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

DENTAL MALFORMATIONS IN RAT EMBRYOS WITH EXENCEPHALY INDUCED BY HYPERVITAMINOSIS A

by

P. A. KNUDSEN

INTRODUCTION

Complete or partial fusion of upper incisor tooth germs has been observed in a large number (68 %) of exencephalic mouse embryos induced by maternal overdosage of vitamin A (*Knudsen,* 1965). Other tooth germs, the remaining oral cavity and adjacent structures were usually normal, only 34 of 272 embryos showing molar agenesia or molar fusions (*Knudsen,* 1966 a). The molar anomalies were frequently accompanied by changes in jaws, temporo-mandibular joints, muscles of mastication and suprahyoid muscles (*Knudsen,* 1966 b). Similar studies were undertaken of exencephalic rat embryos induced by vitamin A overdosage, with the object of establishing whether dental anomalies or anomalous changes in joints and muscles were present.

MATERIAL AND METHODS

Female rats of an inbred strain (L.S.) were placed with males of the same strain, one pair to a cage, for 24 hours. The day when the males were removed was taken as day one of pregnancy. The impregnated females were given 50,000 I.U. vitamin A in oil on days 8, 9 and 10 of pregnancy, by means of a gastric tube. Before

the tube was introduced, the animals were lightly anaesthetized with ether. On day 19, the females were killed and the embryos removed, examined macroscopically and then fixed in Bouin's fluid. After 3 days' fixation, the heads of the embryos were cut off, embedded in paraffin wax and cut in frontal, serial sections. Section thickness was 10 μ and they were stained with haematoxylin-eosin. A Reichert's Visopan microscope was used for examination of the sections, and all important changes registered on a special form.

The shortest distance between incisor germs in each embryo was measured directly on the projection screen of the microscope and the result recorded in Length Units (L.U.) corresponding to the measurement in millimetres at 70 × linear magnification. Finally, a wax plate model of normal upper incisor germs and the epithelium in the roof of the mouth was constructed. The exterior outline of the enamel organ and oral epithelium was traced at 200 × linear magnification.

38 exencephalic rat embryos were examined and compared with 19 normal rat embryos of the same age.

RESULTS

The embryos were divided into three groups on the basis of the most important tooth anomalies.

Group I comprised 10 embryos having 1 or 2 supernumerary tooth germs in the upper jaw. The term "supernumerary" denoted that the germ occurred at a place in the jaw where teeth are not normally found. 9 of these embryos also lacked from 2 to 7 molar germs, the remaining one having all (8) molar germs. Fusio epithelialis of upper incisor germs was found in only one embryo, having 2 supernumerary tooth germs.

Group II contained 7 embryos, without supernumerary tooth germs, but each lacking from 1 to 6 molar germs.

Group III consisted of 21 embryos without important tooth anomalies. There were no supernumerary germs and all the molar germs were present.

Text Figs. I—III show the occurrence of the most important changes in tooth germs, jaws, temporo-mandibular joints, muscles of mastication and some of the suprahyoid muscles.

The most severe tooth anomalies occurred in group I (Text Fig.

Group I

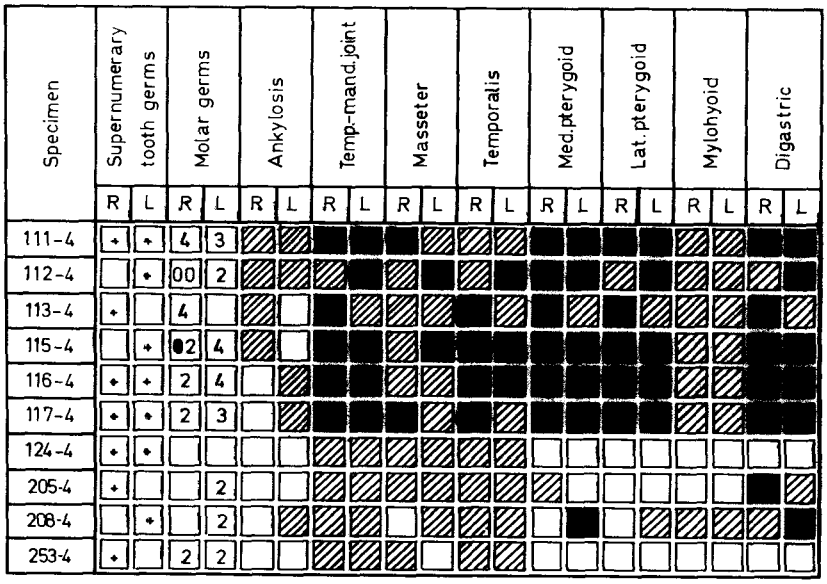


Fig. I Group I: 10 embryos with "supernumerary" tooth germs. The chart indicates for each specimen the number of missing molar germs; it indicates type of fusion of the molar germs, occurrence of maxillo-mandibular ankylosis and the number of the normal, anomalous (incomplete) and missing structures named in the upper line.

R = right
L = left
O = fusio epithelialis
● = fusio totalis
Unshaded: normal structures
Hatched: anomalous structures (incompletely developed).
Solid: agenesia

I), this group also exhibiting the highest frequency of agenesia, incomplete development of the jaws, joints and muscles (Text Fig. IV).

Agenesia of joints and muscles was less common in group II, but incomplete development was frequent (Text Figs. II and IV).

In group III (Text Fig. III) ankylosis did not occur. Incomplete development of temporo-mandibular joints and the muscles of mastication occurred frequently but agenesia was rare (Text Fig. IV).

Incisor germs (all groups). There was a single occurrence in

Group II

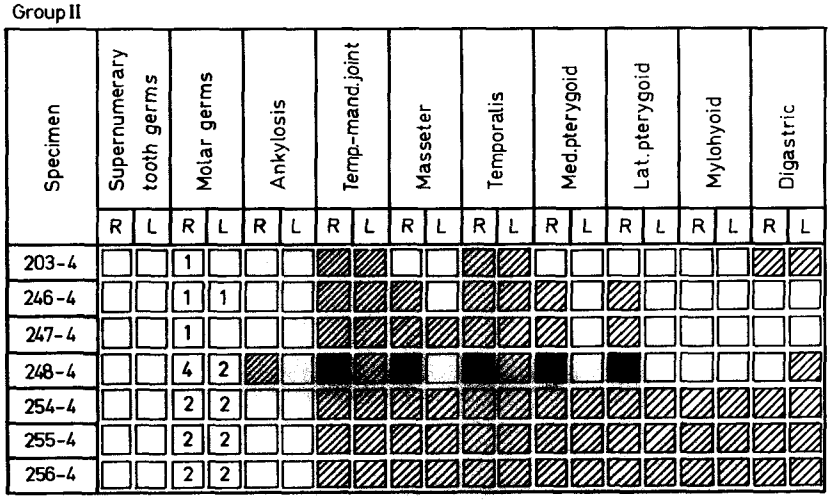


Fig. II Group II: 7 embryos, each lacking from 1 to 6 molar germs. The chart indicates for each specimen the number of missing molar germs, normal, anomalous and missing structures named in the upper line. Ankylosis is seen in one embryo.
Abbreviations, see Text Fig. I.

group I (no. 116-4) of fusio epithelialis of upper incisor germs. Incisal portions of the stellate reticula and outer dental epithelia crossed the mid-line to form a high, Y-shaped bridge (Fig. 2).

Agenesia of incisor germs did not occur in the upper jaw and there were only two instances of one missing incisor germ of the lower jaw. There was a significant reduction in the distance between upper incisor germs, the mean value of the shortest distance being 21 ± 2.42 L.U. in exencephalic, and 23 ± 1.79 L.U. in normal, embryos ($0.01 > P > 0.001$).

Incisor germs of exencephalic embryos resembled those of normal rat embryos of the same age. Oral epithelium between the two germs was quite thick near their lingual surfaces in normal and exencephalic embryos (Fig. 1 b). On frontal sections through the labial part of this epithelial thickening, an oval area of mesenchyme was seen in the epithelium (Fig. 1 a). It decreased in lingual direction and ultimately disappeared. On the wax model (Fig. 5), the epithelial thickening appeared as a transverse crest with a slight labial slant, whereby a shallow niche was formed,

Group III

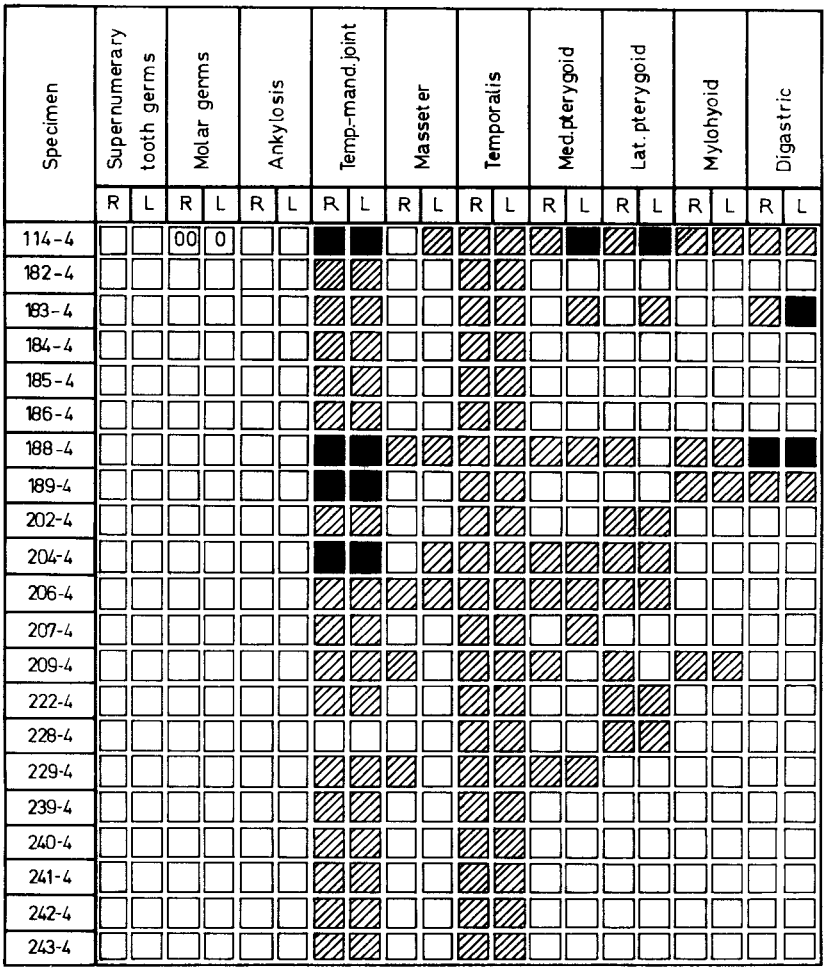


Fig. III Group III: 21 embryos without dental anomalies (except fusio epithelialis in one embryo). The chart indicates for each specimen the occurrence of normal, anomalous and missing structures named in the upper line. There is no maxillo-mandibular ankylosis.
Abbreviations, see Text Fig. I.

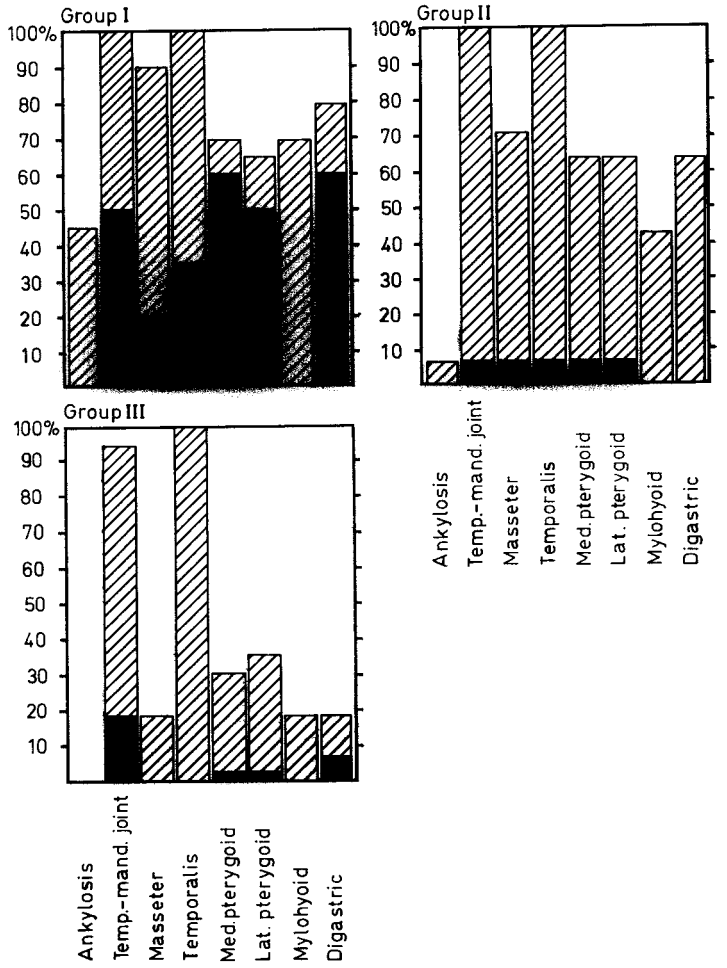


Fig. IV The percentage of anomalous and missing structures in each of the 3 groups shown in Text Figs. I—III.
Solid: missing structures.
Hatched: anomalous structures.

TABLE I

Dental anomalies in 38 exencephalic rat embryos

	Incisor germs		Supernumerary tooth germs			Molar germs missing							
						Upper				Lower			
	Upper Fusio epithelialis	Lower Agenesia (Unilat.)	Upper Unilat.	Upper Bilat.	Lower	First molar Unilat.	First molar Bilat.	Second molar Unilat.	Second molar Bilat.	First molar Unilat.	First molar Bilat.	Second molar Unilat.	Second molar Bilat.
of s	1	1	6	4	0	5	2	4	9	4	1	7	8
of erms		1	6	8	0	5	4	4	18	4	2	7	16

corresponding to the mesenchyme area of the histological sections.

"Supernumerary" tooth germs. In group I, comprising 10 embryos, there were 14 supernumerary tooth germs in the upper jaw. They were situated between incisor and molar germs. They were bilateral in 4 and unilateral in 6 embryos and often demonstrated heterotopic cartilage labially (Fig. 3). In frontal sections, the germs appeared bell-shaped and rather alike while the connection with the oral epithelium was of varying length and direction. There was no connection between supernumerary and adjacent tooth germs. Histodifferentiation had not reached the same degree as the most developed germs in the same embryo (Fig. 4).

Molar germs. A total of 60 molar germs were lacking out of 304 potential germs (in 38 embryos). In groups I and II, 16 embryos were involved, each embryo lacking from 1 to 7 germs (Text Figs. I and II). Molar germs were absent with equal frequency in upper and lower jaws. In both jaws second molar germs were most frequently missing (Table I).

Fusion of molar germs occurred in 3 embryos. Fusio totalis occurred in one embryo of group I (115-4), between first upper and lower molar germs on one side.

Fusio epithelialis was found in one embryo in group I (112-4), between first upper and lower molar germs on one side and between second upper and first lower molar germs on the same side. Finally, in one embryo in group III (114-4), there was a connection between first upper and lower molar germs on each side by way of the vestibular lamina and on one side, also between the second upper and lower molar germs.

Jaws. The commonest bone anomaly in exencephalic embryos with supernumerary tooth germs was maxillo-mandibular ankylosis. The boundary between the upper and lower jaws was erased and the shape and sometimes position of the bones altered. Cartilage was often found in the region of ankylosis. Ankylosis occurred in 7 of 10 embryos in group I and was bilateral in 2 cases. In group II, ankylosis was found unilaterally in one embryo with 6 molar germs lacking. Ankylosis did not occur in group III. Text Fig. IV shows the frequency of ankylotic jaws in the different groups.

Temporo-mandibular joints. The term "agenesia" was used when the joints were missing and "incomplete development" when any morphological deviation from normal joints appeared. Agenesia occurred most frequently in group I, where 50 % of the joints were missing. In group III, 19 % of the joints were missing (Text Fig. IV). Incomplete development usually means that the joint cavities were absent. Sometimes, however, there was a flattening of the mandibular fossa, especially as a result of an incomplete medial margin. In some cases there was also deformation of the head of the mandible. Only one exencephalic rat embryo had normal temporo-mandibular joints on both sides.

Muscles. The term "muscle agenesia" was used when all muscle fibres were lacking where muscles are normally found and "incomplete development" when the muscles were small or consisted of scattered fibres. The latter term was also employed whenever the origin or the insertion was lacking, without taking into account whether the entire muscle or only part of it was involved.

Agenesia and incomplete development of the four muscles of mastication occurred with varying frequency in the three groups (Text Figs. I—III). The temporal muscle was not normal in any embryo and was most frequently missing in group I. The other muscles were severely affected in groups I and II and less so in group III. Agenesia was most common in the pterygoid muscles in group I (Text Fig. IV). The two suprahyoid muscles which have been examined resembled closely the pterygoid muscles as to the total number of anomalies. It was notable that the mylohyoid muscle was always present, although often incompletely developed.

Meckel's cartilage. Meckel's cartilage was missing on one side in 2 embryos (115-4 and 117-4) and in 5 embryos it was markedly asymmetrical. Symmetry and slight asymmetry of Meckel's cartilage occurred with roughly the same frequency in the remainder of the embryos.

DISCUSSION

Exencephaly can be induced with almost equal facility in mice and rats by overdosage of vitamin A, but the size of the dose and the stage of pregnancy at which it is applied have a decisive influence

on the results. The brains in exencephalic mouse and rat embryos resemble each other grossly and histologically. It is therefore remarkable, that the accompanying tooth anomalies, in dentitions so alike as those of the rat and mouse, should differ in location and type. In exencephalic mouse embryos, incisor anomalies are the most common and in most cases molar germs are normal. In rat embryos anomalies of incisor germs are rarely found. It is particularly striking in so far as the type of anomalies is concerned that only one case of fusio epithelialis, the type of fusion previously observed in exencephalic mouse embryos (*Knudsen*, 1965), has been observed in rat embryos under similar experimental conditions. The infrequent occurrence of fusion applies also to the molar germs, where fusion of upper with lower molar germs is rare. Molar agenesia, on the other hand, occurs more often than in mouse embryos. Finally, the occurrence of supernumerary tooth germs is rather frequent in rat embryos, but extremely rare in exencephalic mouse embryos.

It is not possible at the present stage of the investigation, to explain the different patterns of dental malformations in exencephalic rat and mouse embryos. Future teratological experiments should take into account the different reaction of rat and mouse tooth germs to apparently identical stimuli. Therefore, the choice of animal for research should depend on the type of dental anomalies desired.

The niche found in the epithelial thickening between incisor germs of rat embryos has not been observed in normal or exencephalic mouse embryos with separate germs. In frontal sections, the epithelial thickening suggests the modified type of fusio epithelialis first described in exencephalic mouse embryos where exencephaly had been induced by trypan blue (Knudsen, 1966 c). In this type of fusion, the two enamel organs were separated by stratified squamous epithelium, which had only a limited transverse extent, whereas in rat embryos the width of the epithelium is considerable.

While "supernumerary" tooth germs are rare in exencephalic mice, they occur frequently in rat embryos. When both upper incisors and first molars are present, there is no doubt that the intervening tooth germ is "supernumerary". However, in those instances where the first molar germ is absent, there can be doubt

whether a germ is "extra" or represents a rudimentary first molar. As these germs do not resemble molar germs in shape or position, it is more likely that they are supernumerary. This problem has been discussed by Kalter (1960) and Kalter & Deuschle (1966).

Changes in jaws, joints and muscles occurred frequently in rat embryos in the present investigation, in contrast to their infrequent occurrence in exencephalic mouse embryos (34 of 272) (*Knudsen*, 1966 b). Skeletal changes in exencephalic rat embryos resemble those previously described in mouse embryos, apart from the fact that cartilage is seldom found between upper and lower jaws in the mouse. Muscle and joint anomalies likewise resemble those described before. The origin of the temporal muscle will always be abnormal as the cranial roof is absent. Otherwise it is difficult to find any correlation between skeletal and soft tissue changes in rat embryos.

When the dental anomalies of rat embryos are compared with other anomalies, the general rule is that embryos with marked dental anomalies also have marked anomalies of jaws, joints and muscles. As many joint and muscle changes also occur in embryos with normal tooth germs (group III), dental anomalies can hardly be considered as primary in relation to the other changes. Considering that all the structures in which the defects described occur are developed from the mandibular arch (first branchial arch), it is probable that the cause of the anomalies should be sought in an abnormal development of the mesoderm of that arch, in which the neural crest is presumed to play an important part. The frequent occurrence of cartilage in locations where it does not normally occur, also suggests interference with the normal development of the mesoderm. The epithelial disturbances are less pronounced in rat embryos. This is seen in the low incidence of fusion of enamel organs and dental laminae both in the incisor and molar region.

SUMMARY

Exencephaly was induced in rat embryos by maternal hypervitaminosis A. Histologic serial sections of 38 exencephalic embryos were examined and the results compared with previous

investigations of exencephalic mouse embryos. The patterns of dental anomalies were entirely different; in mouse embryos a large number of the upper incisor germs were fused, while only one case of fusio epithelialis occurred in the rat embryos. A number of exencephalic rat embryos exhibited "supernumerary" tooth germs, agenesia of molar germs, maxillo-mandibular ankylosis, agenesia or incomplete development of temporo-mandibular joints, muscles of mastication, mylohyoid and digastric muscles. It seemed unlikely that dental anomalies could be considered an important factor in the development of subsequent anomalies of muscles and joints in that muscle and joint anomalies were observed in embryos with normal tooth germs. Considering that all affected structures are developed from the first branchial arch, it is suggested that the cause of the anomalies should be sought in an abnormal development of the arch mesoderm, probably derived from the neural crest.

RÉSUMÉ

MALFORMATIONS DENTAIRES CHEZ LES EMBRYONS DE RATS EXENCÉPHALES PAR SUITE D'HYPERVITAMINOSE A

L'exencéphalie a été provoquée chez des embryons de rats par hypervitaminose A maternelle. Des séries de coupes histologiques faites sur 38 embryons exencéphales ont été examinées et les résultats ont été comparés aux recherches faites antérieurement sur des embryons de souris exencéphales. Les types des anomalies dentaires étaient entièrement différents; les embryons de souris présentaient un grand nombre de fusions des germes d'incisives supérieures, tandis que, chez les embryons de rats, il ne s'est présenté qu'un seul cas de fusion épithéliale. Un certain nombre d'embryons de rats exencéphales présentaient des germes dentaires "surnuméraires", une agénésie de germes de molaires, une ankylose maxillo-mandibulaire, une agénésie ou un développement incomplet des articulations temporo-mandibulaires, des muscles masticateurs, mylo-hyoïdiens et digastriques. Il semble improbable que les anomalies dentaires puissent être considérées comme un facteur important dans le développement d'anomalies consécutives de muscles et d'articulations, puisque des anomalies musculaires et articulaires ont été observées chez des embryons

dont les germes dentaires étaient normaux. Etant donné que tous les éléments touchés se développent à partir du premier arc branchial, ces anomalies sembleraient devoir être imputées au développement anormal du mésoderme de l'arc, dérivant probablement de la crête ganglionnaire.

ZUSAMMENFASSUNG

DENTALE MISSBILDUNGEN IN EXENCEPHALEN RATTENEMBRYONEN DURCH ÜBERDOSIERUNG MIT A-VITAMIN VERURSACHT

Exencephalie wurde durch Überdosierung der Mütter mit A-Vitamin bei Rattenembryonen hervorgerufen. Histologische Serienschnitte von 38 exencephalen Embryonen wurden untersucht und die Ergebnisse wurden mit früheren Untersuchungen auf Mäuseembryonen verglichen. Die Muster der dentalen Anomalien waren völlig verschiedener Art; bei Mäuseembryonen war eine grosse Anzahl der oberen Schneidezahnkeime verschmolzen, während fusio epithelialis bei Rattenembryonen nur in einem Fall gefunden wurde. Eine Anzahl der exencephalen Rattenembryonen zeigte ''überzählige" Zahnkeime, Agenesie der Molarenkeime, maxillomandibulare Ankylose, Agenesie oder mangelhafte Entwickelung der Kiefergelenke, Kaumuskeln und der kranialen Zungenbeinmuskeln (M. mylohyoideus und M. digastricus). Die dentalen Missbildungen sind wahrscheinlich ohne Bedeutung für die Entwickelung der Muskel- und Gelenkanomalien, da diese Anomalien auch bei Embryonen mit normalen Zahnanlagen beobachtet wurden. Die Neuralleiste beteiligt sich am Aufbau des Mesoderms der Kiemenbogen, und da alle anomalen Strukturen vom ersten Kiemenbogen entwickelt sind wird es angenommen dass die Ursache der Anomalien in einer abnormen Entwickelung des Bogenmesoderms gesucht werden soll.

REFERENCES

Kalter, H., 1960: The teratogenic effects of hypervitaminosis A upon the face and mouth of inbred mice. Ann N. Y. Acad. Sci. *85:* 42—55.

Kalter, H. & F. M. Deuschle, 1966: Congenital malformations experimentally induced by hypervitaminosis A. In: Kreshover, S. J. & F. J. McClure (Eds.) Environmental Variables in Oral Disease. Washington. Amer. Ass. Adv. Sci. Symp. No. 81. p. 273—291.

Knudsen, P. A., 1965: Congenital malformations of upper incisors in exencephalic mouse embryos, induced by hypervitaminosis A. I. Types and frequency. Acta odont. scand. *23:* 71—89.

Knudsen, P. A., 1966 a): Congenital malformations of lower incisors and mo-

lars in exencephalic mouse embryos, induced by hypervitaminosis A. Acta odont. scand. *24:* 55—89.

Knudsen, P. A., 1966 b): Congenital malformations of the jaws and related structures in exencephalic mouse embryos with anomalous molar germs, induced by hypervitaminosis A. Acta odont. scand. *24:* 677—707.

Knudsen, P. A., 1966 c): Malformations of upper incisors in mouse embryos with exencephaly, induced by trypan blue. Acta odont. scand. *24:* 647—675.

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

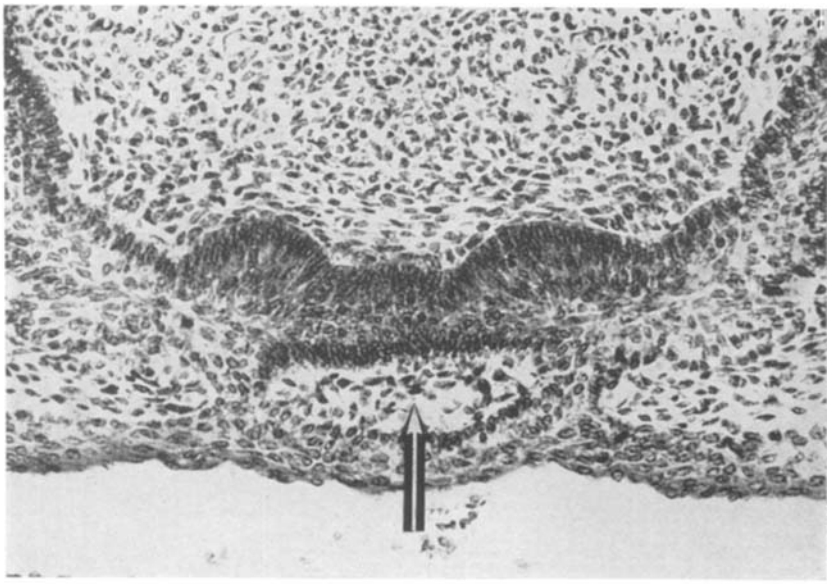
PLATES

PLATE 1

Figs. 1 a and b. Normal rat embryo (209—5). Frontal sections. Oral epithelium between upper incisor germs (x 160).

Fig. 1 a The niche with mesenchyme (arrow).

Fig. 1 b The epithelium lingually to the niche.



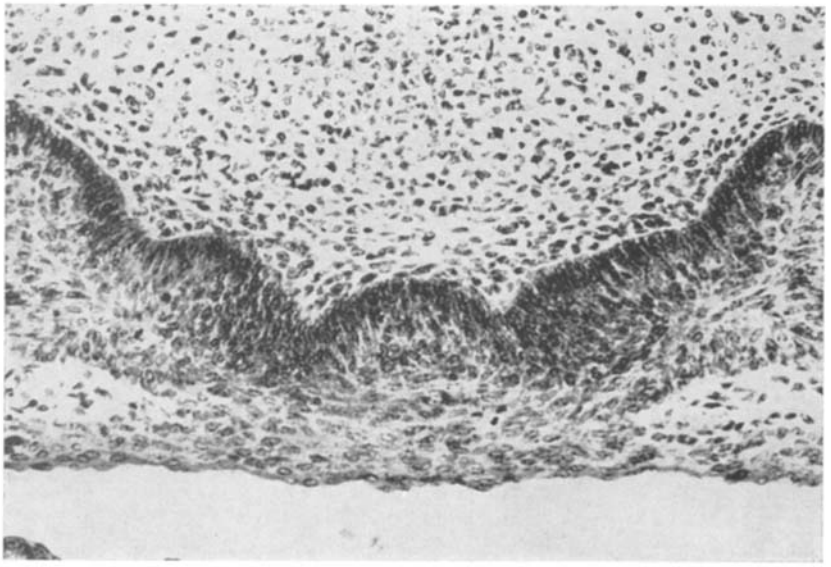
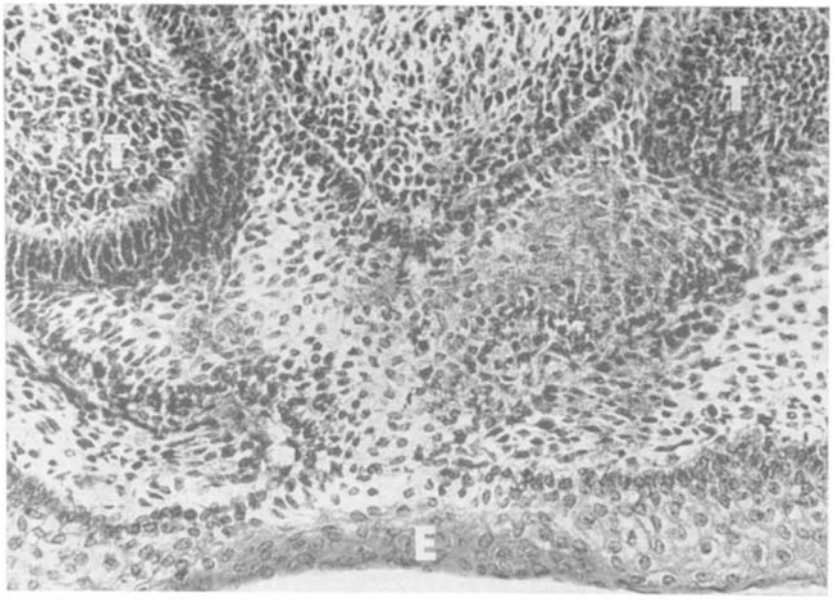


PLATE 2

Fig. 2. Exencephalic rat embryo (116—4). Frontal section.
Fusio epithelialis.
The stellate reticula and outer dental epithelia form a Y-shaped connection between the upper incisor germs and the oral epithelium (x 160).
T = tooth germ.
E = oral epithelium.

Fig. 3. Exencephalic rat embryo (208—4). Frontal section. "Supernumerary" tooth germ. Note the cartilage (C) on the labial side of the tooth germ (x 25).
N = nasal capsule.
T = tongue.

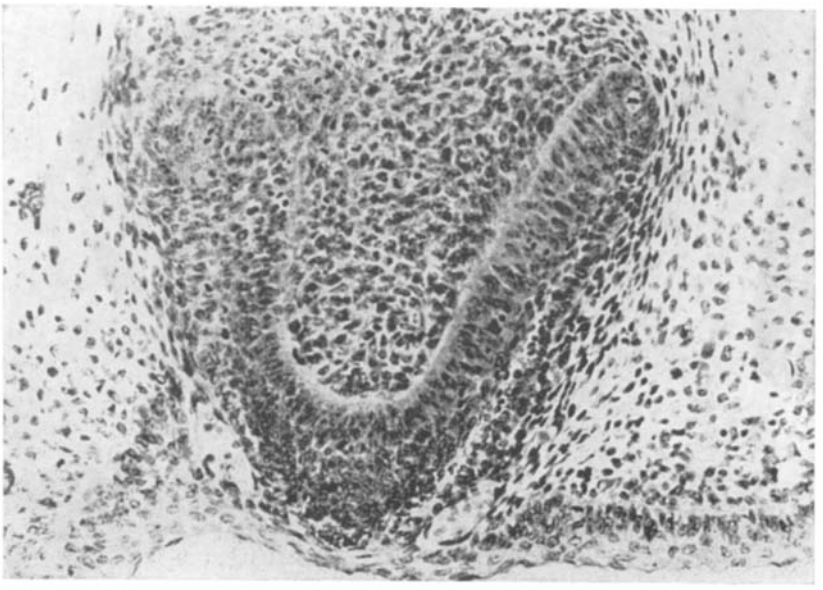

T
T
E

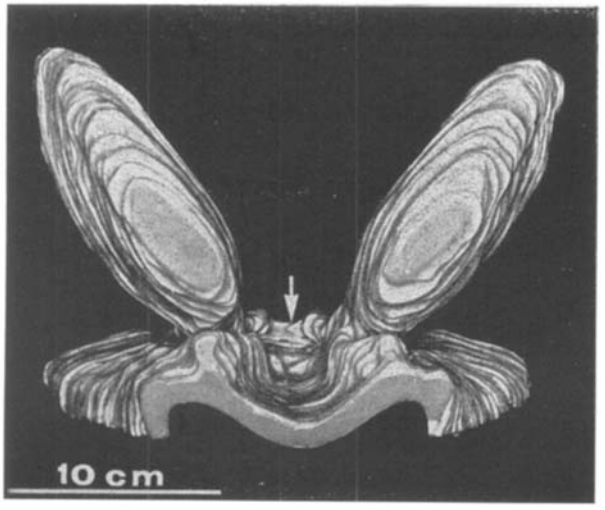

N
C
T

PLATE 3

Fig. 4. Exencephalic rat embryo (204—4). Frontal section. "Supernumerary" tooth germ (x 160).

Fig. 5. Normal rat embryo (209—5).
Photograph of a wax plate reconstruction of upper incisor germs and oral epithelium. Arrow: epithelial crest and niche.




10 cm