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INCISOR GERMS IN MOUSE EMBRYOS WITH EXENCEPHALY INDUCED BY RIBOFLAVIN DEFICIENCY

by

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INTRODUCTION

Experimentally induced exencephaly in mouse embryos is in many cases accompanied by dental anomalies including in particular, fusion of the upper incisor germs. This condition occurs whether the causal agent is the administration of large doses of vitamin A or injection of trypan blue in pregnant mice (*Knudsen,* 1965 and 1966). Exencephaly thus seems to exert a decisive influence on the tooth development.

Kalter & Warkany (1957) and *Kalter* (1963), induced hydrocephalus in mouse embryos by employing a riboflavin-deficient diet adding the riboflavin antimetabolite galactoflavin. This experiment was repeated in this laboratory and resulted in a number of exencephalic embryos resembling those produced by the methods previously used by the present author. Histological serial sections of the embryos were examined in order to decide whether this form of experimentally induced exencephaly was accompanied by the same incisor anomalies as exencephaly induced by hypervitaminosis A or injection of trypan blue.

MATERIAL AND METHODS

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 was taken as the day on which the copulation plug was found in the mouse vagina. The males were then removed. Normal laboratory diet was replaced by a corresponding quantity of riboflavin-deficient food with an admixture of 90 mg galactoflavin*) per kg. This food was administered on a varying number of days in the first half of pregnancy. (For details see Table I). During this period a wire-mesh platform was placed in the cage to prevent coprophagy. The females were killed on day 19 of pregnancy, i.e. about one day before term. The histological preparation and subsequent examination of serial sections followed the standard procedure previously described in detail (*Knudsen*, 1965). This investigation comprised a total of 20 exencephalic embryos. Normal and exencephalic embryos from previous experiments were used in comparison.

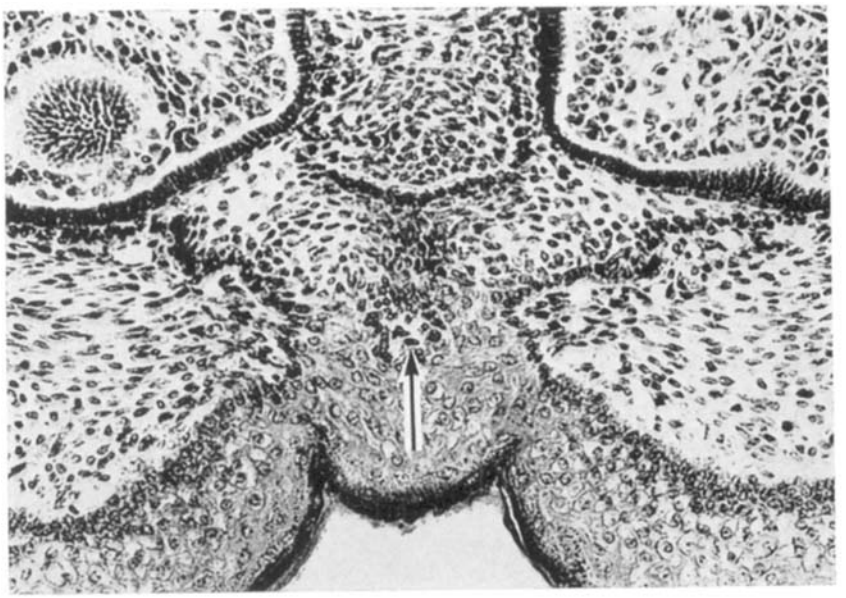
RESULTS

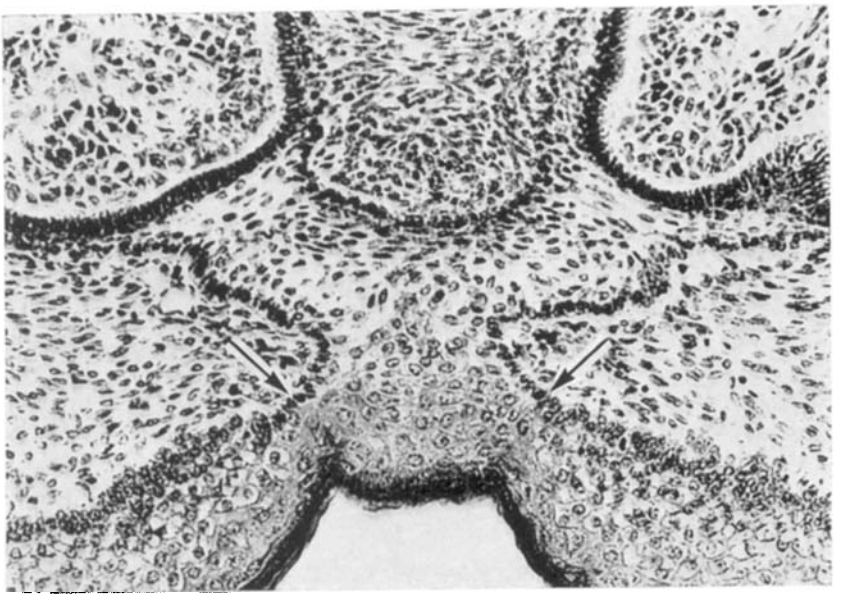
The most important result of this experiment is the demonstration of *fusio epithelialis*, i.e. a fusion of the incisal (oral) parts of the enamel organs, the outer dental epithelia and the stellate reticula crossing the mid-line. *Fusio dentium* which dominates in the exencephalic embryos previously investigated by the present author does not occur here, as the ameloblast layers, dentine and pulps nowhere cross the mid-line. Contact germs are also lacking.

Fusio epithelialis is found in two embryos, where the shortest distance between the incisor germs is reduced to 6 and 8 L.U.°). The distance between the incisor germs in the remaining embryos is, on average, 15 L.U. In *fusio epithelialis* there is no connection between the two germs labially. More lingually the enamel organs are connected by a bridge of stellate reticulum and outer dental epithelium. As each enamel organ also retains a connection with the oral epithelium, a niche is formed (Fig. 1). It contains mes-

*) Kindly supplied by Dr Oswald H. Ganley, Merck Sharp and Dohme Research Laboratories, Rahway, N. J.

°) L. U. (Length Unit) = Number of millimetres measured on the projection screen with 70 times linear enlargement.





Figs. 1 and 2. Exencephalic mouse embryo (422 d I/4. *Riboflavin deficiency*). *Fusio epithelialis.*

The shortest distance between the incisor germs is 8 L. U. Frontal sections (× 160).

Fig. 1. The outer dental epithelia have fused across the mid-line. The fusion of the stellate reticula has nearly finished. The niche is seen (arrow).

Fig. 2. (More lingually). The niche has disappeared and the common stellate reticulum is Y-shaped. There is a smooth transition between the outer dental epithelium and the basal layer of the oral epithelium (arrow).

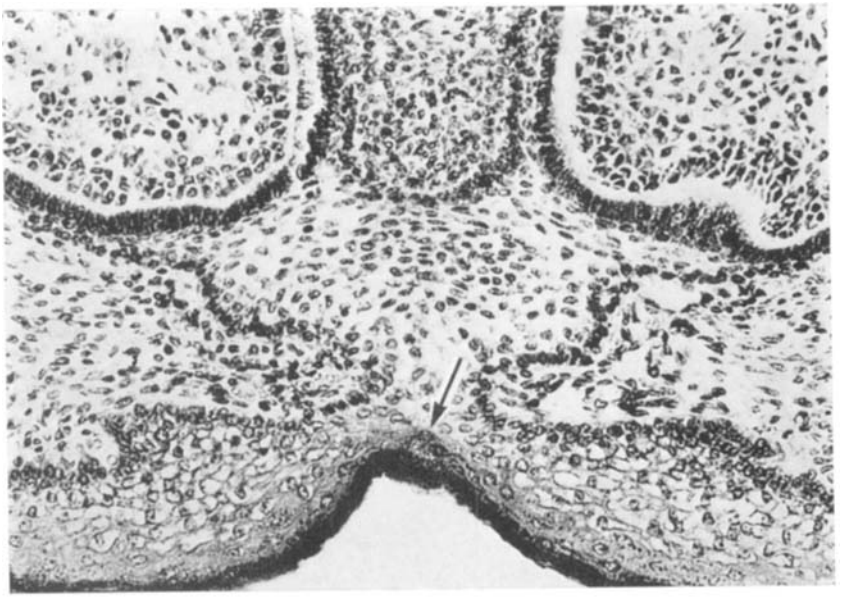
enchyme and disappears more lingually, where the bridge between the enamel organs becomes thicker; at the same time the two connections with the oral epithelium are replaced by a single stem, making the common incisal portion of the enamel organs Y- or V-shaped (Fig. 2). The connection between the stellate reticulum and oral epithelium is sometimes very narrow, as will be seen in Fig. 3. It will also be observed that the stellate reticulum almost reaches the surface as the oral epithelium is very thin at the point of connection. Here the epithelium consists of only a few layers of flat cells lining a median furrow in the roof of the oral cavity. There is a smooth transition between the outer dental epithelium and the basal layer of the stratified squamous epithelium (Figs. 2 and 3).

In four embryos an incomplete type of fusio epithelialis is found, which was first described during the study of embryos with exencephaly induced by trypan blue (*Knudsen*, 1966). In this category, the stellate reticulum is lacking in the middle of the epithelial bridge, where oral epithelium is found in a narrow area around the mid-line (Fig. 4). Both the direction of the "dental laminae" and the limited extent of the oral epithelium in the laminae show that the germs are not normal.

DISCUSSION

Exencephaly in mouse embryos of the same strain has now been produced experimentally by three different methods. In many of the exencephalic embryos, upper incisor anomalies occur in the form of fusions, which have been found in all three categories of exencephalic embryos but never in normal ones. These fusions have been studied in greatest detail in embryos from vitamin A treated mice, where the aim has been a thorough study of the different kinds of fusion in a large sample (272 embryos). The studies of the relatively few embryos from mice treated with trypan blue (47 embryos) or a riboflavin-deficient diet have merely sought to establish whether tooth fusions also occur when exencephaly is induced by these methods.

With reference to the type of incisor fusions, there seems to be greatest similarity between the embryos from vitamin A treated and trypan blue treated mice, as in these two groups both fusio



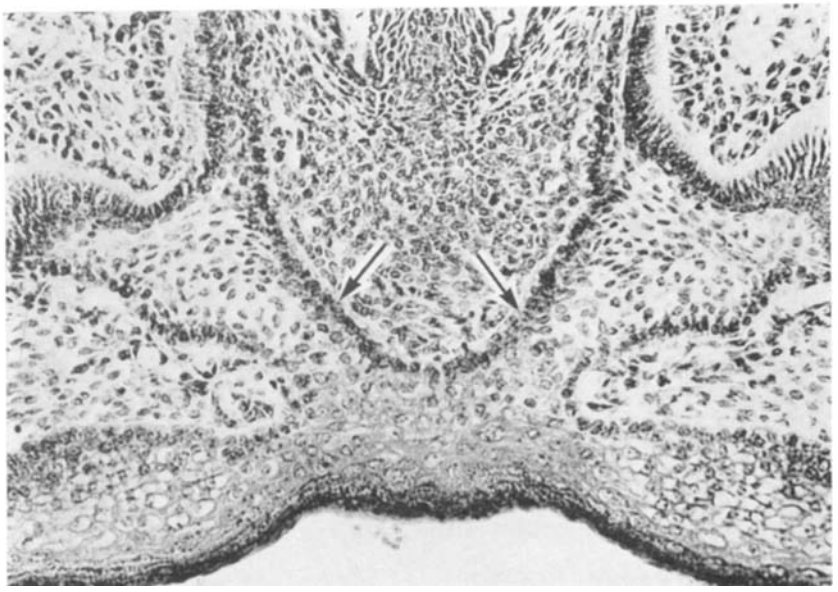


Fig. 3. Exencephalic mouse embryo (446 d I/4. Riboflavin deficiency). *Fusio epithelialis.*

The shortest distance between the incisor germs is 6 L. U. Frontal section. The connection between the stellate reticulum and the oral epithelium is narrow. At the point of connection the oral epithelium consists of a few layers of flat cells (arrow) (× 160).

Fig. 4. Exencephalic mouse embryo (420 d I/4. Riboflavin deficiency). *Incomplete fusio epithelialis.*

The shortest distance between the incisor germs is 12 L. U. Frontal section. Arrows indicate the transition between the oral epithelium and stellate reticula (× 160).

Table I

The length of administration periods of riboflavin-deficient diet with galactoflavin to produce 20 exencephalic embryos

Number of exencephalic embryos	Number of days riboflavin-deficient diet with galactoflavin was administered
10	4 (Day 7—10)
8	5 (Day 6—10)
2	8 (Day 3—10)

Table II

Anomalies of upper incisor germs in mouse embryos with exencephaly induced by various treatments

Upper incisor germs	Vitamin A overdosage	Trypan blue	Riboflavin-deficient diet with galactoflavin
Reduced distance	+	0	+
"Contact"	+	+	0
Fusio epithelialis	+	(+)	+
Fusio dentium	+	+	0
Agenesia	+	0	0

dentium and "contact germs" occur (Table II). In embryos from trypan blue treated mice, fusio epithelialis is rare and differs morphologically from fusio epithelialis found in mice treated with vitamin A. In embryos of mice which have received a riboflavin-deficient diet, fusio dentium is not found, whereas some typical examples of fusio epithelialis are encountered. In the same embryos there is also a reduced distance between the two incisor germs — an anomaly which is not found after treatment with trypan blue. Agenesia of incisor germs only occurs in embryos from vitamin A treated mice. A comparison of the frequencies of the different types of dental anomalies has not been attempted because two groups are relatively small.

Fusion between incisor tooth germs has occurred in all categories of exencephalic embryos. In this respect fusio epithelialis should be considered equally important as fusio dentium even if

it represents a less pronounced degree of fusion. Examination of exencephalic embryos from mice which have received a riboflavin-deficient diet therefore supports the hypothesis that there is a connection between exencephaly and dental anomalies.

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SUMMARY

Exencephaly has been induced in 20 mouse embryos by maternal intake of a riboflavin-deficient diet mixed with galactoflavin. The embryos have been studied with reference to malformations of the upper incisors. Two of the embryos exhibited *fusio epithelialis* (fusion of the incisal parts of the enamel organs) and reduced distance between the upper incisor germs. Previous investigations of embryos with exencephaly induced by hypervitaminosis A or trypan blue led to the conclusion that there is a causal relationship between exencephaly and dental malformation. This concept is supported by the present investigation.

RÉSUMÉ

GERMES DES INCISIVES CHEZ DES EMBRYONS DE SOURIS PRÉSENTANT UNE EXENCÉPHALIE PROVOQUÉE PAR CARENCE EN RIBOFLAVINE

Une exencéphalie a été provoquée chez 20 embryons de souris par ingestion maternelle d'une alimentation déficiente en riboflavine et mêlée de galactoflavine. Les embryons ont été examinés en ce qui concerne les malformations des incisives supérieures. Deux des embryons présentaient une fusion épithéliale (fusion des parties incisives des organes de l'émail) ainsi qu'une réduction de la distance entre les germes des incisives supérieures. Il découle d'une série d'études antérieures sur des embryons présentant une exencéphalie provoquée par hypervitaminose A ou par le bleu trypan qu'il existerait un rapport de cause à effet entre l'exencéphalie et les malformations dentaires. Cette conclusion se trouve confirmée par la présente étude.

ZUSAMMENFASSUNG

SCHNEIDEZAHNKEIME IN MÄUSEEMBRYONEN MIT EXENCEPHALIE DURCH RIBOFLAVINMANGEL VERURSACHT

Exencephalie wurde bei 20 Mäuseembryonen durch Verfütterung von riboflavinfreier Diät gemischt mit Galaktoflavin hervorgerufen. Die Embryonen wurden im Hinblick auf Missbildungen der oberen Schneidezähne untersucht. Zwei der Embryonen zeigten *fusio epithelialis* (Verschmelzung der incisalen Anteile der Schmelzorgane) und verminderten Abstand zwischen den oberen Schneidezahnkeimen. Frühere Untersuchungen an Embryonen mit Exencephalie erzeugt durch A-Hypervitaminose oder Trypanblau, führten zu der Schlussfolgerung, dass eine kausale Beziehung zwischen Exencephalie und dentalen Missbildungen besteht. Diese Deutung wird durch die vorliegende Untersuchung gestützt.

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