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

MALFORMATIONS OF UPPER INCISORS IN MOUSE EMBRYOS WITH EXENCEPHALY, INDUCED BY TRYPAN BLUE

by

P. A. KNUDSEN

INTRODUCTION

In mouse embryos with exencephaly induced by overdosage with vitamin A, the two upper incisors are often fused (68 %) (*Knudsen,* 1965 a). The commonest condition (51 %) is fusio dentium, in which all the layers of the enamel organ, together with the dentine and the pulp, cross the mid-line. The remaining cases (17 %) exhibit fusio epithelialis, i.e. the only connection is between the incisal parts of the enamel organs, where the outer dental epithelia and the stellate reticula cross the mid-line. The direct cause of these malformations is not known, but a connection between brain malformation and dental anomaly must be considered. The frequent occurrence of dental anomalies in patients with cerebral disorders (*Miles,* 1954, *Forrester & Miller,* 1955, and *Via & Churchill,* 1957) points in this direction, as does the rôle of the neural crest in tooth development in lower animals (*Sellman,* 1946) and in mammals (*Pourtois,* 1961).

It is possible, however, that the dental fusions are caused independently of the abnormal brain as a particular effect of vitamin A. The present paper seeks to elucidate this problem by establishing whether incisor anomalies occur in mouse embryos,

in which exencephaly has been induced by another method. In this case trypan blue injection has been employed, which is a well-known means of inducing congenital brain defects, including exencephaly, in animals. Its effect is most certain on rats, whereas in mice it often induces early prenatal death (*Kalter & Warkany,* 1959). Nevertheless, it has been possible to obtain an adequate number of exencephalic embryos for the present investigation, using trypan blue on the same strain of mice as was used in the vitamin A experiments.

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 is taken as the day on which the copulation plug is found in the vagina of the mouse. The males were then removed. On days 7—8 or 7—9 an aqueous preparation of trypan blue*) was administered by subcutaneous injection. For details see Table I. The females were killed on day 18 of pregnancy, i.e. about two days before term. The embryos were removed, examined macroscopically and then fixed in Bouin's fluid for 2—3 days. After the embryos had been photographed, their heads were cut off, embedded in paraffin wax and cut in frontal serial sections. Section thickness was 10 μ and the stain haematoxylin-eosin. All histological sections were examined on Reichert's Visopan microscope and all important changes registered on a special form. In addition, the most important details were studied in microphotographs. The measurements of the distance between the tooth germs were made with a ruler directly on the projection screen. This investigation comprises 47 exencephalic embryos. Normal and exencephalic embryos, previously described (*Knudsen,* 1965 a, b), were used for comparison.

In order to construct wax plate models of "contact germs" and their pulps, the exterior outlines of the oral epithelium and the enamel organ, as well as the inner outlines of the dentine layers, were traced at high magnification (200 ×). The model of the enamel organ is hollow and the labial (anterior) wall detach-

*) Trypanblau stand. (15365), Dr. Theodor Schuchardt.

Table I

Dosage of trypan blue, subcutaneously injected on days 7 to 9 of gestation to effect 47 exencephalic embryos included in the present investigation.

Day of gestation	Trypan blue (0.3 %)	
	ml	ml
Day 7	0.25	0.25
Day 8	0.25	0.25
Day 9	—	0.25
Total dose of trypan blue	0.50	0.75
Number of exencephalic embryos	28	19

able, so that it is possible to study the pulp cavities. The pulp models are solid and mounted in correct mutual position.

RESULTS

The most important result of the experiment is the demonstration of *fusio dentium, fusio epithelialis* and "contact" between the upper incisors. Uni- or bilateral agenesia of the incisors has not been found.

Fusio dentium occurs as fusio totalis in 7 embryos and as fusio partialis in 3, whereas fusio subtotalis is lacking. In comparison, it should be remarked that the commonest type of fusion in vitamin A treated embryos is likewise fusio totalis; 130 embryos with fusio dentium comprise 103 (79.2 %) with fusio totalis, 10 (7.7 %) with fusio partialis and 17 (13.1 %) with fusio subtotalis (*Knudsen,* 1965 a).

Fusio totalis in trypan blue treated embryos cannot be distinguished morphologically from the same condition in embryos which have been affected by vitamin A (Fig. 1). The forms which occur most frequently in a large material (*Knudsen,* 1965 b) are also represented here. Germs are seen with a rectangular or trapezial outline, and the greatest diameter can be either transversal or vertical. Some germs are symmetrical and others lightly asymmetrical, but neither triangular germs nor medium or heavily asymmetrical germs are found. A comparison of the frequency

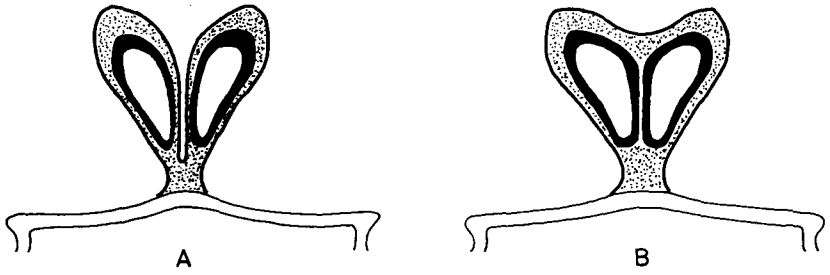

Fig. I. Diagrammatic representation of "contact germs" (frontal sections)
Thick black line: ameloblasts (inner dental epithelium)
Dotted area: stellate reticulum
Black line surrounding dotted area: outer dental epithelium
Thin black line: oral epithelium
A: outer dental epithelia and stellate reticula between the ameloblasts in the area of contact
B: outer dental epithelium is not found in the area of contact

with which the various types occur is omitted on account of the relatively small number of embryos in this experiment.

Fusio partialis does not differ essentially from the same type of fusion found in vitamin A treated embryos, although in embryos which have been treated with trypan blue, the pulp bridge between the right and left sides is unusually narrow (Fig. 2) and the germs in many sections have some resemblance to "contact germs". There is no doubt, however, that the designation fusio dentium is correct and in agreement with the suggested definition, as all the layers of the enamel organ, as well as the dentine and pulp, cross the mid-line.

"Contact" between the upper incisor germs is a peculiar anomaly, occurring in 2 embryos in this experiment. It has previously been found in 2 (out of 272) vitamin A treated embryos (*Knudsen*, 1965 a). Examination of all the sections engaging incisor germs shows that nowhere is there a connection between the dentine layers or pulps of the two sides. In one case the area of contact is of very limited extent, cells of the stellate reticulum being present almost everywhere between the ameloblast layers (inner dental epithelia) (Fig. 3). In the other case (Fig. 4), the

contact surface is of considerable extent, and as the two ameloblast layers are locally pressed tightly together and the cell height simultaneously reduced, it is only possible to distinguish both layers at high magnification (Fig. 5 b).

Comparison of wax reconstructions reveals a close similarity between "contact germs" and fusio partialis, the latter in a vitamin A treated embryo (*Knudsen,* 1965 b) (Figs. 9—12 and 15). On the model of contact germs, the epithelial plate, which represents the oral epithelium opposite the incisor germs, is trapezial, and the relief of both the free oral surface and the basal surface, which is covered with connective tissue, corresponds closely to the relief on the surface of the epithelial plate in fusio partialis. The basal surface has also here four labio-lingual grooves, limited by five crests (Fig. 12). The middle crest is connected with the dental lamina, which is labially narrow and lingually broad. It has, unlike the dental lamina in fusio partialis, no niche on the labial edge. Lingually there is a large flat depression (Fig. 11). It resembles the lingual niche in fusio partialis, except for the border, which is not as sharp in "contact germs". Description of the surface of the tooth germ corresponds to the description for fusio partialis (*Knudsen,* 1965 b), but with the important difference that the contact germs have two entirely separate root openings, whilst fusio partialis has only one, which is hour-glass shaped, with the constriction in the middle of the germ.

The size and shape of the two pulp cavities can be observed, when the labial wall of the hollow model is removed (Fig. 10). The cavities are completely separated by a median "septum", which mainly consists of the ameloblast and dentine layers in the area of contact (Figs. 4 and 5 b). The right and left pulps are consequently separate, whereas there is a pulp bridge across the mid-line in fusio partialis. The surface relief of a single pulp corresponds closely to that of a pulp half in fusio partialis (Figs. 13, 14 and 16). The grooves on the lingual surface are, however, considerably deeper than in fusio partialis, whilst the labial grooves are less pronounced.

Fusio epithelialis occurs in only one case (Fig. 6). The "epithelial bridge" between the two sides is lower than usual, but as both stellate reticulum and outer dental epithelium can be traced across the mid-line, there is no doubt that the incisal parts of the

enamel organs have fused, giving rise to a V-shaped configuration.

In 3 other cases the connection between the incisor germs is likewise V-shaped, but here the stellate reticulum does not cross the mid-line, the central part of the bridge consisting only of oral epithelium (Fig. 7). It is difficult to decide in every case whether the outer dental epithelium crosses the mid-line, or whether it is replaced in the middle by the basal layer of the stratified squamous epithelium. The lack of continuity in the stellate reticulum makes it difficult to use the term fusio epithelialis, in spite of the great similarity with this form, and the obvious difference from normal germs. In the latter the epithelial connection is U-shaped, and the transition between the stellate reticulum and the oral epithelium lies away from the cross-connection and near the tooth germs (Fig. 8).

The shortest distance between separate incisor germs, which is normally 14—20 L.U.*) varies from 11 to 20 L.U. in trypan blue treated embryos, contact germs excluded. In vitamin A treated embryos the variations are much larger, having values from 1 to 20 L.U., a considerable number of which (31 out of 107) lie between 1 and 11 L.U. (*Knudsen*, 1965 a). It is noteworthy that the latter category is entirely absent in trypan blue treated embryos.

DISCUSSION

The investigation has shown that fusion of the upper incisors in exencephalic embryos is not a special reaction to large doses of vitamin A, similar changes occurring in embryos with exencephaly induced by trypan blue.

The question of the connection between tooth anomalies and exencephaly remains unanswered. As exencephaly induced by various methods is frequently accompanied by fusio dentium, it strongly suggests that the abnormal brain development exerts a decisive influence in the abnormal tooth development. However, as exencephaly can be induced by affecting the neural tube at a particular stage in embryonic life with substances as varied as vitamin A and trypan blue, the possibility remains that they also

*) L. U. = Length Unit, i.e. the measurement in millimeters at 70×linear enlargement.

can produce a direct teratogenic effect on the future dental organs, thereby causing fusio dentium independently of exencephaly. If this is correct, one would expect tooth fusions to occur in a number of those embryos which have received large doses of vitamin A, without manifesting exencephaly or other brain defects. This aspect of the problem is being studied at the moment on a larger material, comprising non-exencephalic siblings of exencephalic embryos.

The various types of fusio dentium (partialis and totalis) and "contact" between the incisors, found in this study, resemble those described earlier in embryos which had been affected by vitamin A.

With reference to "contact germs" it has been discussed previously which cells are found between the ameloblast layers in and immediately outside the area of contact (*Knudsen,* 1965 a). The most likely possibilities are shown in Text Fig. I. In one case (A) cells of both the stellate reticulum and outer dental epithelium are found, and in the other (B), only cells of the stellate reticulum. The latter possibility must be considered the most likely on the basis of the histological sections deriving from vitamin A treated embryos. This impression is strengthened by examination of the specimens in this investigation. In Fig. 3 the space between the ameloblast layers is limited at the top by a row of cells perceived as outer dental epithelium both on account of the arrangement of the cells (Fig. 5 a) and of the connection with the outer dental epithelium of one side. The final proof is still lacking, however, as it calls for a certain identification of the cells between the ameloblast layers in the area of contact. Outside the area of contact, outer dental epithelial cells can be distinguished from cells of the stellate reticulum by the difference in position. This is not possible in the contact area, where all the cells are irregularly arranged and where it is also impossible to distinguish with certainty the two cell types on the basis of morphological criteria.

A peculiar transitional form, which has not been mentioned previously, resembles fusio epithelialis, but differs from it in that the stellate reticulum cannot be followed across the low epithelial bridge, the middle part of which consists of oral epithelium. In spite of the absent connection between the stellate reticula, the

germs are best perceived as an incomplete form of fusio epithelialis, as the morphological difference from normal germs is too large to justify an identification with these.

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SUMMARY

47 mouse embryos in which exencephaly has been induced by maternal injection of trypan blue have been studied with reference to malformations of the upper incisors. 7 of the embryos show *fusio totalis*, 3 *fusio partialis* and 2 "contact" between the incisor germs. There is only one case of *fusio epithelialis* (fusion of incisal parts of the enamel organs), and agenesia of the incisors does not occur. The fusions resemble closely those found in embryos where exencephaly has been induced by maternal overdosage of vitamin A. The causal relationship between exencephaly and dental malformations is discussed.

RÉSUMÉ

MALFORMATIONS DES INCISIVES SUPÉRIEURES CHEZ DES EMBRYONS DE SOURIS EXENCÉPHALES PAR SUITE D'INJECTIONS DE BLEU TRYPAN

Chez 47 embryons de souris présentant une exencéphalie provoquée par injection maternelle de bleu trypan, il a été procédé à une étude des malformations des incisives supérieures. Chez 7 des embryons il existe une fusion totale, chez 3 d'entre eux il existe une fusion partielle et chez deux d'entre eux il existe un "contact" entre les germes des incisives. Il n'y a qu'un seul cas de fusion épithéliale (fusion des parties incisives d'organes de l'émail), et aucun cas d'agénésie des incisives. Les fusions présentent une forte ressemblance avec celles qui ont été observées chez les embryons présentant une exencéphalie provoquée par surdosage maternel de vitamine A. Les rapports de cause à effet entre l'exencéphalie et les malformations dentaires font l'objet d'une discussion.

ZUSAMMENFASSUNG

MISSBILDUNGEN DER OBEREN SCHNEIDEZÄHNE IN MÄUSEEMBRYONEN MIT EXENCEPHALIE, DIE DURCH INJEKTION MIT TRYPANBLAU VERURSACHT IST

47 Mäuseembryonen in denen eine Exencephalie durch Injektion von Trypanblau bei den Muttertieren hervorgerufen worden war, wurden im Hinblick auf Missbildungen der oberen Schneidezähne untersucht. 7 der Embryonen zeigten *fusio totalis*, 3 *fusio partialis* und 2 "Kontakt" zwischen den Keimen der Schneidezähne. Es trat nur ein Fall von *fusio epithelialis* (Verschmelzung der inzisalen Anteile der Schmelzorgane) auf. Agenesie der Schneidezähne kam nicht vor. Die Verschmelzungen sind denen sehr ähnlich, die bei Embryonen mit Exencephalie infolge Überdosierung von Vitamin A beobachtet wurden. Die kausale Beziehung zwischen Exencephalie und dentalen Missbildungen wird diskutiert.

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

PLATES

LIST OF ABBREVIATIONS

A: apical end
AM: ameloblast (inner dental epithelium)
AO: apical root opening
C: crest (elevation)
D: distal surface
DL: "dental lamina"
E: epithelium of the roof of the oral cavity
F: fossa
I: incisal end
L: labial surface
LI: lingual surface
M: mesial surface
S: sulcus (depression)
SP: septal cartilage
ST: "septum" between the pulp cavities
TR: tooth germ removed

Plate 1.

Fig. 1. Exencephalic mouse embryo (271 d I/4. Trypan blue). *Fusio totalis.*

Fig. 2. Exencephalic mouse embryo (407 d I/4. Trypan blue). *Fusio partialis.*

Outline: H-shaped. Light asymmetry. Greatest diameter: transversal. The pulp bridge between the right and left sides is unusually narrow. (× 63).

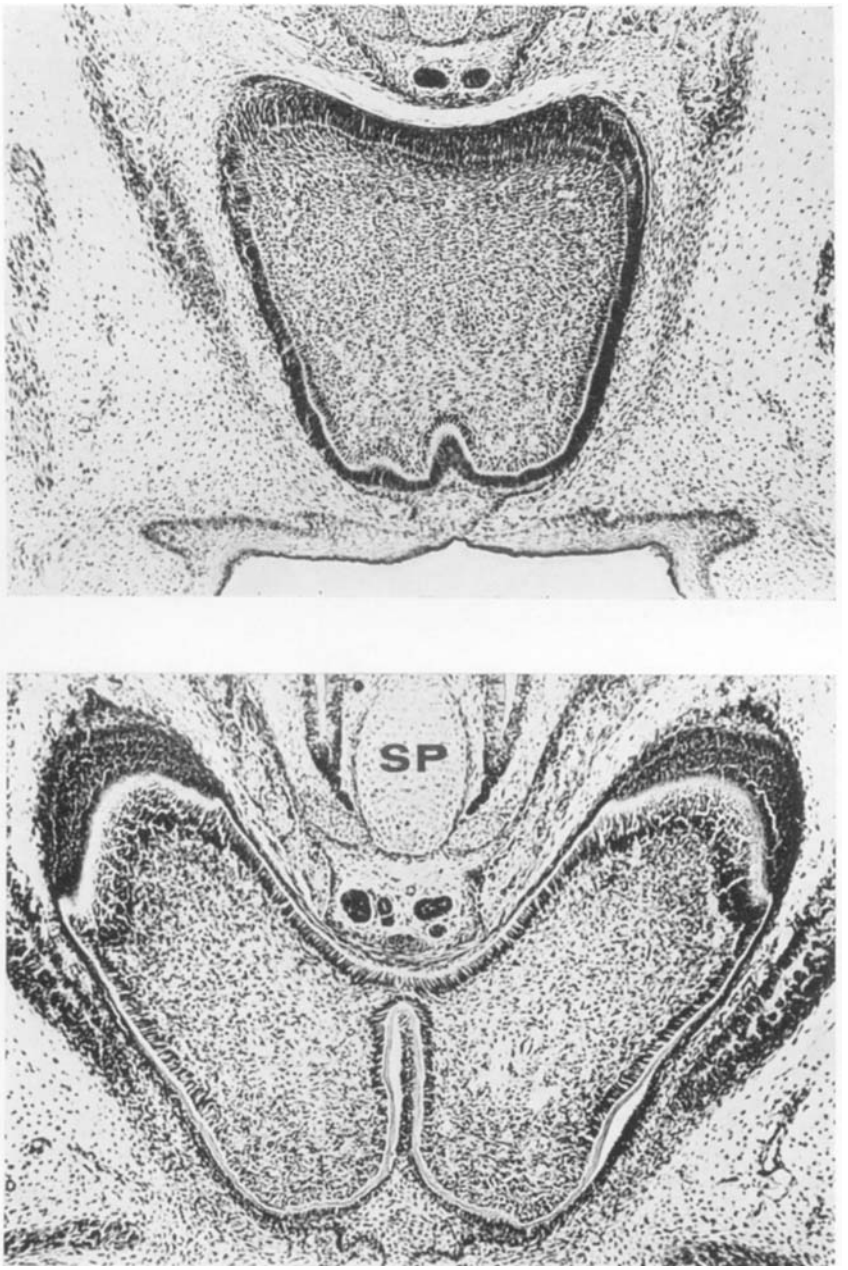
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Plate 2.

Fig. 3. Exencephalic mouse embryo (260 d I/4. Trypan blue). "Contact" between the upper incisor germs. The area of contact is of very limited extent, cells of the stellate reticulum being present almost everywhere between the ameloblast layers. The top of the space between the ameloblast layers is limited by the outer dental epithelium (arrow). (cf. Fig. 5 a) (× 63).

Fig. 4. Exencephalic mouse embryo (404 d I/4. Trypan blue). "Contact" between the upper incisor germs. The two ameloblast layers are locally pressed tightly together. (Arrows: cf. Fig. 5 b). (× 63).


SP
SP

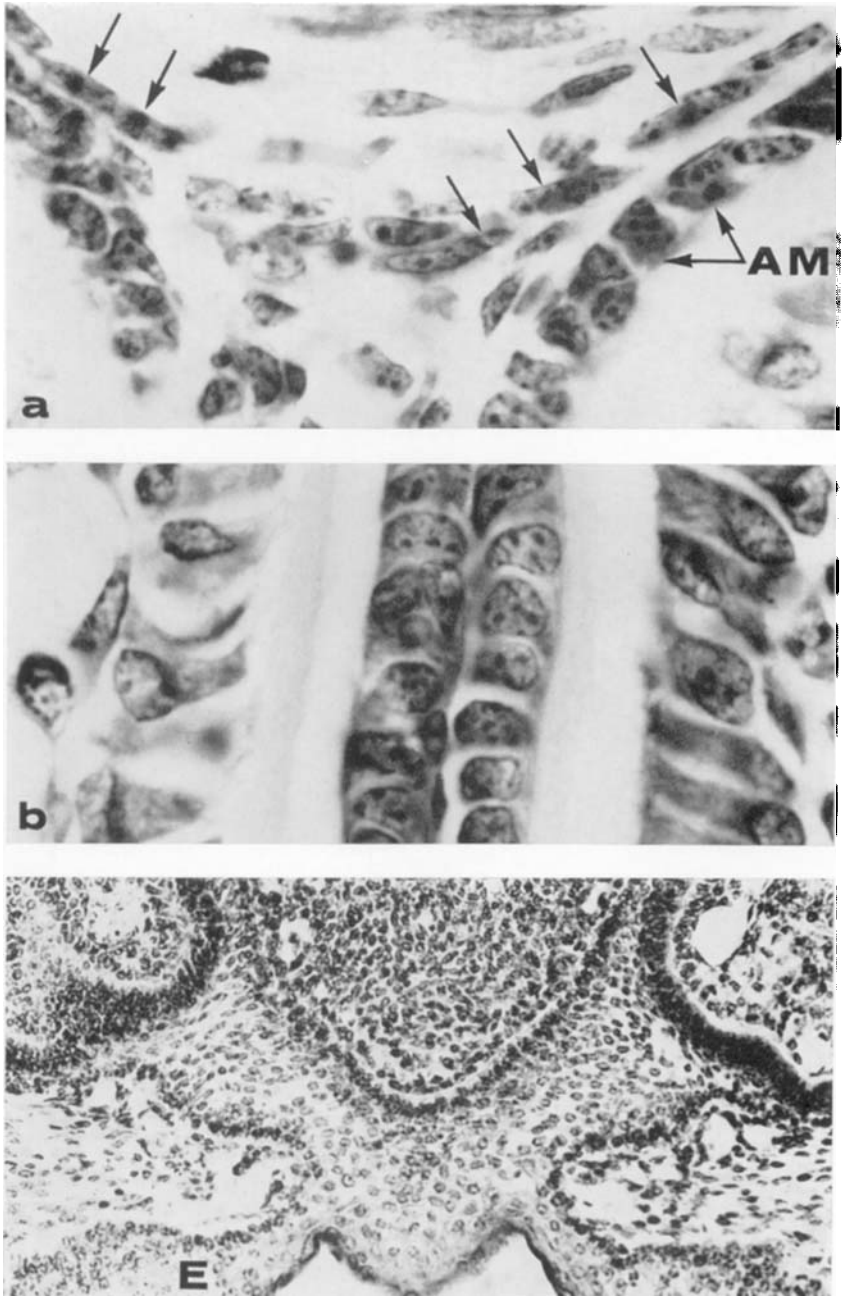
Plate 3.

Fig. 5 a. The top of the space between the ameloblast layers near the arrow in Fig. 3 is limited by the outer dental epithelium. (Arrows indicate the cells). (× 1000).

Fig. 5 b. Part of the contact area between the arrows in Fig. 4. The two ameloblast layers are seen. (× 1000).

Fig. 6. Exencephalic mouse embryo (417 d I/4. Trypan blue).
Fusio epithelialis.

The epithelial bridge between the two sides is lower than usual but the stellate reticulum and the outer dental epithelium can be traced across the mid-line (× 160).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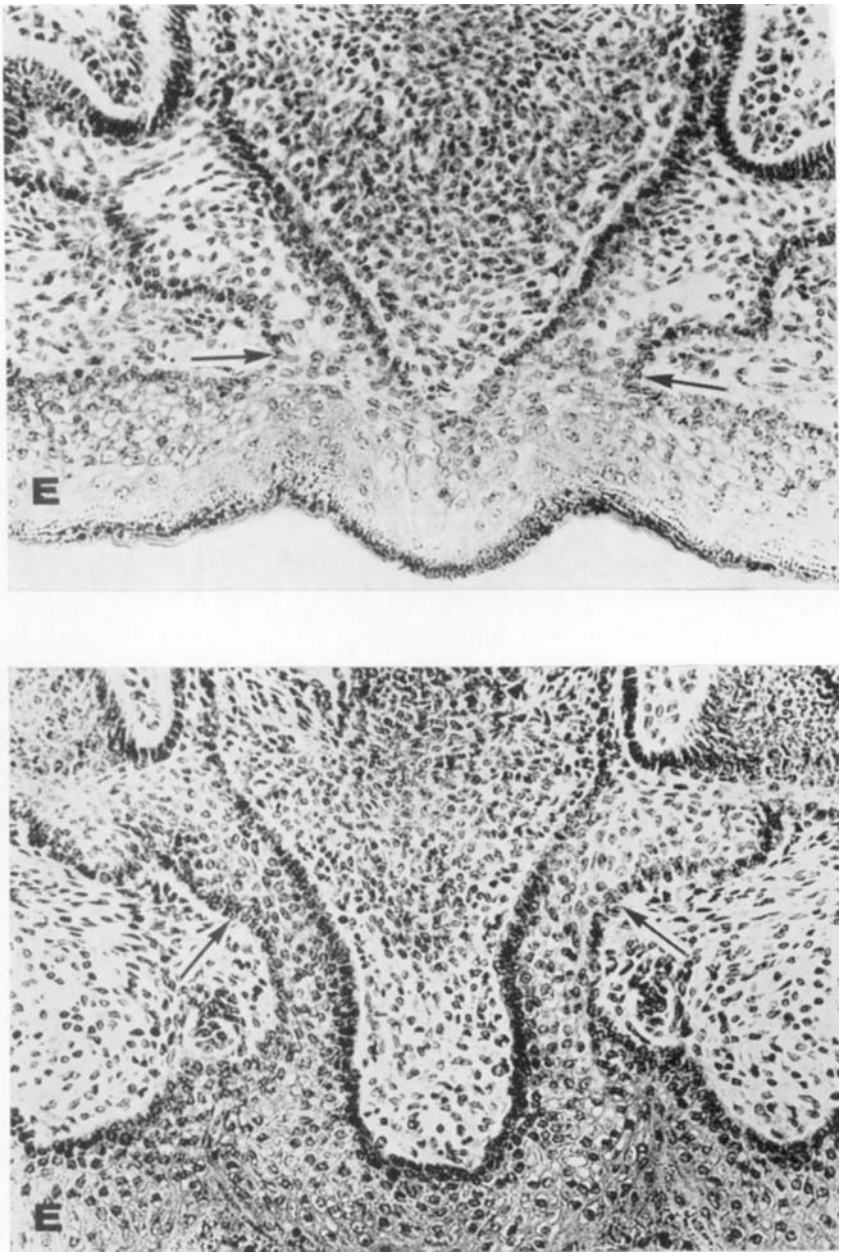
a
b
E

Plate 4.

Fig. 7. Exencephalic mouse embryo (418 d I/4. Trypan blue).
Incomplete fusio epithelialis.

The stellate reticula are separated by oral epithelium. Arrows show the transition (× 160).

Fig. 8. Normal mouse embryo (29 d I/4).
Stellate reticula of the incisor germs and oral epithelium. Arrows show the transition (× 160).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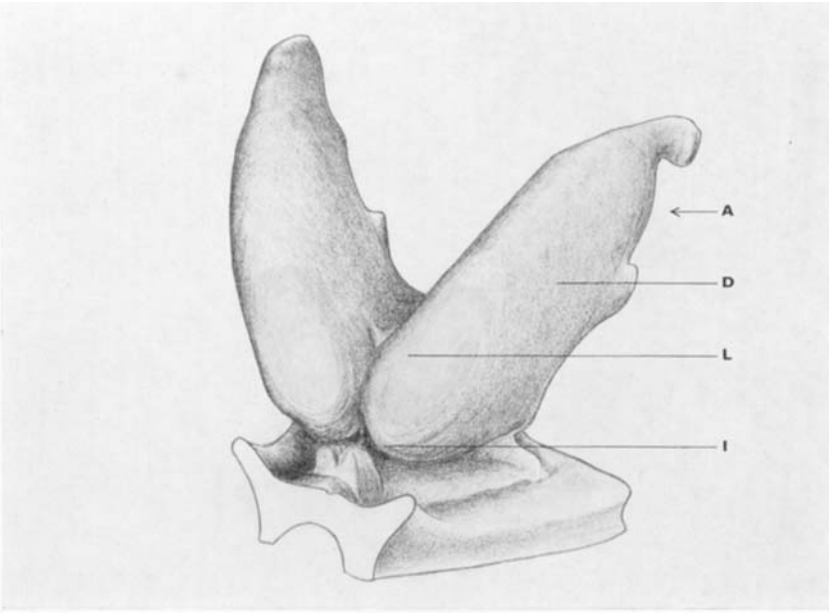
E

Plate 5.

Figs. 9—12. Exencephalic mouse embryo (Trypan blue).
"Contact" between the upper incisor germs.
Drawings of a model of the upper incisor germs and oral epithelium.
Fig. 9. Distal and labial view.

Fig. 10. Labial and distal view. The labial wall of the hollow model has been removed. The pulp cavities are entirely separated by a median "septum".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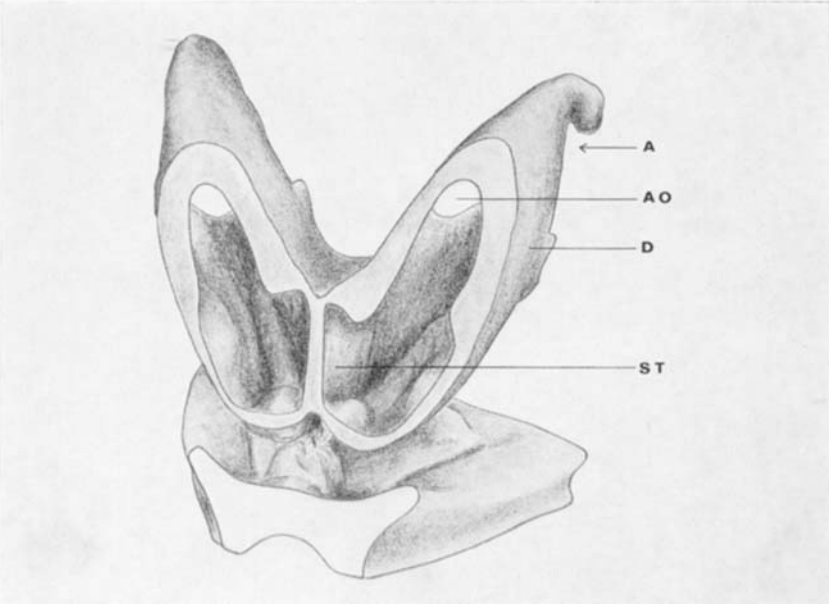
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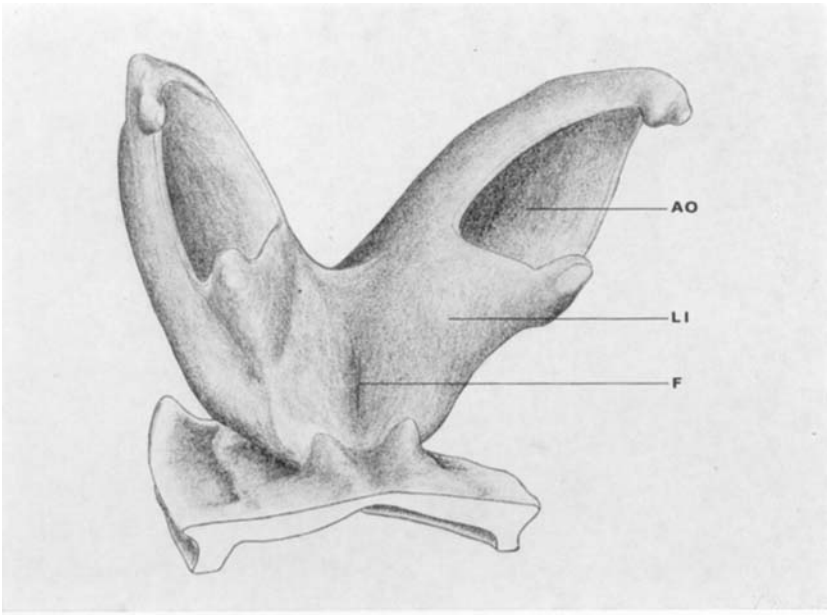
Plate 6.

Fig. 11. Lingual (and distal) view. The apical root openings are separate.

Fig. 12. Oral epithelium. Basal view. The incisor germs have been removed along their common connection with the epithelium.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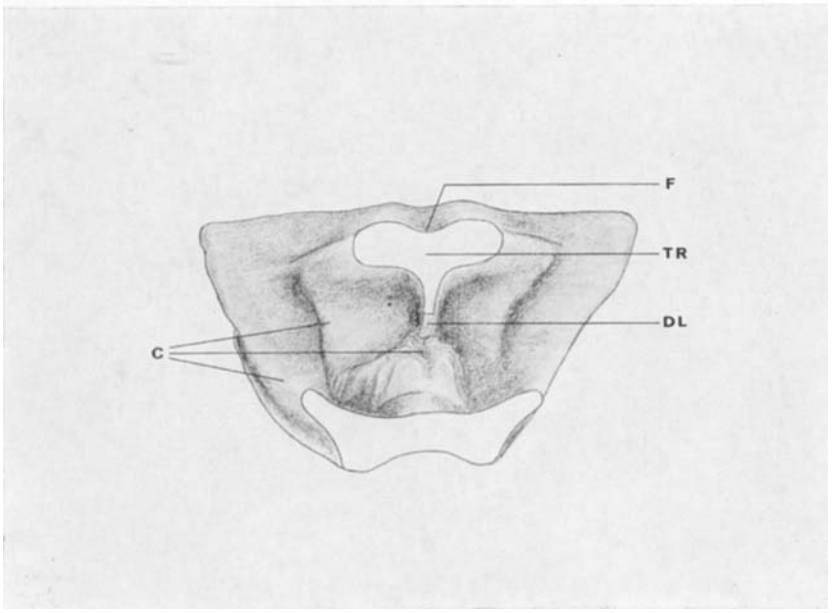
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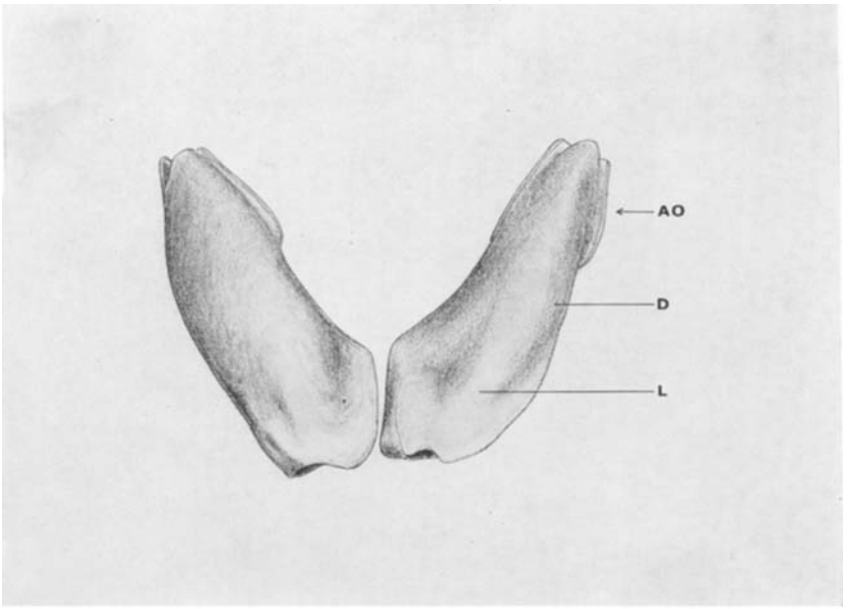
TR
DL
C

Plate 7.

Figs. 13—14. "Contact" between upper incisor germs.
Drawings of a model of the pulps in the incisor germs, shown in Figs. 9—12.
Fig. 13. Labial view.

Fig. 14. Lingual view.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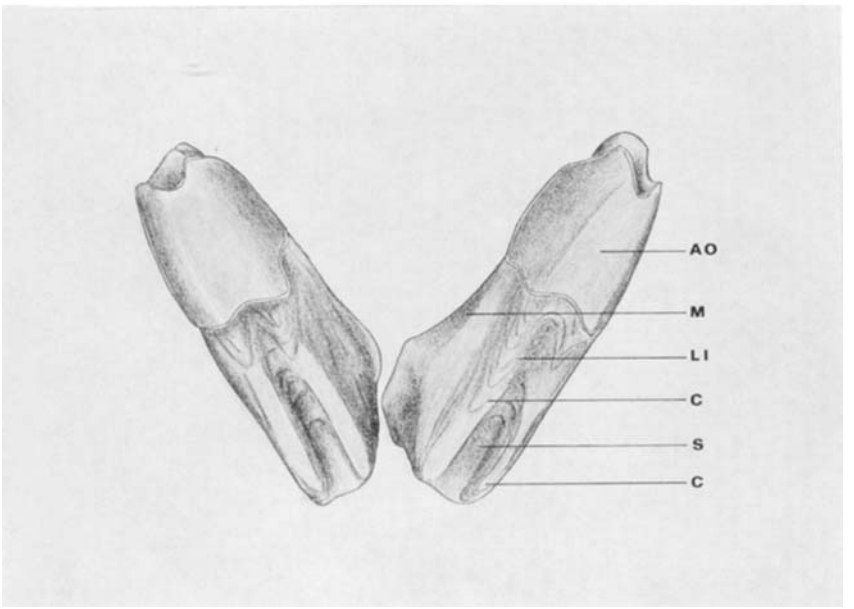
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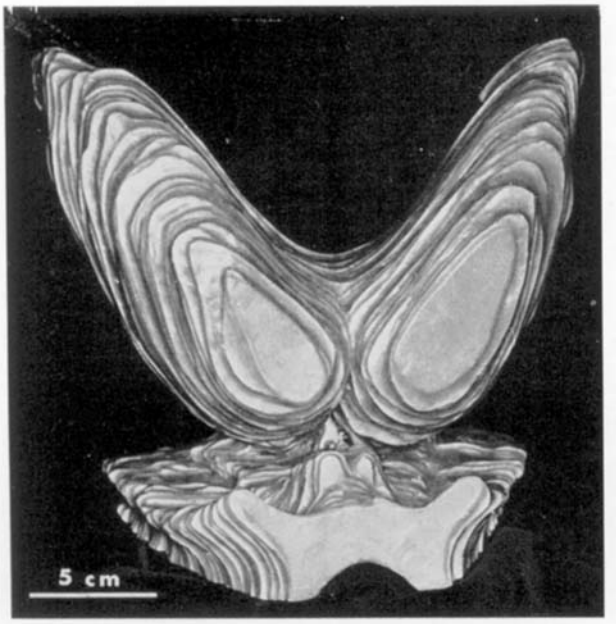
Plate 8.

Figs. 15—16. Exencephalic mouse embryo. 18 days (404 d I/4. Trypan blue). "Contact" between the upper incisor germs. Photographs of wax plate reconstructions.

Fig. 15. Upper incisor germs and oral epithelium; labial (and distal) view.

Fig. 16. Pulps; labial view.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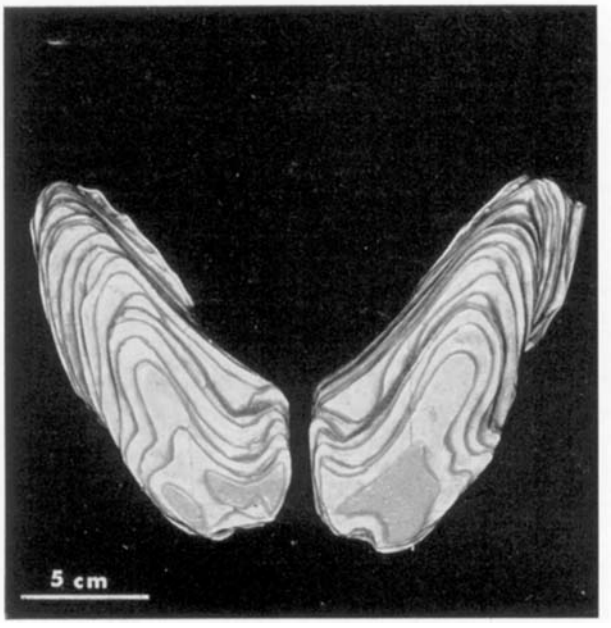
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