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CONGENITAL MALFORMATIONS OF THE JAWS AND RELATED STRUCTURES IN EXENCEPHALIC MOUSE EMBRYOS WITH ANOMALOUS MOLAR GERMS, INDUCED BY HYPERVITAMINOSIS A

by

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INTRODUCTION

Fusion of the upper incisors is the most frequent oral malformation in exencephalic mouse embryos, where the brain defect has been induced by maternal overdosage with vitamin A (*Knudsen*, 1965). A small number of the embryos also shows molar agenesia or abnormal molar germs. Various epithelial connections between germs of upper and lower molars are of special interest. They are found either between the dental laminae alone or between the enamel organs. In the latter case the stellate reticula can be entirely or partly common. Partial and total fusions where both the enamel organs and the dental papillae are common have also been observed (*Knudsen*, 1966). In the embryos where only the incisors are affected, only a few malformations of the oral cavity and surrounding structures are found. The occurrence of molar anomalies, however, is often accompanied by malformations of the jaws, the temporo-mandibular joints and related soft tissues, in particular the muscles. The present paper outlines the nature and frequency of the most important of these

changes, with the object of investigating the connection between molar anomalies and malformations in the surrounding tissues.

MATERIAL AND METHODS

Exencephaly was induced by administering 4,000—15,000 I.U. vitamin A to mice of an inbred strain (AK/a) on the 7th—9th day of pregnancy. Details are given in a previous paper (*Knudsen,* 1965 a). Histological serial sections of 34 exencephalic mouse embryos, 18—19 days old, all with molar anomalies, were examined. All sections of the facial cranium were studied with the aid of a projection microscope (Visopan, Reichert). On a special form were registered all important changes in the jaws, temporomandibular joints, muscles of mastication, mylohyoid muscles, the anterior bellies of digastric muscles and Meckel's cartilage. Serial sections of normal embryos were used for comparison.

RESULTS

The embryos are divided into four groups on the basis of the molar anomalies:

Group I contains 10 embryos with various connections *between dental laminae or enamel organs of molar germs* in the upper and lower jaws, *'fusio epithelialis'*. In two cases the connection has developed into *fusio partialis,* as also the dental papillae have fused in the central part of the germ. All the embryos have a normal number of molar germs.

Group II comprises 10 embryos, with *fusio totalis* between upper and lower molar germs. As each germ of this type is considered equivalent to two normal germs, the number of molar germs is necessarily reduced. Sometimes, in the same embryo, molar germs can be absent for other reasons, and sometimes epithelial connections between other upper and lower molar germs are found. The decisive criterion for all embryos belonging to this type is the presence of fusio totalis.

Group III comprises 7 embryos with *light molar agenesia,* each embryo lacking one or two germs. Other molar changes are absent.

Group IV contains 7 embryos with *heavy molar agenesia,* four

to eight molar germs being absent in each embryo. Other molar changes do not occur in this group either.

Bone anomalies

The commonest bone anomaly in embryos with molar defects is an abnormal connection — maxillo-mandibular ankylosis — between the upper and lower jaws (Figs. 2, 3 and 4 compared with Fig. 1). Simultaneously with the disappearance of the boundaries between the individual bones, considerable changes in the shape of the jaws and sometimes in their position, often occur. These are, in particular, thickenings and lateral displacements.

Maxillo-mandibular ankylosis in an embryo of group I is shown in Text Figs. II—V, which are drawn from histological frontal sections. Text Fig. I shows the jaws of a normal embryo for comparison.

In front of the molar germs, the facial bones of each side form a continuous, solid column, stretching from the cartilaginous nasal capsule to the base of the mandible (Text Fig. II). The boundaries between the individual bones are entirely obliterated.

Further back, the connection between the upper and lower jaws has ended on the right side and the individual bones can be identified (maxilla, mandible, zygomatic arch), whilst on the left side there is still a connection between the bones, although the ankylotic areas are less solid (Text Fig. III).

The following section shows a connection between the dental laminae of the first upper and lower molar germs of each side (Text Fig. IV). Here the maxilla is no longer in connection with the mandible, whereas the latter retains its connection with the zygomatic arch.

Further back again, the zygomatic arch loses its connection with the mandible (Text Fig. V), but the two bones maintain a close relationship.

Ankylosis of the jaws occurs in 8 of the 10 embryos in group I (fusio epithelialis and partialis) (Text Fig. VI). The two embryos (62-2 and 90-2) lacking ankylosis have normal molar germs and sockets and the fusions between the dental laminae are of limited extent.

In group II (fusio totalis), maxillo-mandibular ankylosis is found in all 10 embryos (Text Fig. VII). The close relationship between ankylosis and the various connections between upper and lower molar germs can be seen in Text Figs. VI and VII, which show that uni- and bilateral connections are always accompanied by uni- and bilateral ankylosis respectively, apart from the two exceptions mentioned in group I.

In the embryos of group III (light molar agenesia), no ankylosis is found (Text Fig. VIII), whereas 5 of the 7 embryos in group IV (heavy molar agenesia) exhibit it (Text Fig. IX).

Text Fig. X shows the percentage of ankylotic jaws in each of the four groups.

Temporo-mandibular joints

The term 'agenesia' (agenesis) is used, when the joints are absent, and the term 'incomplete development' comprises any morphological deviation from normal joints (Fig. 6 compared with Fig. 5). The nature and extent of the anomalies vary considerably. There may be marked flattening of the articular fossa, deformation of the head of the mandible (condylar cartilage), displacement of the joint surfaces or absence of the joint-cavities.

Only 7 embryos (out of 34) have normal temporo-mandibular joints on both sides. The frequency of agenesia and incomplete development of the joints in each of the four groups is shown in Text Figs. VI—X. The largest number of normal joints is found in group III. In embryos of group II, only a few (3) are found and in group IV there are no normal joints. In the two latter groups, which have the heaviest tooth anomalies, agenesia is more frequent than incomplete development (Text Fig. X), whereas incomplete development is more common than agenesia in group I.

Muscles

The term 'muscle agenesia' is employed when muscle fibres are entirely absent in locations where muscles are normally developed. The term 'incomplete development' is used when the

muscles are small or consist of scattered thin fibres mixed with much connective tissue, also in cases where the origin or insertion is lacking. The term is used irrespective of whether all or only part of the muscle is incompletely developed.

Agenesia and incomplete development are common among the muscles of mastication. The temporal muscle is in no case normal (Text Figs. VI—X). Agenesia of this muscle is especially marked in group IV and in group II (Text Fig. X), whereas incomplete development is more frequent in group I and in group III. The lateral pterygoid muscle is the most frequently lacking muscle of mastication in all four groups. The changes in the masseter and medial pterygoid muscles are considerable, but variable. Text Figs. II—V show incomplete development of the masseter muscle on the left side and agenesia of this muscle on the right.

The two suprahyoid muscles examined behave differently, the mylohyoid muscle often being defective (Text Figs. V and VI—X) and agenesia being rare, whereas the anterior belly of the digastric muscle is often lacking (Text Figs. II—V and VI—X), but an incomplete development never observed.

If the four groups are compared with reference to the frequency of defects in the bones (maxillo-mandibular ankylosis), joints and muscles, it appears that the fewest changes occur in group III and the most in group II and group IV. It is moreover apparent that agenesia is commoner than incomplete development in groups II and IV. In group I, however, the incomplete development of joints and most muscles dominates (cf. Text Fig. X).

Meckel's cartilage

Meckel's cartilage is often asymmetrical, but otherwise shows remarkably few changes in the embryos examined. Asymmetry occurs especially when the lower jaw is askew as a result of unilateral maxillo-mandibular ankylosis. In one embryo in group IV (179-2) with agenesia of teeth, joints and muscles, Meckel's cartilage is entirely missing (Text Fig. IX). One embryo in group II (43-2) lacks Meckel's cartilage on the left side, together with most of the other structures examined (Text Fig. VII).

Fig. I. Normal mouse embryo (73 d VI/2).
Frontal (= coronal) section of the head through the first molar germs.
Solid: cartilage
Dotted: bone
Hatched: muscle

Abbreviations (Text Figs. I—V):
A: maxillo-mandibular ankylosis
D: digastric muscle (anterior belly)
DL: dental lamina
FU: first upper molar germ
FL: first lower molar germ
G: genioglossus muscle
GH: geniohyoid muscle
M: mandible
MA: maxilla
MC: Meckels cartilage
MM: masseter muscle
MY: mylohyoid muscle
Z: zygomatic arch

Fig. II. Exencephalic mouse embryo (176 d I/2).
Frontal section through the head just in front of the first molars. Maxillo-mandibular ankylosis on both sides. The left masseter is missing and the right masseter is defective. The digastric muscle is missing on both sides.
Abbreviations, see Text Fig. I.

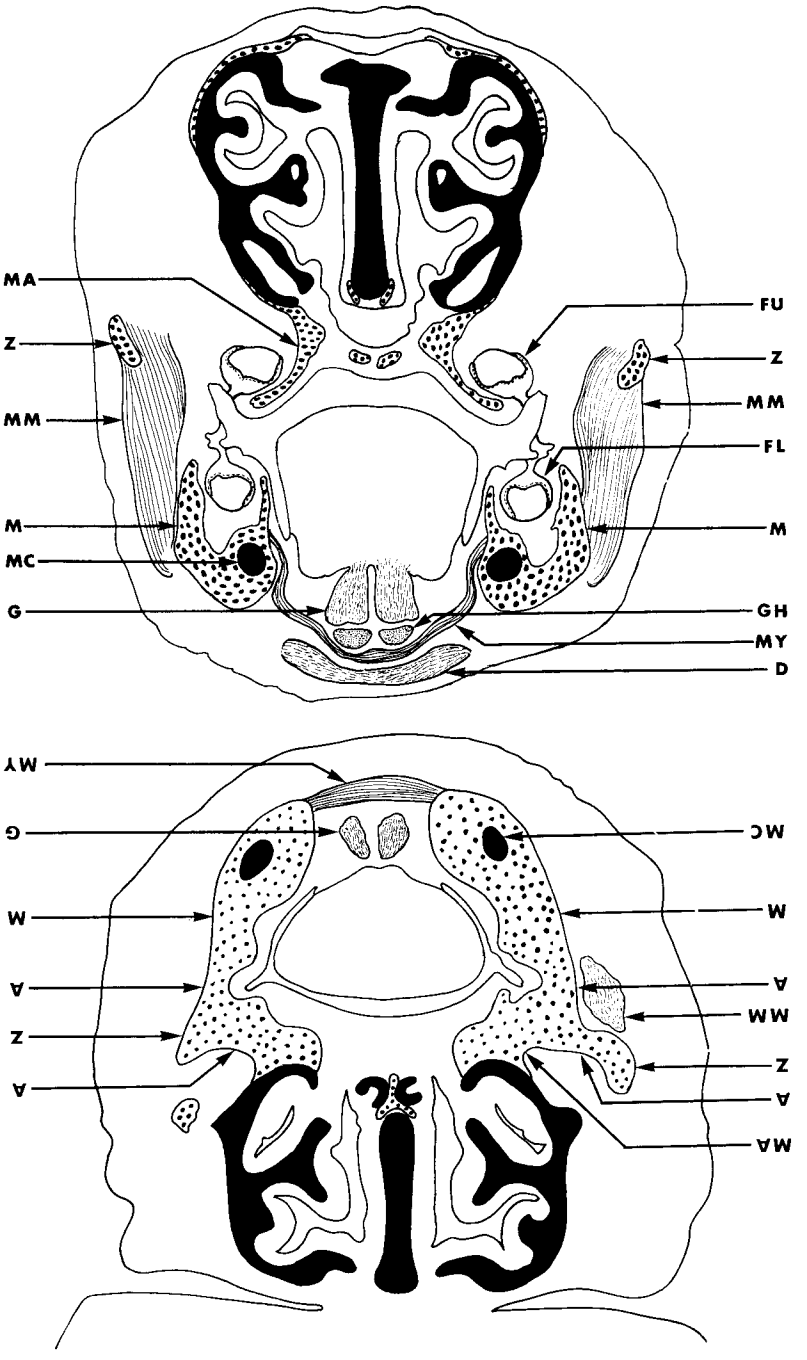
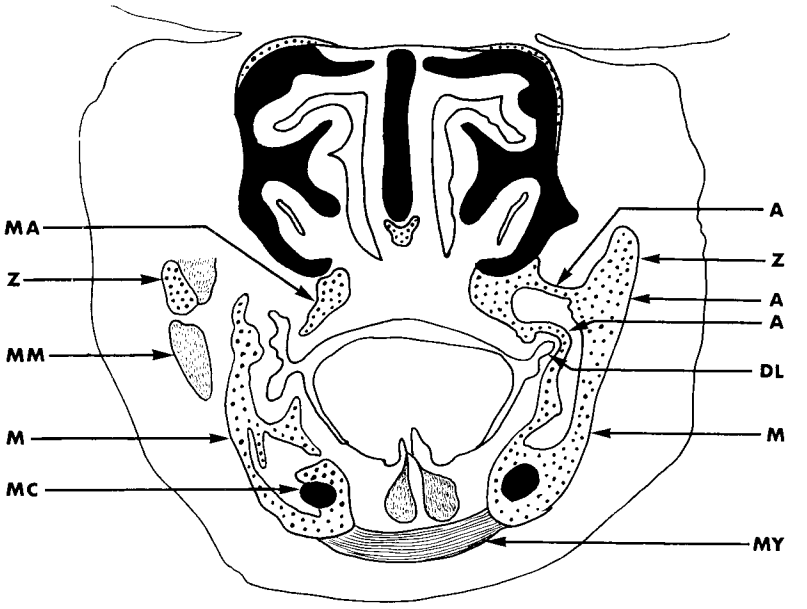

MA
Z
MM
M
MC
G
FU
Z
MM
FL
M
GH
MY
D

Fig. III. Exencephalic mouse embryo (176 d I/2).
Frontal section of the head through the anterior end of the dental laminae.
Left: maxillo-mandibular ankylosis
Right: bones separate
Muscles as in Text Fig. II
Abbreviations, see Text Fig. I.

Fig. IV. Exencephalic mouse embryo (176 d I/2).
Frontal section of the head through the first molar germs. The dental laminae are fused on both sides.
Left: maxillo-mandibular ankylosis
Right: bones separate
Muscles as in Text Fig. II.
Abbreviations, see Text Fig. I.


MA
Z
MM
M
MC
A
Z
A
A
DL
M
MY

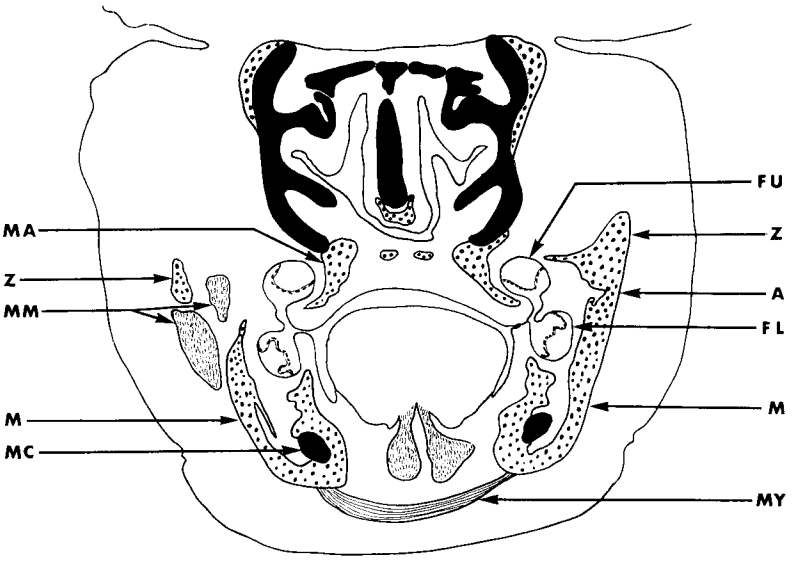

MA
Z
MM
M
MC
FU
Z
A
FL
M
MY

Fig. V. Excencephalic mouse embryo (176 d I/2).
Frontal section of the head through the first molar germs, distally to the section shown in Text Fig. IV.
Ankylosis has ceased on both sides. The mylohyoid muscle is defective. The other muscles are as in Text Fig. II.
Abbreviations, see Text Fig. I.

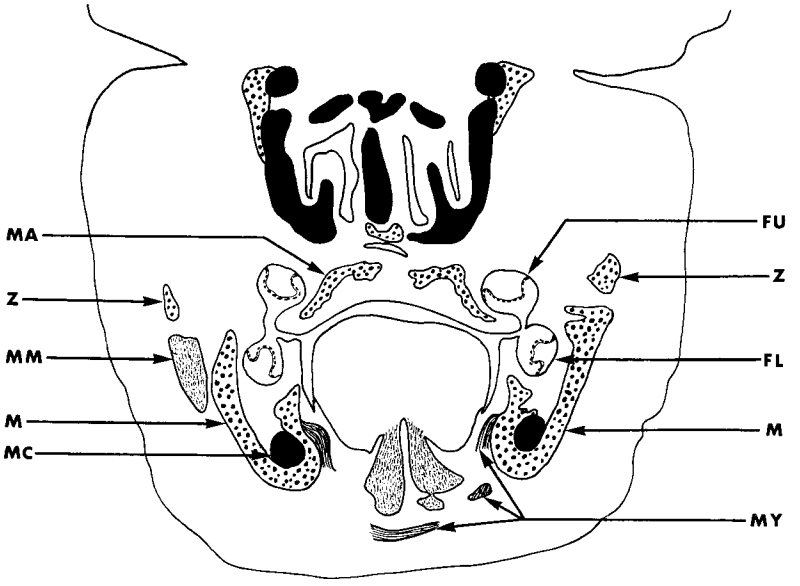

MA
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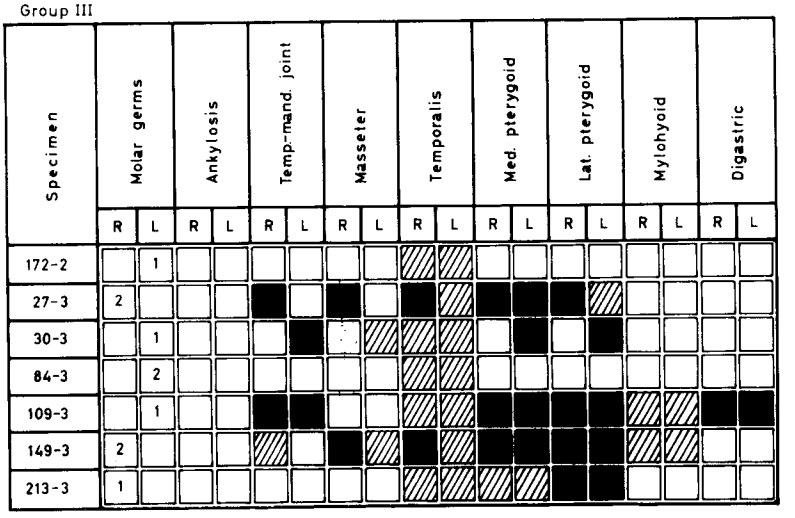
Fig. VI. Group I: 10 embryos with fusio epithelialis and partialis of molar germs. The chart indicates for each specimen the type of fusion of the molar germs, maxillo-mandibular ankylosis, and the normal, anomalous and missing structures named in the upper line.

Text Figs. VI—IX:

R: right
L: left
O: fusio epithelialis of molar germs
◡: fusio partialis of molar germs
●: fusio totalis of molar germs
Unshaded: normal structure
Hatched: anomalous structure
Solid: agenesia

Fig. VII. Group II: 10 embryos with fusio totalis of molar germs. The chart indicates for each specimen the type of fusion of the molar germs, maxillo-mandibular ankylosis, and the normal, anomalous and missing structures named in the upper line.
Abbreviations, see Text Fig. VI.

Group III



Group IV

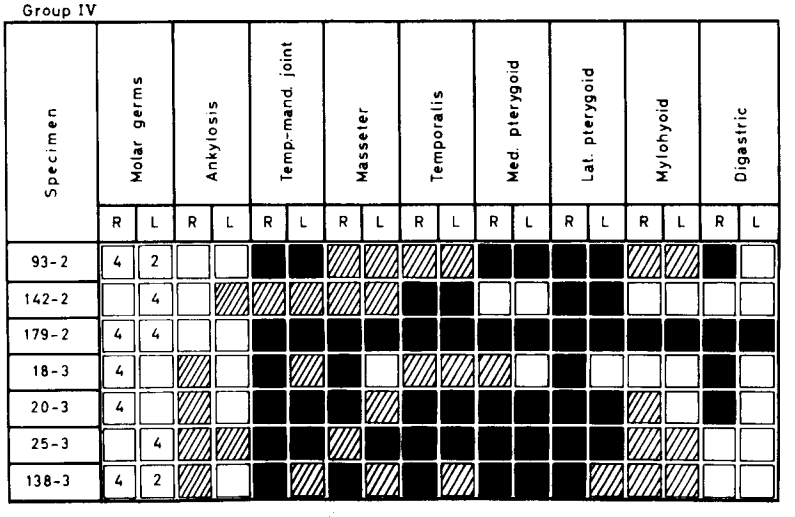
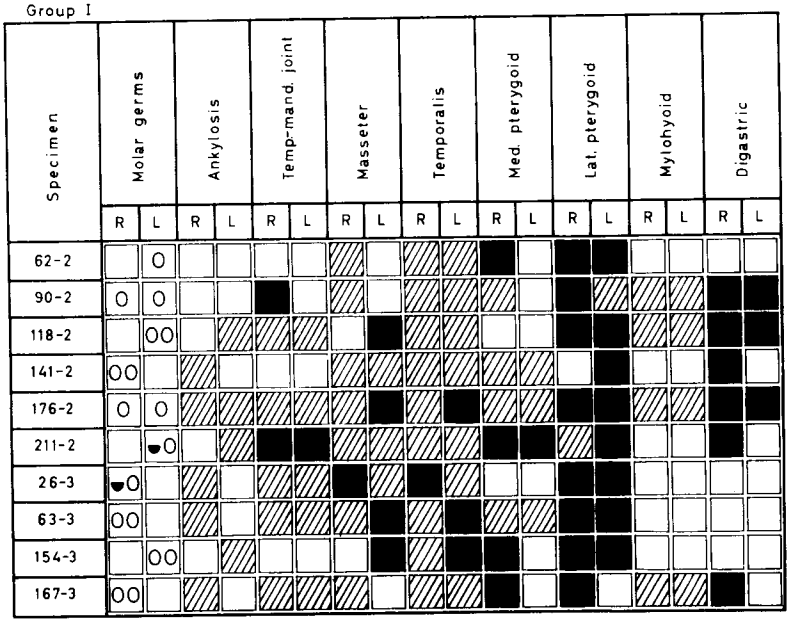


Fig. VIII. Group III: 7 embryos lacking one or two molar germs. The chart indicates for each specimen the number of missing molar germs, and the normal, anomalous and missing structures named in the upper line. There is no maxillo-mandibular ankylosis.
Abbreviations, see Text Fig. VI.

Fig. IX. Group IV: 7 embryos lacking four to eight molar germs. The chart indicates for each specimen the number of missing molar germs, maxillo-mandibular ankylosis, and the normal, anomalous and missing structures named in the upper line.
Abbreviations, see Text Fig. VI.

Group I



Group II

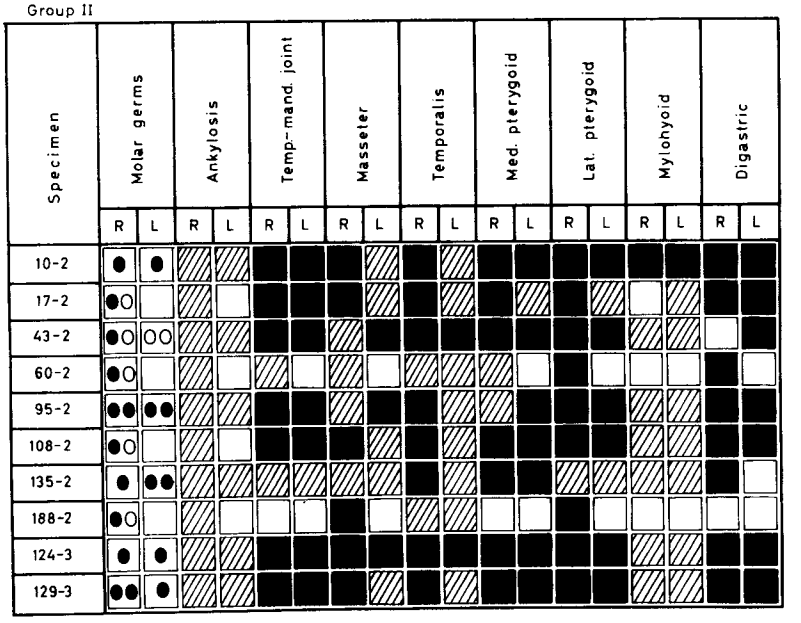
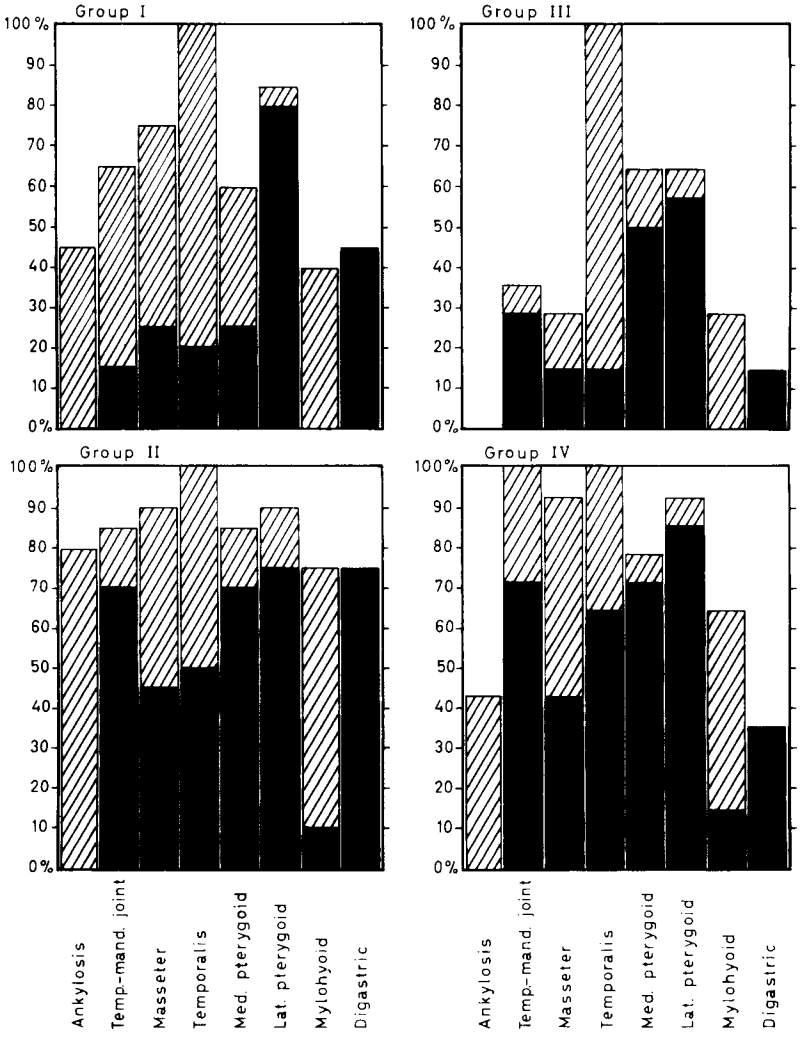


Fig. X. The percentage of anomalous and missing structures in each of the four groups shown in figs. VI—IX.
Solid: missing structure
Hatched: anomalous structure.


Group I
Group III
Group II
Group IV
100 %
90
80
70
60
50
40
30
20
10
0 %
Ankylosis
Temp.-mand. joint
Masseter
Temporalis
Med. pterygoid
Lat. pterygoid
Mylohyoid
Digastric

DISCUSSION

Apart from stating the number of defective jaws, joints and muscles, it is hardly possible to evaluate quantitatively the anomalies described above. An evaluation of the intensity of the defects, on the basis of histological sections, must of necessity be rough, and an attempt at graduation was abandoned. The frequency with which agenesia or incomplete development of the individual structures occurs is different for the different groups of embryos (Text Fig. X). The only exception is the temporal muscle. The fact that this muscle is in no case normal is undoubtedly due to the total or partial absence of the top of the skull in exencephalic embryos, so the normal origin of the muscle has entirely disappeared or has been severely reduced. In the same way, the many cases where the lateral pterygoid muscle is defective or absent, can be explained by the abnormal development of the region of the temporo-mandibular joint, where the muscle is normally inserted.

An interesting result of the present study is the demonstration of a simultaneous occurrence of fused molar germs (dental laminae or enamel organs) and maxillo-mandibular ankylosis in embryos of groups I and II, whilst embryos in group III lack both fusions of molar germs and ankylosis. On this basis, it is tempting to assume that a normal epithelial differentiation, i.e. formation of normal dental and vestibular laminae is a necessary prerequisite for a normal development of upper and lower jaws. Examination of the embryos in group IV would seem to eliminate the possibility of a connection between epithelial fusion and ankylosis, as in this group ankylosis occurs in embryos with heavy molar agenesia without fusions of the upper and lower molar germs. However, group IV may be said to occupy a special position on account of the severe developmental disturbances, which must be the cause of the heavy agenesia. The many defects of muscles and joints which occur simultaneously with the maxillo-mandibular ankyloses, could be considered as secondary consequences of the bone anomalies, especially of the immobilisation of the jaws which the ankylosis is assumed to cause. This conception is, however, difficult to maintain, when one com-

pares the number of joint and muscle defects with the number of ankyloses. There is only an approximate accordance in group II, whilst in the other groups considerably more joint and muscle defects are found than ankyloses. Thus group III comprises a large number of defective joints and muscles, but no ankyloses. In group IV there are numerous joint and muscle defects in proportion to the number of ankyloses. Considering these facts it is not immediately obvious that an incomplete epithelial differentiation should be the primary or even a very important cause of the many defects described above.

With further consideration of the location of the primary change and its cause, it is interesting to note that all the structures in which the defects described occur, are developed from the first branchial arch (the mandibular arch). It is therefore possible that the cause of the anomalies should be sought in an early disturbance of the development of the mesoderm in the first branchial arch, especially in the development of the mesectodermal cell masses deriving from that part of the neural crest, from which the trigeminal ganglion is formed. This part of the neural crest probably makes an important contribution to the mesoderm of the first branchial arch and thereby to the formation of many of its derivatives, including the dental papillae.

As shown by *Hörstadius & Sellman* (1946) on lower animals (Ambystoma), the neural crest plays a decisive role in the formation of large parts of the cranium. *Sellman* (1946) has demonstrated that the cells which form the dental papillae in Ambystoma derive from the neural crest. He obtained rudiments of bones after transplanting neural crest and oral endoderm into the trunk and assumes that the membrane bones of the jaw take their origin from mesectoderm. Studies by *Dalcq* (1953), *Milaire* (1959) and *Pourtois* (1961 and 1964) indicate strongly that the neural crest in mammals also forms mesectodermal cells which can be traced in the mandibular arch. During the closure of the neural groove to form the neural tube, the neural crest is derived from those parts of the neural folds which fuse. The neural crest cells leave the neural tube, and appear dorsally to the tube, lying under the surface epithelium of the back of the embryo (*Hörstadius,* 1950). In exencephalic embryos, however, the neural groove

remains open with a great distance between the neural folds, whereby a normal location of the neural crest is excluded, and a normal development must be considered highly unlikely. If the primary cause of the extensive defects in bones, joints and muscles is to be sought in the development described, the fusion of dental laminae or enamel organs of the upper and lower molar germs becomes secondary in relation to the mesodermal changes.

This investigation was supported by grants from The Danish State Research Foundation and Fonden til fremme af praktisk og videnskabelig odontologi.

SUMMARY

Exencephaly was induced in mouse embryos by administering large doses of vitamin A to the mothers on the 7th—9th day of pregnancy. In histological serial sections of 34 exencephalic mouse embryos with molar agenesia or anomalies, were found maxillo-mandibular ankylosis, agenesia or incomplete development of the temporo-mandibular joints, the muscles of mastication, the mylohyoid and digastric muscles.

Ankylosis occurs in nearly all embryos having fused upper and lower molar germs. Ankylosis is not found in embryos with light molar agenesia (one or two germs absent), but is frequently found in embryos with heavy molar agenesia (4—8 germs absent). It appears that 35 % (light molar agenesia) to 100 % (heavy molar agenesia) of the temporo-mandibular joints are absent or defective. The temporal muscle is lacking or defective in all embryos, whilst the changes in the other muscles of mastication vary. The digastric muscle is often lacking, but has not been found defective, whereas the mylohyoid muscle is most often defective.

It is discussed, whether the anomalies of the jaws and surrounding structures are due to anomalous epithelial differentiation, whereby fused dental and vestibular laminae are formed, or to a disturbed development of the mesoderm of the first branchial arch and particularly the mesectodermal cells derived from the neural crest. The development of the latter is disturbed, as the neural tube does not close in exencephalic embryos.

RÉSUMÉ

MALFORMATIONS CONGÉNITALES DES MACHOIRES ET DES TISSUS VOISINS CHEZ LES EMBRYONS DE SOURIS EXENCÉPHALES PAR SUITE D'HYPERVITAMINOSE A ET PRÉSENTANT DES GERMES DE MOLAIRES ANORMAUX

Une exencéphalie a été provoquée chez des embryons de souris par l'administration de doses massives de vitamine A aux mères pendant les 7ème—9ème jours de la gestation. Des séries de coupes histologiques faites sur 34 embryons de souris exencéphales présentant une agénésie de molaires ou des anomalies au niveau de ces dents ont mis en évidence des ankyloses maxillo-mandibulaires, des agénésies ou le développement incomplet des articulations temporo-mandibulaires, des muscles masticateurs, du mylo-hyoïdien et du digastrique.

L'ankylose se produisait chez presque tous les embryons présentant une fusion de molaires supérieures et inférieures. On ne trouvait pas d'ankylose chez les embryons présentant une agénésie peu marquée de molaires (absence d'un ou deux germes), mais elle était souvent présente chez les embryons ayant une agénésie marquée de molaires (absence de 4—8 germes). Il apparaît que, dans une proportion allant de 35 % (agénésie peu marquée de molaires) à 100 % (agénésie marquée de molaires), les articulations temporo-mandibulaires font défaut ou sont défectueuses. Le muscle temporal est absent ou défectueux chez tous les embryons, tandis que les altérations des autres muscles masticateurs sont variables. Le digastrique est souvent absent, mais n'a jamais été trouvé défectueux, tandis que le muscle mylo-hyoïdien est défectueux dans la majorité des cas.

Les anomalies des mâchoires et des tissus voisins sont-elles dues à une différenciation épithéliale anormale déterminant une fusion des lames dentaires et vestibulaires, ou bien sont-elles dues à la perturbation du développement du mésoderme du premier arc branchial et en particulier des cellules mésectodermiques dérivées de la crête ganglionnaire? Le développement de cette dernière est perturbé, le tube neural ne se fermant pas chez les embryons exencéphales. Ces deux possibilités font l'objet d'une discussion.

ZUSAMMENFASSUNG

ANGEBORENE MISSBILDUNGEN DER KIEFER UND DER UMGEBENDEN STRUKTUREN IN EXENCEPHALEN MÄUSEEMBRYONEN MIT ANOMALIEN DER MOLAREN DURCH ÜBERDOSIERUNG MIT A-VITAMIN VERURSACHT

Exencephalie bei Mäuseembryonen wurde durch Verfütterung grosser Dosen A-Vitamin am 7.—9. Tag der Gravidität bei schwangeren Mäusen hervorgerufen. An histologischen Schnittserien von 34 exencephalen Mäuseembryonen mit Agenesien oder Anomalien der Molaren wurde Ankylose der Kieferknochen, Agenesie oder unvollkommene Entwicklung der Kiefergelenke, Kaumuskeln, des M. mylohyoideus und des M. digastricus gefunden.

Ankylose kommt bei fast allen Embryonen vor, die Verschmelzung der Anlagen der Molaren im Ober- und Unterkiefer aufweisen. Es gibt keine Ankylose bei leichter, aber oft bei schwerer Agenesie der Molaren. Kiefergelenke fehlen oder sind unvollständig entwickelt in 35 % (leichte Agenesie der Molaren) bis 100 % der Fälle (schwere Agenesie der Molaren). M. temporalis fehlt oder ist schlecht entwickelt bei sämtlichen Embryonen, während Veränderungen der sonstigen Kaumuskeln variieren. M. digastricus fehlt oft, wurde aber sonst normal vorgefunden, wogegen M. mylohyoideus meistens nicht normal ist.

Es wird erörtert, ob die beschriebenen Anomalien der Kiefer und der umgebenden Strukturen mangelhafte epitheliale Differenzierung zurückzuführen sind, wodurch sich zusammenhängende Zahn- und Vestibularleisten bilden, oder ob der Grund eine mangelhafte Entwicklung vom Mesoderm des Mandibularbogens und besonders der mesectodermalen Zellen ist, die von Crista neuralis stammen. Die Entwicklung der Crista neuralis wird gestört, da die Neuralplatte bei der Entwicklung der Exencephalie nicht in ein Neuralrohr umgebildet wird.

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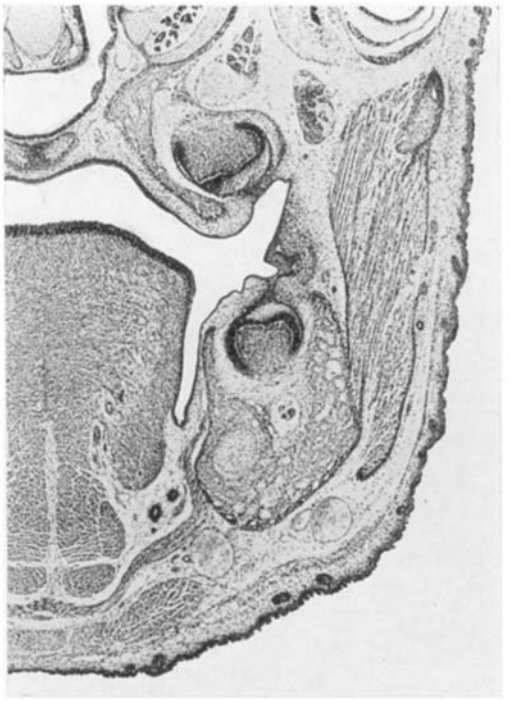
PLATES

Plate 1.

Fig. 1. Normal mouse embryo (73 d VI/2).
Frontal section of the head through the first molar germs (× 25).

Fig. 2. Exencephalic mouse embryo (60 d I/2).
Frontal section of the head through the fused first molar germs.
Arrow: maxillo-mandibular ankylosis (× 25).



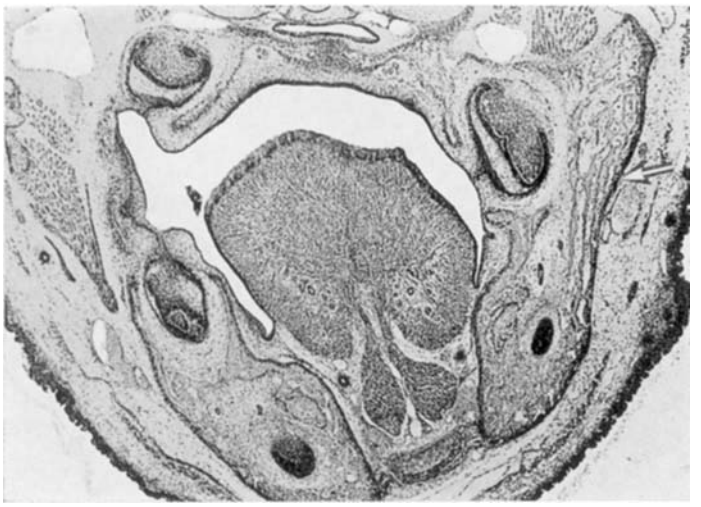
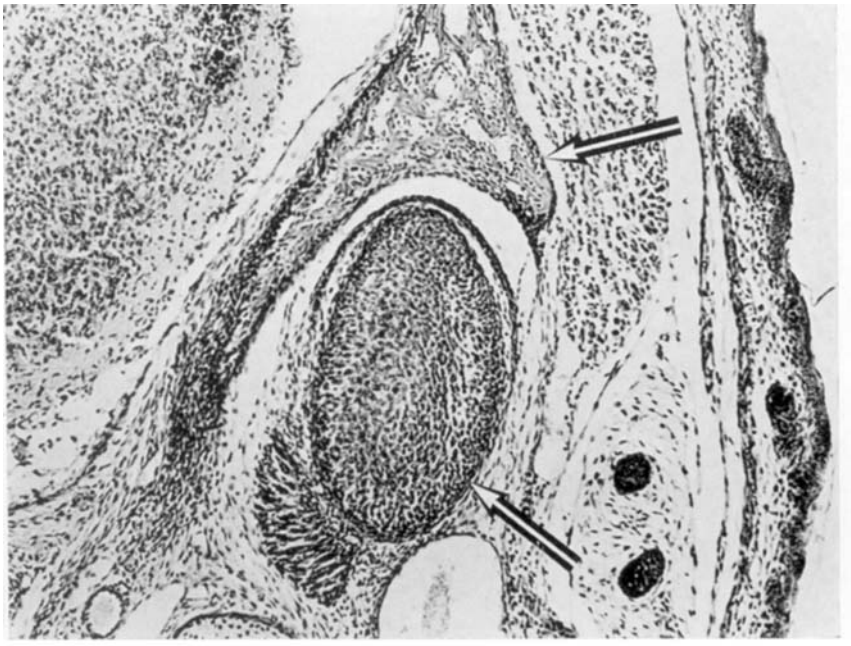


Plate 2.

Fig. 3. Exencephalic mouse embryo (108 d I/2).
Frontal section of the head in front of the first molar germs.
Arrows: maxillo-mandibular ankylosis (× 25).

Fig. 4. Exencephalic mouse embryo (108 d I/2).
Area of maxillo-mandibular ankylosis in Fig. 3 in higher magnification (× 63).



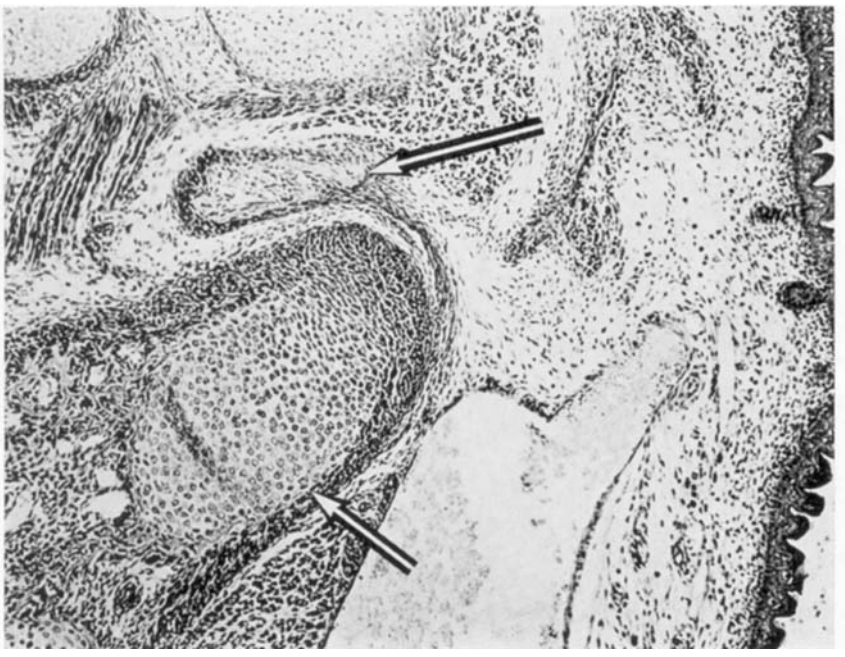


Plate 3.

Fig. 5. Normal mouse embryo (73 d VI/2).
Frontal section of a normal temporo-mandibular joint (× 63).
Upper arrow: articular fossa.
Lower arrow: head of the mandible (condylar cartilage).

Fig. 6. Exencephalic mouse embryo (118 d I/2).
Anomalous temporo-mandibular joint. Note the articular fossa (upper arrow) and the direction of the head of the mandible (condylar cartilage) (lower arrow) (× 63).