

Abnormal neural fold development in trisomy 12 and trisomy 14 mouse embryos

I. Scanning electron microscopy

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SUMMARY

The early development of the exencephalic malformation in trisomy 12 (Ts12) and trisomy 14 (Ts14) mouse embryos was examined by means of scanning electron microscopy and compared with cranial neural tube formation in euploid litter-mates. Embryos from normal laboratory mice were used as additional controls.

The euploid control embryos of the trisomy-inducing breeding system showed a slight delay and some variation in the timing of cranial neurulation. The pre-exencephalic trisomic embryos showed hypoplasia, and lower somite number when compared with euploid litter-mates; there was also a retardation of development of the whole neural tube, when related to the somite stage. External differences from the control embryos were observed at the late pre-somite stage, when the anterior part of the neural plate showed a crumpled appearance. At 6 somites the lateral edges of the forebrain were everted instead of elevated in Ts12 and Ts14 embryos. At later stages, however, the forebrain showed a tendency towards the normal morphogenetic pattern, so that the optic vesicles were eventually formed and the most anterior part fused. The caudal forebrain and the midbrain were more permanently affected by the disturbance of trisomic conditions and grew laterally, failing to appose or fuse in the midline in both Ts12 and Ts14 embryos. Hindbrain morphogenesis was different in Ts12 and Ts14 exencephaly: in Ts12 embryos it did not close rostral to the otic pits, whereas in Ts14 embryos it showed a normal closure up to the hindbrain/midbrain junction.

These observations support the hypothesis that in mammalian embryos the mechanism of neural tube formation of the future brain region is more complex than that of the spinal neural tube and therefore may be more likely to react to a general delay of neurulation with a gross malformation.

Ts12 and Ts14 exencephaly are due to a primary non-closure of the neural tube.

INTRODUCTION

Morphogenetic mechanisms involved in the genesis of exencephaly are not as yet understood. Marin-Padilla (1970) reviewed and classified the available hypotheses according to whether exencephaly resulted from the reopening of a

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previously closed neural tube or from failure of the neural tube to close; he also considered the possible embryonic tissues involved in generating the defect. Since 1970 observations of endogenously or exogenously induced neural tube defects have been published, in support of every existing hypothesis: alterations in the neuroepithelium (Wilson, 1974, 1978; Theodosis & Fraser, 1978; Morriss & New, 1979; Webster & Messerle, 1980), primary mesenchyme (Morriss, 1972, 1973; Marin-Padilla, 1978, 1979) and in the neural crest cells (Poswillo, 1975). Morriss & Steele (1977) observed an involvement of all three germ layers.

Most experimental designs are based on the maternal administration of exogenous teratogens such as vitamin A, trypan blue, or cadmium, given at or just prior to the time of neurulation. The major disadvantage of these methods is that the occurrence of exencephaly is never 100 %, and often less than 50 %. Thus it is impossible to assess whether abnormalities observed early in neurulation are in fact early stages in the development of exencephaly. The two mouse mutants *loop-tail* (Wilson & Center, 1974; Wilson, 1978, 1980) and *curly-tail* (Embury, Seller, Adinolfi & Polani, 1979; Seller, Embury, Polani & Adinolfi, 1979) have the same disadvantage. This may explain why most of the studies deal only with the stages after normal cranial neural tube closure is complete, when exencephaly can be clearly distinguished. Only a few studies have been carried out during neurulation, attempting comparison of control and experimental embryos *in vivo* (Marin-Padilla, 1966: hamster; Morriss, 1972, 1973: rat; Theodosis & Fraser, 1978; Webster & Messerle, 1980: mouse) or *in vitro* (Morriss & Steele, 1977; Morriss & New, 1979: rat; Lee & Nagele, 1979: chicken).

Recently it has been found that the systemic chromosomal disorder trisomy 12 (Ts12) in the mouse displays exencephaly in 100 % of affected embryos. The malformation is not accompanied by facial malformations as is the case with, for example, vitamin A-induced exencephaly in mouse embryos (Putz, Krause, Garde & Gropp, 1980). Thus trisomy 12 embryos are a reliable source of material for observing the early stages of abnormal development in the genesis of an exencephaly. (The *spotch* mutant mouse is a similarly reliable model for studying the genesis of spinal neural tube closure defects: Wilson, 1974; Wilson & Finta, 1979, 1980.) Another trisomy of mouse embryos, trisomy 14 (Ts14), provides a useful adjunct to the study of trisomy 12. About 50 % of affected embryos develop an exencephaly which is slightly different in morphology from that of trisomy 12 embryos, in that the metencephalon is always closed (Gropp & Putz, unpublished observation).

The present study describes a light and scanning electron microscopic investigation of development of the cranial neural folds in Ts12 and Ts14 embryos, together with their euploid litter-mates and controls from unrelated stock. These observations of the external appearance provide the basis for more detailed studies on cellular aspects of the development of exencephaly in these mice, to be reported subsequently.

MATERIALS AND METHODS

Mouse stocks and general procedure

Male mice with the Robertsonian metacentric chromosomes Rb5/Rb9 Bnr or Rb6 Bnr/Rb16 Rma, which were bred on a mixed wild-type background in homozygous lines, were mated with 'all acrocentric' female mice of the outbred laboratory strain MF1. In addition, males and females from the MF1 laboratory strain were mated.

In order to obtain mouse embryos at each stage of the neurulation process, the dams were sacrificed at day 8 and day 9 of pregnancy (appearance of vaginal plug = day 0). After removing the uterus all implants, live and resorbed, were serially recorded. Embryos were dissected from the decidual swellings in PBS and their membranes carefully removed for karyotyping. The embryos were fixed in 2.5% cacodylate-buffered glutaraldehyde (0.1 M; pH = 7.2) after documentary photography and counting of their somites.

Induction of trisomy 12 and trisomy 14, cytogenetic analysis and classification of the embryos

Male mice doubly heterozygous for two partially homologous Robertsonian metacentric chromosomes either

(a) Rb (8.12) 5 and Rb (4.12) 9 Bnr

or (b) Rb (9.14) 6 Bnr and Rb (8.14) 16 Rma

were mated with female laboratory mice MF1. In these breeding designs a considerable rate of nondisjunction of the two metacentric chromosomes, as well as normal segregation, can be expected (Gropp, Kolbus & Giers, 1975). Thus in experimental design (a), trisomy 12, and in (b), trisomy 14 embryos will occur amongst different aneuploid and euploid embryos.

In order to karyotype the embryos their membranes were cultured in medium (Dulbecco 199 plus 20% foetal calf serum) containing colcemid (0.25 ml per 10 ml of a 10 µg/ml solution) for 2 h at 37 °C. The membranes were then transferred to hypotonic saline (1% Na citrate) for 15 min, fixed in 3:1 methanol-acetic acid and spread on warm slides in the usual way as air-dried preparations. The chromosomes of at least five mitotic figures of each embryo were counted and the embryos were classified in the following groups.

Group 1. Embryos with balanced karyotype and heterozygosity of Rb metacentric chromosomes. Cytogenetic marker: 40 chromosomal arms, presence of one metacentric chromosome = 'euploid control embryos'.

Group 2. Trisomic embryos with imbalanced karyotype. Cytogenetic marker: 41 chromosomal arms, two metacentric chromosomes = (a) 'trisomy 12' (Ts12) embryos, if Rb5/Rb9 Bnr males were used, or (b) 'trisomy 14' (Ts14) embryos, if Rb6 Bnr/Rb16 Rma males were used.

Group 3. Other aneuploid embryos with imbalanced karyotype, e.g.

monosomics, trisomics other than Ts12 or Ts14, triploids. These embryos were documented, but not examined in this study.

In addition embryos from 'all-acrocentric' males and females of the outbred strain MF1 were used as:

Group 4. 'MF1 control embryos.' Since no chromosomal disorders are to be expected no karyotypes were made from this group.

Scanning electron microscopy

At least two embryos of the groups 1, 2(a), 2(b) and 4 of each somite stage from 0-30 somites were prepared for scanning electron microscopy. After fixation they were dehydrated in graded acetones, dried in a Polaron critical-point drying apparatus, mounted on aluminium stubs with double-sided Sellotape and coated with gold in a sputter coater. The embryos were viewed and photographed in a JEOL scanning electron microscope.

The term 'somites' refers to pairs of somites throughout this study.

RESULTS

Embryological data, somite stage, and embryological size

Table 1 shows the embryological data from all litters used in this study to obtain Ts12 embryos, Ts14 embryos, euploid litter-mates and MFI controls, on days 8 and 9 of development. Trisomics (Ts12 or Ts14) occurred in about 20 % of the implants at this stage of development. Whereas on day 8 a considerable number of aneuploids other than Ts12 or Ts14 were found, most of these had apparently died by day 9, when only 0.1 % in Ts12 and 2.3 % in Ts14 were recorded. Correspondingly, the resorption rate increased from 12-14 % on day 8 to about 20 % on day 9. The litters of the MFI controls showed a low resorption rate of about 1-2 % on both days of development.

The histograms (Fig. 1) cover all litters in which the somite stage of each embryo was recorded. There was a considerable individual variation of somite stage within one litter: The control embryos of all three experimental groups showed differences of up to 6 somites on day 8 and up to 8 somites on day 9. The same was true for trisomic embryos. However, the average somite number of trisomic embryos was significantly smaller than that of controls, i.e. Ts12 embryos on day 8 had on average about 4 somites, and on day 9, 6 somites, less than their euploid litter-mates; in Ts14 embryos the difference was 3 somites on day 8 and 6 somites on day 9. In addition to this retardation of developmental stage, the embryos showed a hypoplasia which became very marked from the 10-somite stage onwards.

Table 1. Embryological data and karyotype

Progeny of	Stage of development (day)	Number of pregnant females	Total number of implants <i>n</i> (per ♀)	Results of chromosome preparation*					Resorptions <i>n</i> (%)
				Euploid <i>n</i> (%)	Trisomic <i>n</i> (%)	Aneuploid other than Ts12 or Ts14	Unsuccessfully karyotyped <i>n</i> (%)		
♂Rb5/Rb9 Bnr	8	16	192 (12.0)	78 (40.6%)	36 (18.8%)	19† (9.9%)	32 (16.7%)	27 (14%)	
♀MFI (for Ts12)	9	14	171 (12.2)	103 (60.0%)	30 (17.6%)	1‡ (0.6%)	3 (1.8%)	34 (20%)	
♂Rb6 Bnr/Rb16 Rma	8	14	142 (10.1)	56 (39.6%)	21 (14.9%)	15§ (10.6%)	33 (23.3%)	17 (12%)	
♀MFI (for Ts14)	9	15	168 (11.2)	91 (53.6%)	43 (25.6%)	4 (2.3%)	1 (0.6%)	29 (17%)	
♂MFI	8	10	108 (10.8)	106 (98.1%)	—	—	—	2 (1.9%)	
♀MFI (for controls)	9	8	95 (11.9)	94 (98.7%)	—	—	—	1 (1.1%)	

%, percentage of implants.

* No karyotypes in MFI controls supposing all embryos are chromosomally normal.

† 7 Monosomies (Ms), 6 Ts, 2 Triploids, 2 Ts + Ms, 2 Triploid + Ts.

‡ Triploid.

§ 5 Ms, 3 Ts, 5 Triploids, 2 Triploids + Ts.

|| 1 Ms, 1 Ts, 2 Triploids.

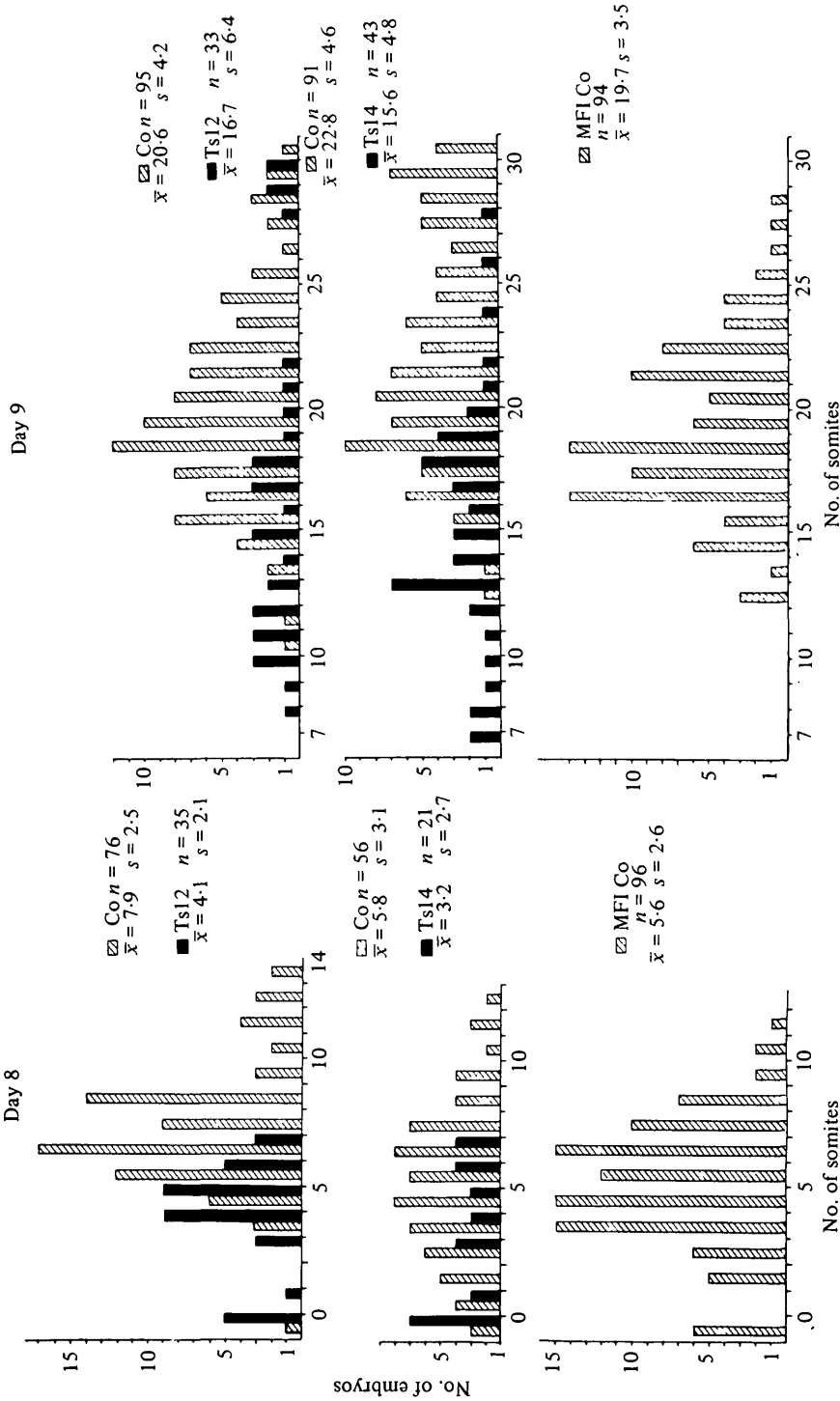


Fig. 1. Histograms showing numbers of embryos of the different embryonic groups used at each somite stage on day 8 and day 9 of development. (\bar{x} = mean somite number; s = standard deviation.) After normal distribution had been tested for all groups ($P = 5\%$) by means of the Kolmogorov-Smirnov test (Massey, 1951) the statistical comparison of somite stage showed significant differences between trisomics and their own controls, but not between different control groups or different trisomic groups.

$$\left(d = \frac{\bar{x}_a - \bar{x}_b}{\sqrt{(s^2/n_a) + (s^2/n_b)}} \right), \text{Bailey, 1969}.$$

*External appearance of neural fold development**(a) Euploid control embryos*

Each stage of development was examined from the late presomite stage to the time of neural tube closure, by observation of living embryos and subsequently by scanning electron microscopy.

In embryos of late pre-somite and early somite stages (0–5 somites) the anterior part of the neural folds, the future brain region, showed a rapidly increasing biconvex shape. The optic pits formed in the anterior part of the forebrain, which showed a slight mediad inclination by 4–5 somites in the hind-brain region, the preotic sulcus appeared and became distinct during this period.

At 6 somites the most obvious changes of morphology were observed in the region of the most anterior part of the forebrain. The inferior part of the optic vesicle formed as the lateral edges of the most anterior part of the forebrain became more medially and dorsally inclined (Fig. 3, b, d). The spinal neural folds at this stage formed a deep groove, fused as neural tube in the upper cervical region (Fig. 3 d).

In the future brain region fusion occurred not only in the myelencephalic (lower hindbrain) region (Fig. 5 a), but also by about 10 somites in the region between fore- and midbrain, extending both rostrally and caudally (Fig. 4 a). There was some variation in the correlation between neural fold morphogenesis and somite stage: e.g. most embryos had a completely closed brain tube by the 16-somite stage, whereas closure was still incomplete in 4 of 32 embryos examined at 18–19 somites. There was a similar variation in spinal neural tube closure: this was usually complete by the 22-somite stage, but 6 of the 23 embryos examined at 24–25 somites still showed slight patency of the posterior neuropore.

(b) MFI controls

Neural fold morphogenesis in MFI controls was similar to the above, but less variable. Closure of the brain tube was always complete by the 16-somite stage, and the posterior neuropore was always closed by the 24-somite stage.

(c) Trisomy 12 embryos

Differences between Ts12 embryos and controls were evident from the late pre-somite stage onwards. At 0–2 somites the neural folds had an irregular crumpled appearance of the anterior and lateral edges, and a slightly flatter and less smooth surface of the biconvex shape, than their euploid litter-mates (Fig. 2 a). At the 4- to 5-somite stage there were no obvious differences in external appearance, although the optic pits were less distinct in Ts12 embryos; the biconvex shape of the cranial neural folds and the pre-otic sulci, however, were as well developed as in controls.

From the 6- to 7-somite stage onwards the Ts12 embryos were again easily distinguishable from euploid control embryos, as the lateral edges of the forebrain

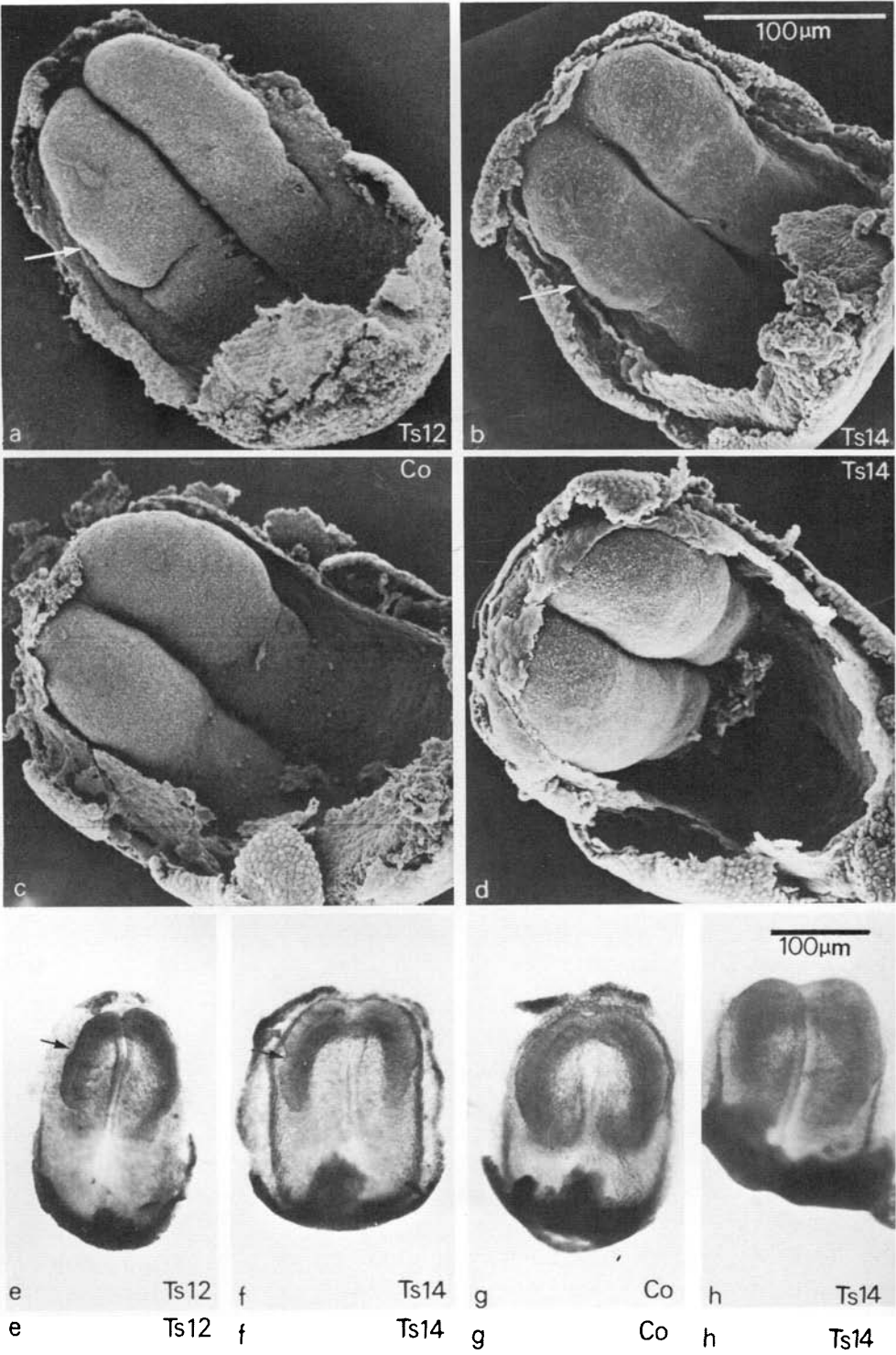


Fig. 2. Comparison of late pre-somite embryos. (a-d) Scanning electron micrographs; (e-h) the same embryos before fixation. (a), (e) Ts12; (b), (f) Ts14 (pre-exencephalic type): irregular cranial neural folds with 'wavy' lateral edges (arrowed). (c), (g) control embryo; (d), (h) Ts14 (normal type): the neural folds are smoother and more convex in appearance.

region developed a lateral eversion instead of the dorsomedial inclination seen in controls (Fig. 3). Initiation of spinal neural tube closure was delayed in most of the Ts12 embryos, six of eight embryos observed at the 6- to 7-somite stage having a completely open spinal neural groove.

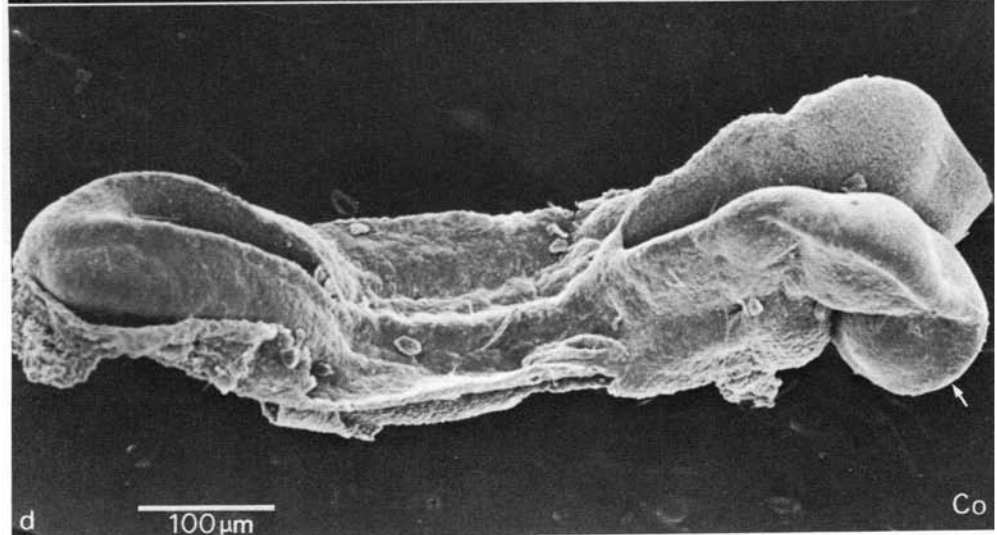
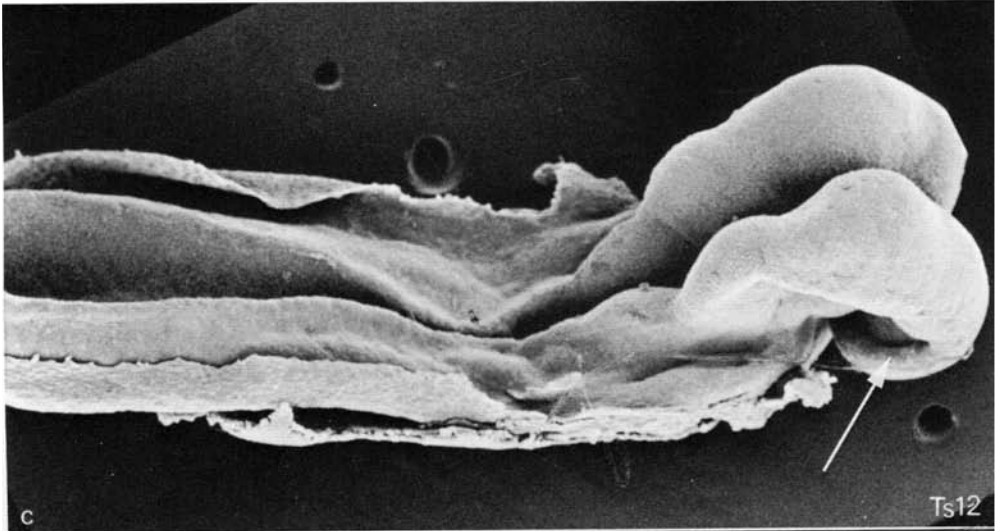
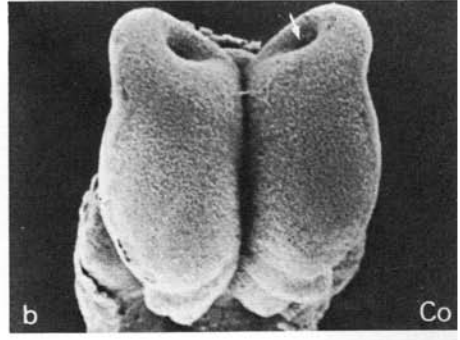
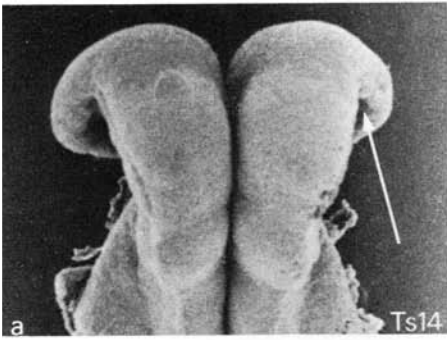
From the 8-somite stage onwards neurulation in Ts12 embryos was characterized by two main features: overall delay of neural fold development, and delay and/or failure of the normal morphogenetic movements in specific regions of the cranial neural folds. The anterior part of the forebrain of 11- to 17-somite stage embryos was observed to undergo morphogenetic movements similar to those seen in control embryos during the 7- to 9-somite period, resulting in the formation of late but well-shaped optic vesicles. However, the two halves of the forebrain failed to appose and fuse, except for the most antero-inferior part, which showed cellular contacts by the 15- to 17-somite stage (Fig. 4 b), and was fused from the 22-somite stage onwards. The hindbrain walls showed an apparently normal elevation and fusion up to the level of the otic pits, but with a 2-somite delay when compared to the morphogenesis of control embryos (Fig. 5b). However, the rhombencephalic walls rostral to the otic pits, and the mesencephalic walls, never showed similar movements to those observed in control embryos; instead they grew laterally or vertically, and failed to appose in the dorsal midline.

Figures 6c, d show a typical example of the fully developed exencephalic malformation, seen here in a 25-somite Ts12 embryo. The rostral part of the 4th ventricle is open. The mesencephalic floor, bulged upwards due to the cephalic flexure, lies on top of the head, as its walls are everted to both sides. In the forebrain region the open 3rd ventricle is recognizable showing on both sides the convex thalamic regions, and anterolateral to them the developing telencephalic hemispheres. The two halves of the median portion of the telencephalon have fused. Closure of the spinal neural tube remained continuously late, being complete by 24–28 somites in five observed Ts12 embryos.

(d) Trisomy 14 embryos

Two different types of Ts14 embryos could be distinguished from the late pre-somite stage onwards, in approximately equal numbers. One type was similar to Ts12 embryos, and were therefore assumed to be pre-exencephalic. The other type showed no gross abnormality of neural fold development when compared with euploid control embryos.

The first group showed crumpled neural folds at the 0- to 2-somite stage (Fig. 2b, f), a flatter biconvex shape than 3-somite controls, no externally visible differences from controls at 4–5 somites, and a lateral eversion of the forebrain by 6–7 somites (Fig. 3a). From the 10-somite stage onwards distinct differences between these Ts14 embryos and Ts12 embryos were recognizable in the hind- and midbrain regions. Unlike Ts12 embryos they showed elevation of the hind-brain neural folds and a closure of this region up to the level of the lower



midbrain by the 14-somite stage (Figs. 5c, 6e). Thus the hindbrain development was similar to that of the euploid control embryos. The midbrain was never observed to form a closed tube; it showed some variation in morphology, some embryos having laterally everted mesencephalic walls like the Ts12 embryos (Figs. 5c, 6e), while in others the walls were more vertical (Fig. 6f). The forebrain showed morphogenetic abnormalities similar to those described for Ts12 embryos. Thus at about the 20-somite stage these Ts14 embryos showed an exencephalic malformation which differed from Ts12 exencephaly only in that the hindbrain was closed (Figs. 6e, f). Development of the spinal neural tube of these Ts14 embryos was slightly delayed but otherwise normal in appearance, being complete by the 28-somite stage. The Ts14 embryos with similarities of neural fold development to euploid control embryos were usually retarded in their overall development. The shape of the neural folds, however, showed a normal appearance at all stages of development.

Table 2 (p. 156) shows the relationship between neural fold development and somite stage of the different embryonic groups compared in this study. Two main characteristics of the abnormal neural fold development in Ts12 and Ts14 exencephaly are apparent: (1) asynchrony between the developing systems with a delay of neural fold development in relation to somite stage when compared with control embryos; (2) abnormal morphogenetic movements of parts of the anterior neural folds, with a tendency to grow laterally instead of dorsomedially.

DISCUSSION

Cranial neural fold development of the control embryos examined in this study showed a morphogenetic pattern generally similar to those described in previous observations in rat, hamster and mouse embryos (Adelman, 1925; Freeman, 1972; Waterman, 1975, 1976; Morriss & Solursh, 1978*a*; Kaufman, 1979). However, previous observations on the relationship between neural fold development and somite number in mouse embryos (Geelen & Langman, 1977; Kaufman, 1979; Greenaway & Shephard, 1979) show considerable differences from our observations. These discrepancies may be due to strain-related differences (Greenaway & Shephard, 1979). In the present study neural tube closure of the future brain of the controls was completed earlier (at about 16–18 somites) than observed in the other studies (20–24 somites). The relationship between neural fold development and somite stage was not found to be as regular in the euploid control embryos (litter-mates of Ts12 and Ts14 embryos, group 1) as in

Fig. 3. 6- to 7-somite-stage embryos. (a) Ts14 (pre-exencephalic type); (b), (d) control embryos (Co); (c) Ts12. In the Ts12 and Ts14 embryos, the lateral edges of the forebrain are distinctly outward-curved and everted (long arrows), c.f. medially directed control forebrain neural folds (formation of the optic vesicles, small arrow). The spinal neural tube of trisomic embryos is unclosed, whereas the fusion process has begun in control embryos.

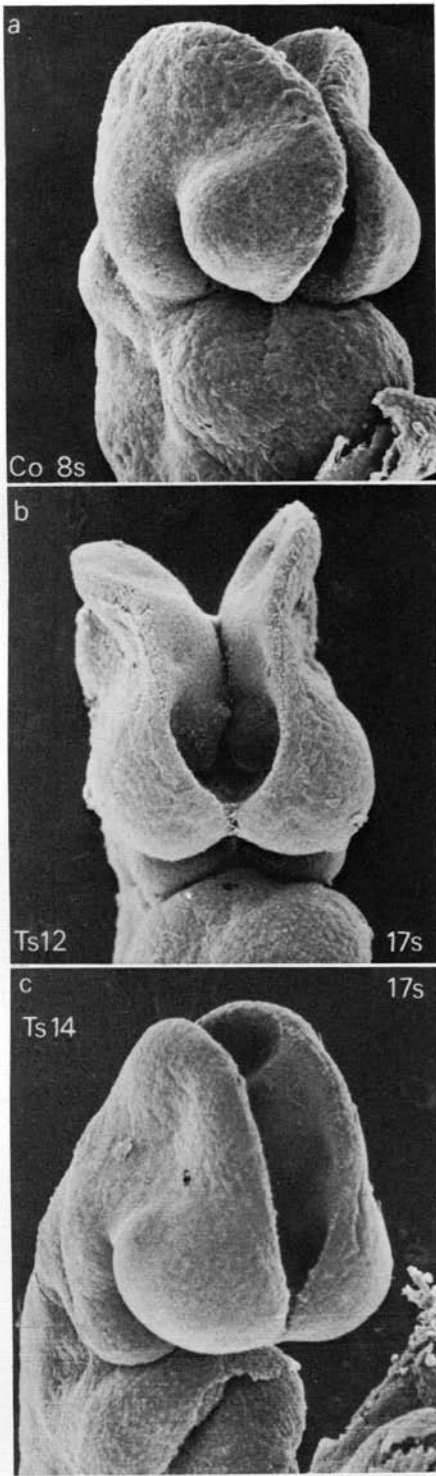


Fig. 4.

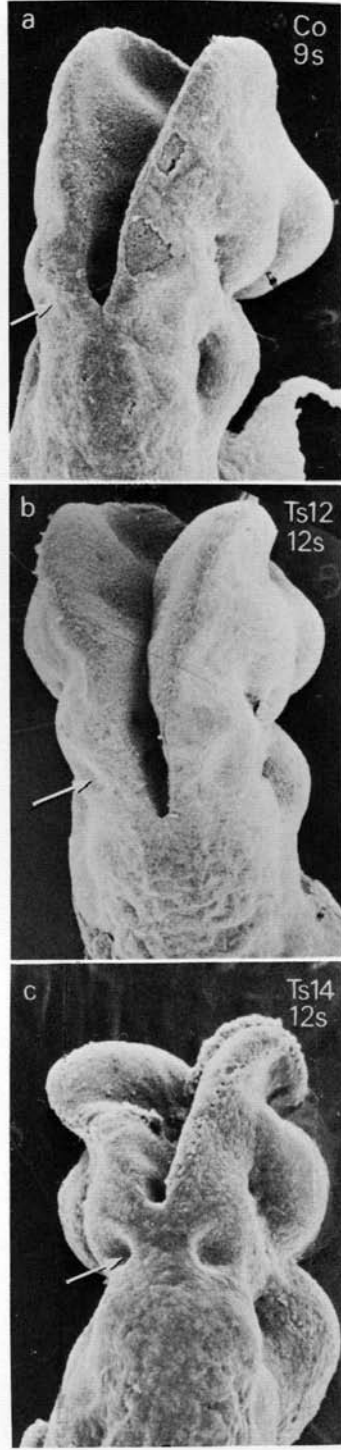


Fig. 5.

the MFI controls (group 4). The first group showed marked individual differences and a slight overall delay in the neural fold development when compared with the latter. This variation in the timing of neural fold morphogenesis may be a reflexion of the genetic heterogeneity of the males used, as they have been bred on a mixed wildtype background (Gropp, Giers & Kolbus, 1974). The embryological data presented in this study, especially the occurrence of different aneuploids and early resorptions, are within the expected range and have been discussed elsewhere (Gropp *et al.* 1974, 1975; Gropp, Putz & Zimmermann, 1976). Our observation of intra-litter differences in the stage of embryonic development is consistent with that of Yamamura (1969).

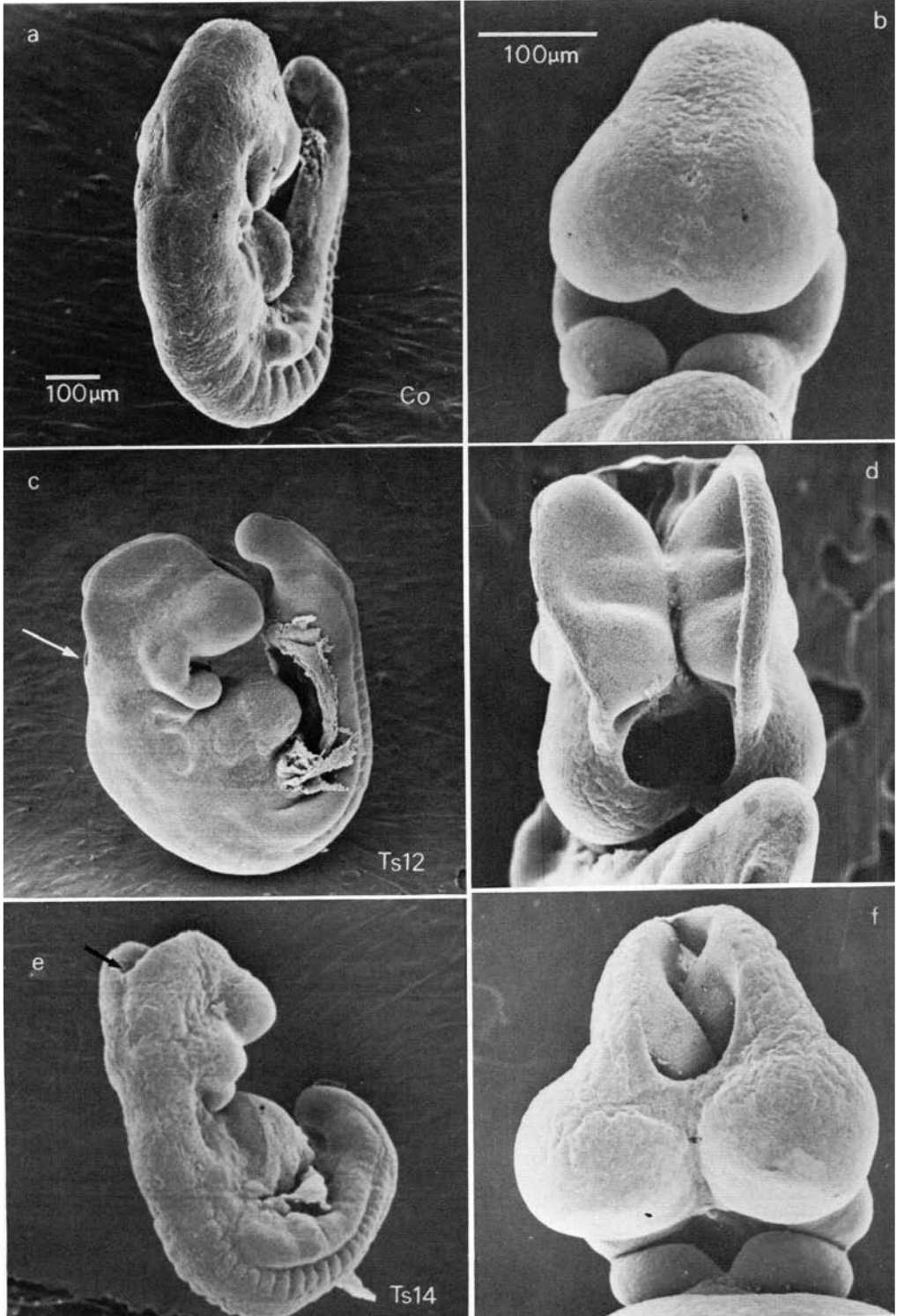
The trisomic embryos showed a hypoplasia and a general retardation of development, with fewer somites than their euploid litter-mates at the same embryological age. All *Ts12* and about 50 % of *Ts14* embryos showed a retardation of neurulation in relation to the somite number, when compared with both categories of control embryos. This delay in development of one organ system corresponds to a report on trisomy 19 in mouse embryos in which asynchrony between different developing systems was observed (Bersu, 1979). We assume that those *Ts14* embryos which resembled *Ts12* embryos in their neural fold development were those which would have developed an exencephaly. This assumption is based on the observation that 50 % of this type of *Ts14* embryos were similar at this early stage to *Ts12* embryos known to be pre-exencephalic, and when examined at later stages, 50 % actually showed exencephaly. Only this type of *Ts14* embryo will be discussed here.

A previous electronmicroscopic study concentrated on the fusion process of the anterior neural folds in mouse embryos as a basis for a better understanding of closure defects, and was able to show differences between the modes of closure of the fore-, mid- and hindbrain regions (Geelen & Langman, 1977, 1979). However, we observed in the pre-exencephalic trisomic embryos alterations of morphology of the neural folds far earlier than the beginning of fusion: externally visible abnormality of the cranial neural folds was observed in late pre-somite stages, i.e. when the cranial neural plate is just beginning to develop its later biconvex form.

Our observations on cranial neural fold morphogenesis in *Ts12* and *Ts14* embryos suggest that specific patterns of morphogenesis are intrinsic to specific areas of the neural epithelium. In the forebrain, even if late and incomplete,

Fig. 4. Forebrain morphology. (a) 8-somite control embryo; (b) 17-somite *Ts12* embryo; (c) 17-somite *Ts14* (pre-exencephalic type) embryo. The optic vesicle region of the forebrain is similar in controls (8s) and trisomics (17s), but there are differences in the midbrain region.

Fig. 5. Hindbrain morphology. (a) 9-somite control embryo; (b) 12-somite *Ts12* embryo; (c) 12-somite *Ts14* (pre-exencephalic type). Arrows indicate otic pits. There are similarities between the hindbrain regions of *Ts12* (12s) and control (9s) embryos and between the midbrain regions of *Ts12* and *Ts14* (both 12s) embryos.



there is a tendency of morphogenesis towards the normal pattern. In the mid-brain any similarity to the normal pattern is lost at an early stage of elevation of the lateral edges of the neural epithelium. This could either mean that there is a greater effect of disturbance due to trisomic conditions on the midbrain than on the forebrain or that the midbrain morphogenesis is more easily and more drastically disturbable than is that of the forebrain. The hindbrain morphogenesis shows differences between Ts12 and Ts14: in Ts14 embryos an apparently normal fusion process occurs along the whole length of the hindbrain, whereas in Ts12 embryos the rhombencephalic walls show delayed but similar morphogenetic movements to those of controls only up to the level of the otic pits, and do not close rostral to this level. These observations suggest that the pattern of morphogenesis of the hindbrain is again less easily altered than that of the midbrain, and that the posterior cranial neural epithelium becomes progressively more vulnerable to morphogenetic disturbances in a posterior to anterior direction.

The observation of two different forms of exencephaly with differences in the extent of the area involved corresponds to observations of different forms of anencephaly in human fetuses (meroacrania, holoacrania, Lemire, Beckwith & Warkany, 1978).

This study has shown that exencephaly in Ts12 and Ts14 mouse embryos is due to a primary nonclosure of the anterior neural folds and not, as discussed by some authors (Gardner, 1968, 1973) to a secondary reopening of the primarily closed neural tube. It is interesting to note that although the development of the whole neural tube is delayed in Ts12 and Ts14 embryos the spinal part always closes, while the cranial part does not. This seems to reflect the more complex pattern of neurulation of the future brain region in mammalian embryos with a two-stage development of (1) biconvex and (2) flat/concave shape of the neural folds compared with the simpler pattern observed in the spinal region (Morriss & Solursh, 1978*a, b*; Morriss & New, 1979). We suggest that there may be a threshold effect whereby the closure process of the future brain region, by involving a greater number of morphogenetic events at the cellular level, is more sensitive to factors causing delay or loss of synchrony than is the closure process of the spinal region.

Our ability to detect pre-exencephalic embryos with this experimental model should facilitate further study of the various cellular and extracellular components during early stages of abnormal cranial neurulation. Ultrastructural studies of these stages will be reported subsequently.

Fig. 6. Comparison of older stages. (a) 20-somite control embryo; (b) 18-somite control embryo; (c, d) 25-somite Ts12 embryo; (e) 22-somite Ts14 embryo (pre-exencephalic type); (f) 18-somite Ts14 embryo (pre-exencephalic type). (c) and (e) show the different degrees of closure of the hindbrain neural tube (arrows) which were observed in Ts12 and Ts14 embryos. (c) (same embryo as d) shows a typical open area of the mid-, fore- and hindbrain regions of a Ts12 embryo; (f) shows the open area of a Ts14 embryo which was a relatively minor defect when compared with other observations of Ts14 embryos at this stage.

Table 2. Relationship between neural fold development and somite stage in the different embryonic groups

Embryonic group	Biconvex shape	Pre-otic sulcus	Somite stage at which specific features of neural fold development could be observed							Spinal neural folds		
			Cranial neural folds (future brain region)				Uppermost part of metencephalon fused			Beginning of fusion in upper cervical region	Posterior neuropore closed	
			Beginning of formation of optic vesicles	Optic vesicles formed	Fusion of hindbrain up to otic pits	Fusion of region between mid- and forebrain	Anterior part of forebrain fused	Midbrain fused	Uppermost part of metencephalon fused			
Euploid controls	0-5	1-2	6	8-9	9-10	10-11	13	14	16-19	6	6	22-25
Ts12	0-5*	1-2	9-10	13-17	12	—	17	—	—	7-9	7-9	25-29
Ts14 (pre-exencephalic type)	0-5*	1-2	9-10	13-17	9-10	—	15-17	—	17-19	7-9	7-9	25-29
Ts14 (normal type)	0-5	1-2	6	8-9	9-10	10-11	13	14	16-19	6	6	24-26
MFI controls	0-5	1-2	6	8-9	8-9	10-11	12-13	13-14	16	6	6	23-24

*Crumpled appearance of the lateral edges of the neural folds at the 0-1 somite stage.

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