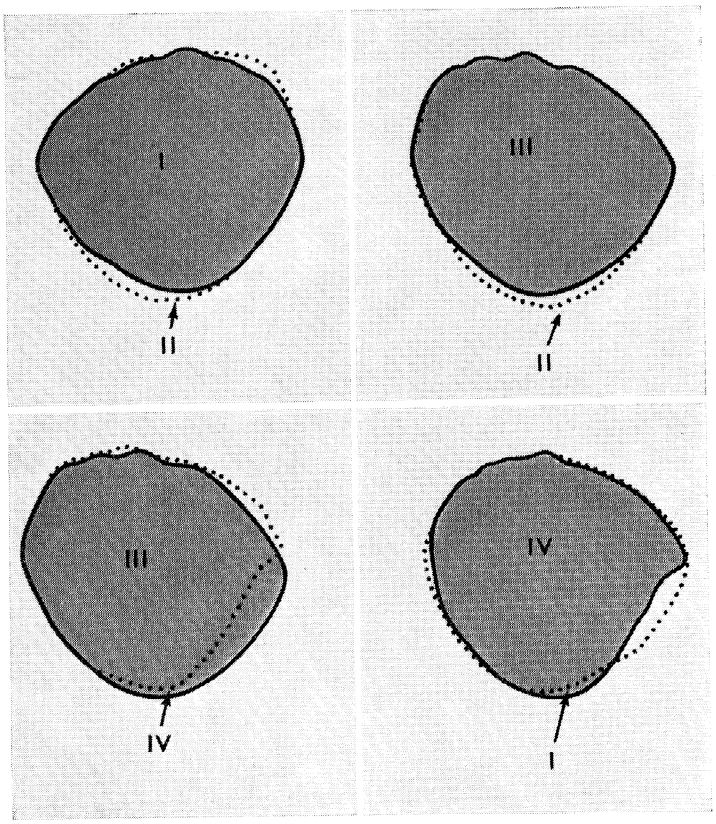

FIGURE 7. Superimposed lingual tracings of the various pairings of maxillary central incisor buds seen in Figure 6. The two figures on the left are same sides; the two on the right are opposite sides. Codes are I, X-4022, right; II, X-4023, right; III, X-4022, left; IV, X-4023, left.

between the right lateral of X-4023 and the left of X-4022—these being on the noncleft side (Figure 9).

Maxillary Supernumerary Teeth. The maxillary right supernumerary tooth bud of X-4022 and the left of X-4023 look very much like lateral incisors (Figure 10) when viewed in lingual profile. The former is the larger and has a mesial and distal shoulder; the latter lacks a high mesial shoulder, and for this reason the two teeth are discordant. Both buds are distinctive in having a high lingual marginal ridge extending from the most mesial to the most distal corner of the incisal edge.

Maxillary Canines. The right canine of X-4023 and the left of X-4022 are identical in morphology and show the same degree of calcification. All four show a pronounced lingual extension of the *zona cingularis,* a normal development at this stage (Figure 11).

Maxillary First Molars. From the occlusal view the upper right of X-4022 is distinct from the other three molars in that it lacks a metacone.

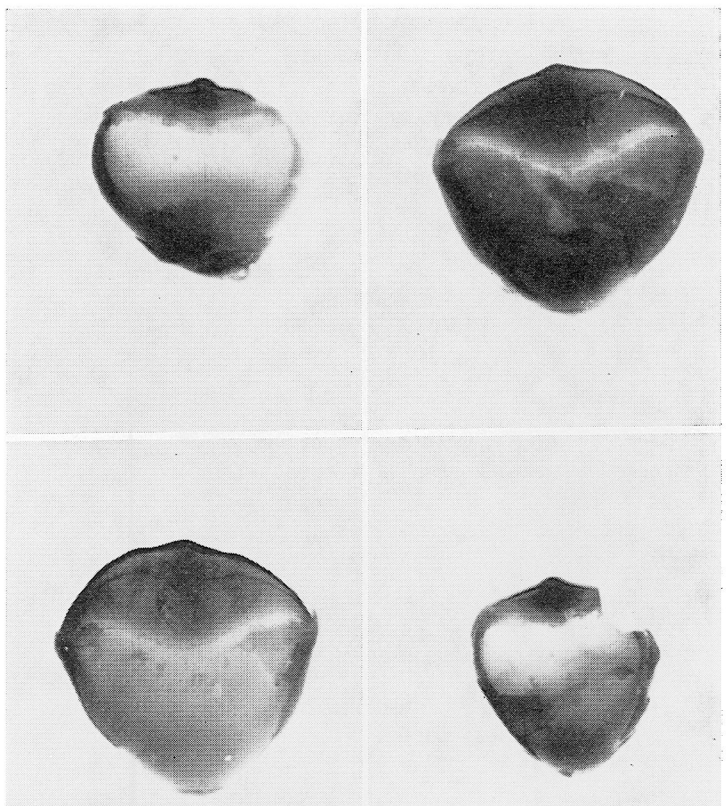

FIGURE 8. Lingual view of the maxillary lateral incisor buds extracted from twin cleft fetuses. The two photographs at the left are for X-4022; upper is right, lower is left side. The two photographs at the right are for X-4023; upper is right, lower is left side.

The calcified margins of the paracone extends along the entire length of the buccal margin in this tooth bud. In the other three molars the small metacones have independent centers of calcification. The right and left molars of X-4023 and the left of X-4022 superimpose almost exactly with regard to the calcified areas and the crown profile. Superimpositioning is very difficult from the lingual view because of the three-dimensional problem and the impossibility of exact orientation. Photographs show that certain profiles are almost identical in three of the molars while that of the maxillary right of X-4022 is different (Figure 12). The mesiobuccal margin on the latter is very flat; the distal slope of the paracone extends all the way down to the distobuccal corner, whereas in the other three molars it dips abruptly before reaching the metacone. The profile of the protocone in all four molars appears to be concordant.

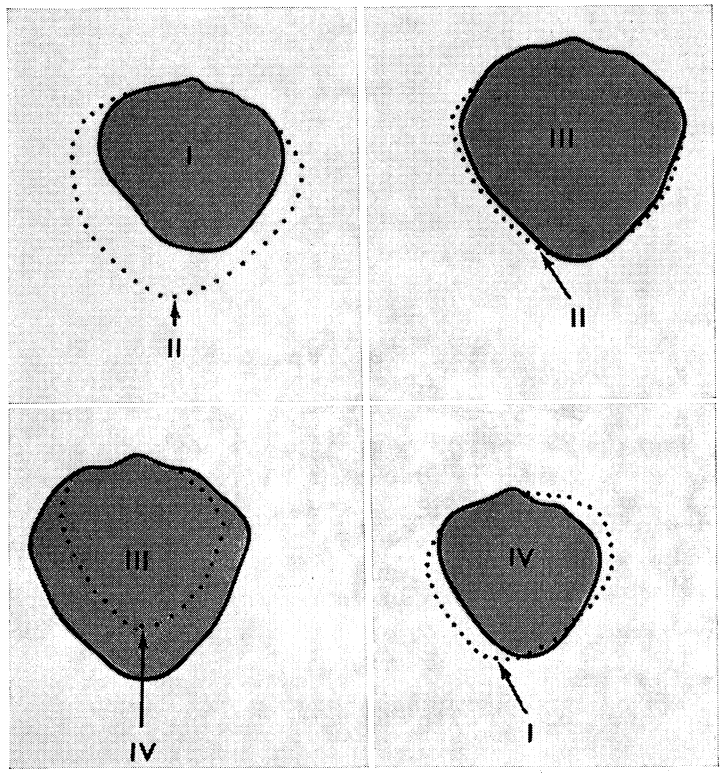

FIGURE 9. Superimposed lingual tracings of the various pairings of maxillary lateral buds seen in Figure 8. The two figures on the left are same sides; the two on the right are opposite sides. Codes are I, X-4022, right; II, X-4023, right; III, X-4022, left; and IV, X-4023, left.

Maxillary Second Molars. The mesial marginal ridges are distinguished on tooth buds of this stage of development by the number and prominence of the cusplets. Normally there are five such cusplets. The maxillary right molar of X-4022 differs from the rest in having a wavy mesial marginal ridge with no distinct cusplets. The right molar of X-4023 shows three cusp-like peaks on the buccal half of the ridge but none on the lingual half. In this respect it is very similar to the left molar of X-4022.

In all four molars the pre-Carabelli cingulum is developed to an equal extent with a slight mound-like precursor of the Carabelli cusp. In the left molar of X-4023 the mound is more cusp-like. In the two molars of X-4022 the mound shows the least development. In general the four molars are concordant with respect to the incipient development of the Carabelli trait.

In terms of relative amount of calcification the four molars are concordant.

Maxillary First Permanent Molars. All are in the same stage of development (Stage V), that is, with the two mesial cusps clearly differen-

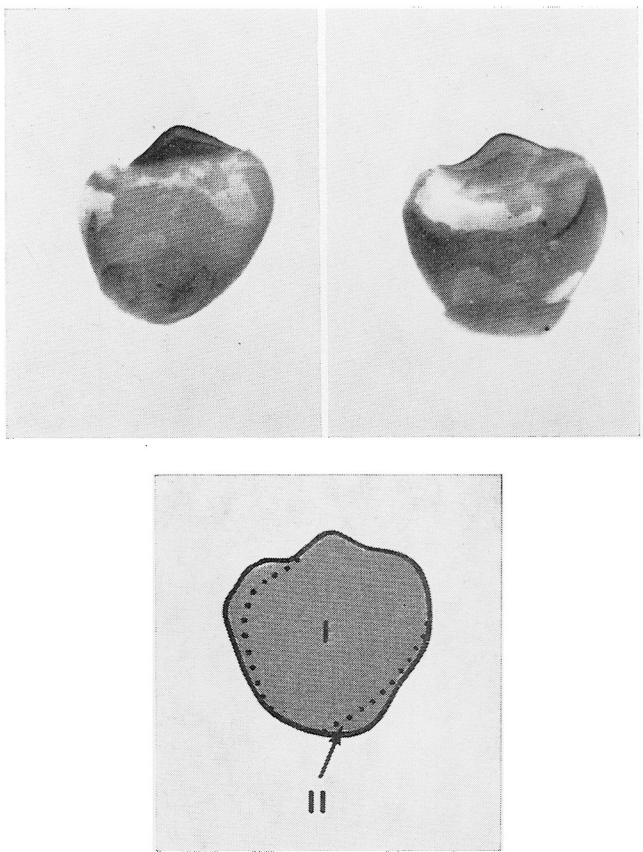

FIGURE 10. Lingual photographs and superimposed tracings of the two maxillary supernumerary buds. Photographs are X-4022, left, and X-4023, right. Tracing shows I, X-4022, and II, X-4023.

tiated and a mound-like eminence where the distobuccal cusp (metacone) will appear. They all appear to be concordant.

Mandibular Central Incisors. Since the right central and lateral incisors of X-4022 and the left central and lateral incisors of X-4023 are fused, only the left of X-4022 and the right of X-4023 can be directly compared (Figure 13). They show complete concordance of the calcified areas in lingual profile (Figure 14). Both teeth are typically normal in appearance.

Mandibular Lateral Incisors. The same limitation applies to the laterals; only the left of X-4022 can be compared with the right of X-4023. With regard to the calcified areas, the two are practically identical (Figure 14).

Fused Central and Lateral Incisors. The right central and lateral incisors of X-4022 are fused, as are the left central and lateral incisors of X-4023 (Figure 13). It is of interest to note that both fusions are on

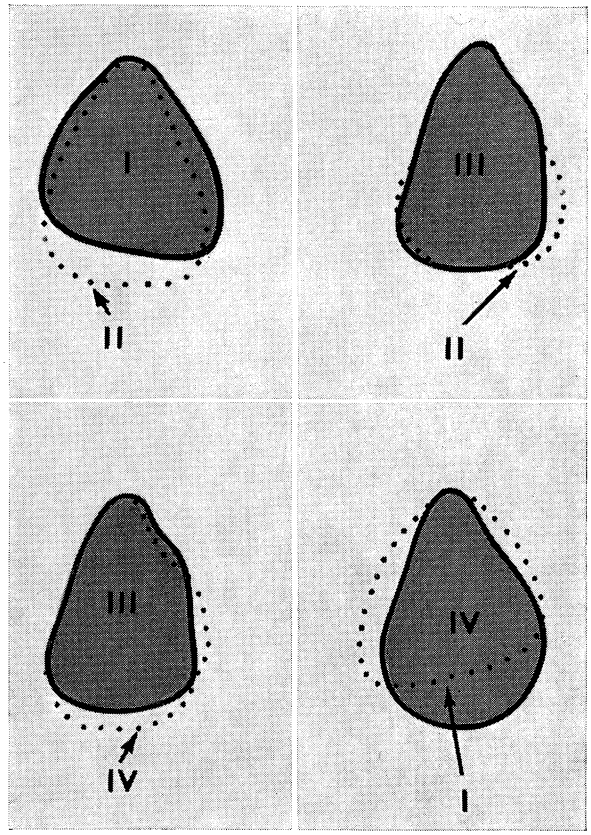

FIGURE 11. Superimposed lingual tracings of the various pairings of maxillary canine buds. The two figures on the left are same sides; the two on the right are opposite sides. Codes are I, X-4022, right; II, X-4023, right; III, X-4022, left; and IV, X-4023, left.

the cleft sides. A comparison of central with central and lateral with lateral shows complete discordance even without superimposing tracings. The central of X-4022 is grossly abnormal while that of X-4023 is normal. The laterals of X-4022 and X-4023 are each grossly abnormal but in different ways. Both fused teeth of X-4022 have but a single lobe and each has a long sloping incisal edge. The lateral of X-4023 has a prominent mesial lobe but a sharply sloping distal margin.

Comparison of the fused left central incisor (normal in morphology) of X-4023 with the normal unfused left central incisor of X-4022 reveals a marked similarity in the profile of the central mammelon and in the height of calcification. The distal and mesial slopes, however, do not superimpose at all: the left central of X-4023 being much the smaller tooth.

Mandibular Canines. The canines are not complicated in shape. Cross comparisons of the mandibular right of X-4022 with the left of X-4023,

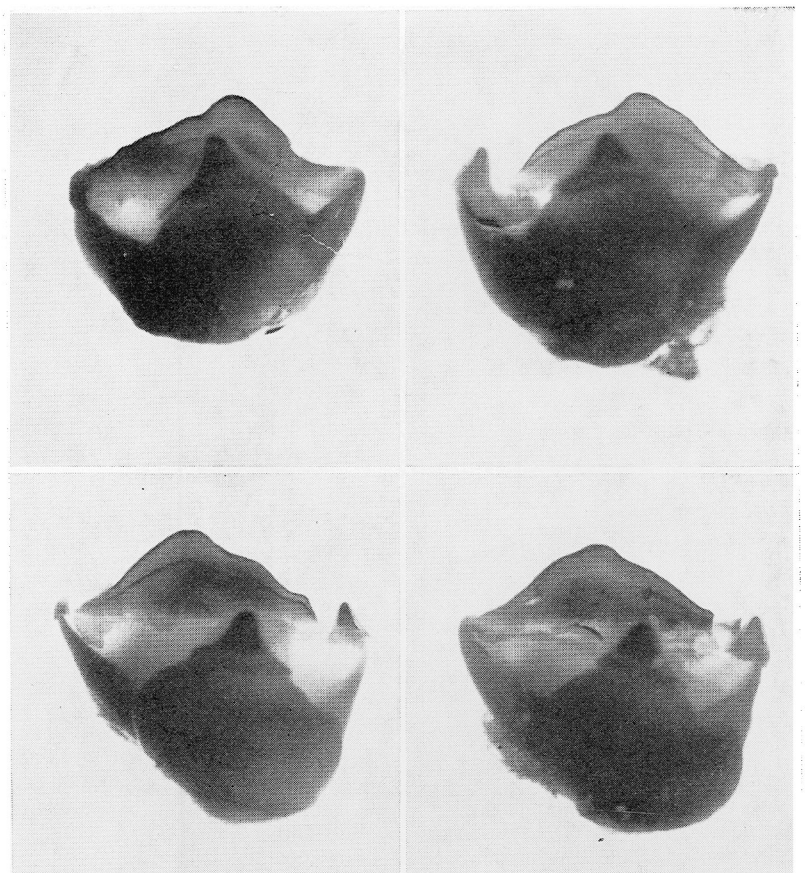

FIGURE 12. Lingual views of the maxillary right first molars of X-4022, top left, and X-4023, top right; maxillary left first molar of X-4022, bottom left, and of X-4023, bottom right.

and the left of X-4022 with the right of X-4023, show concordance of the calcified areas (Figure 15). In addition, the right canine of X-4022 shows concordance with the right of X-4023. The lefts, however, are discordant.

Mandibular First Molars. The lower left molar of X-4022 stands out as the most dissimilar of the four molars (Figure 16). It is the only one that does not have a distal cusplet (future hypoconulid) immediately behind the distobuccal cusp. The protoconid (mesiobuccal cusp) on this tooth is different from the other three in calcification morphology in having a buccal finger on the distal portion creating a forked configuration in this area.

The protoconid is calcified down to the juncture with the marginal ridge in all four molars. Likewise there is a bulge halfway down the distal slope (Figure 17). On the left of X-4022 there is also a slight bulge

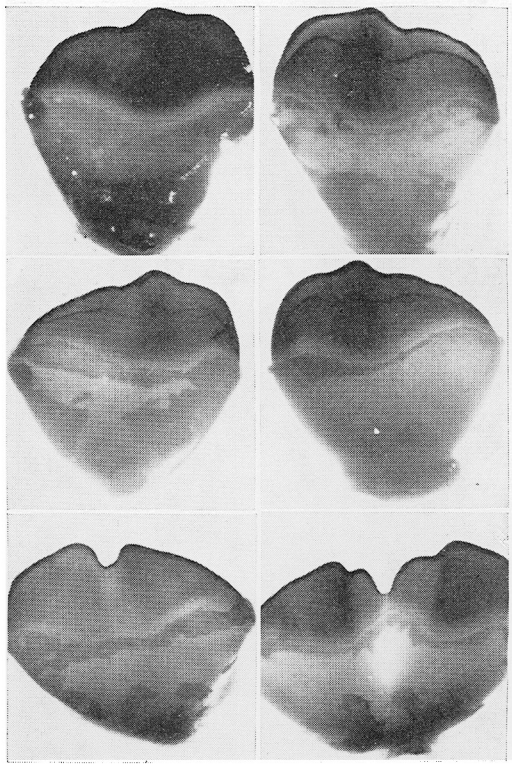

FIGURE 13. The three photographs on the left are left central incisor, top; left lateral incisor, middle; and fused right central and lateral incisor, bottom; all for X-4022. On the right are right central incisor, top; right lateral incisor, middle; and fused left central and lateral incisor, bottom, all for X-4023.

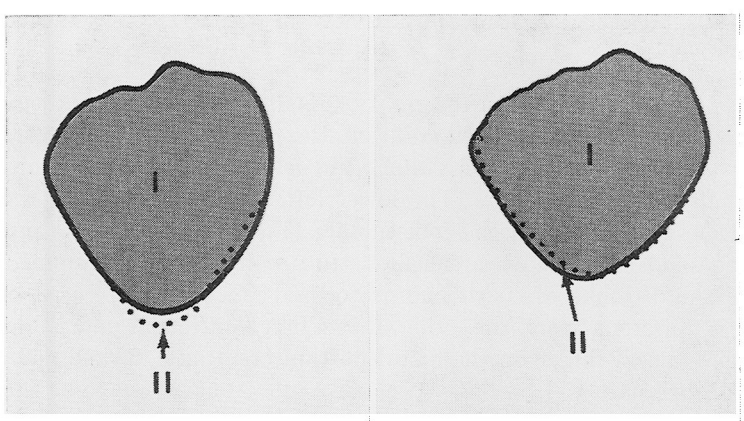

FIGURE 14. Demonstration of concordance of mandibular centrals, left, and mandibular laterals, right, of X-4022 and X-4023. Code is I, X-4022, and II, X-4023.

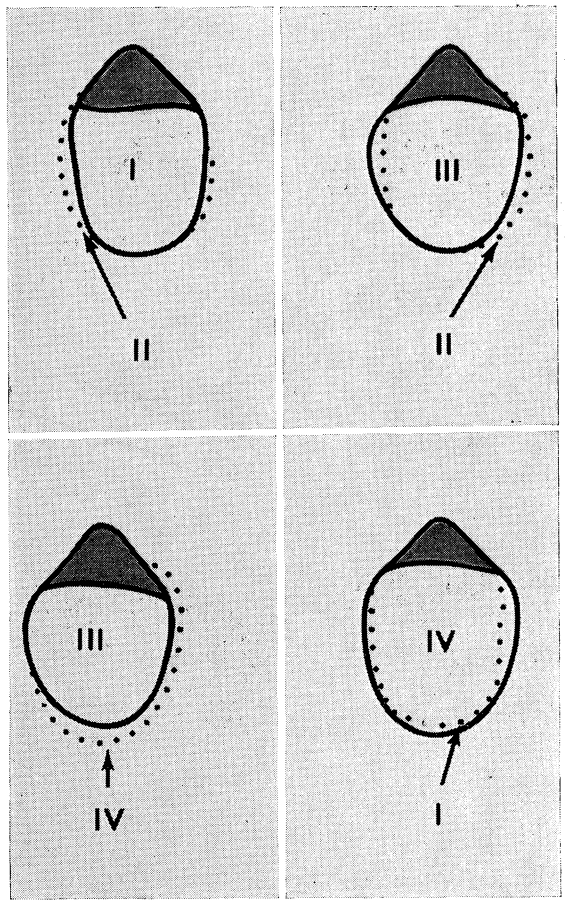

FIGURE 15. Superimposed lingual tracings of the various pairings of mandibular canine buds. The two figures on the left are same sides. The two on the right are opposite sides. Codes are I, X-4022, right; II, X-4023, right; III, X-4022, left; and IV, X-4023, left.

halfway down the mesial slope. In addition this molar has a cusplet on the lower-most distal slope of the protoconid. This cusplet occurs also in the dentino-enamel junction but it is obvious from the enamel thickness in this area that the cusplet did not calcify as an independent center. As far as the protoconid is concerned, the two right molars are concordant. In the left molar of X-4023 calcification has not progressed as far down the protoconid as in the other molars.

There is a single uncalcified hypoconid (distobuccal cusp) on the left molar of X-4022. The molar on the right side of this specimen also has a single uncalcified hypoconid but with a pronounced style on its distal slope near the base (Figure 17). The right molar of X-4023 also shows a single distobuccal cusp bearing two distinct and separately calcified tips

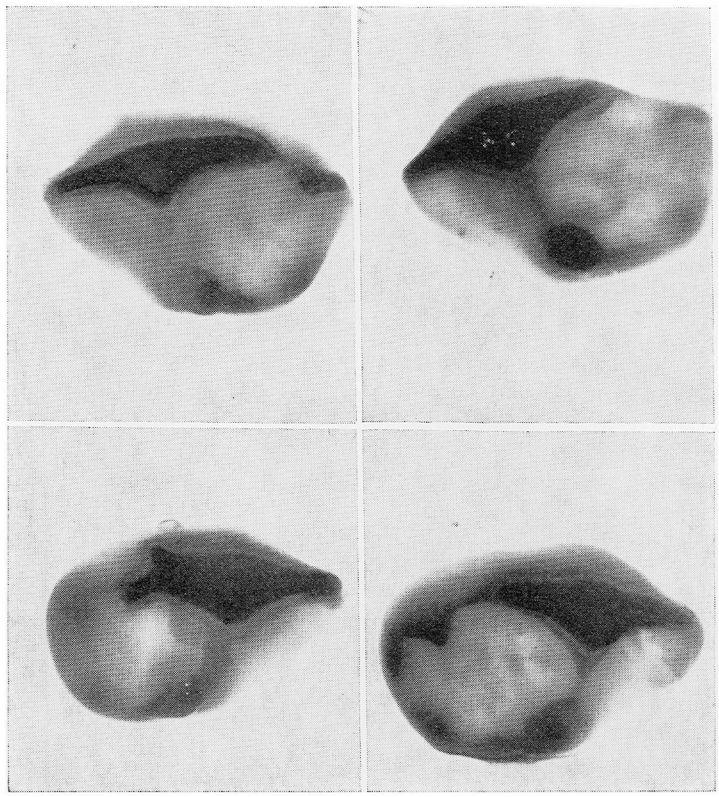

FIGURE 16. Occlusal views of primary mandibular first molars. The two molars on the left side are from X-4022; those in the right column are from X-4023. The right molars are shown on the top photographs, and the left molars are shown on the bottom.

near the apex. The more mesial tip is higher and more calcified. On the left molar of X-4023 there is a single uncalcified hypoconid.

The entoconids (distolingual cusp) of all four molars are equivalent in developmental status. No calcification was evident on any. That on the left molar of X-4023 has a broader base and less elevation than the others, and the distal slope is more convex and broader.

The four molars are unusual in that there is no hypoconulid present on any of them. Normally the mandibular first primary molar shows four main cusps upon eruption. Rarely a small distal cusp may be observed (the hypoconulid). However, during the course of development this molar almost always shows a distinct cusp on its distal ridge, generally making its appearance at about 17 weeks gestational age and persisting through birth. Its absence on the completed crown is a result of enamel build-up over the hypoconid which obscures its presence. The four primary first molars of the twins are in Stage X, at which point

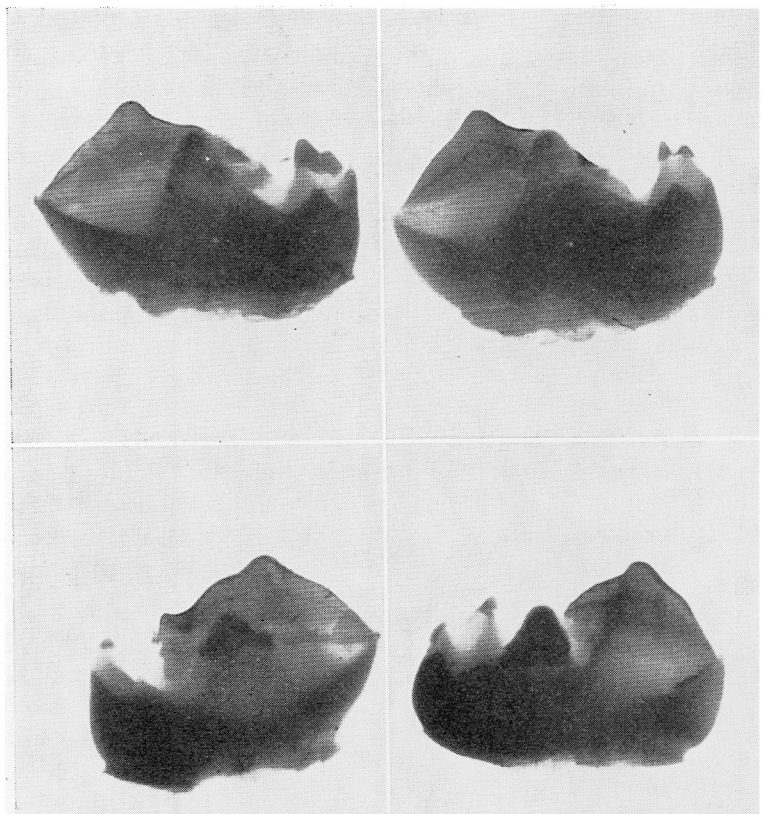

FIGURE 17. Lingual views of the primary mandibular first molars. The arrangements are the same as in Figure 16.

there is normally a well-developed hypoconulid. Its absence in all four molars may be regarded as indicative of abnormal development.

In general size, the left molar of X-4022 is the smallest.

Mandibular Second Molars. All four molars are normal in morphological configuration. There are five cusps present on each, with only the two mesial cusps undergoing calcification. In general size and occlusal profile, they are very similar. They are concordant in all respects but one: the left molar of X-4023 shows a prominent style on the distal slope of the metaconid. In the other three molars there is a slight bulge at this point.

Mandibular First Permanent Molars. The mandibular right molar of X-4023 was not recovered. The other three are in Stage VI of development (*4*), showing two well-defined mesial cusps and a mound-like distobuccal cusp (hypoconid). The talonid is well developed on all three molars. Morphologically they are concordant.

Discussion

One fact clearly emerges from the examination of the dentitions of the cleft twins: the consistent morphological concordance between the teeth on the left side of X-4022 with their analogues on the right side of X-4023. The only exception is in the case of the mandibular first primary molars. With respect to the right side of X-4022 and the left side of X-4023 there is concordance only with respect to the mandibular central incisors and the mandibular canines. Mirror-imaging, therefore, affects only one side of each twin fairly consistently. Since the mirror-imaging occurs between the noncleft sides, it is possible that the cleft itself might have been responsible for the lack of concordance between the two affected sides. On the other hand, this could not explain the lack of concordance between the cleft sides in the *mandibular* dentition.

Earlier studies have pointed out the high frequency of abnormal dental morphology in both fetal and postnatal cleft subjects (*3*). Abnormalities in the tooth buds of the cleft twins have been noted, but are summarized in Table 2 for convenience in discussion, together with observations on concordance and mirror-imaging.

TABLE 2. Summary of concordance and discordance of the dental abnormalities.

Fetus X-4022	*Fetus X-4023*	*concordance*	*mirror-imaging*
1. fusion of mandibular right central and lateral incisors	fusion of mandibular left central and lateral incisors	concordant	mirror-imaged
2. abnormal morphology of mandibular right lateral incisor	abnormal morphology of mandibular left lateral incisor	discordant	mirror-imaged
3. maxillary right supernumerary lateral incisor	maxillary left supernumerary lateral incisor	discordant	mirror-imaged
4. bifurcated hypoconid on mandibular right first primary molar	mandibular right first molar with double-cusped hypoconid	concordant	not mirror-imaged
5. 4-cusped mandibular first primary molars	4-cusped mandibular first primary molars	concordant	all 4 teeth identical
6. bifurcated distal margin of mandibular first primary molar	non-bifurcated	discordant	not mirror-imaged
7. abnormal morphology of mandibular right central incisor	normal	discordant	not mirror-imaged
8. maxillary right lateral incisor reduced in size	maxillary left lateral incisor reduced in size	discordant	mirror-imaged

It is a long-accepted tenet that in any trait that is dictated strictly by the genotype there will be 100% concordance in monozygotic twins. Since the members of monozygotic twin pairs have identical genetic constitutions, any phenotypic differences between the two members of such a pair must be the result of the influence of differing environments. There is ample testimony in the literature that concordance with respect to cleft lip and palate in monozygotic twins is far from being consistent in occurrence. Buzdygan (*1*) reported discordance in this defect for three out of five monozygotic twin pairs. Ross and Coupe (*6*) analyzed six pairs of monozygotic twins, each discordant for cleft lip and/or palate. In his cases, one member of each pair had normal lip and palate. In a comprehensive study of cleft lip and palate in twins, Metrakos, *et al.*, (*5*), in addition to reviewing the studies of others, analyzed ten pairs of twins, two of which were monozygotic. They note total frequency of concordance in twenty-nine pairs of monozygotic twins of 31% and of 6.3% for seventy-nine pairs of dizygotic twins. The present study adds another case, in this instance fetuses, of concordance for cleft lip and palate in a pair of monozygotic twins.

It should be pointed out, as others have, that there is no clear agreement as to the meaning of concordance. How similar must two traits be before they can be termed concordant? Or conversely, how dissimilar must they be before they are labeled discordant? In the twin pair reported upon herein, the morphologies of the two cleft lips and palates are almost identical when superimposed by reversing one of the tracings.

The apparent agreement in the literature that between 70% and 80% of monozygotic twin pairs are discordant with respect to cleft lip and/or palate strongly suggests that the hereditary constitution plays a relatively minor role in the etiology of cleft palate in most cases. On the other hand, the consistent finding of a significantly lower frequency of concordance in dizygotic twins indicates that heredity does, indeed, play some role.

Summary

A pair of aborted 20-week-old monozygotic twin fetuses, both exhibiting cleft lip and palate, were studied. Photographs and tracings of the cleft area showed almost perfect concordance, though mirror-imaged. The tooth buds were extracted and stained with alizarin red S. An analysis of the buds indicate, in general, mirror-imaging of normal crowns on one side only of each twin. Multiple dental abnormalities were present and these are discussed in terms of concordance and discordance.

reprints: *Dr. Bertram S. Kraus*
Cleft Palate Research Center
University of Pittsburgh
Pittsburgh 13, Pennsylvania

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