The Effects of Intraamniotic Injection of Dexamethasone in Rats[1] (35881)

JACK G. CHAMBERLAIN AND MICHIKO KASAHARA
(Introduced by William R. Lyons)

Department of Anatomy, University of California, San Francisco, California 94122

Cleft palate has been produced in mice and rabbits following cortisone administration to pregnant mothers (1, 2). Utilizing the more potent adrenal steroid dexamethasone, Nanda *et al.* (3) and Walker (4) recently produced cleft palate in rats, a species considered genetically less reactive than mice to the teratogenic action of cortisone (1, 4). Since it remained to be shown whether corticosteroids produced their effects as a result of direct action on fetal tissue *in vivo* (5), intraamniotic (ia) injections (6, 7) were employed in testing the efficacy of dexamethasone to affect rat development as well as to provide protection against the nicotinamide antimetabolite 6-aminonicotinamide (6-AN) (6–8).

Methods and Materials. Normal female rats (Long-Evans), averaging 100 days of age and 225 g in weight, were bred with normal males and fed a stock diet of natural foodstuffs.[2] The morning of finding spermatozoa in the vaginal smear was considered day zero of pregnancy. On day 15 of gestation, one day before palate closure,[2] uteri were exteriorized and amniotic sacs were injected with 120, 80, or 40 μg of dexamethasone sodium phosphate[3] at 40 μg/10 μl. Control sites were given corresponding volumes of the diluent[4] or distilled water. Various combinations of the corticoid plus diluent or water were administered in order to observe the effects of volume *per se*. Mothers were sutured, revived, and returned to the stock diet until day 21 of pregnancy. They were then killed, resorbed fetuses were counted, and the living young were weighed, fixed in Bouin's fluid or 95% ethanol and macroscopically (3–70×) examined for congenital malformations. Study of the skeletal defects were based upon alizarin red S-stained specimens (10). Several day-21 control and experimental adrenal glands were embedded in paraffin, sectioned at 8 μ and stained for histological examination. In the countertherapy studies, utilizing dexamethasone as a protective agent, 100 μg/10 μl of 6-AN[5] was injected ia together with 80, 40, or 20 μg of dexamethasone; 138 sites representing 29 litters were thereby doubly injected.

In order to compare the effects of corticoids administered to the mother with those produced by ia application, 16 additional rats were injected subcutaneously (sc) with 0.4 mg of dexamethasone on days 13, 14, and 15 of gestation and the fetuses were similarly examined.

Results. The results are presented in Table I. Since the various parameters of fetal development following ia injection of distilled water or diluent were not significantly different, control data were combined. Injection of control volumes of 10–30 μl did not affect fetal or placental weight gain, yet 14 to 26% of the young had retarded skeletal development, 4 to 9% had full cleft palate and 16 to 30% of the embryos were resorbed. Under the experimental conditions employed, sc or ia injections of dexamethasone notably affected subsequent fetal development; in the case of amniotic application, direct action of the hor-

[1] Supported by U.S. Public Health Service HD 01 503-06.

[2] See Ref. (9) for composition of the diet and timing of palate closure in the Long-Evans rat.

[3] Decadron Phosphate, Merck, Sharp and Dohme, West Point, Pa. 19486.

[4] See Table I for manufactured composition of the diluent.

[5] California Biochemicals, Los Angeles, California.

TABLE I. Effects of Intraamniotic Injection of Dexamethasone in Rats.

Treatment	Litters (no.)	Living embryos (%)	Sites treated (no.)	Fetal resorption (%)	Living young day 21 of pregnancy					
					Wt (g ± SE)	Placental wt (g ± SE)	Abnormal (%)	FCP[a] (%)	PCP[b] (%)	Skel. ret.[c] (%)
Dexamethasone[d]										
120 μg	11	93	63	33	3.77 ± 0.08	0.35 ± 0.01	98	74	17	100
80 μg	20	93	109	26	4.02 ± 0.08	0.39 ± 0.08	94	73	25	88
40 μg	14	91	78	24	4.13 ± 0.07	0.39 ± 0.00	88	34	59	92
Control[e]										
30 μl	10	93	63	30	5.39 ± 0.09	0.50 ± 0.02	21	9	4	26
10 μl	16	89	73	16	5.64 ± 0.06	0.50 ± 0.01	23	15	6	14
Dexamethasone sc[f]	16	87	195	13	3.74 ± 0.15	0.34 ± 0.01	91	45	46	89

[a] Full cleft palate.

[b] Partial (anterior and posterior) cleft palate.

[c] Skeletal retardation.

[d] Intraamniotic injection day 15 (40 μg/10 μl).

[e] Diluent (8 mg of creatinine, 10 mg of sodium citrate, sodium hydroxide to adjust pH, and water for injection, q.s. 1 ml, with 1.0 mg of sodium bisulfite, 1.5 mg of methylparaben, and 0.2 mg of propylparaben added as preservative) or distilled water were similarly injected intraamniotically day 15.

[f] Subcutaneous maternal injections (0.4 mg) on days 13, 14, and 15.

mone on fetal tissues appears to be demonstrated. The most significant observation observation was growth retardation of the skeleton, including cleft palate. At autopsy, experimental young were reduced in size and weight and appeared edematous with pale, smooth skin, some showing small hematomas. In addition, signs of steroid effects on the viscera such as exomphalos and Meckel's diverticulum also were observed. Several fetuses had lens vacuolation, missing digits, kinked tail or clubfoot, the latter defect perhaps representing a myopathic effect of the hormone.

Injections of dexamethasone produced marked decrease in fetal and placental weights ($p = .01$) and high incidence of cleft palate (>90%). Virtually all embryos treated ia with 120 μg of the hormone showed retarded skeletal ossification by term. Although the steroid did not significantly increase fetal mortality, the percentages of both full and partial cleft palate in surviving young were five times higher than the controls. Comparing the data, the incidences of fetal mortality could be accounted for by the ia technique itself; dexamethasone appeared to adversely affect fetal morphogenesis but not toxicity. It is interesting to note that adrenals from control and experimental young were not significantly different histologically by day 21 of gestation, *i.e.*, 6 days after treatment.

Compared to ia administration, sc injections of the steroid reduced fetal mortality but not skeletal retardation or palatal defects. A single ia injection of 80 or 120 μg of dexamethasone on day 15 of pregnancy was more effective in producing full cleft palate than the sc regime started 2 days earlier and totaling 1200 μg; however, more young had partial cleft palate following sc injection than with the two highest doses of hormone given ia. Therefore, regardless of the route of administration, over 90% of the fetuses demonstrated palatal abnormalities (Table I).

Direct effects of dexamethasone on fetal development appeared to be further demonstrated by the results of 6-AN countertherapy studies. Under the conditions employed, combination ia injections of 6-AN and hormone were negative in reducing the slightly toxic effect or preventing the marked teratogenic action of the vitamin antimetabolite. In fact, the adrenal steriod potentiated the action of 6-AN. Injection of 40 to 120 μg of hormone alone resulted in 34 to 74% full cleft palate. When 100 μg of 6-AN was injected ia together with 20 μg of dexamethasone, 75% of the offspring had full cleft palate. If the dose of hormone was increased to 40 or 80 μg the incidence of cleft palate rose to 79 and 100%, respectively.

Injections of the antimetabolite alone produced 37% fetal mortality and 50% full cleft palate. Although counterinjections of graded doses of dexamethasone did not significantly alter fetal death (39%), they progressively increased the incidence of cleft palate to 100% and decreased fetal weight gain during gestation. Reducing the dose of 6-AN failed to modify the potentiation. Fetal mortality resulted primarily from the technique itself.

Discussion. Dexamethasone produced a high incidence of cleft palate when injected into rat amniotic cavities 12 to 24 hr prior to palate closure. Hydrocortisone (200 μg) injected into amniotic cavities on days 12, 13, or 14 of mouse gestation similarly resulted in palatal defects (11). It has been suggested that teratogens have to be administered some time before the teratogenic effect first becomes visible, especially so in glucocorticoid-induced cleft palate (12). Contrary to this hypothesis, the present study demonstrated the relatively short latency period in dexamethasone-induced cleft palate. Such effects of the steroid may reflect rapid growth depression accompanied by inhibition of nucleic acid synthesis as recently postulated for glucocorticoid-induced cleft palate in other systems (13). Glucocorticoids are rapidly absorbed, distributed, and eliminated (13), inhibiting chondrogenesis and myogenesis *in vitro* (14). It has further been reported that corticosteroids act on bone growth by directly inhibiting precursor cell proliferation, especially with high doses (15).

Although the present investigation did not assay the hormone in the fetuses, the number, variety and types of changes observed in the young strongly suggest a direct action

of dexamethasone; it appears to be highly teratogenic but of low toxicity. Lack of correlation between fetal death and malformation has been also observed with 6-AN (7) and 5-bromodeoxyuridine (16).

The results further demonstrate a failure of ia administered dexamethasone to reduce or prevent the teratogenic effects of 6-AN (6). This is noteworthy and in contrast to the therapeutic effects of adrenal steroids and 6-AN in adult tissues (8). Adrenocortical steroids have been reported to prevent the symptoms of nicotinamide deficiency both in rats and in dogs (8, 17). Several glucocorticoids, including dexamethasone, protected against fetal death following administration of 6-AN to pregnant rats and delayed or prevented antimetabolite-induced toxic effects in dogs; adrenal steroids are thought to stabilize tissue levels of nicotinamide-adenine dinucleotide (NAD) under nicotinamide deficient conditions (8, 17). Although this may be true in adult tissues, the potentiation of 6-AN by dexamethasone, as herein reported, may reflect an inability of the hormone to maintain adequate levels of pyridine nucleotides in embryonic tissues; further work is needed to substantiate this hypothesis.

Summary. Direct intraamniotic injections (ia) of the glucocorticoid dexamethasone produced low fetal mortality and a high incidence of cleft palate in rat fetuses. Administration of 120 μg/30 μl of the hormone 12 to 24 hr before palate closure (day 15 of pregnancy) resulted in 100% retarded skeletal development and 91% cleft palate in term fetuses. Subcutaneous maternal injections of 0.4 mg daily for 3 days prior to palate closure resulted in a similar incidence of palatal defects. Intraamniotic injections of dexamethasone and the nicotinamide antimetabolite 6-aminonicotinamide (6-AN) failed to reduce or prevent the development of congenital malformations, potentiating the production of full cleft palate.

1. Kalter, H., and Warkany, J., Physiol. Rev. **39**, 69 (1959).

2. Fava, A., Therapie **19**, 43 (1964).

3. Nanda, R., Van der Linden, F. P. G. M., and Jansen, H. W. B., Experientia **26**, 1111 (1970).

4. Walker, B. E., Teratology **4**, 39 (1971).

5. Milković, S., Milković, K., and Efendić, S., Gen. Comp. Endocrinol. **10**, 240 (1968).

6. Turbow, M. M., and Chamberlain, J. G., Teratology **1**, 103 (1968).

7. Chamberlain, J. G., and Goldyne, M. E., Teratology **3**, 11 (1970).

8. Greengard, P., Sigg, E. B., Fratta, I., and Zak, S. B., J. Pharmacol. Exp. Ther. **154**, 624 (1966).

9. Chamberlain, J. G., Anat. Rec. **156**, 31 (1966).

10. Wright, H. V., Asling, C. W., Dougherty, H. L., Nelson, M. M., and Evans, H. M., Anat. Rec. **130**, 659 (1958).

11. Dostál, M., Teratology **4**, 63 (1971).

12. Zimmerman, E. F., Andrew, F., and Kalter, H., Proc. Nat. Acad. Sci. U.S.A. **67**, 779 (1970).

13. Andrew, F. D., and Zimmerman, E. F., Teratology **4**, 31 (1971).

14. Umansky, R., Pediat. Res. **2**, 85 (1968).

15. Jee, W. S. S., Park, H. Z., Roberts, W. E., and Kenner, G. H., Amer. J. Anat. **129**, 477 (1970).

16. Skalko, R. G., Packard, D. S., Jr., Schwendimann, R. N., and Raggio, J. F., Teratology **4**, 87 (1971).

17. Greengard, P., Kalinsky, H., Manning, T. J., and Zak, S. B., J. Biol. Chem. **243**, 4216 (1968).

Received April 30, 1971. P.S.E.B.M., 1971, Vol. 138.