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# Corticosteroid-induced Cleft Lip in Mice: A Teratologic, Topographic, and Histologic Investigation

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Unlike cleft palate, relatively few teratogens have been found to induce cleft lip in mice. The present study was designed to assess the teratologic, topographic (SEM), and histologic effects on lip morphogenesis following the administration of triamcinolone hexacetonide on the eighth day of gestation. The frequency of cleft lip in treated A/J mice was found to be more than three times greater than the spontaneous frequency in untreated controls. Comparable studies with other murine strains suggest no association between the cleft lip response and either a maternal effect or the H-2 complex. Affected A/J embryos showed a severe reduction in the size of the lateral nasal processes; affected embryos also demonstrated localized cell type-specific alterations, particularly in the epithelia and at the interface between epithelium and mesenchyme.

**Key words: cleft lip, corticosteroid induced; cleft plate, corticosteroid induced; mice, teratological model; corticosteroids, triamcinolone hexacetonide; H-2 complex; embryopathology, teratological; scanning electron microscopy of lip morphologenesis**

## INTRODUCTION

Genetically different inbred strains of mice have different susceptibilities to the induction of isolated cleft plate by a standardized dose of glucocorticosteroids at the critical period of palatal formation, days 11-14 [Fraser and Fainstat, 1951; Kalter, 1957; Walker and Fraser, 1957; Loevy, 1963; Marsk et al, 1971; Bonner and Slavkin, 1975; Biddle and Fraser, 1977]. To wit, A/J mice are highly susceptible, whereas C57B1/6 mice are resistant to cortisone-induced cleft palate [Kalter, 1957; Bibble and Fraser, 1977]. More recent work on mice clearly rules out a polygenic model in the strict sense of many equal and additive genes [Bibble and Fraser, 1977]; this finding parallels recent studies in humans that also suggest that the liability to isolated cleft palate is not likely to include a polygenic component

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[Melnick and Shields, 1976; Melnick et al, 1977, 1980]. One gene complex that appears to be associated with susceptibility to glucocorticosteroid-induced cleft palate is the major histocompatibility complex, H-2 [Bonner and Slavkin, 1975; Tyan and Miller, 1978; Melnick et al, in press]. However, by contrast the production of cleft lip in mice, a more common human malformation, has not as yet met with a similar degree of success.

Recently, Kalter [1979] exhaustively reviewed the biology of the A family of inbred mice. He concluded that, other than 6-aminonicotinamide, phenytoin, and aspirin, teratogens (including corticosteroids) had little or no effect on the frequency of cleft lip and that the development of the lip (unlike the palate) appears to be shielded from developmental interference. However, preliminary studies in our laboratory using corticosteroids suggested the contrary and prompted us to test this hypothesis more extensively.

The present study was designed to assess the teratologic, topographic, and histologic effects on lip morphogenesis following the administration of triamcinolone hexacetonide on the eighth day of gestation in a dose equivalent to that which produces approximately 50% cleft in A/J fetuses when administered on the 12th day of gestation. The frequency of cleft lip in treated A/J mice was found to be more than three times greater than the spontaneous frequency in untreated controls or A/J mice treated on the 12th day of gestation. Furthermore, cleft lip could not be induced with the same dose in C57BL/6, C57BL/10 Sn, B10.A/SgSn, or A/J-C57BL/6 reciprocal $F_1$ hybrid fetuses, suggesting that the tolerance to triamcinolone hexacetonide of the C57BL embryos is greater than that of A/J, that there is no maternal effect, and that susceptibility of A/J embryos to cleft lip is not associated with genes in the H-2 complex. Affected A/J embryos demonstrated a severe reduction in the size of the lateral nasal processes and a lesser reduction in the size of the medial nasal and maxillary processes. In addition, day-8 treated A/J embryos demonstrated localized cell type-specific alterations. The embryopathology following corticosteroid exposure is similar to that described for drug-induced cleft lip using other teratogens [Trasler and Leong, 1974; Sulik et al, 1979]

## MATERIALS AND METHODS

### Mating, Teratogen Administration, and Fetal Phenotype

Virgin female A/J (A), C57BL/6 (B6), C57BL/10 Sn (B10), and cogenic partner B10.A/SgSn (B10.A), from the Jackson Laboratory (Bar Harbor, Maine), were housed by strain in groups of five and were acclimated to the vivarium environment in our institution during a quarantine period of two weeks. When the animals were 12–13 weeks old, groups of three females were placed in a cage with one male overnight. Females were examined daily in the morning for the presence of vaginal plugs. The date of plug detection was designated as day 0 of gestation. A × A, B6 × B6, A × B6, B6 × A, B10.A × B10.A, and B10 × B10 matings were obtained.

Pregnant dams were housed in solid bottom, plastic cages. The vivarium facility is temperature controlled with an average daily temperature of 24°C. Alternate 12-hour periods of light and darkness were maintained daily. All animals were

maintained on Wayne's Mouse Breeder Blox containing 20% crude fat and a maximum of 2% crude fiber, and were supplied with drinking water ad libitum.

Experimental animals received a single intramuscular injection (in the proximal portion of the hindlimb) of 2 mg/kg body weight triamcinolone hexacetonide, a long-acting synthetic analogue of cortisol, at 9:00 AM on day 8 or day 12 of gestation. Nontreated pregnant animals served as controls.

On the morning of day 17 of gestation, all dams were killed by cervical dislocation and the uterus and its contents were exposed. The uterine horns were examined in situ, and the following was noted for each fetus: location (right or left horn); position (from midline union to distal portion of uterine horn); fetal status (live, dead, resorbed). Each live fetus was carefully dissected out of the uterine decidua, and the trophoblast shell and Reichert membrane were removed. Fetuses were measured (crown-rump), weighed, and immediately placed in a glass receptacle with a fixed amount of 10% formalin solution. After fixation for a minimum of 24 hours, the fetuses were examined for all gross external anomalies. Both the developmental staging of the fetuses and the morphologic examinations were performed under a dissecting microscope. All abnormal fetuses and representative normal fetuses were routinely photographed (Fig. 1). It should be noted that each fetus was coded and tagged at the time of fixation, and the staging and examination were done blindly with respect to treatment by one investigator.

## Statistical Analysis

In all experimental groups within each cross (control, day-8 treated, day-12 treated), the following data were collected: 1) mean number of implants; 2) mean number of live fetuses; 3) mean frequency of fetal loss (resorbed + dead

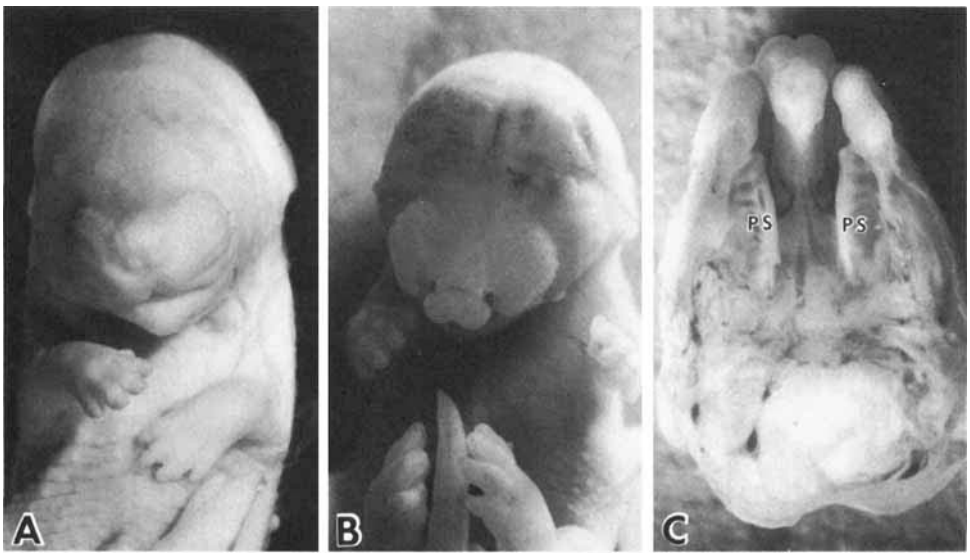


Fig. 1. Theiler stage 25 (17 days) A/J mouse fetuses: (A) control (× 7): (B) bilateral cleft lip (× 7); (C) palatal view (mandible removed) of bilateral cleft palate (PS, palatal shelves that failed to fuse) (magnification, × 10).

fetuses/total implants); 4) mean frequency of normal live fetuses (normal live fetuses/total live fetuses); 5) mean frequency of CL + P (live CL + P fetuses/total live fetuses); 6) mean frequency of CP (live CP fetuses/total live fetuses); 7) fetal weight; and 8) mean frequency of developmental immaturity (less than Theiler stage 25).

Mean frequencies were computed using the general equation

$$\bar{I} = \frac{1}{n} \sum_{j=1}^{n} I_j$$

where $I_j$ is frequency of a given "event" for the jth litter. An unbiased estimate of the sample standard deviation for the frequency of a given "event" is then

$$S_I = \left[ \frac{1}{n-1} \sum_{j=1}^{n} (I_j - \bar{I})^2 \right]^{1/2}$$

Frequency data were "normalized" prior to analysis by arcsin transformation, 0% and 100% values being replaced by ¼n and 100 − (¼n), respectively, where n is the number of fetuses per litter [Dagg et al, 1966]. To test the equivalence of two frequencies, mean weighted arcsins [Dagg et al, 1966] were compared by t or t′ tests, depending on the presence or absence of variance homogeneity [Sokal and Rohlf, 1969; Satterthwaite, 1946; Cochran, 1951].

Means computed for continuous variables were also compared by one- or two-tailed t or t′ tests. Fetal weight was analyzed further by a two-level nested analysis of variance (mixed model) designed for unequal sample sizes [Sokal and Rohlf, 1969]. This allowed us to partition the sources of variation: among treatments, among pregnant dams within treatments, and among the mice of one litter (within litters).

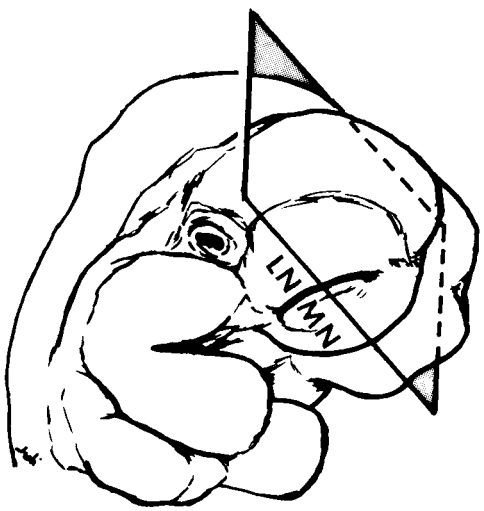


Fig. 2. Diagram of anterolateral view of Theiler stage 17–18 (10½–11 days) indicating selected plane of section, perpendicular to the lateral (LN) and medial (MN) processes.

### Light and Scanning Electron Microscopy

In replicate studies, on the mornings of gestation days 9–13 treated and control dams from A × A crosses were killed by cervical dislocation, and the uterus and its contents were exposed. Embryos were rapidly dissected free of uterine and extraembryonic tissue and immersed in Karnovsky's fixative overnight [Waterman, 1972]. Embryos were staged as described above, rinsed in cacodylate buffer, and postfixed in 0.2 M phosphate buffered 1% osmium tetroxide for one to two hours at room temperature. Dehydration in a graded ethanol series at room temperature was followed by a graded isoamyl acetate series and then critical-point dried [Anderson, 1956]. Specimens were mounted with silver paint on aluminum stubs, sputter coated with gold-palladium, and then examined with an AMR 1000 scanning electron microscope (SEM) at 10–30 kV.

In order to prepare histological sections from embryo specimens previously examined with SEM, they were placed in several changes of propylene oxide and then oriented and embedded in Epon [Meller et al, 1973]. Serial cross sections (1 μm), perpendicular to the nasal processes (Fig. 2), were cut with glass knives on a Sorvall MT2-B ultramicrotome, stained with toluidine blue, and then examined with a Zeiss research photomicroscope.

## RESULTS

### Teratologic Response in A/J × A/J Crosses

**Fetal lethality.** Table I lists the number of litters, implants, live fetuses, and resorbed and dead fetuses for each of the three treatment groups, along with means and 95% confidence limits. There were no significant differences in the mean number of implants between control and day-8 treated ($t_{22} = -0.555$, $P > 0.50$) or day-12 treated ($t_{22} = 0.898$, $P > 0.40$) or between day-8 treated and day-12 treated ($t_{18} = 1.75$, $P > 0.50$). The mean frequency of fetal loss was significantly different between control and day-8 treated ($t'_{162.88} = -4.32$, $P < 0.001$) and between control and day-12 treated ($t'_{146.24} = -3.21$, $P < 0.005$). There were no significant dif-

**TABLE I. Implants, Live Fetuses, Resorptions, and Dead Fetuses by Treatment Group**

| | | Fetal status at gestation day 17 | | |
|---|---|---|---|---|
| Treatment | Number of litters | Number of implants[a] | Number of live fetuses[a] | Number of resorbed fetuses[b] |
| Control | 14 | 131 (9.36 ± 1.21) | 117 (8.36 ± 0.98) | 14 (0.098 ± 0.059) |
| Triam-D8 | 10 | 98 (9.80 ± 1.16) | 79 (7.90 ± 1.14) | 19 (0.178 ± 0.128) |
| Triam-D12 | 10 | 87 (8.70 ± 0.83) | 72 (7.20 ± 1.42) | 15 (0.182 ± 0.116) |

[a]Number in parentheses is the mean number of implants or live fetuses plus or minus the 95% confidence limits.

[b]Number in parentheses is the mean frequency of dead and resorbed fetuses plus or minus the 95% confidence limits.

ferences between day-8 treated and day-12 treated ($t_{183} = 0.919$, $P > 0.20$). Since it is possible that a high frequency of fetal loss in day-8 treated and day-12 treated litters occurs because of a concentration of lethality in only a few litters, we considered the distribution of lethality across litters. There were no significant differences in the distributions between control and day-8 treated ($\chi_1^2 = 0.046$, $P > 0.50$) or between control and day-12 treated litters ($\chi_1^2 = 0.000$, $P > 0.975$).

**Fetal malformations.** Table II lists the number of live fetuses that were "normal" and malformed (CL ± P, CP, other), along with mean frequencies and 95% confidence limits. The mean frequency of malformed live fetuses was significantly lower in control than in day-8 treated ($t'_{97.17} = -10.19$, $P > 0.001$) and day-12 treated litters ($t'_{83.83} = -12.36$, $P > 0.001$). The frequency of malformed fetuses was also greater in day-12 treated than in day-8 treated mice ($t_{149} = -2.80$, $P < 0.01$). The distribution of malformed was uniform across litters for the treated groups, being 90% in day-8 treated and 100% in day-12 treated. Of particular importance, the mean frequency of CL ± P was significantly greater in the day-8 treated group than in the day-12 treated Group ($t_{149} = 9.36$, $P > 0.001$). In fact, the frequency of CL ± P in the day-12 treated group (0.035) was almost identical to that in the control group (0.038). However, the mean frequency of CP was significantly greater in the day-12 treated group than in the day-8 treated group ($t'_{132.61} = -4.79$, $P < 0.001$).

**Fetal group and development.** The mean fetal weights ± standard deviation for the three treatment groups were: control = 0.600 gm ± 0.155, day-8 treated 0.573 gm ± 0.122, and day-12 treated 0.409 gm ± 0.153. The fetal weights were nearly equal in the control and day-8 treated groups, whereas in the day-12 treated group there was about a 30% mean weight reduction. A two-level nested analysis of variance (Table III) revealed a significant component of the total variation among the treatment groups. The variation among pregnant dams within treatment groups was highly significant. However, the variation within litters was comparatively small, even though litters contained both normal and malformed fetuses.

Investigation of immaturity (less than Theiler stage 25) by analysis of $\chi^2$ [Rao,

**TABLE II. Phenotype of Live A/J Fetuses by Treatment Group**

| Treatment | Litters | Number of live fetuses[a] | | | | |
|---|---|---|---|---|---|---|
| | | "Normal" | Malformed = CL ± P | + CP | + Other | |
| Control | 14 | 108 (0.833 ± 0.088) | 9 (0.082 ± 0.061) | 4 (0.035 ± 0.035) | 1 (0.007 ± 0.016) | 4[b] |
| Triam-D8 | 10 | 50 (0.539 ± 0.200) | 29 (0.387 ± 0.200) | 9 (0.114 ± 0.052) | 20[c] (0.270 ± 0.191) | 0 |
| Triam-D12 | 10 | 35 (0.389 ± 0.215) | 37 (0.545 ± 0.225) | 2 (0.038 ± 0.060) | 33[d] (0.484 ± 0.225) | 2[e] |

[a]Number in parentheses is the mean frequency plus or minus the 95% confidence limits.
[b]Includes one micrognathia, one phocomelia, one severe plagiocephaly, and one micrognathia + multiple limb anomalies. These fetuses did not have CL ± P or CP.
[c]Includes one fetus with micrognathia in addition to the CP.
[d]Includes one fetus with bilateral microtia in addition to the CP.
[e]Includes one exencephaly and one phocomelia. These fetuses did not have CL ± P or CP.

**TABLE III. Fetal Weight: Nested Analysis of Variance**

| Source of variation | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Among treatment groups | 2 | 1.75 | 0.875 | 4.93[a] | < 0.025 |
| Among pregnant dams within treatment groups | 31 | 5.17 | 0.167 | 27.83 | < 0.001 |
| Within litters (among fetuses of one dam) | 230 | 1.34 | 0.006 | | |
| Total | 263 | 8.26 | | | |

[a]Note, for reasons that will not be dealt with here, this test of significance is carried out by synthesizing a new denominator mean square (ie, among pregnant dams) against which to test the mean square of among treatment groups. For a more detailed account of this procedure see Sokal and Rohlf [1969].

1952] revealed that the mean frequency of immaturity in live fetuses was independent of both phenotype and treatment groups. In addition, the mean frequency of immaturity by phenotype was independent of treatment group.

## Teratologic Response in Remaining Crosses

Table IV presents the fetal response to triamcinolone hexacetonide administration on day 8 of gestation in all four inbred and two reciprocal (A.B6, B6.A) hybrid, H-2 defined, strains of mice. Although a dose of 2 mg/kg body weight produces a mean clefting frequency of about 11% in A fetuses, there were no apparent cleft lips in any of the B6, A.B6, B6.A, B10.A, or B10 fetuses. This could not be explained on the basis of fetal loss since the mean frequency of fetal loss was greater for A fetuses than for any of the others. Reciprocal hybrid crosses produced equivalent responses among $F_1$ progeny with respect to cleft lip or isolated cleft palate, suggesting no significant maternal effect for corticosteroid-induced clefting when the drug is administrated on day 8 of gestation. In addition, the mean CP frequency of A fetuses is many times greater than that of A.B6 or B6.A fetuses. Comparisons between the congenic pair, B10.A and B10, demonstrate that the association of H-2 with fetal response to corticosteroids is still evident when the assay is isolated cleft palate, not cleft lip ± cleft palate. Doses up to 6 mg/kg body weight failed to produce B10.A embryos with cleft lip; at 8 mg/kg body weight the resorption frequency approached 100%.

## Topographic (SEM) Comparisons Between Treated and Control A/J Embryos

The development of the mouse lip has been described in detail [Pourtois, 1972; Trasler and Fraser, 1977]. Briefly, facial development begins with the appearance of ectodermal thickenings (nasal placodes) on either side of its anterior aspect. Cranial neural crest-derived mesenchyme condenses and proliferates at the borders of each placode, such that their lateral and medial rims expand outward to form nasal pits. The nasal pit becomes deeper as the lateral and medial processes grow, and it is further bounded on its floor by the maxillary process (Fig. 3). Definitive lip formation begins at the bottom of the nasal pit in the isthmus between the lateral and medial nasal processes (Fig. 3A). The medial surface of the lateral process and the lateral surface of the medial process converge above the

**TABLE IV. Response to Triamcinolone Hexacetonide Administration (2 mg/kg) on Day 8 of Gestation in Four Inbred and Two Hybrid, H-2 Defined, Strains of Mice**

| Cross | Fetus | Number | Fetal loss/implants | | CL ± P/live fetuses | | CP/live fetuses | |
|---|---|---|---|---|---|---|---|---|
| dam × sire | (H-2 haplotype) | of litters | Number | Frequency[a] | Number | Frequency[a] | Number | Frequency[a] |
| A × A | A($H\text{-}2^a/H\text{-}2^a$) | 10 | 19/98 | 0.178 ± 0.128 | 9/79 | 0.114 ± 0.052 | 20/79 | 0.270 ± 0.191 |
| B6 × B6 | B6($H\text{-}2^b/H\text{-}2^b$) | 10 | 11/89 | 0.119 ± 0.087 | 0/78 | 0.000 | 2/78 | 0.025 ± 0.057 |
| A × B6 | A.B6$F_1$($H\text{-}2^a/H\text{-}2^b$) | 9 | 6/60 | 0.115 ± 0.201 | 0/54 | 0.000 | 2/54 | 0.028 ± 0.064 |
| B6 × A | B6.A$F_1$($H\text{-}2^b/H\text{-}2^a$) | 9 | 1/60 | 0.028 ± 0.064 | 0/59 | 0.000 | 0/59 | 0.000 |
| B10.A × B10.A | B10.A($H\text{-}2^a/H\text{-}2^a$) | 10 | 8/78 | 0.110 ± 0.112 | 0/70 | 0.000 | 14/70 | 0.200 ± 0.189 |
| B10 × B10 | B10($H\text{-}2^b/H\text{-}2^b$) | 9 | 6/62 | 0.107 ± 0.110 | 0/56 | 0.000 | 3/56 | 0.034 ± 0.053 |

[a]Mean frequency ± 95% confidence limits, calculated as described in the text.

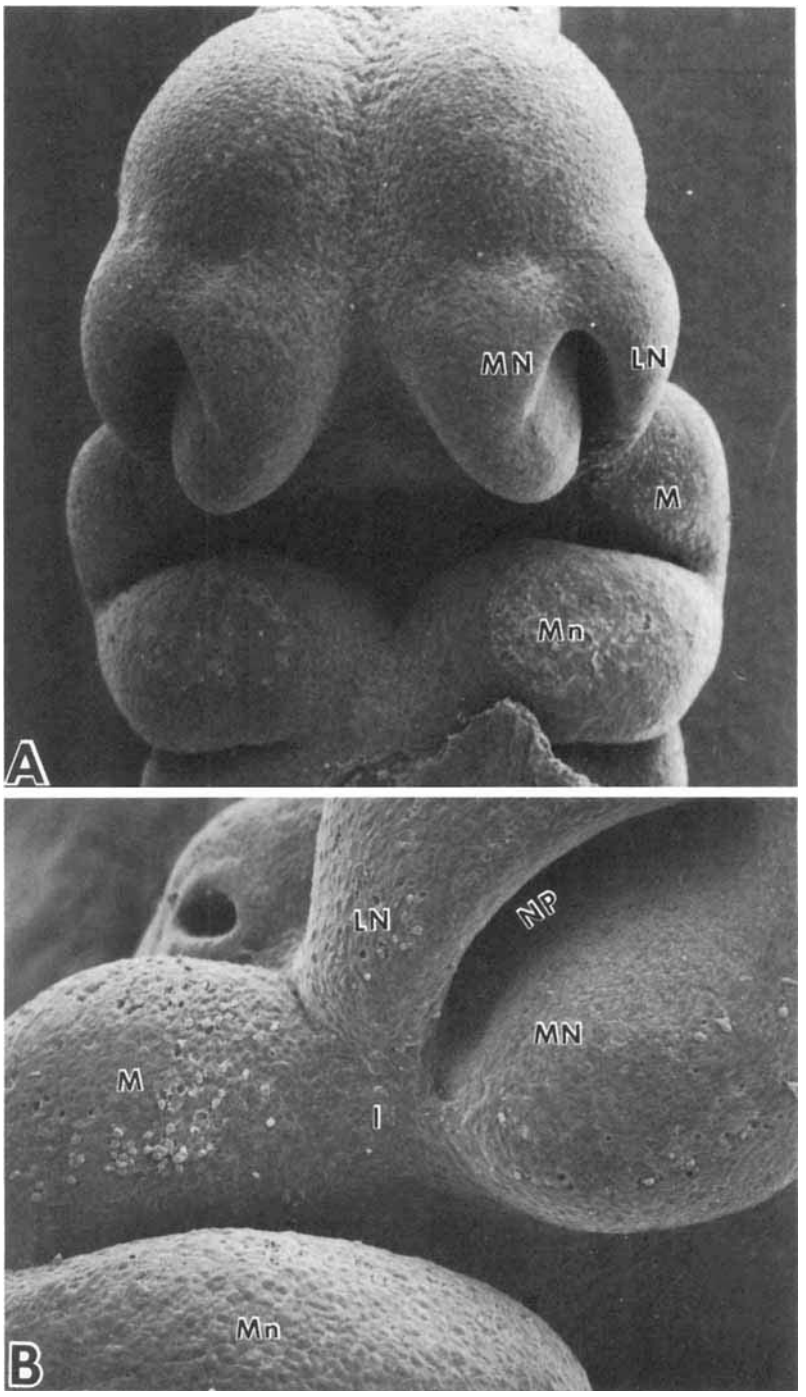


Fig. 3. Scanning electron photomicrographs of Theiler stage 17–18 control embryo. A. The lateral (LN) and medial (MN) nasal processes converge, and each is surrounded by a maxillary process (M) (magnification, × 89). B. The isthmus (I) region; the medial surface of the lateral nasal process and the lateral surface of the medial nasal process converge above the isthmus (magnification, × 220). NP, nasal pit; Mn, mandibular process.

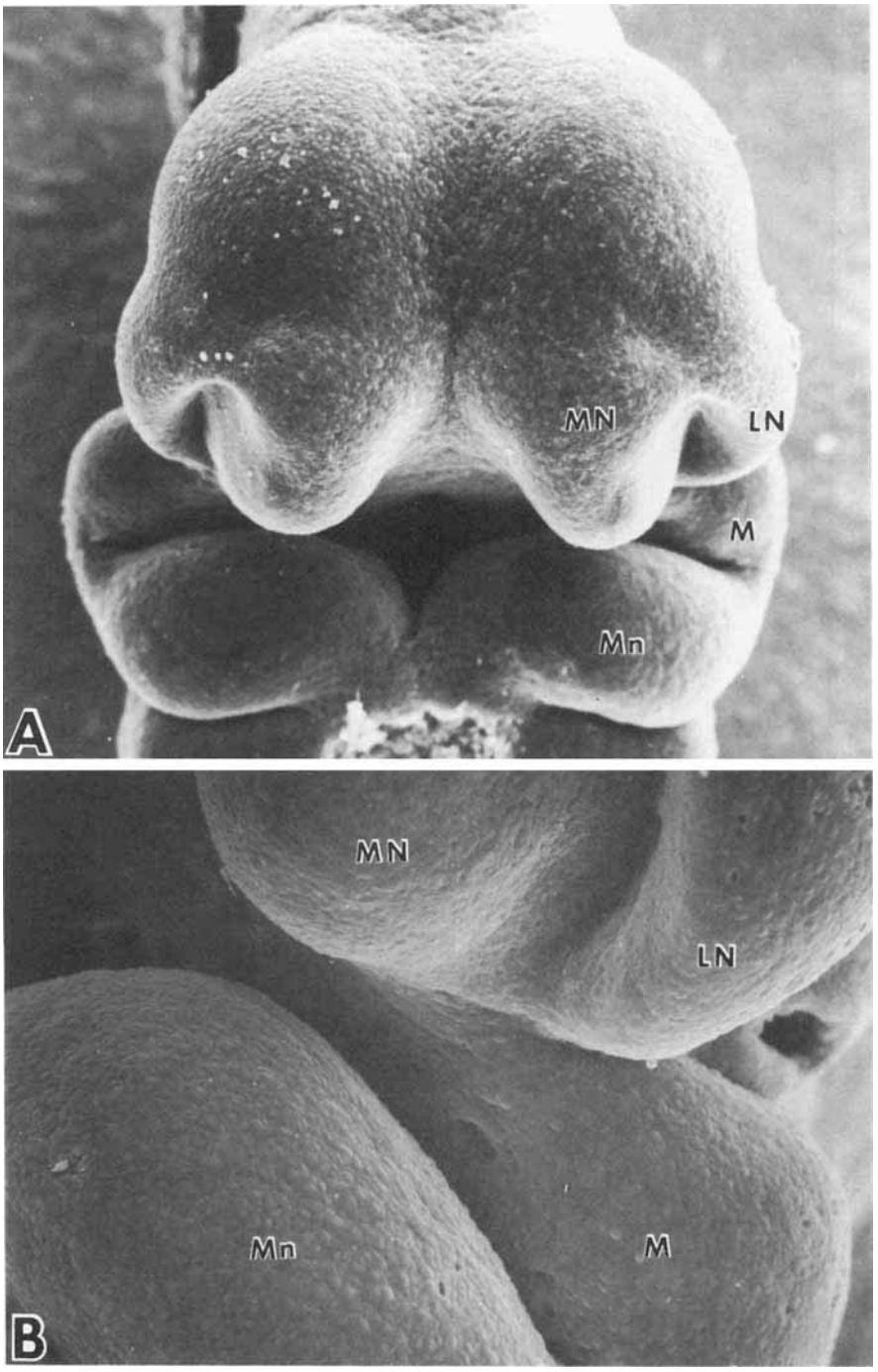


Fig. 4. Scanning electron photomicrograph of a triamcinolone-treated Theiler stage 17–18 embryo. A. The lateral nasal process (LN) was reduced in size; the medial nasal (MN) and and maxillary (M) processes exhibited minimal size reduction (magnification, × 89). B. The isthmus region was significantly reduced; the inferior aspects of the lateral (LN) and medial (MN) nasal processes are separated (magnification, × 220). Mn, mandibular process; M, maxillary process.

isthmus, with their apposed epithelia forming an epithelial plate or "nasal fin." Shortly before the epithelia of the opposing processes make contact, cell degeneration, characterized by condensation and fragmentation, occurs in this epithelium [Garre and Langman, 1977]. Apparently direct contact between the process epithelia is not necessary to cause cell death. After fusion has established the nasal fin, epithelial cells continue to degenerate. The rapidly autolyzed nasal fin is replaced by mesenchyme, and the final contouring of the upper lip begins. The isthmus between the processes progressively widen as the external nasal pit becomes smaller; at the same time the medial aspects of the two medial nasal processes are growing and eventually merge with each other to give rise to the central portion of the lip.

Prior to Theiler stages 17–18 (circa 10.5–11 days, examination by SEM of treated and control embryos revealed no topographic differences. A majority of the Theiler stage 17–18 teratogen-treated embryos demonstrated a considerable reduction in the size of the lateral nasal processes as compared with nontreated controls (Fig. 4). The reduction was most pronounced at the inferior aspect of the lateral processes. Higher magnification showed that the isthmus was exceedingly small and that the most inferior aspects of the lateral and medial nasal and maxillary processes were separated by approximately 100 microns (Fig. 4B). The medial nasal and maxillary processes exhibited minimal size reductions (Fig. 4A). It should be noted that treated embryos either exhibited reduced facial processes or appeared normal. Nontreated control embryos (Fig. 3), in sharp contrast to treated embryos, demonstrated well-developed and converging medial and lateral nasal processes, their isthmuses being of sufficient size. No other topographic differences between treated and control embryos were found.

## Histologic Comparisons Between Control and Treated A/J Embryos

Histologic examination of control (Fig. 5) and treated (Fig. 6) embryos demonstrated differences in their cellular morphology. Compared to the control (Fig. 5) embryos, the reduced size of the lateral nasal processes in treated embryos (Fig. 6) was clearly visualized at comparable planes of section. The epithelium of the control embryos showed a gradual transition from stratified columnar to cuboidal (Fig. 5A,B) in the medial to lateral direction. In contrast, treated embryos did not show a gradual transition (Fig. 6). The epithelium of the medial nasal process in treated embryos showed increased intercellular spaces (Fig. 6B); the epithelium of control embryos was a stratified columnar with few intercellular spaces (compare Figs. 5C & 6B). The interface between the epithelium and underlying mesenchyme in treated embryos was not delineated in localized areas, suggesting the possibility of interrupted or degraded basement membrane (Fig. 6B).

Sections through the maxillary processes of control (Fig. 7A,B) and treated (Fig. 7C,D) embryos also revealed differences. The epithelium of the treated (Figs. 7C,D) embryos exhibited increased basophilia and large intercellular spaces. This occurs in the epithelium, which normally does not fuse and undergo degeneration. In addition, the interface between epithelium and mesenchyme was not distinct in the treated maxillary process, suggesting the possibility that the basement membrane was degraded in localized regions.

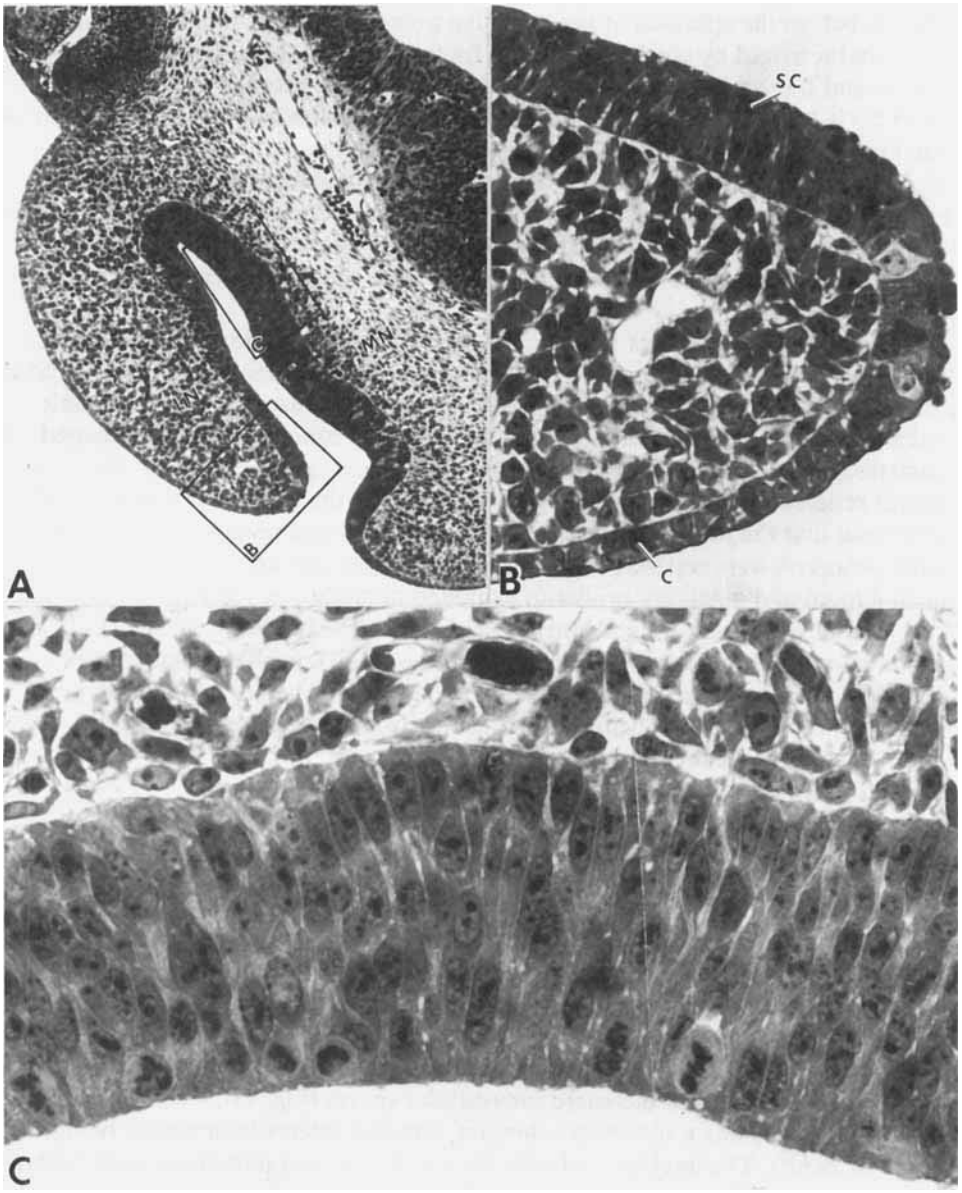


Fig. 5. Representative histologic sections of Theiler stage 17–18 control mouse embryos. The orientation for these 1 $\mu$m thick sections is shown in Figure 2. A. The lateral nasal (LN) and medial nasal (MN) processes converge in control embryos (magnification, × 178). B. The epithelium of the lateral nasal process in control specimens shows a gradual transition from stratified columnar (SC) to cuboidal (C) cells (magnification, × 832). C. The epithelium of the medial nasal process was a stratified columnar with few intercellular spaces (magnification, × 1275).

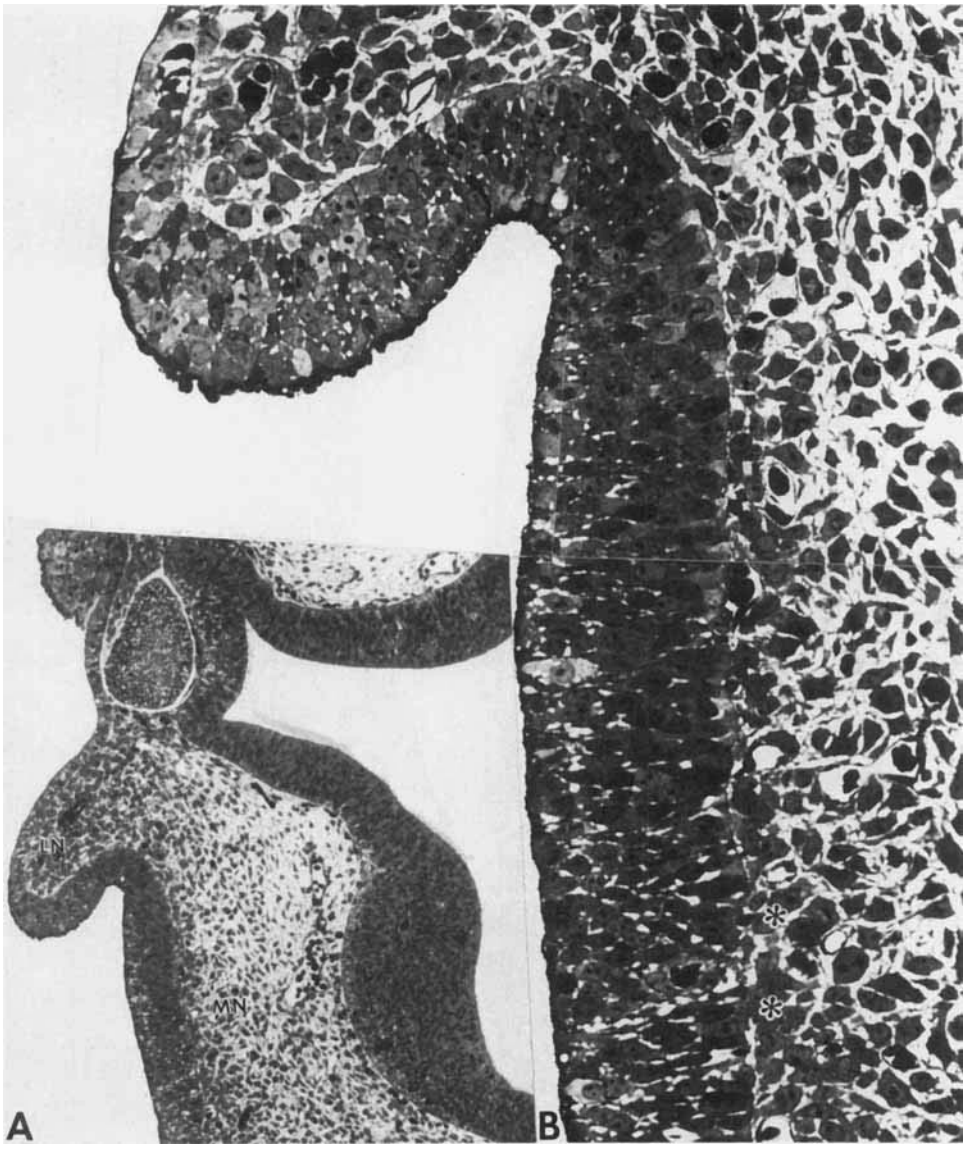


Fig. 6. Representative histologic sections of Theiler stage 17–18 treated mouse embryos. The orientation for these 1 $\mu$m sections is shown in Figure 2. A. The lateral nasal (LN) process was reduced in size and separated from the medial nasal (MN) process in triamcinolone-treated embryos by a greater distance than in controls (compare Fig. 7) ($\times$ 208). B. The nasal process epithelium showed increased intercellular spaces. The interface between the epithelium and underlying mesenchyme was not delineated in localized areas (asterisk) (magnification, $\times$ 780).

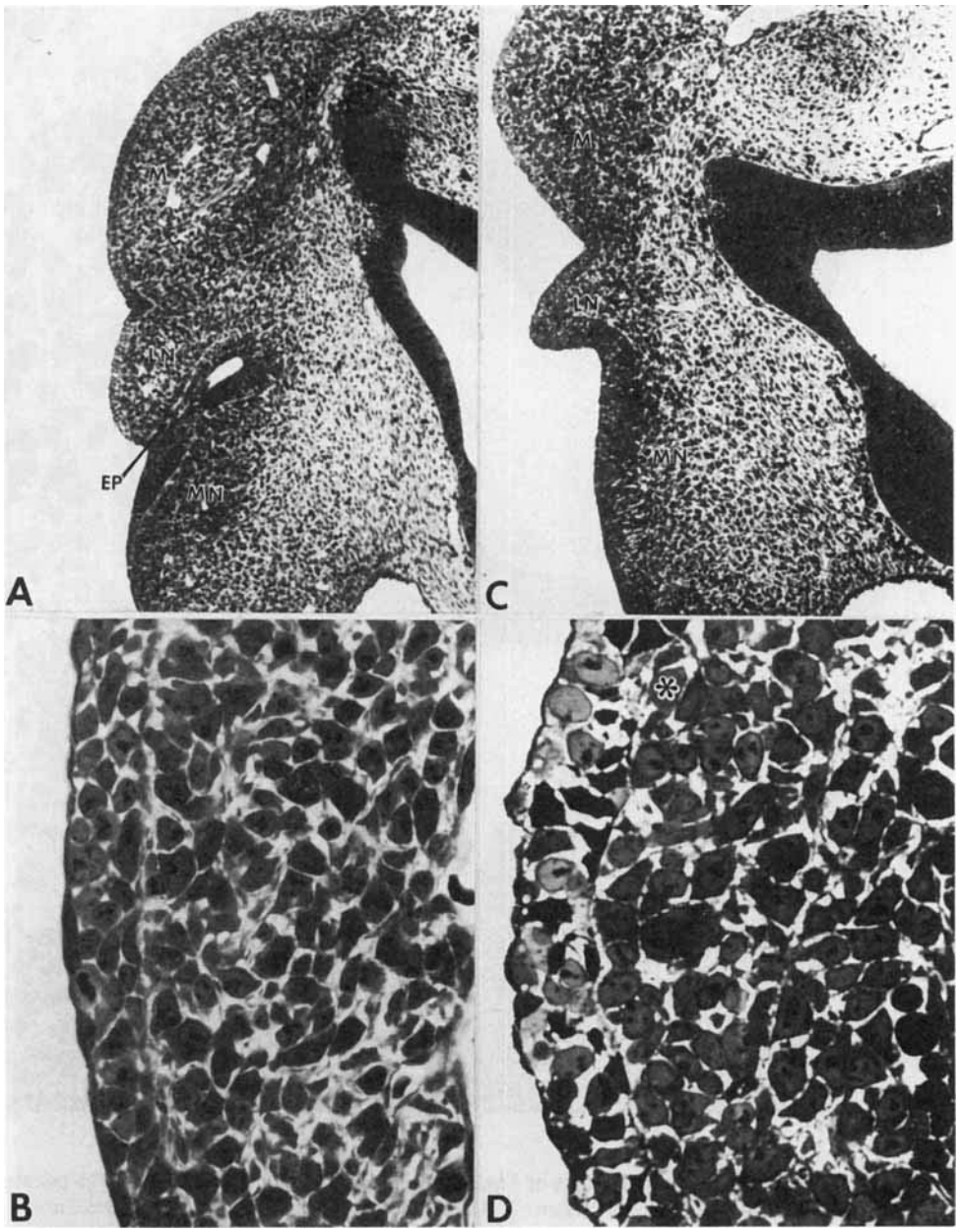


Fig. 7. Histologic sections through the maxillary process. The orientation for these 1 $\mu$m thick sections is shown in Figure 2. A. Control embryo: the lateral (LN) and medial (MN) nasal processes converge forming the epithelial plate (EP) ($\times$ 150). B. The epithelium of the maxillary process was a stratified cuboidal with few intercellular spaces ($\times$ 880). C. Triamcinolone-treated embryo: The lateral nasal (LN) process (magnification, $\times$ 177). D. The epithelium of the maxillary process exhibited increased basophilia and intercellular spaces. The interface between the epithelium and underlying mesenchyme was not distinct in localized areas (asterisk) (magnification, $\times$ 1086). M, maxillary process.

## DISCUSSION

### Cleft Lip Induction

This study demonstrates, for the first time, a significant ($P < 0.001$) increase in the frequency of cleft lip in the A/J mouse strain following maternal treatment with a glucocorticosteroid, triamcinolone hexacetonide, on day 8 of gestation. The frequency of cleft lip among treated fetuses was more than three times greater than that found among nontreated controls. There were also major differences in the phenotypic response to the drug when administered on different days of gestation, 8 vs 12: 1) the mean frequency of normal live fetuses was significantly greater in the day-8 treated group than in the day-12 treated group; 2) the mean frequency of live malformed fetuses was significantly greater for day-12 treated than for day-8 treated; 4) the mean frequency of cleft lip with or without cleft palate was significantly greater in the day-8 treated group than in the day-12 treated, the latter group being equivalent to the "backround" incidence in the control group; and 5) the mean fetal weight in the day-12 treated group was 30% lower than in the day-8 treated group, which was equivalent to the control group. Regarding the cleft lip, these data suggest that the apparent resistance of the A strains to most teratogens thus far tested [Kalter, 1979] may well be a result of the timing of teratogen administration or availability.

A most interesting finding was that in the same day-8 treated A/J litter, littermates with either cleft lip ($\pm$ cleft palate) or isolated cleft palate were identified. This phenotypic discordance among genetically identical, n-chorionic, n-tuplets might be the result of developmental asynchrony within litters. Alternatively, since triamcinolone hexacetonide is long acting, the isolated cleft palates induced by day-8 treatment may result from residual protracted drug action.

Lastly, the studies in other mouse strains (Table IV) have produced some interesting but enigmatic results. Clearly, with respect to cleft lip induction by triamcinolone, there is no clear evidence for a maternal effect nor an association with the H-2 complex; instead, susceptibility of A embryos appears to be associated with alleles at other loci. The enigma is with regard to the isolated cleft palate response. If corticosteroids are administered around day 12 of gestation to A/J and C57BL/6 mice there is a well-established maternal effect [Kalter, 1957; Biddle and Fraser, 1977]. Our results indicate that, for CP, if the drug is administered on day 8 the maternal effect is no longer evident. This difference may be indicative of changes in maternal metabolism as the pregnancy progresses. Furthermore, although the association of cleft palate induction with H-2 haplotype remains with day-8 drug administration, the association is more likely with the embryonic haplotype than with the maternal haplotype, as has been found with the day-12 administration [Bonner and Slavkin, 1975]. Perhaps the embryonic effect is masked at later stages of pregnancy; this embryonic effect may be related to either drug detoxification or unique tissue-specific effects (eg, maxillary process mesenchyme, rather than lateral nasal process mesenchyme). However, it should be noted that the role of cell-surface H-2 antigens in embryonic craniofacial development awaits elucidation. If one may be permitted to extrapolate from mouse to man, the complexities uncovered with mouse models may partially explain the conflicting results from limited studies of the association of HLA and human clefts [Bonner et al, 1978; Van Dyke et al, 1980].

## Lip Morphogenesis and Dysmorphogenesis

Clearly the critical event in lip formation is the convergence of the facial processes to allow fusion or merging. Failure of the lateral and medial processes to fuse results in lateral clefts of the lip; failure of the two medial processes to merge results in the more rare medial clefts of the lip. Trasler and Fraser [1977] have drawn up a list of potential developmental aberrations that would result in cleft lip: 1) alteration of the position and/or time of nasal placode induction; 2) decreased rate of neural crest migration to and mitosis in the facial processes; 3) alteration of the relative sizes and positions of facial processes; 4) changes in the rate of autolysis of the nasal fin; 5) mechanical interference with convergence of facial processes; 6) failure of the facial processes to fuse or coalesce upon converging. Some of these possibilities overlap (eg, 2 and 3). These potential aberrations have been investigated for but a few teratogens known to induce cleft lip in mice: 6-aminonicotinamide is associated with a reduction in mitotic index and results in medial nasal processes of reduced size [Trasler and Leong, 1974]; and phenytoin is associated with a marked reduction in the size of the lateral nasal processes and a less severe reduction in the size of medial nasal and maxillary processes [Sulik et al, 1979].

The lateral cleft lip induced by triamcinolone hexacetonide appears to be associated with a severe size reduction in the lateral nasal process. In addition, there appear to be cellular changes in medial, lateral, and maxillary processes. The gross topographic changes in viewed with the SEM (lateral process reduction) are not unlike those seen with phenytoin-induced cleft lip [Sulik et al, 1979].

The pathogenetic mechanisms induced by these teratogens may be similar. This view is supported by the recent corticoid receptor studies in which the binding of $^{3}$H-dexamethasone in palatal cells is depressed specifically only by other corticoids as well as drugs which are known to induce clefting in sensitive mouse strains, such as phenytoin [Goldman et al, 1978]. This group of teratogens appears to be retained in facial processes by their ability to bind tightly to chromatin, which in turn results in decreased transcription [Bekhor et al, 1978].

The available and somewhat limited evidence indicates that interference with later stages of facial process formation is likely to be the most important alteration for the common forms of nonsyndromic human cleft lip [Johnston and Sulik, 1979]. Although this assertion may be premature, the evidence from mouse studies would seem to point in this direction.

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