Production of Cleft Palate with Dexamethasone and Hypervitaminosis A in Rat Embryos

Cleft palate has been successfully produced in mice[1] and rabbits[2] after the administration of cortisone to pregnant mothers. However, the rat is believed to be genetically resistant to the teratogenic action of cortisone in inducing cleft palate[3]. PINSKY and DIGEORGE[4] employed hydrocortisone, prednisolone and dexamethasone to produce cleft palate in A/Jax mice. They concluded that dexamethasone is 300 times more teratogenic than hydrocortisone regarding the production of cleft palate. On the basis of this conclusion, the present study was undertaken to examine the effect of this highly teratogenic glucocorticoid, in relation to other reported teratogenic agents, on the production of cleft palate in Wistar albino rats.

Materials and methods. 38 adult Wistar albino rats were mated overnight and vaginal plug was looked for in the morning. In case of positive recording, the day following the mating was considered as day 0 of pregnancy. Pregnant rats were divided into 4 groups, 1 control and 3 experimental. The control group comprised 8 rats and the 3 experimental groups consisted of 10 pregnant rats each. The first experimental group was administered 5 mg of cortisone acetate and the second group 0.5 mg of dexamethasone phosphate (Oradexon, Organon, Oss, The Netherlands). Both drugs were given twice a day via s.c. route from day 8 to 13 of gestation. The third group of pregnant rats received 40,000 IU of vitamin A palmitate via gastric tube, once a day, from day 9 to 12 of gestation. The pregnant rats were sacrificed on the 19th day of gestation. The embryos were studied macroscopically for cleft palate and gross malformations and later the heads of 8 control embryos and 9 embryos each from the experimental groups were studied histologically.

Findings and discussion. The Table shows the number of resorbed implantation sites, living embryos and cleft palates recorded after the sacrifice of the pregnant rats. No external anomaly was observed in the embryos belonging to the control and cortisone groups. The embryos of dexamethasone and vitamin A groups showed several external malformations such as micrognathia, microstomia, syndactyly and deformed fore- and hind limbs. 5 embryos from the vitamin A group showed exencephaly which was not observed in the embryos of dexamethasone group. Microscopic examination showed a well fused palate in the embryos of control (Figure 1) and cortisone groups. The embryos obtained from dexamethasone and vitamin A group, however, had unfused palatal processes (Figures 2 and 3). The gap between the unfused palatal processes was wider in the middle and posterior regions. In embryos belonging to the dexamethasone and vitamin A groups, the processes were either rudimentary or completely missing in the posterior region (Figure 3). The tongue also appeared malformed and projected into the oro-nasal cavity. The microscopic features of embryo heads of dexamethasone and vitamin A group were almost similar. The nasal septum of the embryos was bulbous and short (Figure 2). In all embryos the upper molar buds were completely missing. The area of maxillary bone appeared completely malformed and was replaced by a heterotopic cartilage which was seen extending into the mandibular region (Figures 2 and 3). This cartilage was small and tubular in the anterior region while posteriorly it was big in circumference. The mandibular bone was also malformed and several heterotopic cartilages were seen in the ramal area of the mandible.

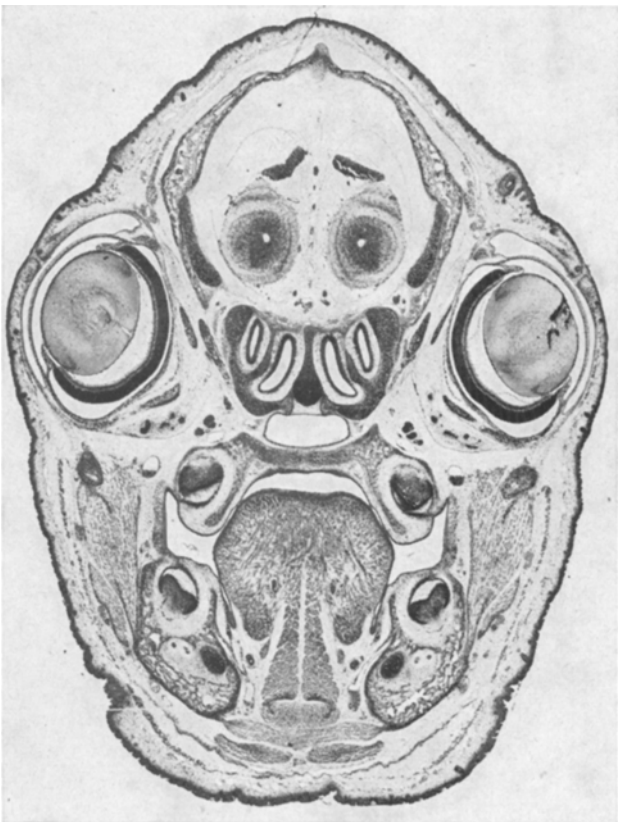

Fig. 1. 19-day-old normal rat embryo. Well fused secondary palate. Haematoxylin-eosin. × 40.

Teratogenic effect of cortisone acetate, dexamethasone and hypervitaminosis A

Group	Dosage	Period of administration (days)	Mothers (No.)	Resorption sites (No.)	Viable embryos (No.)	Cleft palate (No.)	Cleft palate (%)
Control	–	–	8	2	71	0	0.0
Cortisone acetate	5 mg[a]	8–13	10	5	86	0	0.0
Dexamethasone	0.5 mg[a]	8–13	10	9	79	65	82.2
Vitamin A	40,000 IT[b]	9–12	10	13	17	77	74.0

[a] Twice a day. [b] Once a day.

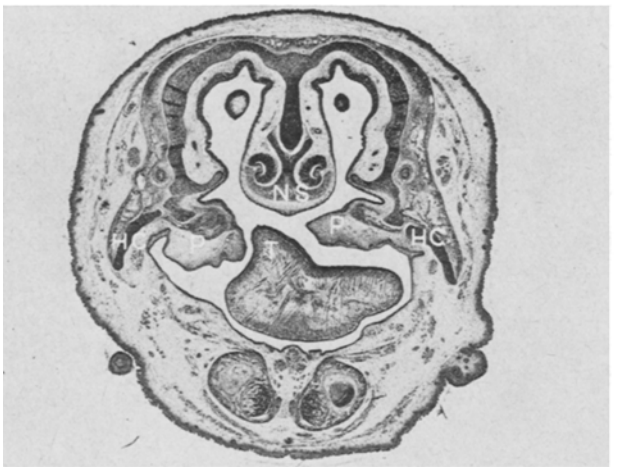

Fig. 2. 19-day-old rat embryo. Hypervitaminosis A group. Both palatal processes (P) are malformed and have unsmooth margins. The tongue (T) is protruding into the oro-nasal cavity. Note the malformed maxillary area and presence of heterotopic cartilage (HC) on both sides. The nasal septum (NS) is bulbous and short in vertical direction. Haematoxylin-eosin. × 38.

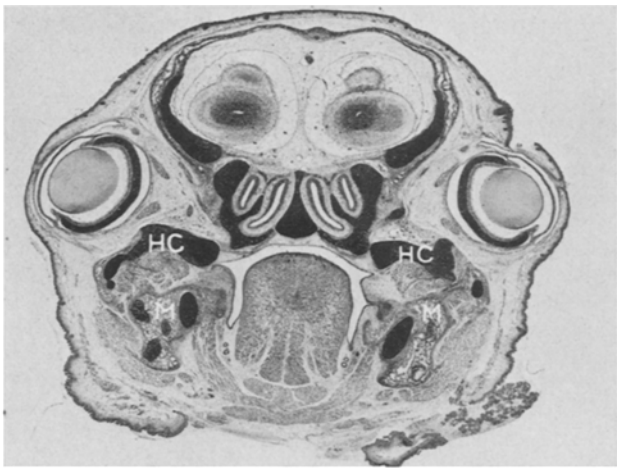

Fig. 3. 19-day-old rat embryo. Hypervitaminosis A group. Posterior region. The palatal processes are missing (compare it with Figure 1). The heterotopic cartilage (HC) is bulbous and several other such cartilages can be seen in the malformed mandible (M). Haematoxylin-eosin. × 40.

It has been reported that dexamethasone supresses the normal adrenals to a low level of activity[5,6] and possibly alters the ovarian secretion[7]. Lerner et al.[8] conducted several experiments employing adrenalectomized male rats and suggested that, regarding granuloma inhibition, liver glycogen deposition, and thymic involution, dexamethasone is 104, 90 and 47 times, respectively, as potent as hydrocortisone. The mechanism of action of hypervitaminosis A in the production of cleft palate is still not fully understood. It has been suggested that it has a direct effect on the foetus[9], disturbs acid mucopolysaccharide metabolism of the ground substance of the palatal processes[10], disturbs the blood concentration of thyroid hormone[11] and interferes with the carbohydrate metabolism[12].

In spite of the reported different modes of action of dexamethasone and hypervitaminosis A, both agents produced cleft palate in rats and almost with similar histological picture. The occurrence of heterotopic cartilage has so far only been reported for hypervitaminosis A[13]. The cause of its occurrence is still unknown but its resultant – maxillo-mandibular-ankylosis – can contribute in the production of cleft palate[10,14].

The difference in the results between the cortisone and dexamethasone groups can be explained by the several times higher teratogenicity of dexamethasone[4] reported. It may be possible that the rat is genetically and physiologically resistant to the action of steroids to a certain level which can be broken by the high potency of dexamethasone, a glucocorticoid. The results of the present study suggest that, although the mode of action of dexamethasone and hypervitaminosis A appears to be different, they nevertheless produce an anomaly with almost similar histological features. Further experiments employing in vivo and in vitro techniques are in progress to study the exact mode of action of dexamethasone and hypervitaminosis A in the production of cleft palate.

Zusammenfassung. Durch Verabreichung von Dexamethason und hohen Dosen Vitamin A bei graviden Wistar-Albinoratten wurden im Embryo experimentell Gaumenspalten erzeugt, nicht aber mit Cortisonazetat. Dexamethason und Vitamin-A-Hypervitaminose ergaben gleiche histologische Bilder.

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[1] F. C. Fraser, H. Kalter, B. E. Walker and T. D. Fainstat, J. Cell comp. Physiol. *43*, 237 (1954).
[2] T. D. Fainstat, Endocrinology *55*, 502 (1954).
[3] H. Kalter and J. Warkany, Physiol. Rev. *39*, 69 (1959).
[4] L. Pinsky and A. M. DiGeorge, Science *147*, 402 (1965).
[5] G. W. Liddle, J. clin. Endocrinol. *20*, 1539 (1960).
[6] S. W. Beaven, E. A. Espiner and D. S. Hast, J. Physiol. *171*, 216 (1964).
[7] W. R. Butt, A. C. Crooke, F. J. Cunningham and R. Palmer, J. Endocrin. *26*, 303 (1963).
[8] L. J. Lerner, A. Bianchi, A. R. Turkheimer, F. M. Singer and A. Borman, Ann. N.Y. Acad. Sci. *116*, 1071 (1964).
[9] A. Giroud, H. Gounelle and M. Martin, Bull. Soc. Chim. biol. *39*, 331 (1957).
[10] D. M. Kochhar and E. M. Johnson, J. Embryol. exp. Morph. *14*, 223 (1965).
[11] S. Takekoshi, J. Embryol. exp. Morph. *12*, 263 (1964).
[12] D. H. M. Woollam and J. W. Millen, in *Congenital Malformations* (Ciba Foundation Symposium, Little Brown Co., Boston 1960), p. 158.
[13] F. M. Deuschle, J. F. Geiger and J. Warkany, J. dent. Res. *38*, 149 (1959).
[14] R. Nanda, J. dent. Res., in press (1970).
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