Orofacial Malformations in the Rat Foetuses Induced by Hypervitaminosis A

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MALFORMATIONS of the face usually occuring as isolated aberrations have been induced teratogenically in mammals e.g. clefts of the lip, palate and mandible; micrognathia and retrognathia; and anomalies of the teeth and eyes (Deuschle and Warkany, 1956; Ingalls and Curley, 1957; Kalter and Warkany, 1959, 1961; Deuschle and Kalter. 1962) Number of oral and facial defects occuring in close association, have been described as a syndrome in mouse foetuses after maternal hyper-vitaminosis A (Kalter, 1960). Similar defects in rat foetuses were reported earlier by Cohlan (1953, 1954) who was the first to use relatively large doses of vitamin A as a teratogenic agent. Eversince, hypervitaminosis A, has been extensively used as a potent teratogen in various laboratory animals, e.g., rats, mice. and hamsters. Although Langman and Welch (1966) have described teratogenic action of vitamin A as neither species nor organ specific but one dependant mainly on the stage of development at which it is active, Cahen (1964) considered its action in producing facial malformation as specific as, that of cortisone for the cleft palate and of thalidomide for inducing limb anomalies. Excessive amounts of vitamin A have been used in repeated doses on several successive days of gestation by most of the workers. However, only a few reports about the teratogenic action of a single excessive dose of vitamin A are available, e.g.. in hamsters (Marin-Padilla and Ferm, 1965; Marin-Padilla, 1966), and mice (Kalter and Warkany, 1961; Kalter and Deuschle, 1966; Ohmori, 1967; Shoji and Kohno, 1969; Hyashi, 1972). The present report concerns the orofacial defects induced in rat foetuses by a single large dose of vitamin A on different days of gestation. Some of the malformations observed, do not seem to be reported earlier.

Material and Methods :

Female wistar rats obtained from the Institute for Research in Reproduction, Bombay, and weighing about 200 gms. each, were placed in oestrus with males of their own strain, one pair to a cage. Presence of sperm in the vaginal smear examined next morning indicated the beginning of pregnancy designated as day 0. On 8, 9 or 10 days after conception, the females were given a single dose of 100,000 IU vitamin A (fat-soluble vitamin A palmitate, Arovit, Roche,

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Bombay) by mouth by means of a blunt-ended needle attach to a tuberculin syringe (Table I). The control animals received no vitamin A, in the corresponding gestation periods. The controls as well as experimental animals were sacrificed 21 days after conception and the foetuses removed after uterotomy were examined for external malformations and preserved in fixatives. Orofacial anomalies were re-examined under the dissecting binocular microscope. The jaws had to be widely separated for detailed examination of the palate.

Observations

As seen from table I, 148 out of 181 (81.7%) living foetuses exhibited orofacial malformations. Treatment on 9th day of gestation produced maximum teratogenic effect, not only in the number of malformations (90%) but also in the severity and type of malformations, e.g., all types of malformations were encountered in this group. But for one case of cleft lip and jaw, found in the 8th day group, all cases of clefts (of lip, tongue, jaw and palate) were confined to the foetuses of the 9th day group (table I). The commonest malformation was a protruded tongue (51.3%) which was least frequent in the 10th day group. Mostly it was a small protrusion beyond the receded lower jaw (micrognathia) but in 5% of these cases the protrusion was severe type. Tongue was fused with the lower lip and jaw (ankyloglossia) in 7 cases in which it also showed a long and deep cleft, The next common anomaly was a highly arched palate which was most frequent in the 10th day group (100%). The actual cleft of the palate was

TABLE 1. Orofacial abnormalities* in rat foetuses induced by maternal administration of single dose of 100,000 IU of vitamin A on different days of gestation.

Day of treatment	8th	9th	10th	Total Exptal.	Controls
No. of litters.	5	11	4	20	6
No. of living foetuses.	42	100	39	181	53
No. of malformed foetuses.	** 33 (78 6%)	** 90 (90%)	** 25 (64.1%)	** 148 (81.7%)	0
1. Microstoma	—	31 (34.4%)	15 (60%)	46 (31%)	—
2. Cleft lip	1 (3%)	9 (10%)	—	10 (6.7%)	—
3. Cleft jaw	1 (3%)	9 (10%)	—	10 (6.7%)	—
4. Arched palate	7 (21.2%)	31 (34.4%)	25 (100%)	63 (42.5%)	—
5. Cleft palate	—	3 (3%)	—	3 (2.0%)	—
6. Protruded tongue	18 (54.5%)	57 (63.3%)	1 (4%)	76 (51.3%)	—
7. Cleft tongue	—	7 (7.7%)	—	7 (4.7%)	—

* Multiple abnormalities in an individual have been counted as separate abnormalities.

** From which percentages for individual abnormalities have been worked out.

rather not common and was present only In the 9th day group but when present it was alway complete cleft (Fig 2). Out of the 10 foetuses showing cleft of the lips and jaw, in seven the cleft continued into the tongue also. Microstoma (Fig. 1) was seen most frequently (60%) in the 10th day group and was absent in the 8th day group. The most interesting malformation was cleft of the lower lip which was deep enough to divide the lower jaw also. It varied from a mere cleft to a wide gap in which the cleft tongue was protruded. Because of its lateral fusion with lips, the protruded tongue folded on itself, giving a false appearance of of multiple clefts (Fig. 3) dividing the lower lip into several parts. In one specimen both upper and lower jaw were underdeveloped on the right side. No abnormality was detected in any of the 53 foetuses of the control group.

Discussion

Though we have focussed our attention on the orofacial malformations, other organs

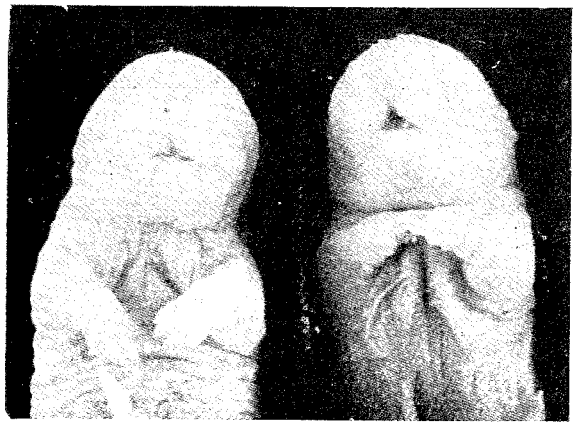

Fig. 1—Malformed foetus showing microstoma. The foetus on the left is a control. (100,000 IU of vitamin A given orally to pregnant rat on 10th day of gestation).

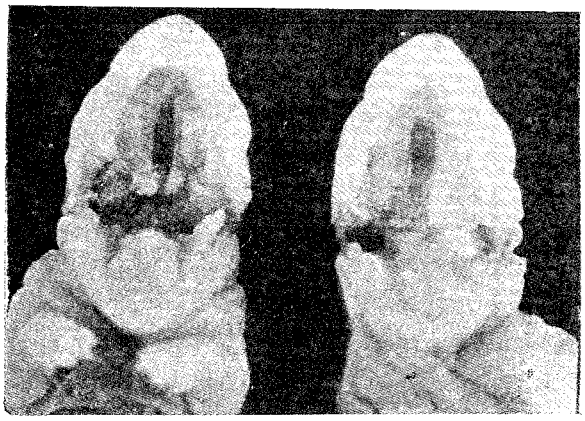

Fig. 2—Malformed foetuses, both showing cleft palate (100,000 IU of vitamin A given orally to pregnant rats on 9th gestation day).

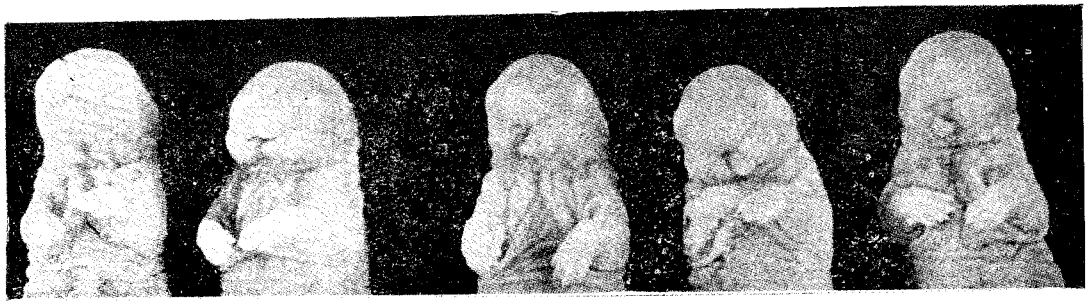

Fig. 3—Malformed foetuses showing a variety of clefts in the lower lips which also divide the lower jaw and tongue. The foetus on the extreme left is a control (100,000 IU Vitamin A given orally to pregnant rats on 9th gestation day).

and tissues were affected by such treatment. Results of this study show that vitamin A given to Wistar rats even in a single large dose on 8th, 9th or 10th day of pregnancy is an efficient teratogenic agent for inducing malformations of the lips, tongue, jaw and palate, which occured in over 80% of the foetuses, the maximum effect (both in number and severity) being exerted on the 9th gestation day. Although interference in this study was made at an early stage of embryonic development as compared to the previous workers who used comparatively later gestation period e.g., Cohlan, 1954 (2-16 days); Kochhar and Johnson, 1965 (9-12 days); Yamaguchi, 1968 (10-13 days); and employed small repeated doses over several successive days. yet the malformations produced have been quite similar. The frequency and severity of these malformations have been somewhat different from the previous reports because of the differences in the dose, time and frequency of administration and due to the different strains of the rats used. That different strains react differently to the same experimental procedures has been repeatedly observed in different teratological studies (Kalter, 1954; Kalter and Warkany, 1957; Ingalls et al, 1953; Smithberg, 1960; Nogami, 1964; Sakurai, 1968) including those using hypervitaminosis A (Walker and Crain, 1960; Kalter and Warkany, 1961; Shoji and Kohno, 1969; Hashimoto et al, 1967).

The ankyloglossia observed in the present study has not been reported in rats although Kalter & Warkany (1961) noted it in mice after maternal hypervitaminosis A. Ankyloglossia in human beings has also been reported several times (See Kalter & Warkany, 1961). The frequency of cleft palate seen in our study (3%) is far below that observed by Cohlan (1954) Kochhar & Johnson (1965) and Yamaguchi (1968) where it was present in 38%, 80% and 61.5% respectively. However, the highly arched palate, an anomaly without actual cleft reported with cyclophosphamide (Singh et al, 1972), was observed in 34% of foetuses in this study. Cleft palate had been found to be the most frequent anomaly in rats when vitamin A was given after 10th gestation day (Giroud & Martinet, 1956). Cleft jaw and cleft lip not reported in mice, have been, however, induced by hypervitaminosis A in rats by Yamaguchi (1968). Although faequency of cleft jaw in his study is in line with the present findings, but the cleft tongue has been observed more frequently in the present study (1.8% Vs 7.7%, table I). Of great interest was the anomaly of cleft lower lip observed by us, the type of which has not been reported earlier in any species after maternal hypervitaminonsis A, although Cohlan (1954) did mention harelip associated with cleft palate in 2 of his severely malformed foetuses. The clefts in the lower lips varied from a mere chink to a wide gap separating the two halves of the lower jaw as well. The protruded and cleft tongue in this gap folded on itself in some cases because of the lateral ankyloglossia, giving thus a false appearence of multiple clefts dividing the lower lip into several parts (Fig. 3). The malformations observed in the present study also occur in certain human pathological conditions forming various clinical syndromes as described by Kalter & Deuschle (1966),

The mode of action of excess vitamin

A in the experimental production of congenital malformations is not well under-stood. Cohlan (1954) suggested that vltamin A like other specific nutrients (folic acid, riboflavine) is essential for enzymatic and metabolic processes vital for normal differentiation, and excess of it may act as an antienzyme or antimetabolite to produce congenital malformations. The experiments of Fell & Mellanby (1952) support the suggestion of Giroud & Martinet (1956) that vitamin A acts on the cells of the embryo and it directly interferes with the metabolism of the differentiating cells in the embroyo (Giroud et al, 1957). It seems now established that excess Vitamin A, at level comparable to those existing in the plasma of patients with hypervitaminosis A, markedly inhiblts cell multiplication in cell cultures (Kochhar, 1968). Interference with mitosis has been noted by Aydelotte (1963) in vitamin A treated epithelial tissues in virto and Langman & Welch (1967) in the neuroepithelial cells of cerebral cortex of mouse embryos after maternal hypervitaminosis A. Walker & Crain (1960) noted that retardation of palatine shelf movement was responsible for cleft plate induced by hypervitaminosis A in mouse foetuses. The morphogenesis of cleft palate and its relation to the retarded growth caused by an antimitotic drug (cyclophosphamide) has been discussed by Singh et al (1972). Hypervitaminosis A may result in hyperplastic palatine shelves having abnormal cartilage and bone formation resulting in nonclosure of the palate (Kochhar & Johnson, 1965). Kochhar (1968) found that the development of face in rat foetuses affected by hypervitaminosis A, was characterized by the appearence of defects in the mesoderm of the maxillary process and in the oral epithelium. He surmised that the initial changes in the oral epithelium subsequently led to disrupted epithelial-mesodermal relation in this region resulting in facial defects e.g. palate, teeth etc. However. morphogenesis of varying clefts in the lower lips, jaw and tongue requires further study.

Summary

Single oral administration of 100,000 IU of vitamin A palmitate in oil to pregnant wistar rats on 8th, 9th or 10th day of gestation resulted in malformations of the tongue, jaw; lower lip and palate in 82% live-born foetuses. The frequency and severity of defects were most marked in the group treated on the 9th day. Clefts of the jaw, lip, tongue and palate were practically confined to the 9th day treated group. Of great interest were the clefts in the lower lips which varied from a mere chink to a wide gap splitting the lower jaw as well. The cleft tongue protruded through the gap and folded on itself, giving a false appearence of multiple clefts in the lower lips.

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