HYPERVITAMINOSIS A IN PREGNANT MICE OF LINE C3HA AND DEVELOPMENTAL ANOMALIES OF THE PALATE AND JAWS IN THEIR OFFSPRING

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Cohlan [1] reported that administration of large doses of vitamin A to pregnant rats leads to the development of cleft palates and anomalies of the upper jaw in some of the surviving embryos. In subsequent investigations [2, 3, 6] it was found that administration of large doses of vitamin A to pregnant rats and mice by mouth for several days or as a single dose causes various anomalies of the facial skull in the embryos, especially cleft palates. Although several investigations have revealed the teratogenic action of vitamin A on the primitive palate and jaws, the periods of development at which the primitive palate and jaws are maximally vulnerable to this agent have not been sufficiently studied.

The object of the present investigation was to determine the periods of development of the primitive palate and jaws of mouse embryos of line C3HA with maximal vulnerability during hypervitaminosis A.

EXPERIMENTAL METHOD

The beginning of pregnancy was determined from the appearance of a vaginal plug. From the 7th to the 14th day of pregnancy a vitamin A concentrate was given by mouth in a single dose of 35,000 i.u. The results were read on the 17th day of development. Under ether anesthesia, the fetuses were extracted from the uterus and studied while alive. The embryos were fixed with Bouin's fluid and then transferred to 70° alcohol. To determine the incidence of cleft palate in the embryos, the lower jaw and tongue were removed, so that the state of the palate could be examined. Altogether 57 mice (567 fetuses) were used as experimental animals and 20 mice (177 fetuses) as controls.

EXPERIMENTAL RESULTS

After administration of vitamin A in a single dose of 35,000 i.u. to the pregnant females, 66.8% of the embryos were found to have cleft palates, 14% anomalies of the upper jaw, and 60.3% anomalies of the lower jaw. All the embryos died after administration of vitamin A on the 7th day of development. After its administration on the 8th day of development 98.5% of the embryos died, and one survivor had developmental anomalies of the jaws and cleft palate. After administration of vitamin A on the 9th day of development cleft palate and developmental anomalies of the lower jaw were found in 88.8% of the surviving embryos and developmental anomalies of the upper jaw in 100%. When vitamin A was given on the 10th day, two types of cleft palate were found in 36 of the 50 surviving embryos: in 16% the palate was cleft throughout its length and in 56% the cleft was in its posterior part (see figure, c). Clefts of the palate situated in its posterior part were segregated as a special type of developmental anomaly in accordance with the data of Warkany and co-workers [7]. Developmental anomalies of the upper jaw were found in 38% of the living embryos, and anomalies of the lower jaw (see figure, d) in 72%; in some of them the lower jaw was absent or was almost vestigial. When vitamin A was given on the 11th day of development, cleft palates were found in 83.5% of the living embryos (see figure, b) and developmental anomalies of the lower jaw in 72.5%. After administration on the 12th day of development, cleft palate was found in 95.6% of the living embryos and was combined in every case with developmental anomalies of the lower jaw. Administration of a single dose of 35,000 i.u. vitamin A to the pregnant female on the 13th day caused development of cleft palate in 25.4% of the living embryos and developmental anomalies of the lower jaw in 7.2%. After administration of vitamin A on the 14th day of development, cleft palate was present in 12.5% of the living embryos.

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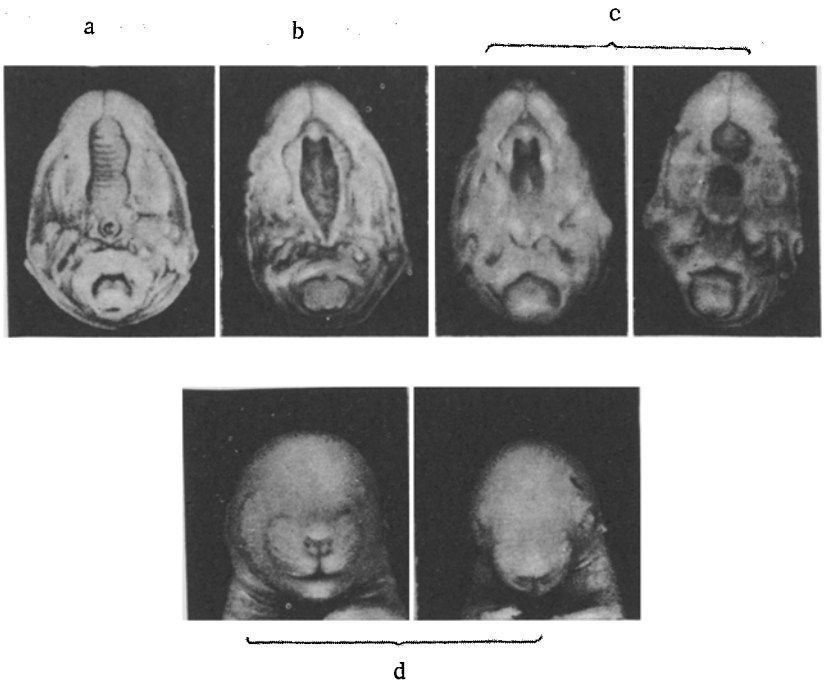

Character of lesion of the palate and lower jaw in 17 day embryos of mothers with hypervitaminosis A. a) Normal palate; b) cleft palate in an embryo after administration on 11th day of development; c) posterior cleft of palate in embryos after administration on 10th day of development; d) partial reduction of lower jaw after administration of vitamin A on 10th day of development (on the left—normal embryo).

It may be concluded from these results that the period of development of the primitive upper jaw of mouse embryos of line C3HA at which they are most vulnerable is on the 9th day, when the maximal number of developmental anomalies of the jaw was observed.

Because the incidence of developmental anomalies of the lower jaw was high from the 9th until the 12th days of development, it was impossible to determine the day of maximal vulnerability of its anlage.

To explain the high frequency of cleft palate after administration of vitamin A on the 9th day of development it should be pointed out that the cleft palate in the embryos treated on this day of development was combined in every case with developmental anomalies of the upper and lower jaws.

As regards the cleft of the posterior part of the palate observed on the 10th day of development, it was suggested that an important role in their pathogenesis was played by mechanical obstructions preventing the closure of the palatal laminae in their posterior parts. This suggestion is in agreement with results obtained by several authors [4, 5] indicating that the tongue may prevent the normal closure of the palate when this anomaly is combined with micrognathia and agnathia. In the present experiment on the 10th day a posterior cleft of the palate was always combined with absence or marked reduction of the lower jaw; in cases of this type of cleft palate the embryo's head was pressed against the thorax. This means that the development of a posterior cleft of the palate on the 10th day of development was not due to injury of the palatal anlagen, but to mechanical obstacles. It may accordingly be concluded that only 16% of cleft palates running along the whole length can be counted as resulting from injury to the palatal anlage itself; consequently, in hypervitaminosis A the sensitivity of the palatal anlage to this agent is reduced on the 10th day of development.

On the 11th and 12th days of development the incidence of cleft palate in the embryos after administration of vitamin A again rose temporarily. The appearance of cleft palate on the 11th-12th and 9th days of development was evidently due to different causes. Throughout the 9th day the whole complex of the facial skull was injured, along with the anlagen of the palate and jaws, which had not yet differentiated at this period. It may be concluded from the results obtained that the differentiation of the palate into a separate anlage as a physiological unit takes place after the 9th day of development; the period of development with maximal vulnerability of the palatal anlage to the action of vitamin A in a dose of 35,000 i.u. occurs on the 11th-12th day.

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