From: Department of Anatomy, The Royal Dental College, Aarhus, Denmark.

TOOTH GERMS IN NON-EXENCEPHALIC SIBLINGS OF MOUSE EMBRYOS WITH EXENCEPHALY INDUCED BY HYPER-VITAMINOSIS A.

by

P. A. KNUDSEN

INTRODUCTION

By the administration of large doses of vitamin A to pregnant mice, exencephaly was induced in some of the embryos. The number of exencephalic embryos varied, often beting confined to one or two in each litter. Many embryos were resorbed and others were without visible malformations. In about 68 % of 272 exencephalic embryos, various degrees of fusion of the upper incisor germs were ascertained (*Knudsen*, 1965 a and b). In a small number of embryos (34 out of 272) molar anomalies with or without incisor anomalies were found (*Knudsen*, 1966). The frequent occurrence of dental anomalies in exencephalic embryos suggested that exencephaly was a decisive factor in their development. This apparent connection, however, might be due to a teratogenic effect of large doses of vitamin A acting independently on the embryonic brain and dental germs. If this was the case, dental fusions should also occur in non-exencephalic embryos. The aim of the present study was to elucidate this problem by investigating wheter dental anomalies occurred in embryos which had been subject to vitamin A overdosage without developing

exencephaly. Embryos with normal brains, from litters with one or more exencephalic embryos, were studied.

METHODS AND MATERIALS

Female mice of an inbred strain (AK/a) were placed with males of the same strain one pair to a cage, and examined in the morning 24 hours later for a vaginal plug. Day one of pregnancy is taken as the day on which the copulation plug is found in the vagina of the mouse. The males were then removed and the impregnated females given from 4,000—15,000 I. U. vitamin A, on day seven to nine of pregnancy. (For details, see *Knudsen,* 1965 a).

The females were killed on days 17 to 19 of pregnancy, i. e. about one to three days before term. The embryos were removed and examined macroscopically. Exencephalic specimens were fixed in Bouin's fluid as described elsewhere (*Knudsen,* 1965 a). Apparently normal embryos were fixed in Lillie's fluid (acetic alcohol formalin). The heads of the embryos were embedded in paraffin wax and cut in serial sections. The plane of sectioning was frontal and section thickness 10 μ. Sections were stained with haematoxylin-eosin. The final selection of the material was based on histological examination of the brains, all embryos with abnormal brains being discarded. Embryos with enlarged cerebral ventricles were not included in the examination. All histological sections engaging tooth germs were examined and important changes registered on a special form. Reichert's Visopan microscope was used for the examination. Measurements of the smallest distance between the upper incisor tooth germs were made with a ruler directly on the projection screen of the microscope. Distance measurements were given in Length Units (L. U.) corresponding to the number of millimetres measured on the projection screen with 70 times linear enlargement. A total of 102 non-exencephalic embryos were examined. The results of the present investigation were compared with previous investigations of 272 exencephalic embryos (*Knudsen,* 1965 a, b and 1966). In addition, 30 normal mouse embryos from mothers who had not been treated with vitamin A were used for comparison (*Knudsen,* 1965 a).

RESULTS

Various anomalies of incisor and molar germs were found in a number of the non-exencephalic embryos. The most important anomaly was fusion, fusio dentium, of the upper incisor germs that occurred in 13.7 % of (102) non-exencephalic embryos and in 51.5 % of (272) exencephalic embryos. The difference is significant ($P < 0.001$). Fusio totalis was the commonest type of fusion encountered in both groups (Fig. 1). Connection across

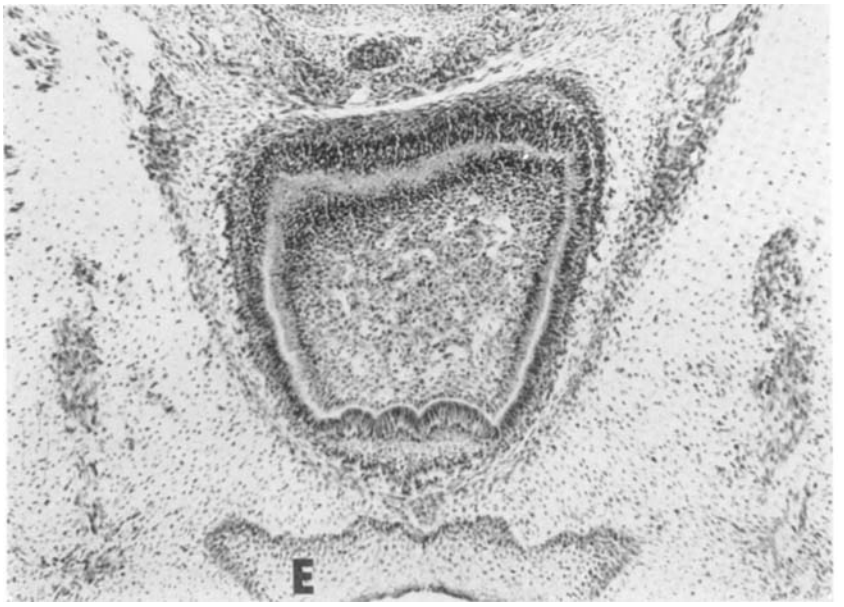

Fig. 1. Non-exencephalic mouse embryo (339 d 1/4).
Frontal section (× 63).
Fusio totalis of upper incisor germs.
(Oral epithelium = E).

the mid-line between the incisal parts of the stellate reticula of the upper incisor germs, *fusio epithelialis*, occurred in 7.9 % of the non-exencephalic embryos (Fig. 2). This figure includes one embryo with "contact" between the upper incisor germs and two embryos where the stellate reticula approached the mid-line without crossing it, the two germs being connected by a narrow thickened bridge of oral epithelium (Fig. 3). In exencephalic embryos, *fusio epithelialis* occurred in 16.8 % (of 272), this

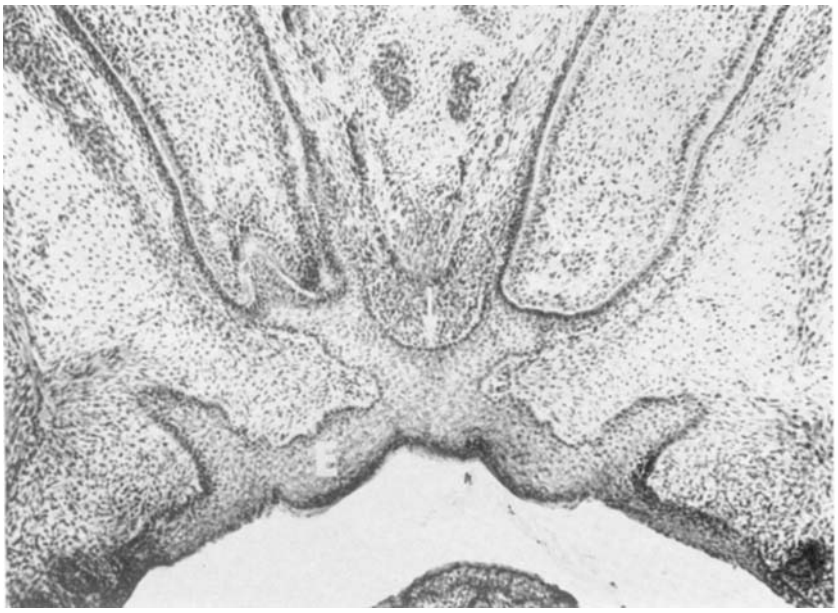

Fig. 2. Non-exencephalic mouse embryo (165 d I/4).
Frontal section (× 63).
Fusio epithelialis of upper incisor germs, i.e. stellate reticula and outer dental epithelia cross the midline (arrow).
(Oral epithelium = E.)

figure including two embryos with "contact" between the upper incisor germs. New types of fusion were not observed in non-exencephalic embryos, and the shape of fused incisor germs was rather similar in exenphalic and non-exencephalic embryos.

The shortest distance between the upper incisor germs was measured in 88 non-exencephalic embryos without fusio dentium. The mean value was 16.3±3.53 L. U., compared with a mean of 16.7±1.18 L. U. in 30 normal AK/a embryos which had not been treated with vitamin A. The two means do not differ significantly ($P>0.1$).

Agenesia of upper incisor germs was not found in non-exencephalic embryos, whereas 8.5 % of the exencephalic embryos exhibited uni- or bilateral agenesia.

Distally to the normal or fused upper incisors, supernumerary tooth germs occurred in 13.8 % of the non-exencephalic embryos. The germs occurred either bilaterally (11.8 %) or unilaterally

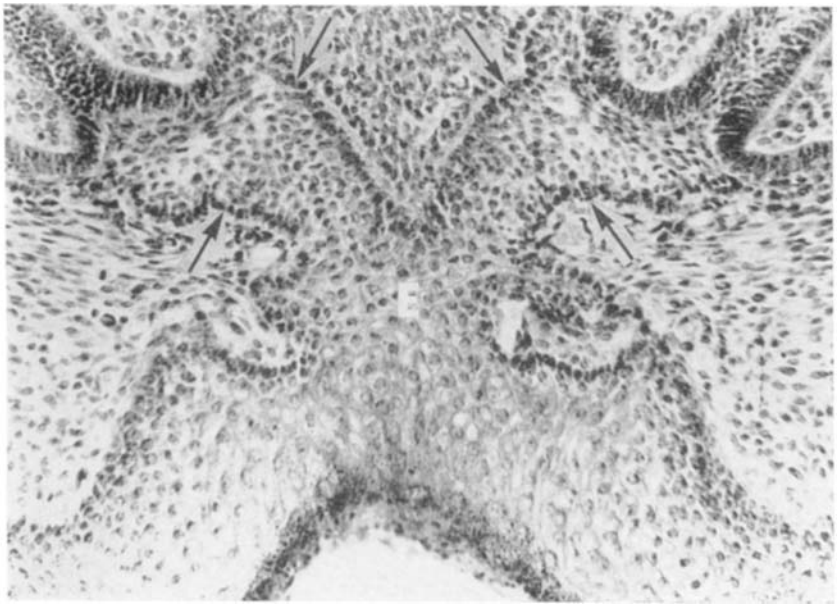

Fig. 3. Non-exencephalic mouse embryo (58 d 1/4).
Frontal section (× 160).
Stellate reticula approach the mid-line without crossing it (arrows).
The two upper incisor germs are connected by a narrow thickened bridge of oral epithelium (E).

(2.0 %). In exencephalic mouse embryos supernumerary tooth germs were extremely rare.

The most frequent anomaly of molar germs in non-exencephalic embryos was *agenesia*, 19.0 % of all germs being absent (155 out of 816 potential germs). In exencephalic embryos 3.4 % were lacking (74 out of 2176 potential germs). This difference is significant ($P<0.001$).

Fusion of upper and lower molar germs occurred in one non-exencephalic embryo. In exencephalic embryos the number of fusions of molar germs was 21. Epithelial connections between upper and lower molar germs also occurred in non-exencephalic embryos (12 in non-exencephalic and 24 in exencephalic embryos).

Lower incisor germs were anomalous in four non-exencephalic embryos. Fusio dentium (subtotalis) was found in one and fusio epithelialis with contact in three embryos. Agenesia did not occur.

In exencephalic embryos fusio dentium was found in five and bilateral agenesia in four cases, but fusio epithelialis was not encountered. Distally to the lower incisor germs, supernumerary germs occurred in 9.8 % of non-exencephalic embryos, whereas they did not occur in embryos with exencephaly.

DISCUSSION

The present investigation has demonstrated that dental anomalies occurred in embryos which had been subjected to large doses of vitamin A vithout developing visible malformations of the central nervous system. Considering this fact, the concept of a simple causal relationship between exencephaly and dental anomalies cannot be maintained without qualification. The results described seem to suggest that vitamin A in large doses can produce an independent teratogenic effect on the tooth germs. It is difficult, however, to accept this explanation as the only possible one, because the mode of occurrence of dental anomalies in non-exencephalic and exencephalic embryos differed essentially. Particularly striking were the relatively few changes in the upper incisor region in non-exencephalic embryos, where agenesia did not occur, and where considerably fewer fusions were encountered than in exencephalic embryos. On the other hand, anomalies often occurred in the molar region in non-exencephalic embryos. Particularly noteworthy in that region were the frequent occurrence of agenesia and the small number of fusions. Finally, the supernumerary tooth germs between the incisor and molar germs were peculiar to non-exencephalic embryos. The considerable quantitative and qualitative differences strongly suggest that the mechanism underlying the development of dental malformations in the two groups is different. The cause of this is not known.

The present investigation, in conjunction with a study of hydrocephalic embryos (*Knudsen*, 1967), showed that the most important changes, for instance fusions of the upper incisor germs, presented almost the same appearance in all groups of embryos. As the dental malformations occurred in various combinations, however, definite patterns particular to each group were evolved.

SUMMARY

The tooth germs of 102 mouse embryos with normal brains were investigated. The embryos belonged to litters where exencephaly had been induced in one or more of the embryos by maternal hypervitaminosis A. Fusions of upper incisor germs and of upper and lower molar germs were found, but the occurrence was less frequent than in exencephalic embryos. Agenesia of incisor germs did not occur, whilst agenesia of molar germs was more common than in exencephalic embryos. Supernumerary tooth germs occurred most frequently in non-exencephalic embryos. The widely different pattern of dental anomalies in non-exencephalic and exencephalic embryos suggested that the mechanism underlying the development of the anomalies was different in the two groups.

RÉSUMÉ

LES GERMES DENTAIRES CHEZ LES COLLATÉRAUX NON EXENCÉPHALES D'EMBRYONS DE SOURIS PRÉSENTANT UNE EXENCÉPHALIE PROVOQUÉE PAR HYPERVITAMINOSE A.

Les germes dentaires de 102 embryons de souris au cerveau normal ont été examinés. Ces embryons provenaient de portées dans lesquelles une hypervitaminose A chez la mère avait provoqué une exencéphalie chez au moins un des embryons. La fusion de germes d'incisives supérieures, celle de germes de molaires supérieures et de germes de molaires inférieures ont été observées, mais la fréquence en était moins élevée que chez les embryons exencéphalis. L'agénésie de germes de incisives n'a pas été observée, mais l'agénésie de germes de molaires était plus courante que chez les embryons exencéphales. La fréquence des germes dentaires surnuméraires était plus élevée chez les embryons non exencéphales. La grande différence existant entre les types des anomalies dentaires chez les embryons non exencéphales et chez les embryons exencéphales semble indiquer que le mécanisme déterminant le développement des anomalies était différent dans les deux groupes.

ZUSAMMENFASSUNG

ZAHNKEIME BEI NICHT-EXENCEPHALEN GESCHWISTERN VON MÄUSE-EMBRYONEN MIT EXENCEPHALIE DURCH HYPERVITAMINOSIS A HERVORGERUFEN

Die Zahnkeime von 102 Mäuseembryonen mit normalen Gehirnen wurden untersucht. Die Embryonen gehörten zu Würfen bei denen Exencephalie bei einem oder mehreren Embryonen durch Hypervitaminosis A bei den Muttertieren hervorgerufen war.

Verschmelzungen der Keime der oberen Schneidezähne und der oberen und unteren Molaren wurden gefunden; es kam aber seltener vor als bei den exencephalen Embryonen. Agenesie der Schneidezahnanlagen kam nicht vor, wogegen Agenesie der Molarrenanlagen kam häufiger vor als bei exencephalen Embryonen. Überzählige Zahnkeime kamen bei nicht-exencephalen Embryonen am häufigsten vor.

Das sehr verschiedene Muster der dentalen Anomalien bei nicht-exencephalen Embryonen deutete an, dass der der Entwicklung der Anomalien zugrundeliegende Mechanismus in den beiden Gruppen verschieden wäre.

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Address: *Aarhus Tandlægehøjskole,*
Vennelyst Boulevard,
Aarhus C, Denmark.