Influence of Cleft Lip Upon Palatal Closure in A/Jax Mice

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Spontaneous clefts of primary, as well as secondary, palate can be observed in mice populations (*2, 4, 6*). Most often, the two malformations are associated and seldom occur separately. In the A/Jax strain, from a series of 100 embryos with cleft lip, examined on day 15 of pregnancy (when the palate has normally closed), only four achieved successfully palatal closure.[1] In the most frequent type of cleft, (cleft lip associated with cleft palate), a question arises as to whether both malformations are resulting from a) a systemic alteration of the capability of fusion, first in the primary palate, then in the secondary palate, or b) whether the palatal cleft would be a mechanical consequence of the cleft lip. In the first hypothesis, the palatal processes would be unable to fuse upon approximation; in the second, they would be unable to approximate.

This paper represents an attempt to determine which hypothesis is the more probable explanation. Palatal shelves of 14 day, 12 hour old A/Jax mice embryos were dissected as shown in Figure 1, then approximated by pairs, and cultivated *in vitro*. A total of 20 embryos were used. Among them, ten had a cleft lip and ten others showed normal lip closure. Among the cleft embryos, four were bilateral and six unilateral clefts. The cleft specimens were from ten different litters. One normal embryo was also taken at random from each of these litters to provide the ten controls.

The two palatal processes of each embryo were laid down in contact (Figure 2) on lens paper at the surface of a liquid nutrient medium (NCTC 109: 9 vol.; Fetal Bovine Serum: 1 vol.; Penicillin-streptomycin; 100 units/ml.), following a technique described previously (*3*). The incubation proceeded for 28 hours at 37°C, within an atmosphere saturated with humidity and containing 5% CO_2. The explants were studied by direct as well as by histological observation.

Of the ten pairs of palatal processes originating from the cleft lip embryos, all fused *in vitro*. Nine of these pairs underwent a mesenchymal

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[1] Trasler, D. G., Personal communication.

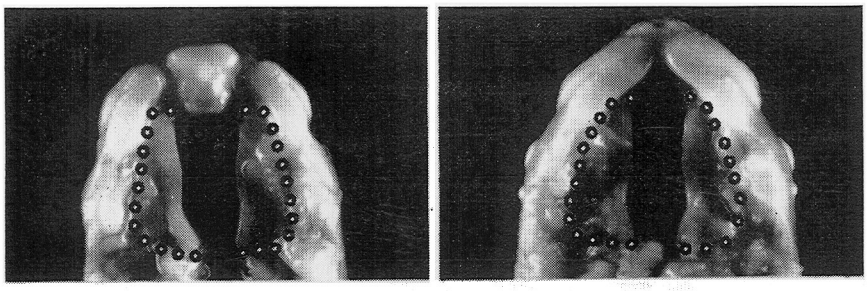

Fig. 1

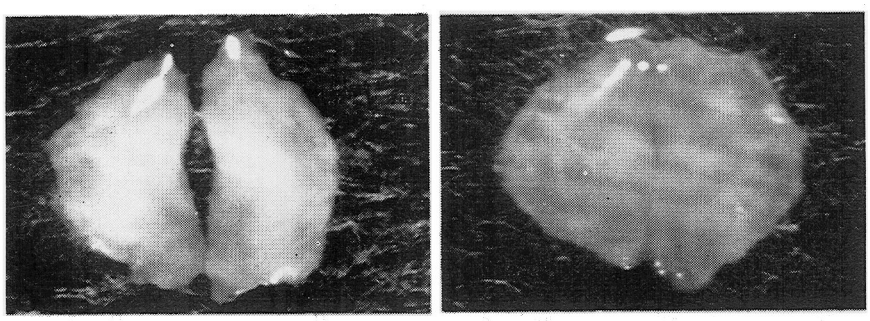

Fig. 2

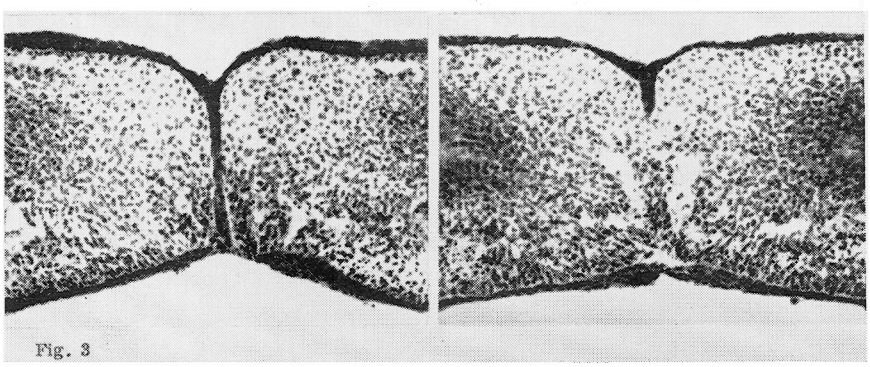

Fig. 3

FIGURE 1. Left, head of a 14 day, 12 hour old A/Jax embryo with lower jaw removed, showing a bilateral cleft lip; right, head of a 14 day, 12 hour old A/Jax embryo with lower jaw removed, showing a normal lip closure. The dotted lines indicate the tracing of the dissection.

FIGURE 2. Left, position of two palatal shelves *in vitro* after explanation; right, view of the same palatal shelves after 28 hours *in vitro*.

FIGURE 3. Left, section across two palatal shelves which underwent epithelial fusion *in vitro;* right, section across two palatal shelves having fused *in vitro*. Most of the epithelial wall has disappeared, showing a mesenchymal fusion between the both parts of the explant.

TABLE 1. Behavior *in vitro* of twenty pairs of palatal shelves.

Origin of the explants	*Behavior in vitro*		
	no fusion	*epithelial fusion*	*mesenchymal fusion*
Cleft lip embryos	0	1*	9
Normal embryos	1*	0	9

* Not litter mates.

fusion with resorption of the epithelial barrier, while one pair underwent only epithelial fusion (Figure 3). Of the ten pairs of palatal processes dissected from normal embryos, nine underwent mesenchymal fusion but one pair did not fuse. These results are summarized in Table 1.

Obviously, there is no significant difference between the normal and cleft series in the relative frequency of complete palatal fusion, since in both series nine explants out of ten underwent complete fusion. Both the incomplete fusion obtained in the cleft lip series, and the complete failure recorded in the normal lip series, might reasonably be attributed to a slightly earlier stage of development of the embryos from which these explants were removed. Such individual variations in development often occur within a litter or between the embryos of two different litters recorded as being of the same stage (*1*). They can explain a lower level of differentiation in some of the palatal shelves removed from a sample of embryos killed at a given age (*3*).

Since both normal and cleft lip embryos demonstrated a comparable potentiality for palatal fusion, the failure of the palate to close, observed in 96% of the cleft lip A/Jax embryos (*1*), must be attributed to some other factor. One of these factors might be the failure of the tongue to displace itself downward and forward, thus preventing the palatal shelves from assuming their horizontal position (*5*).

Summary

Palatal shelves of twenty 14 day, 12 hour old A/Jax mice embryos were dissected, then approximated by pairs, and cultivated *in vitro*. Ten of the embryos had a cleft lip, ten were normal. All of the ten pairs of processes from cleft lip embryos fused; nine of the pairs of processes of normal embryos fused. The presence of the cleft lip obviously did not affect the propensity of the palate processes for fusion.

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