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# Cleft palate induction in hamster fetuses by glucocorticoid hormones and their synthetic analogues

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## SUMMARY

The effect of prenatal administration of different doses of cortisone, corticosterone, dexamethasone, triamcinolone and prednisolone on the fetus and its palatal development was studied. All the glucocorticoids, except cortisone, produced cleft palate in the fetuses. Both the total frequency and morphologically different types of cleft palate were related to the dose of the teratogen. Triamcinolone appeared to be more potent than other glucocorticoid in inducing cleft palate. An association was noted between fetal growth inhibition, the dose of the teratogen and the frequency and type of cleft palate.

## INTRODUCTION

For the past several decades, hormones have been used during pregnancy in order to improve fetal survival and as therapy of maternal disease. Many of these hormones are shown to produce lethal and teratological effects on the fetus and newborn (Reilly, 1958; Sutherland & Light, 1965; Herbst, 1973). Glucocorticoids and their synthetic analogues have been used during human pregnancy primarily to treat maternal disease. However, their use during pregnancy has occasionally been reported to be complicated by the development of cleft palate in the offspring (Harris & Ross, 1956; Doig & Coltman, 1956; Bongiovanni & McFadden, 1960; Popert, 1962).

In 1950, Baxter and Fraser reported that administration of cortisone to pregnant mice produces cleft palate in the fetuses. Since then, teratogenicity of glucocorticoids has been extensively studied primarily in different strains of mice (Fraser & Fainstat, 1951; Chaudhry, Schwartz, Schwartz & Schmutz, 1967; Dostal & Jelinek, 1970, 1971*a*; Blaustein, Feller & Rosenzweig, 1971; Hackman & Brown, 1972). Scattered reports are also available for other species (Walker, 1967, 1971; Shah & Chaudhry, 1973). It appears from these studies that the teratogenic potency of glucocorticoids differs from species to species

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Table 1. *Incidence of induced fetal cleft palate and resorption following administration of different drugs to pregnant hamsters at day 11 of gestation*

| Drug | Dose (mg) | No. of animals | No. of live fetuses | Resorption (%) | Cleft palate (%) |
|---|---|---|---|---|---|
| Cortisone in water | 10 | 3 | 39 | 7 | 0 |
| | 20 | 3 | 38 | 5 | 0 |
| | 30 | 3 | 34 | 5 | 0 |
| | 40 | 3 | 41 | 3 | 0 |
| | 60 | 3 | 38 | 0 | 0 |
| Corticosterone in oil | 5 | 3 | 29 | 0 | 7 |
| | 10 | 3 | 27 | 7 | 63 |
| | 20 | 3 | 25 | 0 | 100 |
| | 40 | 4 | 35 | 3 | 100 |
| | 60 | 2 | 17 | 10 | 100 |
| Prednisolone in oil | 5 | 3 | 30 | 3 | 0 |
| | 7 | 3 | 27 | 0 | 59 |
| | 10 | 3 | 27 | 10 | 93 |
| | 15 | 3 | 26 | 10 | 100 |
| | 20 | 3 | 18 | 18 | 100 |
| Triamcinolone in water | 0·25 | 3 | 29 | 3 | 24 |
| | 0·5 | 3 | 26 | 0 | 42 |
| | 1·0 | 3 | 25 | 17 | 100 |
| | 2·5 | 3 | 25 | 8 | 100 |
| | 5·0 | 4 | 30 | 30 | 100 |
| Dexamethasone in water | 0·5 | 4 | 35 | 8 | 32 |
| | 1·0 | 4 | 39 | 7 | 75 |
| | 2·5 | 3 | 26 | 16 | 100 |
| | 5·0 | 2 | 11 | 42 | 100 |
| Control (oil) | 1 ml | 5 | 47 | 6 | 0 |
| Control (water) | 0·5 ml | 3 | 31 | 0 | 0 |

and also in different strains of the same species. Walker (1971) suggested that teratologic drug testing should be done with a group of closely related compounds in several species.

In an earlier study, Shah & Travill (1976*a*) described the effect of hydrocortisone on the palatal development in the hamster fetus. The present study describes the effect of cortisone, corticosterone, prednisolone, triamcinolone and dexamethasone on hamster palatogenesis.

## MATERIALS AND METHODS

Male and female Golden Syrian hamsters were caged singly and maintained under controlled environmental conditions of temperature (24 ± 1 °C), humidity (50 ± 5 %) and an alternate cycle of light (6 a.m. to 6 p.m.) and dark. Food and water were made available to the animals at all times. Following a minimum of

Table 2. *Estimation of actual effective dose to produce 0·5 probability of cleft palate in the fetuses*

| Drug | Effective dose (mg) | Confidence interval at 95 % | $\chi^2$ (D.F.) |
|---|---|---|---|
| Triamcinolone | 0·484 | 0·378–0·621 | 6·486 (3) |
| Dexamethasone | 0·693 | 0·583–0·823 | 5·799 (2) |
| Prednisolone | 6·846 | 5·909–7·931 | 9·725 (3) |
| Corticosterone | 9·349 | 7·458–11·719 | 8·041 (3) |

$\chi^2$, chi-square test; D.F., degrees of freedom.

one week of acclimatization, females weighing approximately 84 ($\pm$ 3) gm were mated with males from 7 to 9 p.m. on the appropriate day of the estrous cycle. The midpoint of the mating period (8 p.m.) was designated as day 0.

On day 11 of gestation, pregnant animals were given a single intramuscular injection of drugs, in the doses shown in Table 1. A group of animals was similarly treated with the appropriate vehicle at each time. Both experimental and control animals were killed on day 15 of gestation. The viable fetuses were weighed and then immersed in Bouin's fluid for fixation. Subsequently the fetuses were examined for the status of cleft palate development. For statistical evaluation of data on cleft palate development, a computer program of minimum NORMIT chi-squared analysis (Berkson, 1957) was performed. An integrated normal curve was obtained for each teratogen to describe the relationship between the amount of drug and the probability of cleft palate.

## RESULTS

The data presented in Table 1 show that four of five glucocorticoids given to female hamsters during pregnancy produce cleft palate in the fetuses. Only cortisone administration did not induce cleft palate.

A single intramuscular injection of 20–60 mg corticosterone produced cleft palate in all fetuses. Similar observations were made following injection of 1–5 mg triamcinolone, 2·5–5 mg dexamethasone and 15–20 mg prednisolone. The frequency of cleft palate decreased, however, as the dose of the teratogen was reduced (Table 1).

An integrated normal curve indicated a close association between the dose and the probability of cleft palate following dexamethasone, triamcinolone, prednisolone and corticosterone administration. In order to compare the teratogenic potency of different drugs, the actual effective dose that would give 0·5 probability of cleft palate in the fetuses was determined at 95 % confidence interval. The results for each drug are summarized in Table 2. One may deduce from the table that triamcinolone appears to be more potent than other

Table 3. *Incidence of induced fetal complete and partial cleft palate following administration of different drugs to pregnant hamsters at day 11 of gestation*

| Drug | Dose (mg) | No. of fetuses with cleft palate | Complete cleft palate | Partial cleft palate | | | |
|---|---|---|---|---|---|---|---|
| | | | | Total | AP* | A† | P‡ |
| Corticosterone | 5 | 2 | 0 | 2 | 0 | 2 | 0 |
| | 10 | 17 | 8 | 9 | 0 | 9 | 0 |
| | 20 | 25 | 21 | 4 | 3 | 1 | 0 |
| | 40 | 35 | 35 | 0 | 0 | 0 | 0 |
| | 60 | 17 | 17 | 0 | 0 | 0 | 0 |
| Prednisolone | 7 | 16 | 5 | 11 | 2 | 9 | 0 |
| | 10 | 25 | 2 | 23 | 6 | 17 | 0 |
| | 15 | 26 | 25 | 1 | 0 | 1 | 0 |
| | 20 | 18 | 15 | 3 | 3 | 0 | 0 |
| Triamcinolone | 0·25 | 7 | 5 | 2 | 1 | 0 | 1 |
| | 0·5 | 11 | 2 | 9 | 1 | 8 | 0 |
| | 1·0 | 25 | 15 | 10 | 0 | 10 | 0 |
| | 2·5 | 25 | 23 | 2 | 1 | 1 | 0 |
| | 5·0 | 30 | 30 | 0 | 0 | 0 | 0 |
| Dexamethasone | 0·5 | 8 | 4 | 4 | 1 | 3 | 0 |
| | 1·0 | 30 | 28 | 2 | 2 | 0 | 0 |
| | 2·5 | 26 | 26 | 0 | 0 | 0 | 0 |
| | 5·0 | 11 | 11 | 0 | 0 | 0 | 0 |

* Cleft of the anterior and posterior part of the secondary palate.
† Cleft of the anterior part of the secondary palate.
‡ Cleft of the posterior part of the secondary palate.

glucocorticoids in inducing cleft palate since a relatively small amount is required to produce the desired effect in the fetus.

The resorption rate, following triamcinolone and dexamethasone administration, was generally higher than that of controls (Table 1). However, the rate of resorption following injection of corticosterone and prednisolone did not differ significantly from that of controls, except following 20 mg prednisolone when the rate was significantly higher than that of the controls. Also, doses that produce cleft palate in all fetuses following triamcinolone and dexamethasone administration showed a significantly higher rate of resorption than that following corticosterone and prednisolone with the exception of 20 mg prednisolone.

The morphology of the observed cleft palate varied, some being complete and others incomplete or partial. The complete cleft extended through the length of the secondary palate. The partial cleft palate involved either the anterior part of the secondary palate only or a combination of clefts of the anterior and the posterior part of the secondary palate with fusion in the middle. Occasionally, a cleft of only the posterior part of the secondary palate was also observed.

Table 3 shows the incidence of morphologically different types of cleft palate

Table 4. *Mean fetal weights (in grams) following administration of different drugs to pregnant hamsters at day 11 of gestation*

| Drug | Dose (mg) | Mean fetal weight (±S.D.) |
|---|---|---|
| Corticosterone | 5 | 1·7 (0·11) |
| | 10 | 1·6 (0·20) |
| | 20 | 1·5 (0·16) |
| | 40 | 1·3 (0·12) |
| | 60 | 1·1 (0·17) |
| Prednisolone | 7 | 1·6 (0·05) |
| | 10 | 1·5 (0·17) |
| | 15 | 1·3 (0·14) |
| | 20 | 1·3 (0·07) |
| Triamcinolone | 0·25 | 1·5 (0·09) |
| | 0·5 | 1·7 (0·23) |
| | 1·0 | 1·2 (0·43) |
| | 2·5 | 1·1 (0·28) |
| | 5·0 | 0·6 (0·20) |
| Dexamethasone | 0·5 | 1·6 (0·20) |
| | 1·0 | 1·2 (0·28) |
| | 2·5 | 1·2 (0·42) |
| | 5·0 | 0·6 (0·09) |
| Control (oil) | 1 ml | 1·95 (0·15) |
| Control (water) | 0·5 ml | 2·0 (0·15) |

as related to the doses of various drugs. One may deduce that the total frequency of partial cleft palate increases as the teratogenic doses were reduced. Further generalization of data indicates that the frequency of combined cleft in the anterior and posterior part is directly related, and that of cleft in only the anterior part is inversely related to the dose of teratogen.

Following treatment with various teratogens, the mean fetal weight declined significantly when compared with controls ($P < 0.05$), suggesting a generalized growth retardation (Table 4). Furthermore, the weight reduction is more pronounced as teratogenic doses are increased, indicating a direct relationship between the dose and frequency of cleft palate and the degree of fetal growth retardation.

## DISCUSSION

Results of our study concur with those of Fraser & Fainstat (1951), Chaudhry and associates (1967) and Walker (1967, 1971) that frequency of glucocorticoid induced cleft palate is dependent on the amount of drug administered to pregnant mothers. With the exception of cortisone, both naturally-occurring glucocorticoids and their synthetic analogues tested in the present investigation produced increasing frequency of cleft palate with concomitant increase in dose.

There appears to be certain species differences with regards to the teratogenicity of various glucocorticoids. For example, amongst natural glucocorticoids, cortisone produces cleft palate in the mouse (Fraser & Fainstat, 1951) and rabbit (Fainstat, 1954; Walker, 1967) but not in rat (Gunberg, 1957; Walker, 1971) and as shown in the present study, hamster fetuses. Similarly, corticosterone and hydrocortisone induce cleft palate in fetuses from mice (Blaustein *et al.* 1971; Hackman & Brown, 1972) and hamsters (Shah & Travill, 1976*a*) but not in rats (Shah, unpublished observations). Amongst synthetic analogues of glucocorticoids, dexamethasone and triamcinolone produce cleft palate in fetuses from all the aforementioned species whereas prednisolone is effective in all species but the rat (Pinsky & DiGeorge, 1965; Walker, 1965, 1967, 1971; present study). These observations lend further support to Fraser's hypothesis (1964) that the teratogenic effect of a compound is sporadic and unpredictable between species. However, since specific teratogenic effects of glucocorticoids and their synthetic analogues as a group applies to a wide variety of rodents in which they produce cleft palate, their untoward effect on the human fetuses cannot be ruled out. Palmer (1969) has suggested that a drug should be considered teratogenic or non-teratogenic only after evaluating dose relationships of embryonal parameters in several species. Thus, in the light of proven teratogenicity of glucocorticoids in the aforementioned laboratory animals and their suspected effect on the human embryo (Harris & Ross, 1956; Doig & Coltman, 1956; Bongiovanni & McFadden, 1960; Popert, 1962), we suggest that prior to therapeutic recommendation, all glucocorticoids should be tested for their teratogenic potency to induce cleft palate in at least two species, preferably mouse and hamster.

Therapeutic potency of dexamethasone is several times greater than that of hydrocortisone, prednisolone and triamcinolone (Laurence, 1966). As seen in the present study, however, and with observations made earlier by Shah & Travill (1976*a*), the teratogenic potency of triamcinolone seems to be greater than that of the other three, indicating that the teratogenic and therapeutic potentialities of a drug may be different. Teratogenic potency of a drug depends on such factors as maternal homeostasis, metabolism of drug and placental barrier (Saxén & Rapola, 1969) which may modify the ultimate effect of the drug on the fetus. For example, natural glucocorticoids do not produce cleft palate in rats when given by parenteral route but do so when given intra-amniotically (Dostal & Jelinek, 1971*b*). This would suggest that it is not the resistance of the fetus *per se*, as noted by Walker (1971), but some other factor, such as the placental barrier. Blackburn, Kaplan & McKay (1965) have observed that administration of glucocorticoid to pregnant rats inhibited placental growth and caused morphological changes resembling premature ageing of the placenta, and significantly increased the frequency of resorption.

Morphologically different types of cleft palate have been observed earlier by Giroud & Martinet (1956), Buresh & Urban (1964) and Dostal & Jelinek

(1971*a*). These authors, however, did not observe any relationship between the dose of the teratogen and type of induced cleft palate. Different morphological varients of cleft palate observed in our study were dose related. Both severity and frequency of cleft palate increased with increasing dose. Incomplete cleft palate showed high frequency at low teratogenic doses indicating that small teratogenic doses selectively inhibit palatogenesis and induce various types of clefts. The hard and soft palate have different types of adult tissue which may differ in their growth rates, differentiation and sensitivity to a teratogen. Perhaps maintenance of teratogenic concentration in the maternal serum, for a precise interval, may be required in order to produce a particular type of cleft palate. Also, fetuses show intra-litter differences in their stage of palatal development (Shah & Travill, 1976*b*), which may explain the morphological variety of cleft palate with the same dose.

A close association was observed in the present study between fetal weight and cleft palate following glucocorticoid treatment. Moreover, inhibition in fetal growth was directly related to the dose and the frequency and type of cleft palate (Tables 1, 3 and 4). The correlation between fetal weight and cleft palate has also been observed in the mouse by Fraser & Fainstat (1951) and Chaudhry *et al.* (1967). We may, therefore, suggest that (1) duration of growth inhibitory effects of glucocorticoid play an important part in cleft palate induction and (2) following larger doses of glucocorticoid the retarding effect is faster and of longer duration than that of smaller doses.

In brief, the foregoing discussion clearly suggests that since glucocorticoids are teratogenic in several rodents, and since their therapeutic and teratogenic potentialities seem to be different, their use during human pregnancy should be considered only following adequate medical justification. Small doses may produce less severe or non-observable defects which may in turn affect various physiological functions in later life. Therefore, negative results from experiments on rodents should not rule out possible teratogenic hazards of any environmental agent on the developing human embryo.

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