Studies on the Production of Cleft Palate and Cleft Lip in the Embryos of Pregnant Mice Treated with Corticosterone: A Pilot Study

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Production of congenital malformations in laboratory animals by various modalities has long been a subject of investigation. Various agents implicated and studied include anoxia (1), hypervitaminosis (2, 3) and steroid hormones (4, 5, 6, 7). Since 1950 when Fraser and Baxter (8) demonstrated the teratogenic effect of cortisone in mice, considerable interest has been directed toward the possible ability of certain steroid hormones to induce cleft palate in offspring of various animals.

Selye (9) was among the first to describe a “general adaptation syndrome.” In this syndrome, environmental stress provokes the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. The ACTH, in turn, provokes the release of adrenal cortical hormones which allow the individual to better cope with the stressful situation.

Fraser and Selye’s work provided the impetus for others to examine the role of ACTH (10) administration and the ability of environmental stress to provoke cleft palate formation in laboratory animals (11). Results of these studies have shown that ACTH administration to pregnant females is capable of producing a significant increase in the percentage of cleft palate embryos. Studies involving stress as an etiologic factor are quite variable due to the fact that “stress” is difficult to qualify and quantify in laboratory animals.

In many instances, studies carried out during the past twenty years have used rodents as the experimental animal. Attempts have been made to correlate stress, cortisone and ACTH administration in these animals with the spontaneous occurrence of cleft palate in humans (12).

However, papers such as that by Bush (13) have apparently been overlooked. Bush was able to show that corticosterone is the primary

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This work was conducted when Robert M. Hackman, the junior author, was a high school student. He has a continuing interest in this area. Dr. Bennett is Associate Professor of Anesthesiology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pa.
adrenal steroid hormone produced by the rodent and that practically no cortisone secretion occurs.

Although a study of this nature would seem important, no literature describing the teratogenic effect of corticosterone can be found. In view of the fact that cortisone is foreign to the rodent, this study was undertaken to determine the teratogenic effect of corticosterone, the naturally occurring steroid hormone, in mice.

**Material and Methods**

An attempt was made to duplicate the study of Fraser, et al. using corticosterone (corticosterone acetate, Calbiochem) instead of cortisone. In addition, one group of animals received double the dose while a second group received half the drug dose used by Fraser.

Pregnant animals of the A/Jax strain (Jackson Laboratories, Bar Harbour, Maine) were injected subcutaneously at 3:00 P.M. on the eleventh through the fourteenth days of the gestation period. Day of pregnancy was certified by the supplier, with plug day designated as day 0.

Nine animals served as controls and were injected with 0.1 cc. sesame oil. Experimental group S1 consisted of seven animals injected with 1.25 mg. corticosterone acetate in 0.1 cc. sesame oil, group S2 received 2.50 mg. corticosterone and group S3 received 5.00 mg. corticosterone per day. All animals were fed a standard laboratory chow and tap water ad libitum.

In order to prevent cannibalism by the mother, the animals were sacrificed on day eighteen and the embryos removed. The head of each embryo was detached and the mandible and tongue removed. The heads were examined grossly for the presence of either cleft palate only, cleft lip only, or both cleft palate and cleft lip.

**Results**

The effect of the three dose levels of corticosterone on the embryos is shown in Table 1.

Of the 77 control embryos, 7 had a facial deformity. The 47 embryos whose mothers received 1.25 mg. of corticosterone showed the highest percentage of embryos having both a cleft palate and cleft lip, with 9

<table>
<thead>
<tr>
<th>treatment</th>
<th>no. of females</th>
<th>no. of embryos</th>
<th>CP only</th>
<th>CL only</th>
<th>CP and CL</th>
<th>re-sorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>9</td>
<td>77</td>
<td>2 (3%)</td>
<td>0 (0%)</td>
<td>5 (7%)</td>
<td>5</td>
</tr>
<tr>
<td>1.25 mg.</td>
<td>7</td>
<td>47</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>9 (19%)</td>
<td>2</td>
</tr>
<tr>
<td>2.5 mg.</td>
<td>7</td>
<td>59</td>
<td>4 (7%)</td>
<td>0 (0%)</td>
<td>5 (9%)</td>
<td>3</td>
</tr>
<tr>
<td>5.0 mg.</td>
<td>6</td>
<td>59</td>
<td>5 (9%)</td>
<td>0 (0%)</td>
<td>3 (5%)</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Injections given once every 24 hours on days 11–14 of gestation.
(19%) qualifying. The percentages for cleft lip and palate then taper off, with 5 of the 59 embryos of the 2.5 mg. group having them, (9%), and 3 of the 59 fetuses of the 5.0 mg. group being classified in this group (5%). No cases of cleft lip not associated with cleft palate were noted.

The percentages of the group having isolated cleft palate tends to increase as the dosage is increased. There is an increase between the 1.25 mg. and the 2.5 mg. groups (2% to 7%). Another rise can be noted between the 2.5 mg. (7%) and 5.0 mg. (9%) group.

However, there seems to be no evidence that the administration of corticosterone to pregnant mice had any effect on the incidence of cleft palate and cleft lip in the embryos.

Discussion

The fact that cortisone is teratogenic in mice (4, 5, 6, 7) will not be disputed. The process whereby cortisone causes cleft palate in mice has been thought of as a laboratory model of what happens in man (12).

However, since cortisone does not naturally exist in the mouse, it becomes apparent that administration of this foreign agent may be producing cleft palate by virtue of a toxic effect. It would seem therefore that the works involving cortisol-induced cleft palate in mice provide a means or a research tool to study palate mechanism and closure rather than providing an explanation for spontaneous cleft formation in man.

Another implication of this work might be that ACTH is capable of producing malformations by an effect other than its steroid releasing ability, since the steroid it releases does not appear to be teratogenic. Perhaps ACTH is acting directly on the embryo, as has been suggested.

A more detailed study is apparently in order. Plans include more animals in the drug groups, more extensive controls, elimination of factors such as shipping, and experimentation with different vehicles. It may then be more accurately determined what role, if any, corticosteroids have in stress-induced cleft palate formation in mice.

Summary

A study was conducted to determine the teratogenic effects of corticosterone on the offspring of pregnant A/Jax mice. At the three dose levels tested, no significant difference was noted in the incidence of facial deformities between control and drug treated animals.

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References