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A Comparison Between Trisomy 12 and Vitamin A Induced Exencephaly and Associated Malformations in the Mouse Embryo*

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Summary. Morphological and morphometric comparison of exencephaly in 13–15 day old mouse embryos caused either by chromosomal trisomy (Ts 12) or the teratogenic action of Vitamin A disclosed significant similarities as well as distinct differences: similarities included an open cranial vault and laterally everted brain, shortening of the sagittal and transversal axis of the skull base, caudal shift of the optic axis, deformation and dislocation of the hypophysis and inner ear due to a change of growth vectors of the skull base and its surrounding tissue. The differences were that in Ts 12 the caudal shift of the eyes causes enophthalmia, while after Vitamin A application additional malformations of the facial bones and defective formation of the orbits result in exophthalmia; the inner ear shows developmental retardation and hypoplasia only in Ts 12, but severe impairment of the development of all parts of the ear with complete lack of the outer and middle ear characterizes Vitamin A damage. Similarly, marked inhibition of hypophyseal development, the residues of Rathke's pouch, and cleft of the basal sphenoid occurs after Vitamin A, but is absent in Ts 12.

The differences in the exencephalic malformation complex suggest that the area affected in early organogenesis which determins the later malformation, is limited to the paraxial mesoderm in Ts 12, whereas Vitamin A treatment produces additional head mesenchyme abnormalities, in particular of neural crest derivatives. Although Vitamin A-damaged embryos show more extensive malformations, Ts 12 embryos have a shorter lifespan. This is a consequence of the fact that all the cells of the embryo are endowed with the abnormal chromosomal condition.

Key words: Mouse embryo – Exencephaly – Skull base – Hypophysis – Inner ear – Experimental Trisomy – Teratogenesis – Vitamin A.

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Introduction

Defects of closure of the neural tube are among the most common congenital malformations in man. Their aetiology is unknown in the majority of cases. However, it is well established that in several animal species malformations of the neural tube may be induced experimentally by a great variety of exogenous teratogens (Giroud, 1960). Moreover, neural tube defects, especially exencephaly, occur in certain mouse mutants (Bonnevie, 1943; Grüneberg, 1953), and as a consequence of chromosome inbalance in the mouse, as shown by Snell and Picken (1935) in the offspring of X-irradiated males and by Gropp and Kolbus (1974) by systematic induction of trisomy of chromosome No. 12.

One has to conclude that, in general, the aetiology of neural tube defects is multifactorial, involving environmental agents as well as endogenous or genetic factors. There is also the possibility of overlapping of different causative factors in individual cases. Undoubtedly the fusion of the neural wall is complex (Geelen and Langman, 1979), and several tissue components are involved (Marin-Padilla, 1970, Morriss and New, 1979). One can anticipate therefore that the pathogenesis and the actual expression of neural tube defects may show distinct differences according to their specific aetiology. Relatively little work has been devoted to these comparative aspects of neural tube malformations.

It is the aim of the present paper to examine the morphological patterns of exencephaly caused by two different aetiological factors, namely exencephaly found in mouse embryos with trisomy of chromosome No. 12 (Ts 12) and the malformation complex resulting from Vitamin A given to the mother. Particular attention was paid to the involvement of the skull base, the neighbouring cranial structures and organs such as the orbits, the hypophysis and the inner ear.

This study provides an opportunity to consider the biological differences between the chromosome abnormality which, as a systemic disorder, affects all cells of a developing organism, and the effects of an exogenous teratogen upon an embryo whose intact tissues are exposed only temporarily to the agent causing damage. The scope of the present investigation is determined by a special interest in the late developmental stages of day 13 to 15 when the malformation is fully expressed. It is understood that the primary causative factors leading to the exencephalic malformation act upon the much earlier steps of morphogenesis when, on day 8 to 9, the neural tube normally closes (Schneider and Norton, 1979).

Material and Methods

1. Mouse Stocks and General Procedures

Mice with the Robertsonian metacentric chromosomes Rb5/Rb9Bnr (Gropp et al., 1972) are bred on a mixed wild type background in homozygous lines. For the experimental matings with "all acrocentric" mice, females of the outbred strain NMRI (Han) were used.

The appearance of vaginal plug after mating was counted as day 1. The pregnant females were killed on days 13 to 15 of gestation.

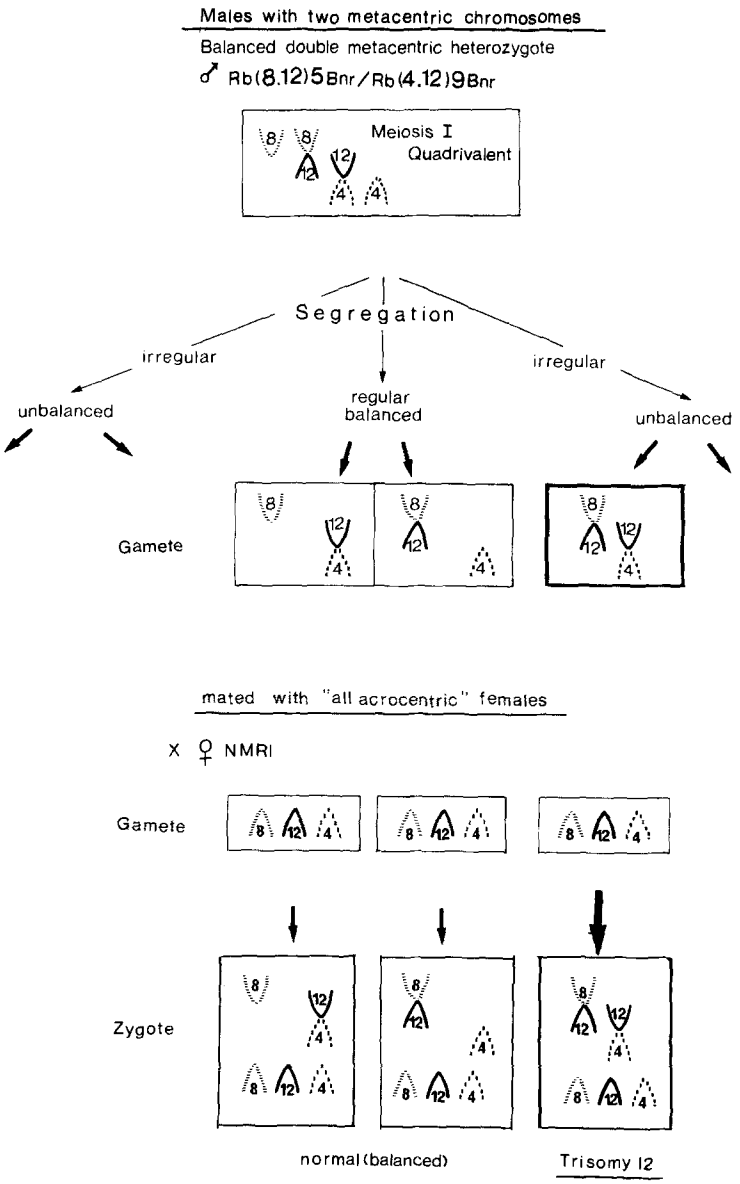

Fig. 1. Breeding design for the induction of Ts 12 in mouse embryos. Arrows without assignment refer to other types of malsegregation of Rb5/Rb9 heterozygote (not relevant for the present study)

2. Induction of Trisomy No. 12

Male mice doubly heterozygous for the two partially homologous Robertsonian metacentric chromosomes Rb(8.*12*)5Bnr and Rb(4.*12*)9Bnr (Rb5Bnr and Rb9Bnr) were mated with female laboratory mice NMRI. A considerable rate of meiotic nondisjunction of the two metacentric chromosomes

is expected to occur, besides normal segregation, in the gametogenesis of the heterozygous male. From previous observations (Gropp et al., 1975), it is apparent that the fetal progeny surviving day 10 includes only normal embryos and embryos trisomic for chromosome No. 12 (see Fig. 1).

3. Induction of Exencephaly by Treatment of the Mother with Vitamin A

Excess Vitamin A administered to a pregnant mouse during the critical period of neural tube closure (day 8 to 9) leads to nonclosure of the neural tube in some of the embryos (Giroud and Martinet, 1960). Using the breeding system explained above (2), 35 of 69 females were treated on day $8^1/_2$ of gestation with 12,500 i.u. Vitamin A, i.p., whereas 34 of 69 females remained untreated. According to morphological and karyological criteria the following 5 types of fetal progeny were available.

Type 1. Normal embryos from untreated mothers. Balanced karyotype with heterozygosity of Rb metacentric (cytogenetic marker: presence of one metacentric).

Type 2. Exencephalic embryos from untreated mothers. Unbalanced karyotype with Ts 12 (cytogenetic marker: presence of two metacentrics).

Type 3. Exencephalic (non-trisomic) embryos from Vitamin A treated mothers. Balanced karyotype with heterozygosity of Rb metacentric (cytogenetics as in type 1).

Type 4. Non-exencephalic (non-trisomic) embryos from Vitamin A treated mothers. Balanced karyotype with heterozygosity of metacentric (like type 1 and 3), some of them without macroscopically visible malformations (*Type 4a*), and others with malformations of the face and/or the caudal spinal cord (*Type 4b*).

Type 5. Embryos with Ts 12 from Vitamin A treated mothers. Trisomic karyotype as in type 2.

Type 1 fetuses were used as controls and those belonging to type 2, 3 and 4b as experimental groups. Fetuses of type 4a and 5 were not considered for the present study.

4. Cytogenetic Analysis

In order to facilitate chromosome preparations, Colcemid (0.25 ml CIBA®) was injected i.p. to the mother 2 h before sacrifice. After opening the uterus and the individual chorionic sacs, the extraembryonic membranes of each live implant were quickly transferred to hypotonic saline for 30 min, and further processed by standard techniques of 3:1 alcohol – acetic acid fixation, air-dried preparation and Orcein or Giemsa staining. For ascertainment of euploidy versus Ts 12 the demonstration of the presence of one rather than two metacentrics, and the total count of 40 versus 41 chromosome arms is sufficient.

5. Morphological Studies

All implants, live and resorbed, were serially recorded. The embryos were fixed in Bouin's fluid. Documentary photography and weighing were done in 80% alcohol 3 weeks later.

At least four embryos of the groups 1, 2, 3 and 4b were selected for careful examination. They were cut in serial frontal sections of 4–5 μ and stained with hematoxylin-eosin. The morphometric evaluation of the areas occupied by the orbits and ocular bulbs was performed with the aid of M.O.P., KM II (manual optic picture analysis system, Kontron®). The volume of these organs was calculated on the basis of the measured areas using the formula (Blinkov and Glezer, 1968):

$$V = \frac{A \times n}{(m)^2} \times d.$$

V = volume; A = measured area of the organ; n = number of sections; d = distance between sections; m = magnification.

By the same method the volume of the total embryo was determined as a reference value for the volume of the organs.

In addition to frontal sections at least two embryos of each type were cut serially in sagittal sections.

Results

1. Ts 12 in the Fetal Progeny of Rb(8.12)5Bnr/Rb(4.12)9Bnr Males

After day 11/12 the fetal progeny of males doubly heterozygous for the metacentrics Rb5Bnr/Rb9Bnr mated with "all acrocentric" females comprises chromosomally balanced normal implants (Type 1) and Ts 12 embryos (Type 2). The upper part of Table 1 records the progeny on days 13, 14 and 15 of a total of 34 NMRI females belonging to the experimental material of this study in the proper sense. Additional data of similar composition from separate experimental series covering the longer developmental period between day 10 and day 19 are collected in the lower part of Table 1.

Some of the exencephalic embryos were autolytic, and chromosome analysis sometimes failed in these cases, mainly when autolysis affected advanced developmental stages. In other embryos with exencephaly the karyological analysis was successful. They invariably showed two metacentric chromosomes and 41 chromosome arms in metaphase spreads. They were, therefore, trisomic for chromosome No. 12. Only one out of 120 successfully karyotyped embryos with Ts 12 did not show exencephaly. The proportions of embryos found to be both trisomic and exencephalic varied between 22% (day 10) and 11% (day 19) of all live embryos, and between 20% and 7% of all implants.

2. Fetal Progeny of Rb5Bnr/Rb9Bnr Males with Females Treated with Vitamin A During Pregnancy

The observations made in the 13 to 15 day progeny of 35 females mated with metacentric heterozygotes, and treated on day $8^1/_2$ with 12,500 i.u. Vitamin A are listed in Table 2. The chromosomally balanced (=balanced heterozygous) implants are subdivided according to whether the embryos do (Type 3) or do not (Type 4) show exencephaly.

No further analysis of Ts 12 embryos from treated mothers (Type 5) was attempted to this study. The percentage of these trisomic embryos varied between 4% to 2% of all implants, and 4% to 0% of the live progeny. The low number of trisomic embryos on day 15 (for comparison see Table 1) and the respective increase of the number of resorptions can be explained by earlier death of Ts 12 embryos following additional Vitamin A treatment of the mother.

Among the chromosomally balanced embryos, only a minority, namely 3.5% of all implants or 6% of all live embryos on day 15, exhibited exencephaly and spina bifida (Type 3). The non-exencephalic embryos (Type 4) showed varying degrees of abnormality of the face (see below). Survival of the malformed fetuses from Vitamin A treated females was not impaired until day 19, as revealed by additional experiments from day 17 to day 19.

Table 1. Fetal progeny of ♂ Rb5/Rb9 Bnr × ♀ NMRI

Stage of development	Number of pregnant females	Number of implants	Exencephalic embryos				Non-exencephalic embryos			Resorptions (moles)
			Total number	Autolytic	Chromosome preparation		No visible malformation	Euploid	Trisomy 12	
					successful	Trisomy 12				
(A) Data evaluated for the present study										
day 13	11	132	18 (14%)	5	18/18	18	84 (63%)	84	–	30 (23%)
day 14	12	124	14 (11%)	6	12/14	12	83 (67%)	82	1	27 (22%)
day 15	11	142	14 (10%)	8	11/14	11	96 (68%)	96	–	32 (22%)
(B) Data from additional experiments: The developmental profile of Trisomy 12										
day 10	5	61	12 (20%)	–	12/12	12	41[a] (67%)	36	–	8 (13%)
day 11	5	71	13 (18%)	–	13/13	13	41 (58%)	41	–	17 (24%)
day 12	8	109	14 (13%)	–	14/14	14	72 (66%)	72	–	23 (21%)
day 13	6	72	10 (14%)	3	10/10	10	46 (64%)	46	–	16 (22%)
day 14	8	101	13 (13%)	4	10/13	10	67 (66%)	66	–	21 (21%)
day 15	7	91	11 (12%)	5	10/11	10	60 (66%)	60	–	20 (22%)
day 16	8	93	15 (16%)	10	7/15	7	53 (57%)	53	–	25 (27%)
day 17	5	56	6 (11%)	5	1/6	1	32 (57%)	32	–	18 (32%)
day 18	5	60	5 (8%)	4	2/5	2	38 (63%)	38	–	17 (28%)
day 19	4	53	4 (7%)	4	0/4	–	31 (59%)	31	–	18 (34%)

[a] 5 other aneuploid embryos on day 10: 3 triploidies, 2 combined triploidies with monosomy

Table 2. Fetal progeny of ♂ Rb5/Rb9 Bnr × ♀ NMRI treated with 12,500 i.u. Vitamin A i.p. on day $8^1/_2$ of pregnancy

Stage of development	Number of pregnant females	Number of implants	Exencephalic embryos					Non-exencephalic embryos			Resorptions (moles)
			Total number	Autolytic	Chromosome preparation			No visible malformation	Malformations[a]	Euploid	
					successful	Euploid	Trisomy 12				
(A) Data evaluated for the present study											
day 13	10	139	8 (6%)	3	8/8	4	4	35 (25%)	31 (22%)	66	65 (47%)
day 14	11	145	9 (6%)	5	8/9	4	4	38 (26%)	35 (24%)	73	63 (44%)
day 15	14	170	9 (5%)	6	9/9	6	3	27 (16%)	59 (35%)	86	75 (44%)
(B) Data from additional experiments: Developmental profile after Vitamin A treatment											
day 17	2	21	1 (5%)	–	1/1	1	–	–	11 (52%)	11	9 (43%)
day 18	5	57	2 (4%)	–	2/2	2	–	–	26 (45%)	26	29 (51%)
day 19	2	25	1 (4%)	–	1/1	1	–	–	12 (48%)	12	12 (48%)

[a] All of them with craniofacial malformations and sometimes with exomyelia of caudal spinal cord

Table 3. Weight increase of normal and exencephalic fetuses (day 13 to day 15)

Stage of development	Non-exencephalic normal embryos			Exencephalic embryos with Trisomy 12			Exencephalic embryos due to Vitamin A		
Day	Number of weighed embryos	Mean weight $\bar{x}$ (g)	Standard deviation s	Number of weighed embryos	Mean weight $\bar{x}$ (g)	Standard deviation s	Number of weighed embryos	Mean weight $\bar{x}$ (g)	Standard deviation s
13	25	0.052	0.005	7	0.05	0.01	4	0.034	0.006
14	53	0.112	0.017	11	0.063	0.017	4	0.065	0.015
15	79	0.168	0.033	9	0.111	0.046	6	0.128	0.025

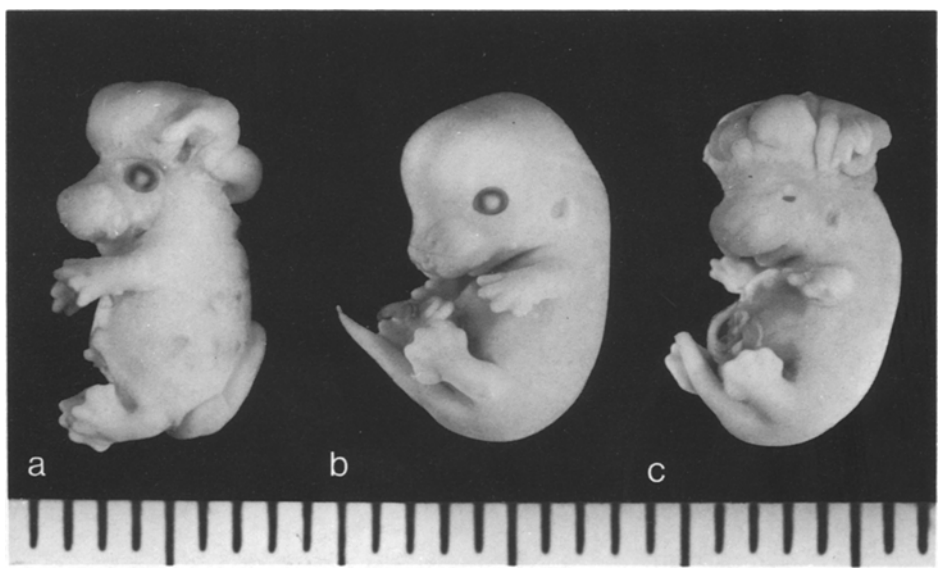

Fig. 2a–c. Macroscopical features of mouse embryos on day 15 (mm scale). **a** Vitamin A induced malformation including exencephaly and spina bifida; **b** normal embryo, in utero-mate to **c** Ts 12 with encephaly

3. Macroscopical Characteristics of Fetuses with Exencephaly. Comparative Description

Fetuses with exencephaly due to Ts 12 (Type 2) or to Vitamin A treatment of the mother (Type 3) showed hydramnios which was absent in non-exencephalic fetuses. A special feature of Ts 12 embryos is a soft, sometimes almost deliquescent consistence, apparently due to an increased water content of the tissues. The weight increase was considerably slowed down in both types of exencephalic fetuses, with a more distinct inhibition in Ts 12 (Table 3).

The malformation of the brain shows very similar features in Ts 12 and in Vitamin A-induced exencephaly (Fig. 2). The everted lobes of the telencepha-

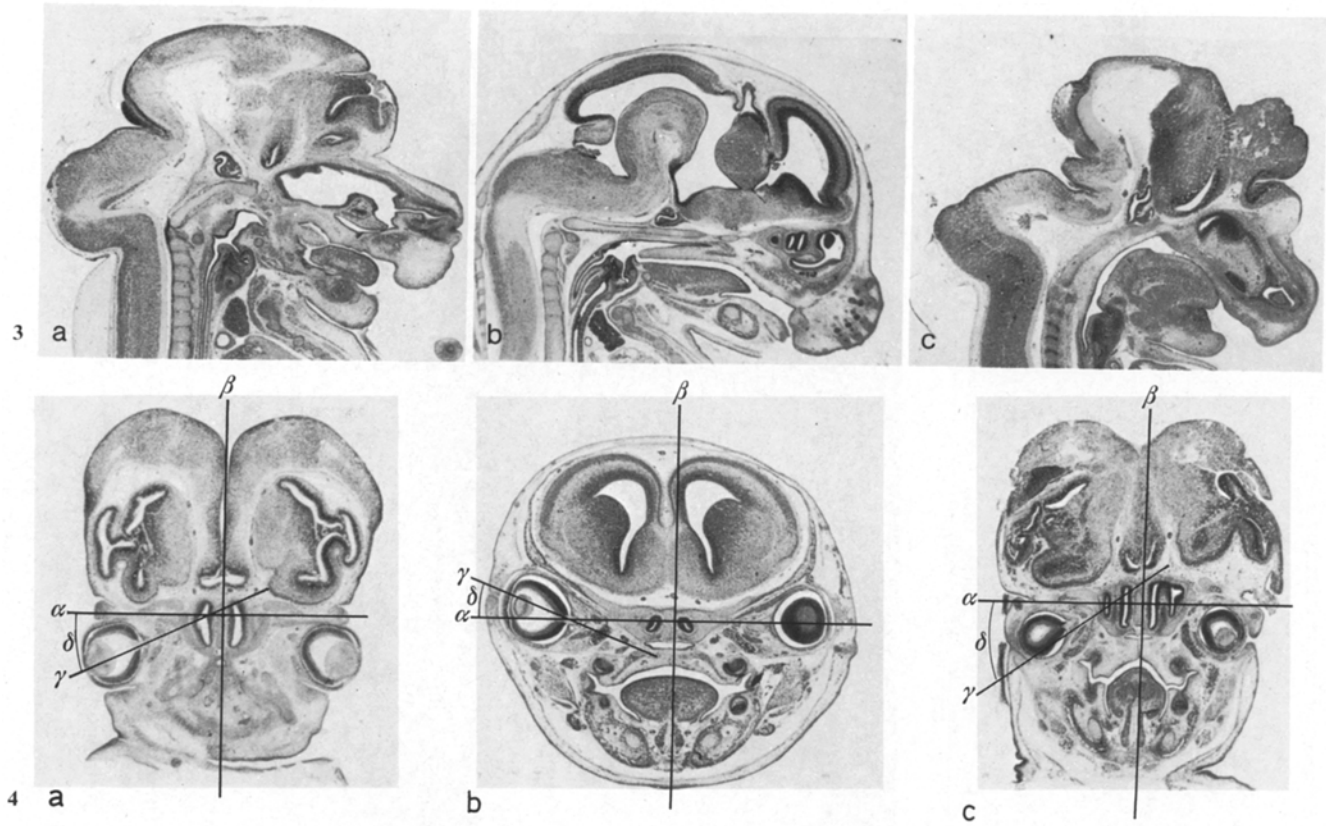

Fig. 3a–c. Sagittal sections of mouse embryos on day 15 (H.E., 3,2×). **a** Vitamin A induced exencephaly; **b** normal embryo; **c** Ts 12 with exencephaly. Note about 90° angle of vertebra and basioccipitale in **b** and abnormal flexion in **a** and **c**

Fig. 4a–c. Frontal sections of mouse embryos on day 15 on eye level (H.E., 3,2×). **a** Vitamin A induced exencephaly; **b** normal embryo; **c** Ts 12 with exencephaly. α, horizontal axis; β, vertical axis; γ, axis of the optic nerve; δ, angle between γ and α. Note displacement of the orbita in **a** and **c** and abnormality of the orbital wall in **a**

lon, separated by a deep diencephalic grove, protrude beyond the lateral edges of the skull base. They are overtopped by a variably expanded mesencephalic area. Non-closure of the cephalic segments involves the mesencephalon and the anterior part of the myelencephalon. As a consequence, the chorioid plexus of the fourth ventricle forms a border line between the everted parts of the brain and the adjacent closed posterior segment of the myelencephalon. In both cases, the head shows a brachycephalic shape and is in an upright position due to a considerably increased angle between the skull base and the spine (see below).

In contrast to these similarities, certain considerable differences of the skull base, the viscerocranium and the face between both types of exencephalic malformation are noticeable. In Ts 12, the ocular bulbs seem to be small to variable extent and hidden behind narrow lids. The longitudinal axis of the face is only slightly shortened. In contrast to the Ts 12 embryos which rarely display cleft palate, fetuses with Vitamin A induced exencephaly show conspicuous facial dysmorphy with exophthalmia, mandibular hypoplasia, microstomia, small circumoral skin evaginations, and hypoplasia or aplasia of the pinna. Moreover, exomyely (spina bifida) of a caudal segment of the spinal cord was common in cases of Vitamin A dependent exencephaly, but was never observed in Ts 12 fetuses.

4. Comparative and Histomorphometric Analyses of Cranial Structures in Ts 12- and Vitamin A-Type Exencephaly

(a) Skull Base. Mid sagittal sections of normal fetuses on day 15 (Fig. 3b) show that the angle formed by the bent line of the basisphenoid (bs) and the basioccipitale (bo) on the one hand, and by the vertebral column (vc) on the other hand, is about 100%. This angle is almost 120° in Vitamin A induced exencephaly, and about 160° in Ts 12-exencephaly (Fig. 3a and c). In addition, the respective lines of bs and bo intersect at more acute angles in exencephaly. As a result, a considerable shortening of the sagittal axis of the head, and an anomalous spatial arrangement of the skull base cartilages are characteristic of both types of exencephaly, but these changes are more conspicuous in Ts 12.

Similarly, a shortening of the transverse axis of the skull base is found in frontal sections of exencephalics. Yet, in Ts 12 the compression of the transverse diameter is more distinct in the occipital plane, while in Vitamin A exencephaly the rostral parts are more affected, and a cleft corresponding to a canalis craniopharyngeus (Fig. 5a) is regularly present within the bs.

(b) Orbits. For comparative studies of the orbital region frontal sections at the level of the ocular bulbs, aided by insertion of the following orientation lines were used (Fig. 4): a horizontal line (α) through the lowest points of the greater wings of the sphenoid bone, and a vertical line (β) through the central point of the horizontal line. An additional (δ) line in prolongation of the optic nerves to the centre of the ocular bulbs forms an acute angle

Table 4. Mean volumes of different head regions in per cent of total volume of the embryo (measured on day 15)

Embryonic feature	Number of embryos	Mean volume of the bulb	Mean volume the orbita	Mean volume of the hypophysis
Normal (control) embryo	6	0.135%	0.112%	0.075%
Vitamin A induced exencephaly	4	0.131%	0.075%	0.061%
Trisomy 12 induced exencephaly	4	0.15%	0.166%	0.055%

(λ) with the horizontal line (α) which shows, in normal embryos, a range between 1° to 7° in upward direction (Fig. 4b).

In exencephaly of both types (Fig. 4a and c), the optic nerve is directed downwards forming an (negative) angle of 10° to 25°. The shift of these angles reflects the dislocation of the orbits with a horizontalization of their normally inclined roofs. This gives the impression of a downward movement of the bulbs in relation to the skull base. But it appears from closer morphological analysis that the central part of the skull base primordium is raised increasing the volume of midline cranial structures. The resulting decline of the axis γ causes a median dislocation of the orbits. These changes are further reflected by the reduction of the horizontal distance between the ocular bulbs and the vertical line (β) in both types of exencephaly, though slightly more conspicuous in the Ts 12 case. The shape and volume of the orbits show more distinct differences in both types of exencephaly. In Ts 12 the ocular bulbs take a position deep in the orbits, while their relative size, in comparison to the whole embryo, is not markedly affected (Table 4). In contrast, in Vitamin A-induced exencephaly the volume of the orbits is distinctly reduced (Table 4) due, in part, to extensive developmental disturbances in the oropharyngeal area. These are associated with the occurrence or irregular cartilaginous islets, which may cause, on the one hand, a narrowing or even atresia of the pharyngeal cavity, but which on the other hand may also replace the median wall of the orbits, thus diminishing its size. In addition, a defect of the primordium of the maxillary part of the zygomatic arch leads to incomplete formation of the lateral wall of the orbits. As a consequence of these Vitamin A-induced changes, the ocular bulbs protrude beyond the plane of the lids, while they are displaced deep behind the lid plane in Ts 12-exencephaly.

(c) Hypophysis. The hypophysis was found to occupy the normal site irrespective of the type of exencephaly. On day 15 no differences of the grade of histological differentiation of the adenohypophysis existed in exencephalic and in normal fetuses. However, shape and volume of the hypophysis were considerably affected in exencephalic fetuses. Normally, this organ has, in frontal sections, a flat skifflike shape (Fig. 5b), whereas in exencephaly the hypophysis is of pyramidal (Vitamin A), or apple-like (Ts 12) shape with shortened transverse

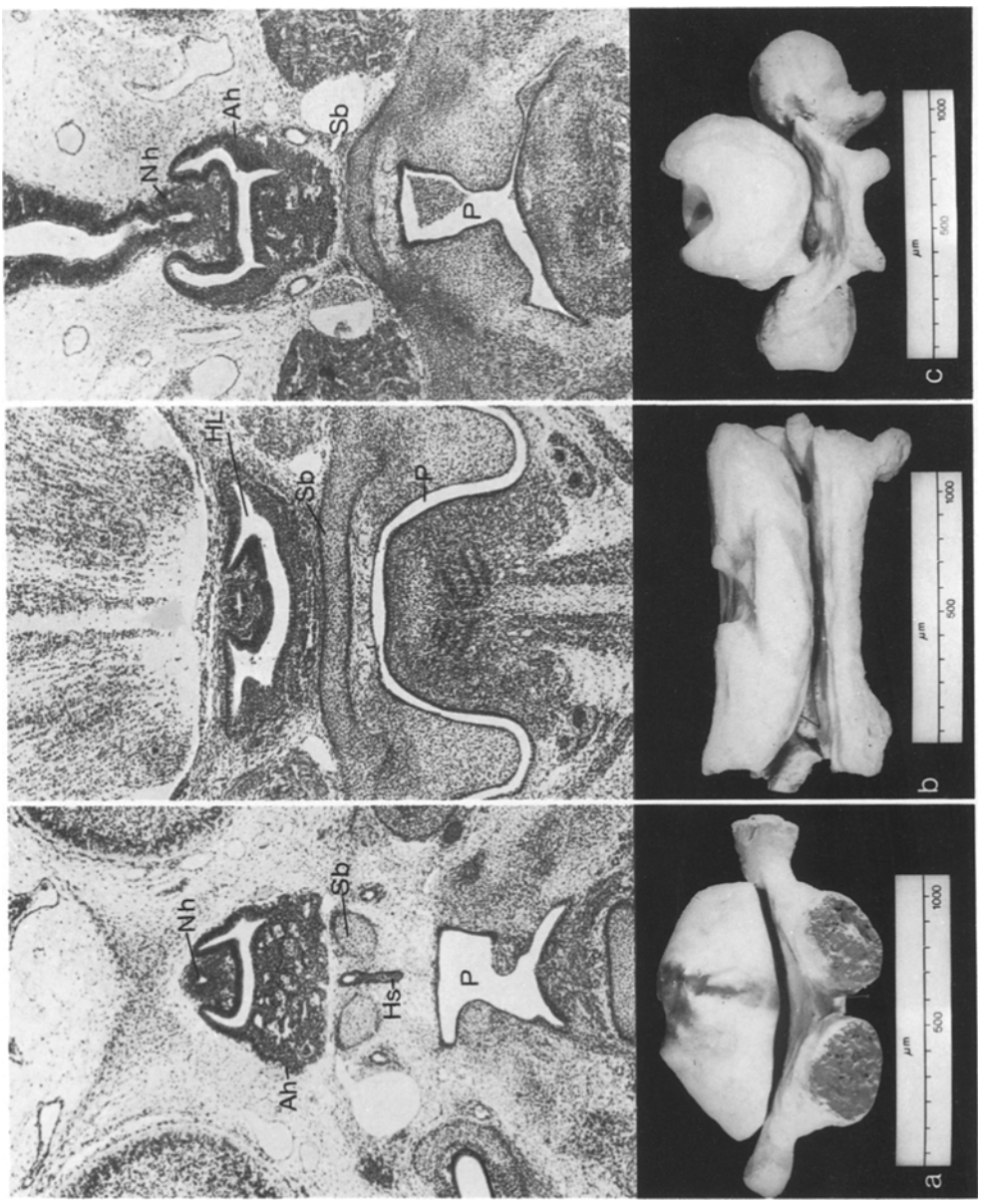

Fig. 5a–c. Upper part: Frontal sections of mouse embryos on day 15 on the level of hypophysis. Lower part: Styropore reconstruction models of this area based on serial sections. **a** Vitamin A induced exencephaly; **b** normal embryo; **c** Ts 12 with exencephaly. *Ah*, Adenohypophysis; *Nh*, Neurohypophysis; *Hs*, Hypophyseal stalk (Rathke's pouch); *P*, Pharyngeal cavity; *Sb*, Skull base. Marked change of hypophyseal shape (see text) in **a** and **c**

diameter and increased height (see Fig. 5a and c). In comparison with the normal hypophysis (Table 4), the volumes of the adenohypophysis were reduced in both types of exencephaly. The abnormal shape of the hypophysis in exencephaly is associated with a considerable enlargement and a vertically directed elongation of midline space, filled with loose arachnoidal mesenchyme which also extends between the skull base and the brain. The characteristic deformation

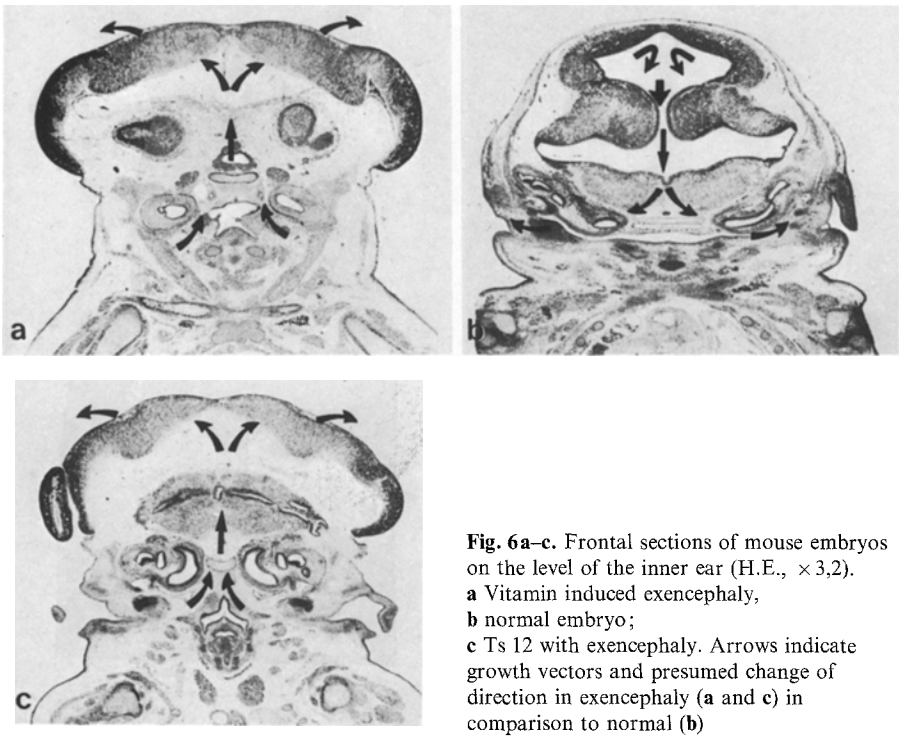

Fig. 6a–c. Frontal sections of mouse embryos on the level of the inner ear (H.E., ×3,2).
a Vitamin induced exencephaly,
b normal embryo;
c Ts 12 with exencephaly. Arrows indicate growth vectors and presumed change of direction in exencephaly (**a** and **c**) in comparison to normal (**b**)

of the hypophysis connected with exencephaly can, at least to some extent, be understood as an adaptation to these spatial changes.

A special feature of Vitamin A induced exencephaly is the occurrence of remnants of Rathke's pouch (Fig. 5a) associated with a persistent canalis craniopharyngeus.

(d) Inner Ear. The developmental patterns of the ear show considerable differences between Ts 12- and Vitamin A-induced exencephaly (Fig. 6a–c). In the Ts 12 embryos outer, middle and inner ear are present, and the main developmental anomalies of the inner ear are (1) reduced size, (2) abnormal shape and displacement, (3) delay of differentiation of the vestibular derivatives, and (4) impairment of development of the cochlea. The labyrinthal organ is dislocated and the orientation of the axis is changed. The whole organ in its cartilaginous capsule is more medially located than normally, while the vestibular part of the labyrinth is moved in a caudal direction. Cochlear and vestibular parts of the labyrinth are misshapen and embedded into the abnormally flattened capsular cartilage and petrous bone (Fig. 6). Along with these changes, retarded differentiation of the vestibular primordia and derivatives leads to a delayed appearance of the neural cells of the utricular and saccular macula (macula statica). The number of turns of the cochlear duct is reduced, and the ganglion spirale of the cochlea, which is located on the floor of the internal auditory meatus, shows a reduction of the nerve fibers belonging to the more severely affected upper parts. The lumen of the less impaired basal part of the cochlea

is enlarged. On the whole, the changes of Ts 12 inner ears bear similarity to the Mondini type of malformation in man.

In Vitamin A-induced exencephaly, the outer and middle ear were totally absent, and the inner ear was severely malformed. Also, the ganglion of N. VII and N. VIII were missing. The labyrinthal organ persisted as an irregular saclike structure without further development of the primordia of the utriculus and sacculus. Apart from incomplete differentiation and developmental arrest of the derivatives of the auditory vesicle, the torsion and a considerable caudal displacement of the inner ear cause an increase in the distance from the skull base.

5. Observations on Non-Exencephalic Fetal Progeny from Vitamin A-Treated Mothers

As shown in Table 2, some malformed fetuses from Vitamin A treated mothers did not exhibit exencephaly. They correspond to the type 4b experimental group (see: Material and Methods) with balanced heterozygous karyotype, in which, nevertheless, damage caused by Vitamin A, other than exencephaly, could be expected.

In these cases brain and skull were morphologically normal and the eyes showed regular differentiation, although exophthalmia was more or less distinct. All embryos of this group had severe malformation of facial structures however, though displaying differences of the grade of manifestation. In the face and in the oropharyngeal area similar tissue disturbances and irregularly situated cartilages occurred, leading to microstomia, oropharyngeal atresia, and exophthalmia as described in Vitamin A-induced exencephaly.

In the inner ear region vestiges of the vestibulum, utriculus and sacculus, and the semicircular canals were present, but were often small and retarded. The utriculus and sacculus were not clearly delineated, fusing into one area of varying distinctness. The number of turns of the cochlear duct was reduced. The developmental defect of the ganglion of the N. statoacusticus (VIII) parallelled the underdevelopment of the inner ear. Similarly, in cases with severe malformation of the inner ears, the middle and outer ear were virtually absent, while in more mildly affected cases the middle and outer ear anlagen were only hypoplastic and frontally displaced.

Discussion

Comparison of the brain and head in Ts 12- and Vitamin A-dependent exencephaly illustrates the following conformities on the one hand and differences on the other: The cerebral malformation, with eversion of the brain is similar in both types of exencephaly. The same is true of the characteristic horizontalization and shortening of the transversal and sagittal axes. Similar alterations have also been described in human anencephaly (Marin-Padilla, 1970; Fields et al., 1978) and in Vitamin A-treated rat embryos (Geelen, 1973). Differences in the pattern of abnormal developmental features associated with both types of exencephaly in mouse fetuses concerned the face, the ears, the oropharynx, and the caudal spinal cord. The involvement of the craniofacial area and oropha-

rynx, caused by severe morphogenetic disturbances of the viscerocranium, seems to be characteristic of Vitamin A-induced damage of the embryo, as shown earlier by Kalter (1960). Additional malformations of these regions were absent in Ts 12, except of rare cases with cleft palate.

On the basis of such a comparative analysis it becomes clear that apparent microphthalmia in Ts 12 corresponds to a variable degree of enophthalmia, caused by a downward inclination of the orbits and concomitant inward movement of the ocular bulbs. In contrast, similar changes in Vitamin A exencephaly lead to exophthalmia because of the supplementary effect of the developmental abnormalities of the viscerocranium and of the oropharyngeal area on the orbits. It is interesting to note that these latter malformations, which characteristically accompany Vitamin A-induced exencephaly, were also found in non-exencephalic fetuses from Vitamin A-treated mothers. Thus, it seems possible to distinguish changes accompanying the exencephalic malformation alone and other changes which are superimposed and also observed after Vitamin A damage without exencephaly.

In fact, the fundamental traits of exencephaly, though present in both types, occur in Ts 12 in an almost pure form. They comprise a defect of the cranial vault, eversion of the uncovered brain, and changes of the transverse and sagittal cranial axes. There is also a change in the spatial arrangement of the skull base leading to a cone-like shape of the hypophysis. The latter abnormality seems to be the result of formative forces which, with the eversion of the brain, change from centripetal to centrifugal vectors (see Fig. 6). They account for the rotation of the inner ear anlagen in Ts 12, and are effectively modelling the shape of structures and organs of the skull base. Thus, the horizontally compressed and vertically lengthened shape of the hypophysis in exencephaly, irrespective of whether it is caused by Ts 12 or Vitamin A, is explainable by this assumption. Similarly, the lifting of the skull base and the corresponding decline of the orbital axis in both types of exencephaly may be a consequence of such changes of growth vectors.

The developmental disorders caused by Ts 12 are probably effective in a very narrow time range between day 8 and 9 of embryonic development. Interestingly, a few other trisomies of the mouse may also show exencephaly, though not with the same regularity as in Ts 12. Thus, in Ts 14 about half of the embryos exhibit exencephaly which, however, differs slightly from the Ts-12-dependent malformation: in Ts 12 the metencephalon is constantly included in the defect area and remains unclosed. In contrast, this part of the brain is always closed in Ts 14 (Gropp and B. Putz, unpubl.). These observations hint at some semispecificity of chromosomal determinants. They are only understandable in terms of narrow time and region specific limitation of the effects of such determinants. By definition, these conditions correspond to a vulnerable, age-related and tissue-specific phase of teratogenic susceptibility. One can assume that, in trisomy, the epigenetic crisis (Waddington) in the target area is brought about by growth impairment due to the chromosome anomaly. Although all parts of a trisomic embryo may be affected by growth lag (Gropp, 1978) the deficiency of cell number can, in certain blastemas, fall below a critical threshold level otherwise necessary for the normal course of development.

If part of the changes of the skull base, in particular those brought about by the disturbances of morphogenetic vectorial gradients (see Fig. 6), constitute the pattern of exencephaly sensu stricut, then the Vitamin A-type exencephaly is characterized by additional craniofacial malformations. These are, collectively, the consequence of less specific damage affecting a more extended target area, involving paraxial mesoderm and in particular, neural crest derivatives, as postulated by Poswillo (1975). One can assume that also the severe ear malformation in case of Vitamin A-damage to the mouse embryo, comparable to similar malformations observed in Vitamin A-treated rat embryos by Baba and Kuzukuwa (1962), can be explained by involvement of neural crest cells. Yet, present results do not lend support to the more general assumption of Poswillo (1975) that damage of neural crest derivatives is the main morphogenetic mechanism in the origin of either anencephaly or of mandibulo-facial dysostosis (depending on the period of action of the teratogenic agent). The evidence against the general and prevalent role of neural crest derivatives emerges in particular from the following facts: (a) the fundamental malformations of the brain and skull base are similar in both types of exencephaly, although an involvement of neural crest derivatives is unlikely in Ts 12 exencephaly, and (b) the additional cranio-facial and oropharyngeal malformations, that are caused, at least in part, by damage to neural crest cells, are observed after Vitamin A treatment irrespective of the presence or absence of exencephaly. These findings and interpretations support the concept of complexity of factors determining varying kinds of neural tube defects. Other factors contributing to this complexity are, for example, the disturbances affecting the cranial paraxial mesoderm, playing a role in both – Ts 12- and Vitamin A-dependent – types of exencephaly and the developmental interdependence of different organs, such as the brain and inner ear (Deol, 1966) in Ts 12.

The observation that trisomic embryos have a shorter life span than embryos with Vitamin A induced exencephaly is explicable by the fact that the disorder in trisomy is systemic and irreparable, whereas extrinsic damage is accidental and to some extent reparable. The embryo with a chromosome abnormality is, from the beginning of the formation of the zygote, a defective system, since all cells, blastemas and organs of the fetus, including the fetal part of the placenta, are involved in the chromosome triplication. This condition may cause eventual breakdown of the development of the fetus by effects independent of the exencephalic malformation.

In man, the meroacrania type of anencephaly can be distinguished from holoacrania, the latter showing in many cases increased vertebral flexion and additional malformations (see Lemire et al., 1978; Rehder, 1979). These two manifestations of anencephaly in man bear some resemblance to "pure" (or Ts 12-dependent) and Vitamin A-induced exencephaly, respectively. However, in absence of sufficient knowledge of aetiology in man, attempts at an aetiological classification must be postponed until a better understanding of the significance of the different types of neural tube defects is possible.

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