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CONGENITAL MALFORMATIONS OF LOWER  
INCISORS AND MOLARS IN EXENCEPHALIC MOUSE  
EMBRYOS, INDUCED BY HYPERVITAMINOSIS A

by

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INTRODUCTION

Malformations of tooth germs occur frequently in exencephalic mouse embryos, where the brain defect is induced by administering large doses of vitamin A to pregnant mice. The upper incisors in particular are affected and the commonest deformity is fusion, *fusio dentium*, of the two incisor germs. Fusion occurs in 51.5 % of the embryos examined and has been described in detail, together with other changes in the incisors of the upper jaw (Knudsen, 1965 a, b). In the same embryos, occasional malformations are found in the incisor germs of the lower jaw and in molar germs of the upper and lower jaws. The present paper summarizes these malformations and gives a short description of some morphological details.

METHODS AND MATERIALS

Exencephaly is induced by the administration of 4,000—15,000 I.U. vitamin A to mice of an inbred strain (AK/a) on the 7th to the 9th day of pregnancy. Details are described in previous papers. Histological serial sections of 272 exencephalic embryos, 17

**Table I**  
*272 exencephalic mouse embryos divided into four groups according to the state of the lower incisors. For comparison the corresponding figures for the upper incisors are added (in brackets)*

	Two incisors		Fusio dentium	Agenesis dentium		Total
	Separated by connective tissue	In contact		Unilateral	Bilateral	
	Group I	Group II	Group III	Group IV		
Number of embryos	263 (107)	0 (2)	5 (140)	0 (3)	4 (20)	272 (272)
Per cent	96.7 (39.3)	0 (0.7)	1.8 (51.5)	0 (1.1)	1.5 (7.4)	100 (100)

—19 days old, were examined, every section engaging tooth germs being studied with the aid of a projection microscope (Visopan, Reichert). All important changes with regard to the number of germs, and their form and location, were registered on a special form. Serial sections of 100 normal embryos, which have been employed in earlier studies, were here too used in comparison.

RESULTS

Lower Incisors

Of the embryos examined (272), only nine show changes in the lower incisors. Table I shows the frequency of *fusio dentium* and *agenesia* in the lower, compared with the upper jaw. Fusions, which are divided into the same categories as for the upper jaw, are found in five embryos: two having *fusio totalis*, two *fusio partialis* and one *fusio subtotalis*. The central part of the fused germs is the same for all three degrees of fusion. In *fusio totalis*, the germ is found without sign of cleavage, whilst the germ is divided both labially and lingually in *fusio partialis* and only lingually in *fusio subtotalis*.

A series of frontal sections through a lower incisor with *fusio subtotalis* is illustrated in Figs. 2 a—g. (Fig. 1 shows normal in-

Table II

*Agenesis of molar germs in 24 exencephalic embryos*

Number of molar germs lacking	Number of embryos	Per cent
1	9	37.5
2	3	12.5
3	1	4.2
4	5	20.8
5	2	8.3
6	3	12.5
7	0	0.0
8	1	4.2
	24	100.0

Table III

*Number of absent first and second molar germs in 24 exencephalic embryos*

Molar germs lacking	Number	Per cent
First molar (upper and lower)	35	47.3
Second molar (upper and lower)	39	52.7
	74	100.0

cisors for comparison). First, a tangential section through the labial end of the enamel organ is seen (Fig. 2 a). In the following sections, all the layers in the enamel organ, together with the pulp and dentine, cross the mid-line, in agreement with the definition for *fusio dentium* (Figs. 2 b and c). It is further apparent that the "dental lamina" consists almost entirely of stellate reticulum and outer dental epithelium. The next sections demonstrate the decisive influence of Meckel's cartilage on the shape of the tooth germ. In contact with the cartilage, the inner dental

Table IV

*Normal and malformed upper incisors in 248 exencephalic embryos with all (8) first and second molar germs present and in 24 exencephalic embryos with reduced number of molar germs*

Upper incisors	Embryos with 8 molar germs present		Embryos with reduced number of molar germs		Molars absent	
	Number	Per cent	Number	Per cent	Number	Per cent
Separate (normal)	61	24.6	2	8.3	6	8.1
Connection between stellate reticula	44	17.8	2	8.3	7	9.5
Fusio dentium	130	52.4	10	41.7	18	24.3
Agenesis	13	5.2	10	41.7	43	58.1
	248	100.0	24	100.0	74	100.0



epithelium disappears and the odontoblast layer is reduced in height (Fig. 2 d). More lingually, the area of contact of the dental epithelium with Meckel's cartilage becomes strongly concave, and the central part of the organ becomes thinner (Figs. 2 e and f). The dental germ and Meckel's cartilage show a simultaneous cleavage (Fig. 2 g).

*Fusio epithelialis* (i.e. the only connection being between the stellate reticula in the incisal part of the enamel organs) is not found in lower incisors, whilst this type of connection occurs frequently between upper incisors (in 46 out of 109 embryos).

Two embryos exhibit fusion of the incisors in both upper and lower jaws, whilst one embryo with *fusio dentium* in the lower jaw has bilateral agenesis of the upper incisors (Table V). Two embryos have fused lower incisors and normal upper incisors (not represented in Table V, as the number of molars is normal). Agenesis of the lower incisors is found in only four of the 272 embryos examined and is in all cases bilateral (Table I and V). Bilateral agenesis of the upper incisors is found in the same embryos (Table V).

#### Molar germs

At the end of intra-uterine life, mouse foetuses usually have eight molar germs, the first and second upper and lower molars being present whilst the third molars are still lacking. Of 2176 potential molar germs in 272 embryos, 74 germs are lacking (3.4 %). As it will be apparent from Table II, 24 of the embryos show reduction in the number of molar germs, and frequently (9 embryos) only one germ is lacking in each embryo. In the material studied, the first and second molar germs are lacking with roughly the same frequency (Table III). An attempt to determine the frequency of molar agenesis in the upper and the lower jaw respectively had to be abandoned, as in 19 (out of 74) cases it is not possible to determine in which jaw the germ is lacking. This is because the remaining molar germ cannot be assigned to the upper or the lower jaw, being located between the two jaws, with the apical end directed laterally towards the cheek. The germ is often large and is connected to the oral epithelium by a horizontal dental lamina (Figs. 15, 16 and 17).

**Table VI**  
*Various connections between molar germs or their dental laminae in embryos with all molar germs present and with the number reduced*

	"Epithelial connections" (number)		Fusio partialis (number)		Fusio totalis (number)	
	First upper and lower molars	Second upper and lower molars	First upper and lower molars	Second upper and lower molars	First upper and lower molars	Second upper and lower molars
All molar germs present	9	8	2	0	0	0
Reduced number of molar germs	1	6	0	0	12	7

These conditions may, as will be discussed, indicate a complete 'fusion' of the upper and lower molar germs with consequent reduction of the total.

The frequency of fusion and agenesis of the upper incisors in embryos with a normal number of molar germs, and in embryos where the number of germs is reduced, is given in Table IV. It should be noted that only two out of 24 embryos with a molar reduction have normal incisors in the upper jaw. 10 of the 23 embryos with agenesis of the upper incisors are found in the group with reduced number of molar germs, and this reduction is considerable, 43 out of 80 potential germs being absent in these 10 embryos. It is further apparent that *fusio dentium* of the upper incisors occurs in 41.7 % of embryos with molar reduction and in 52.4 % with the normal number.

Table V shows how many molar germs are lacking in each of the 24 embryos where the number is reduced, also whether normal or malformed upper and lower incisor germs are found. In four embryos, bilateral agenesis of both upper and lower incisors is found in connection with a considerable reduction in the number of molar germs, 24 of the potential 32 germs being absent. In two embryos with *fusio dentium* (*partialis* and *totalis*) of the lower incisors and *fusio totalis* of the upper incisors, five molar germs are missing in each case.

In embryos (248) with the normal number of molar germs, 17 cases are found with epithelial connections of varying extent between the molar germs of the upper and lower jaws (Table VI). The least pronounced connections occur between the dental laminae alone, with the enamel organs quite separate; whereas in more pronounced cases the stellate reticula are common too. Finally, *fusio partialis* is found in two cases, all the layers of the enamel organs together with the dental papillae being common, except mesially and distally.

In embryos (24) with a reduction in the number of molar germs, 7 purely epithelial connections and 19 cases of *fusio totalis* are found between molar germs in the upper and lower jaws (Table VI). In the same embryo, several epithelial connections or an epithelial connection and a *fusio partialis* or *totalis* may be found.

In the following, a short description is given of the most im-

portant connections between the molar germs in the upper and lower jaws. Figs. 3 and 4, showing normal molar germs and dental laminae, and the normal vestibule in 15- and 18-day old embryos, are available for comparison. It is remarkable that the vestibule is well developed when the molar germs are still in the bud stage (Fig. 3).

Connection between parts of the dental laminae for the first upper and lower molar germs is shown in Figs. 5 a—d. At the mesial end the two laminae are separated by the vestibule (Fig. 5 a). More distally, parts of the epithelial surfaces are connected and the vestibule is represented by a recess only (Fig. 5 b). In the following sections it almost entirely disappears (Fig. 5 c), to enlarge again near the distal end of the germ (Fig. 5 d). The connection between the laminae of the second upper and lower molar germs of the same embryo has roughly the same appearance and the vestibule is found here too in the form of a recess.

In another embryo, the vestibular lamina and the dental laminae of the first upper and lower molar germs have a common origin from the oral epithelium (Fig. 6). In this case there is no separation between the laminae; the vestibule is absent, whilst the vestibular lamina is clearly seen laterally.

The epithelial connection between the first upper and lower molar germs illustrated in Fig. 7, resembles those described, but the two germs lie closer and are more intimately connected. The vestibular lamina cannot be identified with certainty.

An even more pronounced connection between the first upper and lower molar germs is seen in Figs. 8 and 9. These germs are close and there is no trace of either vestibular lamina or vestibule.

A broad epithelial connection between the germs of the second upper and lower molars is seen in Fig. 10. The outer dental epithelium in the two germs continues in the peripheral cell layers of the epithelial connection, whilst the stellate reticulum is developed only adjacent to the inner dental epithelium.

An example of proximity of the second upper and lower molar germs with common stellate reticulum in the majority of sections is seen in Figs. 11 a and b. At the extreme mesial end, the stellate reticulum is divided by a layer of undifferentiated epithelium (Fig. 11 a). This layer has disappeared in Fig. 11 b.

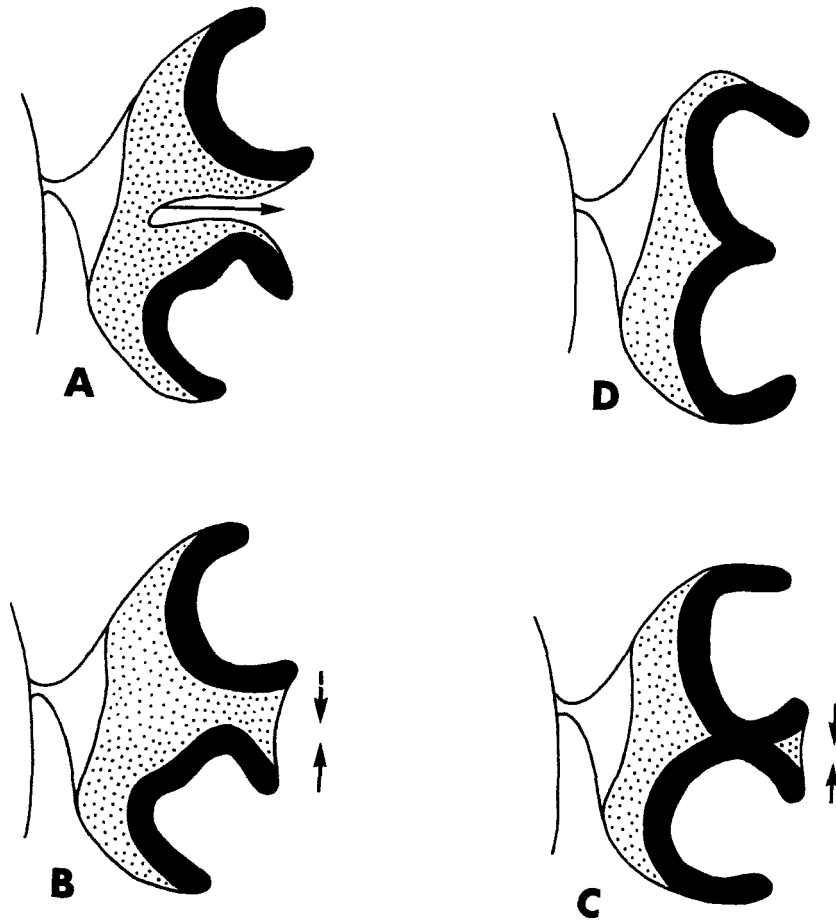


Fig. 1. Diagrammatic representation of the transition area between the mesially divided and centrally undivided parts of a molar germ with fusio partialis.

- A: Frontal section of the mesial parts of the germ. Stellate reticula and outer dental epithelia are common while inner dental epithelia and dental papillae are separate, being on each side of the 'occlusal plane'. (arrow).
- B: Stellate reticula are common to a greater extent than in A. The arrows indicate the direction of approach, shown in the next figure.
- C: Contact has been established between the inner dental epithelia. Note part of the stellate reticulum and outer dental epithelium apically for the area of contact.
- D: All layers of the enamel organs and the dental papillae are common, crossing the 'occlusal plane'. This figure thus represents 'total fusion'.

In the next case the stellate reticulum is common in all sections of the second upper and lower molar germs (Fig. 12), but the inner dental epithelia and dental papillae of the two germs are still separate.

A complex system of epithelial connections is illustrated in Fig. 13, where the dental laminae of the first lower and the first and second upper molar germs have a common origin from the oral epithelium.

A well-developed connection between the first upper and lower molar germs, corresponding to *fusio partialis* of the incisors, is seen in Fig. 14. A large germ is found, cleft mesially and distally (Figs. 14 a, b and i), whilst centrally there is a connection across the 'occlusal plane' between all the layers of the enamel organ and the dental papilla (Fig. 14 f). The gradual transition between the cleft and fused parts of the germ is seen in Figs. 14 c, d, e, g and h. The transition is illustrated diagrammatically in Text Fig. I. A and B show connections of different extent between the stellate reticula. C shows 'contact' between the inner dental epithelia in the two parts of the germ. On each side of the area of contact the stellate reticulum and outer dental epithelium are seen and when the apical part of these layers disappears, the inner dental epithelia and the dental papillae form continuous layers across the occlusal plane as illustrated in D. If this occurs everywhere in the germ, the connection is a *fusio totalis*.

In several cases 'giant molars' are found. It is assumed that each of these corresponds to two normal germs, most often to the first upper and lower molars (cf. Table VI and Figs. 15, 16 and 17). This interpretation is supported as mentioned above by a simultaneous reduction in the number of molar germs and by the location of the large germs in relation to the jaw skeleton. Examination of all histological sections reveals an absence of all signs of cleavage in these germs, which thus correspond to incisors with *fusio totalis*.

#### DISCUSSION

There is a considerable difference in the frequency of malformations of the upper and lower incisors in exencephalic mouse embryos. *Fusio dentium* of the lower incisors is found in only

1.8 %, whereas a connection between the two upper incisors is demonstrated in 68.4 %, either in the form of *fusio dentium* (51.5 %) or of *fusio epithelialis* (16.9 %), where there is a connection only between the incisal parts of the stellate reticula. As the latter form is not found in the lower jaw there is a qualitative as well as a quantitative difference between the malformations of the upper and lower incisors. Fusion of the lower incisors has an incidental character in contrast to the regularly occurring fusions of the upper incisors. This impression is supported by the occurrence of *fusio partialis* in the lower jaw of an otherwise normal mouse embryo which has not received large doses of vitamin A. Connection between the incisors of the upper jaw has never been found in normal embryos. It has not been possible to find definite changes in Meckel's cartilage in cases of *fusio dentium* in the lower jaw in spite of the close relationship between the cartilage and the fused germs.

In embryos lacking a single molar germ, it is often difficult to determine whether it is one of the normal upper or lower germs which is missing, or whether the reduction is due to 'fusion' of a germ in the upper and a germ in the lower jaw. The answer to this question is determined by the position of the germ in relation to the jaw skeleton. A plane roughly corresponding to the occlusal plane will here be just as relevant for the molars as the mid-line is for the incisors, where one can speak of unilateral agenesis if the whole of the remaining germ is found on one side of the mid-line, and *fusio dentium*, if the germ crosses the mid-line. If a molar germ lies on both sides of the 'occlusal plane' the term *fusio totalis* is employed. It cannot be entirely excluded that agenesis can occur in one jaw whilst a normal germ in the other assumes an abnormal position due to skeletal changes. It seems justified to speak of fusion, however, since germs with *fusio partialis*, where the two 'original' enamel organs can still be seen, have the same position in relation to the jaw skeleton as germs with presumed *fusio totalis*.

Varying degrees of epithelial connection, as well as true fusions of enamel organs and dental papillae occur in molar as in incisor germs. In the case of the incisors, the epithelial connection always consists of stellate reticulum (and outer dental epithelium), whatever the distance between the germs, whereas in

the case of the molars there is only connection between the stellate reticula when the distance between the upper and lower germs is small. With increased distance, there is connection only between the dental laminae. This difference is perhaps due to the different structure of the dental laminae. In the incisors, the stellate reticulum is the most important part of the epithelial lamina connecting the germ with the oral epithelium, whilst in the molars, the stellate reticulum is only found in the bell-shaped portion of the enamel organ. An attempt to divide the epithelial connections of molar germs into two groups, one comprising fusion between dental laminae alone and the other between the stellate reticula, had to be abandoned, as in several cases it proved difficult to determine where the exact border is between the epithelium of the dental lamina and the stellate reticulum of the enamel organ. The problem is further complicated by the fact that the vestibular lamina often has an intimate connection with the dental laminae of the molar germs.

As demonstrated by *Warkany and Deuschle* (1955), changes in lower incisors can be induced in rats by maternal riboflavin deficiency. Molar agenesis and ectopy have been induced by vitamin A overdosage in rats and mice by *Deuschle, Geiger and Warkany* (1959) and *Kalter* (1960) respectively. *Ritter* (1963) has induced molar fusion by X-ray treatment of mice. It is difficult to compare the present material with the results of earlier studies, however, partly because these do not describe the molar changes in detail, and partly because the embryos in the present investigation are all exencephalic. Furthermore, the majority (68 %) show characteristic malformations of the upper incisors. A similar selection of material has not been made before as the aim of previous investigations has been to note the deformities accompanying the use of various teratogenic methods.

In a study of fused incisor germs at the bud stage, it has been shown that no fusion takes place, but that a single germ is formed from the beginning (*Knudsen, 1965 c*). In the material used to investigate the bud stage of the incisors, fused molar buds have not been found and only one case of connection between the dental laminae of molar germs. It has not been possible, therefore, to determine whether development proceeds as for the incisors, although it seems most likely. The fact that molar fusions are ac-

accompanied by considerable skeletal changes, whilst these are rare with fusio of incisors, suggests however, that the processes involved in the development of molar defects are deeper and more extensive. This, and the fact that rodent incisors represent a special type of tooth, call for caution in drawing too many conclusions on the basis of comparison between incisors and molars. The defects in the skeleton and muscles of the jaws are being studied in order to investigate the type and extent of the changes in the masticatory apparatus that accompany molar fusions.

The question of whether the tooth malformations described are a result of the brain defect or of the cranial deformities which accompany it, or whether they are due to the direct effect of vitamin A on the primordial tooth germs is still open.

#### SUMMARY

272 mouse embryos in which exencephaly has been induced by maternal overdosage of vitamin A were investigated with reference to dental malformations. Fusions of upper incisors in 51.5 % of the embryos have been described in detail in a previous article. The present paper reports malformations of lower incisors and of upper and lower molars in the same embryos. In spite of its rare occurrence (1.8 %), fusion of the lower incisors exhibited the same degrees as found in the upper jaw: fusio partialis, sub-totalis and totalis. However, fusion of the incisal parts of the stellate reticula only was never found in the lower jaw, in contrast to its frequent occurrence in the upper jaw. Agenesis of lower incisors, too, occurred only rarely.

Only 24 embryos show reduction in the number of molar germs. Most frequently (18 embryos), one to four germs are lacking in one embryo. The total number of absent molar germs is 74. In the case of only one molar being absent it is often impossible to decide whether the remaining giant germ belongs to the upper or lower jaw. Owing to its position and size it is considered to be similar to *fusio totalis* in the incisor region. *Fusio partialis* also occurs (in two embryos) and is described in detail. Various epithelial connections are found and described, partly between dental laminae and partly between stellate reticula. Transitional forms are frequent. The difference between epithe-

lial connections found between incisor and molar germs respectively is briefly discussed. The frequent occurrence of malformations of the jaw skeleton is mentioned.

RÉSUMÉ

**MALFORMATIONS CONGÉNITALES DES INCISIVES INFÉRIEURES ET DES MOLAIRES CHEZ DES EMBRYONS DE SOURIS EXENCÉPHALES PAR SUITE D'HYPERVITAMINOSE A**

272 embryons de souris présentant une exencéphalie produite par surdosage de vitamine A ont été examinés en ce qui concerne les malformations dentaires. Les fusions des incisives que présentaient 51,5 % des embryons ont été décrites dans un article antérieur. Le présent article rend compte des malformations des incisives inférieures et des molaires supérieures et inférieures chez les mêmes embryons. Bien qu'ayant une fréquence peu élevée (1,8 %) les fusions des incisives inférieures présentaient les mêmes degrés que les fusions trouvées à la mâchoire supérieure: fusion partielle, subtotala et totale. Cependant, la fusion limitée aux parties incisives des pulpes de l'émail n'a jamais été observée à la mâchoire inférieure, contrairement à ce qui se passe à la mâchoire supérieure où ce type de fusion a une fréquence élevée. De même, l'agénésie des incisives inférieures ne se produisait que rarement.

Chez 24 embryons seulement on observe une réduction du nombre des germes de molaires. Le plus souvent (18 embryons), il manque de un à quatre germes chez un embryon. Le nombre total de germes de molaires absents est de 74. Dans les cas où il ne manque qu'une molaire, il est souvent difficile de décider si le germe géant restant appartient à la mâchoire supérieure ou à la mâchoire inférieure. En raison de sa position et de sa taille, il est considéré comme analogue à la fusion totale observée dans la région incisive. La fusion partielle se produit aussi (chez deux embryons) et se trouve décrite en détail. Différentes connexions épithéliales sont observées et décrites, d'une part entre les lames dentaires, et d'autre part entre les pulpes de l'émail. Les formes de transition sont fréquentes. Les différences entre les connexions épithéliales observées entre les germes d'incisives et celles obser-

vées entre les germes de molaires font l'objet d'une brève discussion. Mention est faite de la fréquence des malformations du squelette des mâchoires.

#### ZUSAMMENFASSUNG

#### ANGEBORENE MISSBILDUNGEN DER UNTEREN SCHNEIDEZÄHNE UND MOLAREN IN EXENCEPHALEN MÄUSEMBRYONEN DURCH ÜBERDOSIERUNG MIT A-VITAMIN VERURSACHT

272 Mäuseembryonen, bei welchen mittels einer mütterlichen Überdosierung des A-Vitamins Exencephalie hervorgerufen wurde, wurden auf dentale Missbildungen untersucht. Fusionen der oberen Schneidezähne bei 51,5 % der Embryonen wurden in einer früheren Arbeit in Einzelheiten beschrieben. Der vorliegende Aufsatz berichtet von Missbildungen der unteren Schneidezähne sowie der oberen und unteren Molaren derselben Embryonen. Trotz ihres seltenen Vorkommens (1,8 %) ergab eine Fusion der unteren Schneidezähne dieselben Stufen, die im Oberkiefer vorgefunden wurden: *fusio partialis*, *subtotalis* und *totalis*. Eine Fusion der oralen Teile der Schmelzpulpen allein gab es aber im Unterkiefer nie im Gegensatz zu deren häufigem Vorkommen im Oberkiefer. Mangelhafte Entwicklung der unteren Schneidezähne kam auch nur selten vor.

Nur 24 Embryonen zeigen eine Reduktion der Anzahl von Molarenanlagen. Am häufigsten (18 Embryonen) fehlen 1-4 Anlagen in demselben Embryo. Die Totalanzahl der fehlenden Molarenanlagen ist 74. Im Falle, wo nur ein Molar fehlt, ist es öfters unmöglich zu entscheiden, ob der übriggebliebene "Riesenkeim" zu dem Ober- oder Unterkiefer gehört. Wegen seiner Position und Grösse wird er der *fusio totalis* der Schneidezahnregion gleichgestellt. *Fusio partialis* kommt auch (bei zwei Embryonen) vor und wurde in Einzelheiten beschrieben. Verschiedene epitheliale Verbindungen, teils zwischen den Zahnleisten und teils zwischen den Schmelzpulpen, wurden auch beobachtet und beschrieben. Übergangsformen kommen häufig vor. Der Unterschied epithelialer Verbindungen, die zwischen den Schneidezahn-, bzw. den Molarenanlagen vorgefunden wurden, wurde kurz erörtert. Das häufige Vorkommen von Missbildungen im Kieferskelett wird erwähnt.

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