Postimplantation development of tetraploid mouse embryos produced by electrofusion

M. H. KAUFMAN* and S. WEBB
Department of Anatomy, University Medical School, Teviot Place, Edinburgh EH8 9AG, UK

*Author for correspondence

Summary
Despite the fact that a variety of experimental techniques have been devised over the years to induce tetraploid mammalian embryonic development, success rates to date have been limited. Apart from the early study by Snow, who obtained development to term of a limited number of cytochalasin B-induced tetraploid mouse embryos, no other researchers have achieved development of tetraploid embryos beyond the early postimplantation period. We now report advanced postimplantation development of tetraploid mouse embryos following electrofusion of blastomeres at the 2-cell stage, and subsequent transfer of these 1-cell 'fused' embryos to appropriate recipients. Cytogenetic analysis of the extraembryonic membranes of all of the postimplantation embryos encountered in the present study has provided an unequivocal means of confirming their tetraploid chromosome constitution. A preliminary morphological and histological analysis of the tetraploid embryos obtained by this technique has revealed that characteristic craniofacial abnormalities particularly involving the forebrain and eyes were consistently observed, and these features were often associated with abnormalities of the vertebral axis and heart. The most advanced viable embryo in this series was recovered on the 15th day of gestation, and its morphological features suggest that it was developmentally equivalent to a normal embryo of about 13.5–14 days p.c.

Key words: tetraploid, electrofusion, 2-cell embryo, mouse, postimplantation development, craniofacial abnormalities, vertebral and cardiac abnormalities.

Introduction
Various experimental techniques have been devised over the years to induce the development of tetraploid mammalian embryos (Niemierko and Opas, 1978; Dyban and Baranov, 1987) and limited degrees of postimplantation development of tetraploid mouse embryos have been achieved, following either the experimental inhibition of an early cleavage division, after exposure to either colchicine (Pincus and Waddington, 1939; Edwards, 1958) or cytochalasin treatment (Snow, 1973; Tarkowski et al. 1977), or by blastomere fusion (Graham, 1971; Eglitis, 1980; Eglitis and Wiley, 1981; Kubiak and Tarkowski, 1985; Ozil and Modlinski, 1986; Kato and Tsunoda, 1987; O'Neill et al. 1990). Limited success has also been achieved when nuclei isolated from fertilised mouse embryos have been microsurgically transplanted into other mouse embryos (Modlinski, 1978, 1981). Previously, Snow (Snow, 1973, 1975, 1976) obtained development to term of a limited number of cytochalasin B-induced tetraploid mouse embryos, but no other researchers have achieved development of tetraploid embryos beyond the early postimplantation period.

In our own recent studies (O’Neill et al. 1990), tetraploid mouse embryos, which developed to the early somite stage, were produced following the exposure of 2-cell-stage embryos (previously incubated in medium containing colcemid and cytochalasin D) to inactivated Sendai virus. While the rates of blastomere fusion achieved were relatively modest (21.1%), high rates of implantation (88.2%) and a limited degree of early postimplantation embryonic development (49.6% of those that implanted) were nevertheless achieved. However, the majority of the embryos in this study failed to progress beyond the presomite headfold stage.

The technique of electrofusion provides an alternative experimental means of achieving blastomere fusion in vitro, which has also been used in recent attempts by others to explore, albeit unsuccessfully to date, the postimplantation development potential of tetraploid mammalian embryos (Kubiak and Tarkowski, 1985; Ozil and Modlinski, 1986). This technique has, potentially, important advantages over cytochalasin-induced inhibition of cell division in the production of tetraploids, in that the fusigenic stimulus involved in electrofusion is of very short duration, and does not
require that the cells to be fused should be at any particular stage of the cell cycle. In addition, the embryos whose blastomeres are to be fused need not be exposed to potentially toxic chemicals such as cytochalasin and/or colcemid.

Materials and methods

(i) Isolation of 2-cell stage embryos

8- to 12-week-old (C57BL x CBA)F1 hybrid female mice were injected with 5 i.u. PMSG and then 48 h later with 5 i.u. HCG to induce ovulation. After the HCG injection, the females were caged individually with fertile homozygous Rb(1.3)1Bnr male mice. The presence of a vaginal plug the next morning was taken as evidence of mating and this was considered to be the first day of gestation. Early on the morning of the second day of gestation, the female mice were killed by cervical dislocation and the oviducts removed and flushed with phosphate-buffered saline containing 4% bovine serum albumin in order to recover the 2-cell-stage embryos. The embryos were then transferred into drops of tissue culture medium (Whittingham, 1971) under paraffin oil, and retained in an incubator maintained at 37 °C in an atmosphere of 5% CO2 in air.

(ii) Electrofusion and transfer of fused tetraploid embryos to pseudopregnant recipients

The embryos that were to be fused were then transferred into a non-electrolyte solution consisting of 0.3 M mannitol (Kubiak and Tarkowski, 1985) and a similar solution was also present in the fusion chamber. The latter consisted of a plastic tissue culture dish which had two platinum wires of 250 μm diameter fixed parallel to each other on the bottom of the dish with a space of about 600 μm between them. The ends of the platinum wires were connected to a digitimer pulse stimulator set at 200 V, with a pulse duration of 50 μsec. The embryos, in batches of 10, were then placed between the two platinum wires in the chamber and the pulse stimulator was triggered. The embryos were removed immediately and washed through 4 drops of tissue culture medium and then returned to the incubator maintained at 37 °C in an atmosphere of 5% CO2 in air.

Electrofusion and transfer of fused tetraploid embryos to pseudopregnant recipients

The recipients were anaesthetised with tribromoethanol (Avertin, Winthrop; dose 0.02 ml g−1 body weight of a freshly prepared 1.2% solution of Avertin dissolved in 0.9% saline).

(iii) Analysis of postimplantation stages of development

The recipients were subsequently autopsied between the 10th and 18th days of gestation, and the contents of their uterine horns examined in order to establish the total number of resorptions and viable embryos present. The embryos were then isolated from within their extraembryonic membranes. Details of the external morphology of the viable embryos were recorded, and these were fixed in Bouin's solution for subsequent histological examination. The embryos were serially sectioned in the transverse plane, cut at a nominal thickness of 8 μm and stained with haematoxylin and eosin.

The exact developmental stage of these embryos was established by comparing their histological features with those of material in the author's (i.e. MHK) reference collection of serially sectioned mouse embryos. The extraembryonic membranes of these embryos meanwhile were incubated in medium containing colcemid and mitotic preparations made (Evans et al. 1972) in order to confirm their tetraploid chromosome constitution.

Results

(1) General observations

Following the transfer of 1-cell ‘fused’ tetraploid embryos to the oviducts of pseudopregnant recipients, high rates of implantation were consistently achieved (Table 1). Highest rates of embryonic recovery occurred when autopsies were carried out on the 10th and 11th days of gestation, with substantially lower rates thereafter. Thus, out of a total of 230 tetraploid embryos transferred to recipients that were autopsied on the 10th day of gestation, 181 successfully implanted, and a total of 129 embryos that appeared on gross inspection to be viable were recovered. The majority of these consisted of either extremely small presomite headfold-stage embryos, or embryos that possessed a limited number of somites. Almost all of these embryos possessed a disproportionately enlarged allantois. In fact, only 14 embryos that were isolated at this stage of

<table>
<thead>
<tr>
<th>Day of gestation at autopsy*</th>
<th>No. recipients</th>
<th>No. embryos transferred</th>
<th>No. implants (% transferred)</th>
<th>No. resorptions (% implants)</th>
<th>No. embryos recovered (% implants)</th>
<th>No. healthy embryos recovered (% implants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>24</td>
<td>230</td>
<td>181 (78.7)</td>
<td>52 (28.7)</td>
<td>129 (71.3)</td>
<td>126 (69.6)</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>48</td>
<td>38 (79.2)</td>
<td>19 (50.0)</td>
<td>19 (50.0)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>70</td>
<td>67 (95.7)</td>
<td>48 (71.6)</td>
<td>19 (28.4)</td>
<td>6 (9.0)</td>
</tr>
<tr>
<td>13</td>
<td>104</td>
<td>87 (83.7)</td>
<td>73 (83.9)</td>
<td>14 (16.1)</td>
<td>10 (11.5)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>68</td>
<td>60 (88.2)</td>
<td>57 (95.0)</td>
<td>3 (5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>70</td>
<td>48 (68.6)</td>
<td>46 (95.8)</td>
<td>2 (4.2)</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>18</td>
<td>15 (83.3)</td>
<td>15 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>30</td>
<td>25 (83.3)</td>
<td>25 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

* Day of transfer to pseudopregnant recipient considered as first day of gestation.
† Heart beating at time of isolation.

Table 1. Postimplantation development of electrofused tetraploid embryos transferred to pseudopregnant recipients analysed at various stages of gestation
gestation had between 10 and 25 pairs of somites present. In all of these embryos, whatever the other abnormalities present, the cephalic region, and particularly the forebrain and its derivatives, was invariably grossly abnormal. It was also clearly apparent that the craniofacial region of all of these embryos had certain characteristic features in common (detailed below). Similarly, while relatively high rates of implantation were observed when recipients were autopsied on the 11th day of gestation, the total number of viable embryos recovered was more modest. Thus out of a total of 38 embryos that implanted, a total of 19 embryos were recovered, and of these only 5 appeared on gross inspection to be healthy at the time of their isolation. All five had both fore- and hindlimb buds present, though the vertebral axis was abnormal in four of these embryos. The caudal part of the vertebral axis in two of these embryos appeared to be substantially reduced compared to normal embryos at a similar developmental stage, while the vertebral axis in a further two embryos displayed a severe degree of lateral deviation caudally from the mid-trunk region. In only one embryo in this group did the vertebral axis appear to be grossly normal. One of the two embryos in this group, which displayed a reduced caudal vertebral axis, also had situs inversus. The crown–rump lengths of the two largest embryos in this group measured 3.6 mm and 3.5 mm, respectively.

Out of a total of 70 embryos transferred to recipients that were autopsied on the 12th day of gestation, 19 limb-bud-stage embryos were recovered, but of these only 6 appeared to be viable at the time of their isolation. All members of this group had characteristic craniofacial abnormalities of varying degrees of severity. Apart from possessing an abnormal craniofacial region, only one embryo in this group appeared on gross inspection to have an otherwise normal postcranial axial morphology. A second embryo displayed a reduced caudal part of its vertebral axis, while the vertebral axis of a third embryo displayed a severe degree of lateral deviation caudally from the mid-trunk region. The three other embryos recovered at this time were substantially smaller and less well developed than the former group, but had no obvious axial abnormalities. The developmentally most advanced embryo in this group had situs inversus, while one of the smaller of the viable embryos had an overexpanded pericardium in which was located a morphologically abnormal heart. The crown–rump lengths of the three largest embryos in this group measured 5.7 mm, 5.3 mm and 4.3 mm, respectively. The two largest embryos from this group are illustrated in Fig. 1A and 1B.

Out of a total of 104 embryos transferred to recipients that were autopsied on the 13th day of gestation, a total of 44 limb-bud-stage embryos were recovered, 10 of which were healthy at the time of their isolation. All possessed characteristic craniofacial abnormalities of varying degrees of severity. Two of these embryos, on gross inspection, appeared otherwise to be morphologically normal. Two additional embryos, one of which possessed an overexpanded pericardium, were grossly retarded, while a further six embryos in this group had severe vertebral axis abnormalities. Two of the embryos in this group, while clearly viable at the time of their isolation, had an extensive open neural tube defect which extended from the midbrain–hindbrain junction to the midtrunk region. In one of these embryos, a large omphalocele, which contained the liver and gut tube, was also present. The crown–rump lengths of the three largest embryos in this group measured 7.5 mm, 6.5 mm and 6.2 mm, respectively. A selection of embryos from this group are illustrated in Fig. 1C–I, and Fig. 2A–G.

Out of a total of 68 embryos transferred to recipients that were isolated on the 14th day of gestation, only three limb-bud-stage embryos were recovered, and only one of these appeared to be healthy at the time of its isolation. While possessing an abnormal craniofacial region and overexpanded pericardium, this embryo appeared otherwise on gross inspection to have a normal postcranial axial morphology. The crown–rump length of this embryo was 8.2 mm (see Fig. 2H, I). A similar situation was observed in relation to the 70 embryos that were transferred to recipients that were autopsied on the 15th day of gestation. A total of two viable embryos were recovered, only one of which appeared to have a normal postcranial axial morphology. In this embryo, a single midline forebrain vesicle was present. However, there was no evidence of any intraocular structures and the pituitary gland was also absent. The crown–rump length of this embryo was 9.3 mm. The second embryo in this group had a diminished caudal vertebral axis and an enlarged, morphologically grossly abnormal and protruding heart.

If the crown–rump length and external morphological features of the developmentally most advanced tetraploid embryo isolated in this study are compared with those of normal diploid embryos, it is seen to be approximately equivalent to a normal embryo of about 13 days p.c. (see Fig. 3). A detailed histological examination of this embryo, however, strongly indicates, from an analysis of a variety of organs and the degree of differentiation of its limbs, that it is developmentally equivalent to a normal embryo of about 13.5–14 days p.c.

When autopsies were carried out on the 17th and 18th days of gestation, no embryos were recovered. In about half of the implants, while no evidence of an embryo was seen, large discarded placentas were recovered, suggesting that, in these instances, pregnancy had probably progressed beyond the 14th or 15th day of gestation.

Cytogenetic analysis of the extraembryonic membranes of all of the embryos isolated in this study revealed that they were all homozygous tetraploids. All of the mitotic preparations contained two paternally derived Rb(1.3) 'marker' chromosomes.

(2) Craniofacial features
In all of the tetraploid embryos isolated that developed
paralleled that of the optic cup. A selection of the optic abnormalities encountered is illustrated in Fig. 5. Differentiation, or otherwise, of the lens usually closely bore no clear relationship to the degree of differentiation, or otherwise, of the optic apparatus on one side, often minute or absent. The degree of differentiation, or otherwise, of the optic nerve was present, the eye was either clearly abnormal. In some instances, while the proximal vesicle is also present. Crown-rump length 7.5 mm (See Table 2, no. 5). (D) An embryo with similar features to those seen in the embryo illustrated in 1(C). Crown-rump length 6.5 mm (See Table 2, no. 4). (E,F) Frontal and lateral views of an embryo with reasonably normal craniofacial features, but note the presence of a swelling at the forebrain–midbrain junction. The olfactory pits are well differentiated. Crown–rump length 5 mm. (G–I) Posterior (G), lateral (H) and frontal (I) views of a grossly abnormal embryo with an extensive open neural tube defect that extends from the midbrain–hindbrain junction to the mid-trunk region. In addition to possessing particularly abnormal craniofacial features, this embryo also displays an abnormal, laterally deviated, vertebral axis. Crown–rump length about 4.5 mm.

beyond the forelimb bud stage, a syndrome complex of craniofacial abnormalities was consistently observed. In all instances, the forebrain vesicles of the embryos isolated after the 10th day of gestation were smaller than those of normal embryos analysed at similar developmental stages. More specifically, the forebrain vesicles showed only a moderate degree of symmetrical enlargement. In some instances the enlargement was clearly asymmetrical, and in a few embryos there was no evidence of normal differentiation to form the paired telencephalic vesicles. The above features were confirmed when these embryos were analysed histologically. A representative selection of histological sections through the forebrain region of tetraploid embryos at various stages of development is illustrated in Fig. 4.

In the majority of these embryos, the infundibular recess of the diencephalon and its principal derivative the neurohypophysis appeared to be normal. However, by contrast, the optic evaginations and their derivatives were found to be substantially smaller than expected, and in the majority of cases were clearly morphologically abnormal. In some instances, while the proximal part of the optic nerve was present, the eye was either minute or absent. The degree of differentiation, or otherwise, of the optic apparatus on one side, often bore no clear relationship to the degree of differentiation observed on the other side. The degree of differentiation, or otherwise, of the lens usually closely paralleled that of the optic cup. A selection of the optic abnormalities encountered is illustrated in Fig. 5.

In a proportion of the embryos isolated on the 12th and subsequent days of gestation, a prominent enlargement was clearly apparent, which protruded in the midline from the dorsal surface at the forebrain–midbrain junction, and may represent an enlarged epiphysis (see, for example, the embryos illustrated in Fig. 1C and D).

(3) Vertebral axis features
In about two-thirds of the embryos that successfully developed beyond the forelimb-bud stage, major abnormalities were present in relation to the vertebral axis, which often showed a severe degree of lateral deviation (scoliosis) in the midtrunk region, principally at the lower thoracic/upper lumbar level (see, for example, the embryos illustrated in Figs 1B; 1G–I; 2A–C; 2D,E; 2F,G). The latter defect was almost invariably associated with a moderate to severe degree of lordosis due to a dorsal axial deviation at the same level. In several of these embryos, extensive neural tube defects, with widely everted neural folds, were also present that extended caudally from about the level of the midbrain–hindbrain junction to the midtrunk region. Two examples of embryos with this type of lesion are illustrated, namely in Figs 1G–I, and 2A–C. Occasionally, a forking and a lateral duplication of the caudal part of the neural axis was observed in the midtrunk region (see, for example, the embryo illustrated in Fig. 2D,E).

(4) The crown–rump lengths of tetraploid embryos and those of developmentally matched control diploid embryos
In order to establish whether the tetraploid embryos obtained in this study were of similar size to, or were possibly smaller than, normal diploid embryos, it is essential that developmentally matched stages are compared. To undertake this exercise, the exact developmental stage of a representative selection of seven tetraploid embryos, all of which had a normal vertebral axial morphology and had been serially sectioned in the transverse plane, was established. In each case, the unfixed crown–rump length was known, and the fixed crown–rump lengths were established from analysis of the numbers of sections cut at a nominal thickness of 8 μm. The embryos ranged in size from an unfixed crown–rump length of 3.6–9.3 mm, and had been isolated between the 11th and 15th day of gestation. The detailed findings are presented in Table 2. The degree of shrinkage observed following the fixation and serial sectioning of this material ranged from 66–83 % of the original crown–rump length. When the histological features of the tetraploids were examined in detail it was then possible to individually match them with controls of known gestational age from the author’s (i.e. MHK) reference collection of serially sectioned material. This manoeuvre then allowed the overall size, as assessed by the crown–rump length, of the individual tetraploid embryos to be compared with that of developmentally matched controls (see Table 2). In six out of seven instances, the
Fig. 2. External morphological appearance of a representative selection of tetraploid embryos isolated at either day 13 (A–C; D,E; F,G) or day 14 (H,I) of gestation. (A–C) Frontal (A), anterolateral (B) and posterior (C) views of a grossly abnormal embryo that has a similar extensive open neural tube defect to the embryo illustrated in Fig. 1G–I. The telencephalic vesicles are asymmetrically enlarged, and the midline facial features are reduced in extent. Crown–rump length about 6.2 mm. (D,E) Frontolateral (D) and posterior (E) views of an embryo with both severe craniofacial and vertebral axial abnormalities. Note that the neural tube forks in the mid-trunk region, giving rise to a Y-shaped (duplicated caudal) spinal cord. Crown–rump length about 5 mm. (F,G) Frontal (F) and posterior (G) views of an embryo with similar vertebral axial abnormalities to those seen in the embryo illustrated in D,E. As in the latter embryo, the forebrain vesicle appears not to have differentiated into two telencephalic vesicles. Crown–rump length about 4.5 mm. (H,I) Lateral (H) and frontal views (I) of an embryo with a normal vertebral axis, but moderately severe craniofacial abnormalities. The two telencephalic vesicles, though present, are incompletely separated. Crown–rump length 8.2 mm (see Table 2, no. 6).

Crown–rump lengths of the tetraploids were moderately to slightly smaller than those of the matched controls. What did emerge from this study was the fact that, with a single exception (embryo 7), in which a small volume of the right lobe of the liver was present in the physiological umbilical hernia, the histological features of all of the intraabdominal organs and limbs appeared to be normal. In a second embryo (no. 6) there was an extreme degree of vascular engorgement which was particularly marked in relation to the anterior and posterior cardinal venous systems.

A preliminary morphometric analysis of the cellular and nuclear volume of the primitive red cells, and the nuclear volume of a variety of tissues, has revealed that, in all of the tissues studied, the volume was significantly greater in the tetraploids than in comparable tissues in developmentally matched diploid controls (C. Henery, personal communication).

Discussion

When appropriate experimental conditions had been established (Kubiak and Tarkowski, 1985; Ozil and Modliński, 1986; Kato and Tsunoda, 1987), we observed that electrofusion was an extremely efficient and simple means for producing blastomere fusion when 2-cell-stage embryos are exposed to this form of fusigenic stimulus. The fact that extremely high rates of blastomere fusion and of tetraploid development could be achieved without exposure of embryos to cytochalasin or colcemid, or the inevitable microinjection and micromanipulatory steps involved when inactivated Sendai virus is used, has had the additional advantage

Fig. 3. Lateral views of normal embryos isolated on the 13th (A) and 14th (C) days of gestation (12.5 and 13.5 days p.c., respectively), illustrated at the same magnification as a tetraploid embryo (B) isolated on the 15th day of gestation. The external morphological features of the tetraploid embryo suggest that it is developmentally equivalent to a normal embryo of about 13 days p.c. However, a detailed histological analysis of this embryo indicates that it is developmentally equivalent to a normal embryo of about 13.5–14 days p.c. Note the presence in the tetraploid of a variety of craniofacial abnormalities including a very small/absent eye on the side illustrated. This was associated with partial fusion of the cerebral hemispheres, and absence of the philtrum. Crown–rump length 9.3 mm (see Table 2, no. 7).
Fig. 4. Representative series of histological sections through the cephalic region of tetraploid embryos at various stages of development to illustrate the abnormal morphological appearance of the forebrain region invariably seen in these embryos. The developmental ages of the tetraploids are typical of normal diploid embryos isolated at about 10.5 days p.c. (A,B), 12–12.5 days p.c., (C,D,E) and 13 days p.c. (F). All sections stained with haematoxylin and eosin. (A) Note the abnormal appearance of the forebrain and hindbrain. While olfactory placodes are present, the optic apparatus is absent bilaterally. x63. (B) Despite the asymmetry of the section, it is possible to see that while the hindbrain, otocyst and trigeminal ganglion appear to be normal, the forebrain vesicle is grossly distorted and has failed to differentiate into the paired telencephalic vesicles (lateral ventricles). x40. (C) While the hindbrain in this embryo, as well as the otocysts and trigeminal ganglia, appear to be normal, the two telencephalic vesicles (lateral ventricles), while present and symmetrical, are much smaller than would be expected at this stage of development. x25. (D) This embryo has an extensive neural tube defect which extends from the midbrain–hindbrain junction to the mid-trunk region, and the exposed neural ectoderm in the hindbrain is clearly evident in this section. Note in addition the gross distortion of the forebrain, and asymmetrical and grossly abnomral telencephalic vesicles (lateral ventricles). x40. (E) While the hindbrain in this embryo appears to be normal, the telencephalic vesicles (lateral ventricles), though present, are asymmetrically enlarged and much smaller than would be expected at this stage of development (for external morphology of this embryo, see Fig. 1C). x25. (F) While the hindbrain in this embryo appears to be normal, the forebrain is clearly grossly abnomral. An unsuccessful attempt has been made to form telencephalic vesicles, and the lumina of the lateral ventricles are only just evident (for external morphology of this embryo, see Fig. 3B). x25.
of substantially decreasing the technical difficulties that we previously encountered (O’Neill et al. 1990) in the production of tetraploid embryos. Moreover, electrofusion appears to have a less harmful effect on the development potential of fused embryos than other experimental procedures, and consequently we have been able to recover and undertake a preliminary analysis of advanced postimplantation stages of tetraploid mouse embryonic development.

The present findings have consequently confirmed, after a period of almost 15 years, Snow’s original observation (Snow, 1975) that genetically homozygous tetraploid mouse embryos are capable of development to advanced postimplantation stages. It was also apparent from our own studies that those embryos that survived beyond the early postimplantation period consistently developed characteristic craniofacial abnormalities, often, though not invariably, associated with cardiac and vertebral axial lesions. While a few of Snow’s embryos displayed craniofacial abnormalities similar to those observed in this study (see Snow, 1975), this was clearly not a consistent finding in his study. Indeed, according to Snow (1975) ‘the external morphology of all the live 4N embryos except the 17.5-day embryo was entirely normal and consistent with their gestational age. The 17.5-day embryo had externalised viscera but was otherwise normal. They were however, small in comparison with diploids of similar age...’. This difference between Snow’s and our own findings reported here is of considerable interest, and may be a manifestation of strain variability. The latter suggestion is consistent with observations by others in the survival of mouse trisomies of different genetic backgrounds (for reviews, see Epstein, 1986; Dyban and Baranov, 1987). Despite the presence of craniofacial and other abnormalities, we believe that genetically homozygous mouse tetraploids produced by electrofusion are probably capable of more advanced embryonic development than we have so far obtained. This would be consistent with the situation in man, where pure tetraploid infants have very rarely survived to term. Cleavage anomalies during early development, however, can also lead to the development of diploid–tetraploid mosaic embryos, and to date at least eight infants, both male and female, of this type have survived to term. Both pure tetraploid and diploid–tetraploid mosaic infants invariably have multiple congenital abnormalities, and are severely mentally retarded (Golbus et al. 1976; Pitt et al. 1981; Veenema et al. 1982; Scarbrough et al. 1984; Shiono et al. 1988; Wilson et al. 1985).

The overall morphology of the craniofacial region in the tetraploid embryos encountered in our study was invariably associated with a rostral neural tube syndrome complex. The abnormal features of the latter varied from mild to severe in their manifestation, though a reduction in the volume, and failure of differentiation of the forebrain and its derivatives was always observed. In a few of the limb-bud-stage embryos recovered, two symmetrical and reasonably well-developed telencephalic vesicles were present. In others, the telencephalic vesicles though present were asymmetrically formed, while in yet others little or no evidence of differentiation of the primitive forebrain (prosencephalic) vesicle to form two telencephalic vesicles was apparent. These forebrain abnormalities were frequently associated with bilateral either partial or complete failure of differentiation of the optic apparatus. Some of the abnormalities observed resemble those seen in certain mutant states, such as in homozygous Sey/Sey embryos (Hogan et al. 1984; Shiono et al. 1988), though in the tetraploids the failure of lens induction, and the other eye abnormalities, was not usually associated with abnormalities of the nasal pits. In a small proportion of embryos, the pituitary gland was also absent.

The type of abnormalities encountered here, therefore, closely resembles the holoprosencephaly sequence, with its associated arhinencephaly-cebocephaly-cyclopia defects encountered in about 1 in 5000 newborn human infants, though the incidence in spontaneous abortions is said to be substantially greater (Cohen, 1982; Smith and Jones, 1982). It has been

Table 2. A comparison of the crown–rump lengths of a representative selection of serially sectioned tetraploid embryos with normal postcranial vertebral axial morphology with those of developmentally matched diploid controls

<table>
<thead>
<tr>
<th>Tetraploid series</th>
<th>Diploid control series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref. no.</td>
<td>Day of gestation* at autopsy</td>
</tr>
<tr>
<td>1</td>
<td>9021</td>
</tr>
<tr>
<td>2</td>
<td>9027</td>
</tr>
<tr>
<td>3</td>
<td>9095</td>
</tr>
<tr>
<td>4</td>
<td>9031</td>
</tr>
<tr>
<td>5</td>
<td>9033</td>
</tr>
<tr>
<td>6</td>
<td>9094</td>
</tr>
<tr>
<td>7</td>
<td>9039</td>
</tr>
</tbody>
</table>

*Calculated from total number of sections in series×nominal section thickness (8 μm).
Fig. 5. Representative series of histological sections through the cephalic region of tetraploid embryos at various stages of development to illustrate the abnormal morphology of the optic apparatus generally seen in these embryos. The developmental ages of the tetraploids are typical of normal diploid embryos isolated at about 10.5 days p.c. (A), 12–12.5 days p.c. (B–G) and 13 days p.c. (H). All sections stained with haematoxylin and eosin.

(A) A small optic cup and lens vesicle are present. A short blind-ending optic stalk was present on the opposite side. ×160. (B,C) A minute optic cup is present on one side, with no evidence of a lens (B) ×100. A somewhat similar arrangement was present on the opposite side, associated, however, with a moderately well-differentiated lens vesicle (C). ×160. (D,E) The optic apparatus in this embryo was very rudimentary. On both sides a minute optic cup was present, though no evidence of a lens was seen. ×100. (F,G) A relatively normal eye was present on one side, but no evidence of an eye was present on the opposite side (for external morphology of this embryo, see Fig. 1,C). ×160. (H) In this embryo, a blind-ending optic stalk was present bilaterally. Reasonably well formed eyelids were however, apparent (for external morphology of this embryo, see Fig. 3B). ×40.

suggested that these features may result from a failure of migration of the prechordal mesoderm into the area anterior to the notochord. This is believed to be essential for the normal development of the midfacial region, as well as having an inductive role in the morphogenesis of the forebrain (Smith and Jones, 1982). Alternatively, the craniofacial features observed might be a consequence of interference with neural crest cell migration. It is believed that certain facial malformations may arise by this mechanism (for reviews, see Le Douarin, 1982; Hall, 1988), and much work has been undertaken, using a variety of sophisticated histochemical techniques and more recently using appropriate gene probes, to map the pattern of neural crest cell migration, and establish its ultimate fate in a variety of mammalian species (for recent review, see Hall, 1988). Less information is, however, available on the factors that influence the development of the forebrain, though recent chick–quail chimera studies have been informative in this regard (Couly and Le Douarin, 1985, 1987, 1988).

Similar craniofacial abnormalities to those observed in this study have also been induced experimentally in animals by a variety of teratogenic agents (Adelmann, 1936a,b; Giroud et al. 1963; Sulik and Schoenwolf, 1982; and for review of the earlier literature, see Warkany, 1971) including ethanol (for recent review, see Sulik et al. 1988, and for observations on ocular manifestations of ethanol teratogenicity, see Cook et al. 1987). These abnormalities have also occurred in sheep as a result of the ingestion of the plant Veratrum californicum between the fifth and fifteenth days of gestation (Babbott et al. 1962). However, while there is no specific environmental teratogenic influence known to be consistently associated with this anomaly in man, this condition is frequently associated with trisomy 13, in which midline facial defects are often encountered, and is occasionally seen in individuals with a deletion of the short arm of chromosome 18, though other individuals with similar craniofacial defects are reported to have had a normal karyotype (Bishop et al. 1964; Uchida et al. 1965; Warkany et al. 1966).

Unlike the situation observed in triploid mouse embryos, which are also capable of developing to at least the forelimb bud stage (Kaufman et al. 1989), but in all probability not much further, possibly due to their genetically unbalanced state, there seems no obvious explanation for the relatively poor development potential and consistent abnormalities in those tetraploids encountered in the present study that survived to midgestation. It has been suggested that, in these polyplody states, one possible factor that might account for their restricted development is the enlarged size but decreased total number of individual cells and their abnormal relationship to each other (Epstein, 1986). We consequently plan in the near future to undertake a detailed histological and morphometric analysis of this material, to investigate this possibility. Our own findings to date indicate that tetraploid embryos are generally moderately to slightly smaller than developmentally matched diploid controls, and that their nuclear volume is significantly greater, when the cells within matched tissues and organs are compared. Until detailed morphometric studies are undertaken, however, it is unclear whether the apparent histological normality of their postcranial tissues and organs results from an increased cell packing density, with the tetraploids having the same total number of cells in their organs as controls. Alternatively, there may be an overall decrease in the cell population of these organs, and this might provide a compensatory mechanism to accommodate any possible increase in the volume of their component cells.

We also plan to analyse the expression of certain homebox-containing genes (such as HOX-1.1, HOX-7.1 and retinoic acid receptor-gamma transcripts (Ballantyne et al. 1989; Hill et al. 1989; Ruberte et al. 1990)) that are expressed, for example, in facial and precartilaginous mesenchyme and neural crest tissue, in the hope that this may shed important new light on pattern formation in relation to the differentiation of the craniofacial region, the heart and the postcranial vertebral axis.

We thank Mr J. Cable for photographic assistance, and Miss C. Arnott for histological assistance.

References


