Cleft Palate in the Fetuses of Lathyric Rats and Its Relation to Other Structures: Nasal Septum, Tongue and Mandible

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Introduction

"Spontaneous" cleft palates in the human species, as well as those obtained experimentally by means of teratogenic environmental factors, have been related to the development of other structures.

Lutz (*27*) found that when the cleft palate was associated with other anomalies in human subjects, the mean birth weight was significantly lower than normal. Several authors (*4, 24, 30, 33*) linked cleft palate in man to Pierre Robin's syndrome. Linthicum and Body (*25*), Kelemen (*22*) Gosepath and Haym (*17*) found that the cleft palate was often related to ear diseases. Trasler and Fraser (*37*), Ross (*31*), Ross and Walker (*32*), Humphrey (*20*) and Walker (*39*) related the production of cleft palate to movements of the tongue. Long et al. (*26*) resected the nasal septum cartilage in young rabbits and found marked underdevelopment of the snout and relative mandibular prognathism. Kremenak (*23*), studying growth of the maxillofacial region of Beagle pups whose nasal septum had been removed, concluded that the latter might play more than a passive role in development of the midfacial area. Burston et al. (*7*) attributed an important role in the relation between the upper and lower jaws; jaws to Meckel's cartilage, developed in the anterior region of chondrocranium and serving as skeletal support for the first visceral arch and added that when the anomalies were compatible with life, they would produce deformities in the new born so profound that no clinical procedure could hope to correct them, as in the achondroplastic child. Poswillo (*29*), who carried out amniotic puncture in rats, found that severe chin-sternum compression maintained the tongue between the palatal shelves, micrognathia resulted from postural molding and ossification of the body of the mandible was delayed.

Mandibular development has repeatedly been related to occurrence of cleft palate. Whereas many workers have found cleft palates associated with shorter mandibles (*5, 9, 10, 11, 13, 14, 21, 28, 34*), others have observed no difference (*15, 16, 38*). Recently, Hart and Smiley (*19*) found

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no difference between the mandibles of A/Jax mice with spontaneous cleft palate and those of controls.

Deuschle and Kalter (*12*) found that the mandibles in mice with cortisone-induced palatal clefts were larger than in normal offspring and ascribed this increase to a relative increase in length of premolar corpus.

Lathyrus odoratus is one of the substances which interferes with normal closure of the palate when added to the mother's diet (*1, 2*). In the present paper, the relation between congenital cleft palate induced by Lathyrus odoratus and the development of neighboring structures (nasal septum, tongue and mandible) is described.

Material and Methods

Twenty four mature female rats from the Histology and Embryology Department of the University of Buenos Aires were mated. The age of the fetuses was determined by means of vaginal smears. The morning on which spermatozoids were found in the vaginal plug was counted as the zero day.

Six pregnant rats, used as controls, received the standard diet[1] during gestation. The remaining eighteen rats received the same diet, except from the 10th to 13th days of gestation, during which it was replaced by a diet made up of the standard diet and ground Lathyrus odoratus mixed in equal amounts. Both groups were given water "ad libitum".

The fetuses were obtained by laparotomy on the 18th or 21st day of gestation. After decapitation, the heads were fixed in 15% formaldehyde and included in paraffin in order to obtain semi-serial frontal sections, which were stained with hematoxylin-eosin and Von Kossa's method.

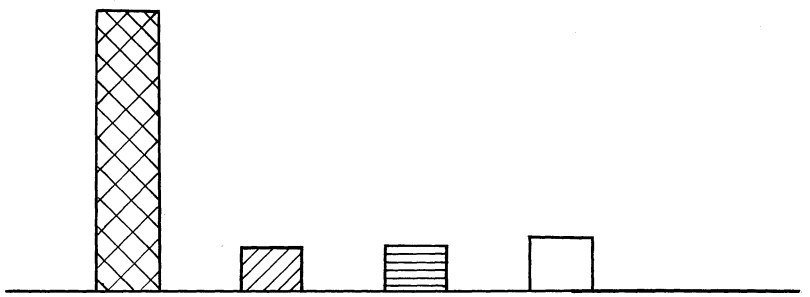
The following micrometric measurements were carried out on the frontal sections: a) total length of the nasal septum at a plane passing behind Jacobson's organ; b) length of the cartilage contained on the previous measurement. Measurements were then carried out at a plane passing through the first molars, taking into account: c) the distance between the external surfaces of the mandible; d) the distance between the internal surfaces of the mandible; e) the distance between the source of the dental lamina of the lower molar tooth germ; f) the width of the tongue at the lingual furrow; the height of the tongue from the geniohyoid muscles to its dorsal surface. When the tongue was irregularly shaped, several measurements were made in order to obtain a mean for each fetus.

Results

Whereas the 6 control rats gave 44 normal fetuses, the 18 treated rats produced 61 viable fetuses and 65 macerated or resorbed fetuses. The latter were not used in the present work, since their state of disorganization did not permit application of the usual histological techniques. Out of the 61 experimental fetuses studied, 47 (77%) presented cleft palate, 41 (67%) displayed mandibular alterations; and 6 (10%) had normal

[1] Forramez Lab., Molinos Rio De La Plata, Buenos Aires.

TABLE 1. Relation between cleft palate and mandibular alterations in treated fetuses.



CLEFT PALATE WITH MANDIBULAR ALTERATIONS	41 FETUSES	67%
CLEFT PALATE WITH NORMAL MANDIBLES	6 FETUSES	10%
CLOSED PALATE WITH MANDIBULAR ALTERATIONS	6 FETUSES	10%
CLOSED PALATE WITH NORMAL MANDIBLES	8 FETUSES	13%

jaws. The remaining 14 fetuses (23%) did not present cleft palate, and 6 (10%) had mandibular alterations whereas 8 (13%) had normal jaws (Table 1).

In most of the fetuses with cleft palate, the anterior part of the palatine shelves was found in a horizontal position, while the posterior part maintained its vertical position. In some cases, one shelf was horizontal and the other vertical. In some 21-day fetuses, both shelves were vertical and appeared as small lateral appendixes adjacent to the region of the upper molar tooth germs. In spite of their scant development (even in these cases), Von Kossa's method for calcium salt precipitation showed the presence of such salts within the palatine shelves (Figure 1).

Macroscopic examination of the material obtained from treated rats showed decreased antero-posterior length of the mandible. This fact was corroborated histologically by the presence of Meckel's cartilage at a plane passing through the bucconasal conduct, and in many fetuses, at the level of the eyes. Frontal sections of the mandible presented various alterations in size, shape and structure. The statistical results are summarized in Table 2. At a plane passing through the first molars there was a 10% incrementation in the distance between the external surfaces ($P < .001$) and a 40% decrease in that between the internal surfaces ($P < .001$), showing a marked increase in thickness of the jaw. The shape was altered by expansions, which were very pronounced on the lingual expansions of the mandible which appeared as a result of a single or double cartilaginous masses, more or less circular in cross-section (Figure 2). These additional cartilages were found closer to the midline than Meckel's cartilage, characteristically in the region adjoining the lower first molars, and were

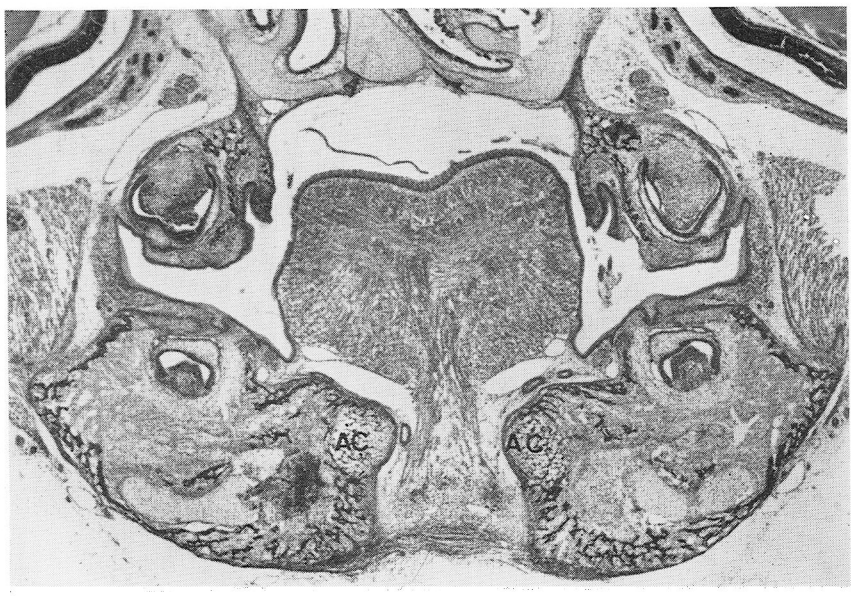


FIGURE 1. 21-day-old treated fetus with cleft palate and mandibular alterations. Jaw increased in thickness; additional cartilaginous masses (AC); delayed ossification. (Von Kossa's method & H. orig. mag. × 25.)

TABLE 2. Mean values of frontal section measurements (measurements are expressed in arbitrary units).

	total length of nasal septum	*length of nasal septum cartilage*	*distance between external surfaces of mandible*	*distance between internal surfaces of mandible*	*distance between first lower molar tooth germs*	*distance between lingual furrow*	*height of tongue*
control fetuses 18 d	26.6±0.68 (1)	22±0.67	49.4±0.83	22.4±1.04	31.8±0.51	18.7±0.81	23.8±0.55
treated fetuses 18 d	$\bar{X} \pm Es$ 25.2±1.4	17±0.77	55±0.86	8.8±1.06	37±1.12	20±0.68	18.9±0.80
P <	.3 [1] $\bar{X} \pm Es$	.001	.001	.001	.001	.3	.001

absent from the anterior and posterior parts of the mandible. In some cases, the additional cartilages became completely detached from the mandible, appearing as small masses among the muscles of the tongue (Figure 3). Because of the intense development of its lingual portion, the mandible occupied the anterior part of the submaxillary gland cell, approaching the hyoglossus muscles (Figure 4).

With Von Kossa's method, the first centers of calcium precipitation were found in the 18-day-old control fetuses (Figure 5); whereas in fetuses of the same age from the treated group, this process had not yet started. In 21-day-old control fetuses, calcium salts precipitated immediately

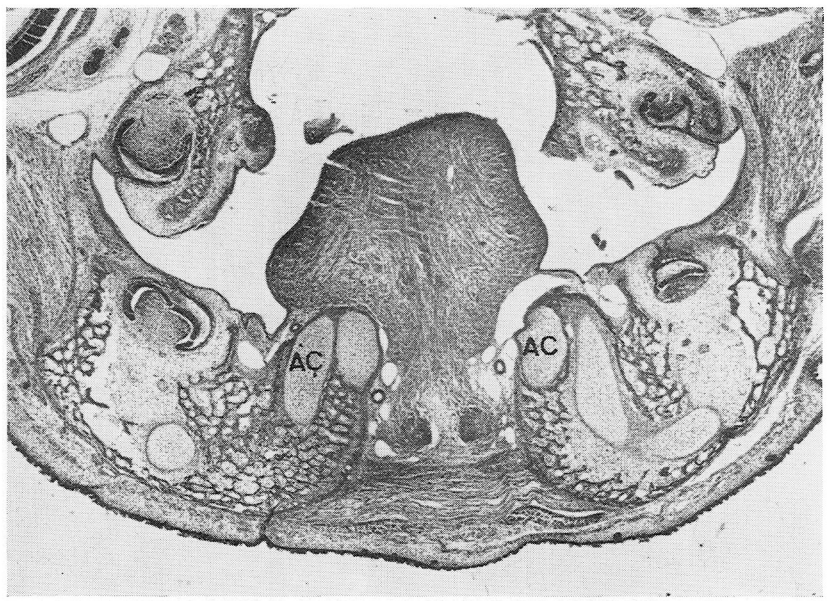


FIGURE 2. 18-day-old treated fetus with cleft palate and mandibular alterations. Jaw increased in thickness; additional cartilaginous masses (AC). Stain H.&E., orig. mag. × 25.)

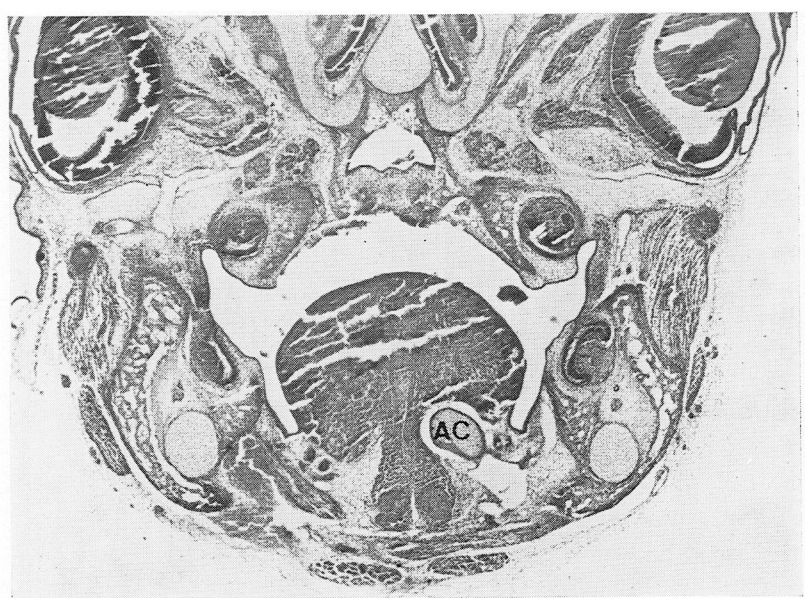


FIGURE 3. 18-day-old treated fetus with closed palate. Additional cartilaginous masses within the tongue (AC). (Stain H.&E. orig. mag. × 25.)

FIGURE 4. 18-day-old treated fetus with closed palate and mandibular alterations. Additional cartilaginous masses (AC). (Stain H.&E., orig. × 25.)

FIGURE 5. 18-day-old control fetus. (Stain H.&E., orig. mag. × 25.)

after formation of the organic matrix; while in the experimental animals, numerous bone trabeculae without calcium salt precipitation were found. Most of these trabeculae were located in the center of the mandible; at the edges, a mineralized bony lamella was observed. There was a 13%

increase in the distance between the first molar tooth germ, in comparison with the controls.

Although the increase in width of the tongue at the level of the lingual furrow was not significant, the previously mentioned decreased intermandibular distance provoked diminished development of the part of the tongue interposed between the two halves of the jaw. The decrease in height of the tongue from the geniohyoid muscles to the dorsal surface was highly significant ($P < .001$). In fetuses with cleft palate the tongue was asymmetric when one palatine shelf was horizontal and the other vertical. However, the buccal part of the tongue was more frequently triangular with its median vertex adjacent to the inferior part of the nasal septum, and the intermandibular part was found as a small muscular stem, representing the hyoglossus muscles.

The decrease in length of the nasal septum was not statistically significant ($P < .3$). The length of its cartilage, on the other hand, showed a highly significant decrease ($P < .001$). In most experimental fetuses, this cartilage was "S" shaped and less differentiated than in the controls: its intercellular substance had scarce staining affinity and the cellular elements conserved long prolongations.

In experimental fetuses with closed palate and mandibular alterations (Figures 3 and 4), the latter were similar to those described for fetuses where such alterations were associated with cleft palate. In experimental fetuses with closed palate, the nasal septum developed normally.

Discussion

The results of this study show that Lathyrus odoratus has a teratogenic effect on various parts of the maxillofacial region, producing cleft palate and perturbing development of the nasal septum, tongue and mandible. Although most of the fetuses studied presented anomalies in different areas simultaneously, mandibular and tongue alterations could be observed even when the palate had closed normally. Shortening of the nasal septum cartilage, on the other hand, was only associated with cleft palate. We find it noteworthy that only the skeletal structure of the nasal septum should have decreased in size, whereas the soft tissues showed no significant decrease.

Cleft palates induced by Lathyrus odoratus were associated with much shorter mandibles than the control. Several teratogenic agents produce the same result. Giroud and Boisselot (*14*) reported similar findings in rats treated with a diet lacking vitamin B_2; Cohlan (*10*), with large doses of vitamin A; Fraser and Fainstat (*13*), in mice treated with cortisone; Kalter and Warkany (*21*), in riboflavin-deficient mice; Asling et al. (*5*), in pteroylglutamic acid-deficient rats; Chamberlain (*9*), in rats treated with 6-amino-nicotinamide; Morgan (*28*), in rats treated with meclizine; Poswillo (*29*), in rats, by means of amniotic puncture; Schwartz and Chaudhry (*34*), in cortisone-treated rats. However, other authors (*15, 16,*

38) found no difference between the length of the mandible of animals with cleft palate and control animals. Hart and Smiley (*19*) obtained similar results in the A/Jax strain of mice, with spontaneous cleft palate. Deuschle and Kalter (*12*) found that the cortisone-induced cleft palate in rats was associated with a longer mandible than normal and that the increase resulted from greater development of the premolar mandibular corpus, but they observed no structural difference between this region and the rest of the jaw.

Cameron (*8*) showed that inoculation of one of the active principles of Lathyrus odoratus (semicarbazide hydrochloride) during incubation of the turkey produced a bilateral L-shaped deformity of the lower beak, without describing any structural alterations.

We have been unable to find any description in the literature of a teratogenic agent acting independently on the mandible, without affecting normal closure of the palate. Our results show that a high percentage of mandibular alterations were associated with cleft palates and a low percentage were not. However, we consider these facts important because they give evidence against the existence of a cause-effect relationship between cleft palate and mandibular alterations. The fact that the mandibular alterations described were localized in the region of the first molar shows that sensitivity to an environmental factor may be more intense in different parts of the same structure. The greater sensitivity of the area of the first molar with respect to the anterior and posterior regions of the jaw may be related to its more intense development in comparison with the latter regions.

Avery and Devine (*6*) found that in human embryos with cleft lip and cleft palate, all the facial bones were smaller, in all dimensions, than in normal embryos and that the jaws of fetuses with cleft palate showed a marked decrease in anterior-posterior length, while remaining normal in shape. Our results showed not only an increase in the thickness of the mandible in the region of the first molar, but also other changes: the occurrence of additional masses of cartilage and a delay in the precipitation of calcium salts on the bone trabeculae. The presence of ectopic cartilages in the maxillofacial region has been pointed out as a consequence of high doses of vitamin A (*15, 18, 21, 36*); they appear preferentially in a certain place with a well-defined shape: small cartilaginous bars in an antero-posterior position in the commissural zones.

The cartilages found in experimental fetuses proved to be independent of Meckel's cartilage, appearing to be adjacent to the internal surface of the mandible and capable of detaching themselves and becoming isolated within the muscular mass of the tongue. The mandible derives from the first branchial arch. Its marked increase in thickness has the secondary effect of reducing the size of the lower portion of the tongue. These facts, together with the presence of ectopic cartilages within the tongue, lead us to assume that Lathyrus odoratus not only has a teratogenic effect on

the first arch branchial, but also on the branchial arches from which the tongue develops.

Moreover, our study was limited to fetuses, but undoubtedly under these conditions these rats would not have been able to masticate and swallow normally in post-natal life, even though closure of the palate had been normal. Usually, precipitation of calcium salts and differentiation of bone tissue within the palatine shelves takes place after rotation and fusion of the palatine shelves in the midline. In experimental animals with cleft palate, calcium salt precipitation was found in the palatine shelves of 21-day-old fetuses, even when development of the shelves was almost insignificant.

These facts lead to the assumption that in organogenesis, a series of events take place which are not necessarily related to each other and that some of these events may occur even though stages which normally precede them have not taken place (*1*). In the mandible, the delay in calcium salt precipitation on the bone trabeculae could be related to a delay in collagen maturation, a fact in agreement with the findings of Shoshan et al. (*35*) who maintain that calcium salt precipitation depends on the protein nature of the matrix. With the methods used, no change was observed in structures of the tooth germs.

Summary

Fetuses from rats receiving a 50% lathyrogenic diet between the 10th and 13th day of gestation were studied. Lathyrus odoratus is a teratogenic agent which inhibits normal development of the maxillofacial region. 67% of the treated fetuses presented cleft palate and 41%, in addition, showed mandibular and lingual alterations. The mandible of the treated fetuses was shorter and thicker at the level of the first molar, than that of the controls. The increase in thickness resulted from marked development of the internal part. As a secondary result, the inferior part of the tongue was compressed. Structural alterations were also found in the area of the first mandibular molar. The same mandibular and lingual alterations were found in treated fetuses with or without cleft palate. The nasal septum cartilage was shorter in the treated fetuses than in the controls.

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