The development of monosomy 19 mouse embryos

By TERRY MAGNUSON,1 SANDRA SMITH AND CHARLES J. EPSTEIN

From the Departments of Pediatrics and of Biochemistry and Biophysics, University of California, San Francisco

SUMMARY

In general, autosomal monosomy is lethal much earlier in mammalian development than autosomal trisomy. In an attempt to understand why monosomy is so deleterious, we have begun to characterize the development of mouse embryos monosomic for chromosome 19. A dramatic loss of monosomy 19 embryos was found to occur between days 3 and 4 of development. This loss occurred both in vivo and in vitro and with intact blastocysts or isolated inner cell masses. Experiments with inbred strains showed that this loss was not due to the expression of recessive lethal genes. While monosomic embryos were found to have fewer cells than normal and trisomic litter-mates beginning at the early morula stage, the ability to form blastocysts is not interfered with. Electron microscopy revealed no difference in the cellular ultrastructure of monosomic when compared with diploid embryos. Furthermore, two-dimensional gel electrophoresis did not reveal any differences in the proteins synthesized by monosomic, trisomic or diploid litter-mates when examined at day 3 of development. These results indicate a lack of gross genomic disturbances in monosomic embryos. When monosomy→diploid chimaeras were made, viable monosomic cells were found in day-9 post-implantation embryos, well past the lethal period. Thus, in chimaeric embryos, the normal cells appear to be able to provide whatever is lacking, suggesting that monosomy 19 is not a cell lethal. Instead, death may be due to a dosage alteration in specific gene products needed during early development.

INTRODUCTION

When aneuploid progeny are produced in mice by breeding techniques that result in a high rate of non-disjunction, trisomic embryos are found to survive well past implantation (White, Tjio, Vandewater & Crandall, 1974; Gropp, Kolbus & Giers, 1975). In contrast, monosomic embryos generally die before or during implantation (Dyban & Baranov, 1978; Epstein & Travis, 1979; reviewed by Magnuson and Epstein, 1981). Studies in man on spontaneous abortions also reveal that while a large number of trisomic foetuses are identified, monosomic foetuses are rarely found (Boué, Boué & Lazar, 1975; Hassold et al. 1978). Therefore, in attempting to further our understanding of the pathogenesis of conditions resulting from aneuploidy, it is of interest to determine why autosomal monosomy is lethal so much earlier in development than is trisomy.

1 Author's address: Room 1421 HSW, Department of Pediatrics, University of California, San Francisco, California 94143, U.S.A.
Fig. 1. Production of mouse embryos aneuploid from chromosome 19. When a mouse is doubly heterozygous for two different Robertsonian metacentric chromosomes, each of which has a chromosome in common, non-disjunction occurs during meiosis about 20% of the time. Thus, when a male carrying two of these metacentric chromosomes is mated with a normal female, the resulting litter will consist of monosomic, trisomic and diploid embryos.

In an earlier study (Epstein & Travis, 1979) using males carrying both the Rb(8.19)1Ct and Rb(9.19)163H translocations as fathers, we observed that mouse embryos monosomic for chromosome 19 have a very well-defined time of death. The frequencies of trisomy and monosomy are equal (approximately 15–20% each) on day 3 (early blastocyst) of preimplantation development, but on day 4 (late blastocyst), the frequency of monosomic embryos decreases greatly while that of the trisomic embryos remains the same. We have now repeated these studies using males carrying a different combination of translocation chromosomes and have obtained similar results. We have also extended these studies in an attempt to begin to understand why monosomy 19 die so early in development. Our results suggest that monosomy 19 is not a cell lethal, and that death may be due directly to the reduction by half of the synthesis of specific gene product(s) needed at that time in development.

MATERIALS AND METHODS

A. Breeding scheme for the production of aneuploid embryos. Male mice homozygous for the Rb(5.19)1Wh Robertsonian translocation chromosome (obtained from Dr D. Trasler, McGill University, Montreal, Canada) were mated with females homozygous for the Rb(9.19)163H Robertsonian translocation (obtained from Dr A. Gropp, Abteilung für Pathologie der Medizini-
Fig. 2. Identical twin half embryos obtained by the twin embryo technique. The two blastomeres of a 2-cell embryo were separated from one another as described in the text. Each half embryo was individually cultured for 4 days. One twin embryo was then spread (A) allowing for karyotypic identification. Once identified, the other twin (B) was used for experimental purposes. Magnification: B = 400 x.

To obtain monosomy 19, trisomy 19 and diploid embryo litter-mates, the doubly heterozygous Rb(5.19)1Wh/Rb(9.19)163H males were mated with superovulated ICR Swiss Albino or C57BL/6 females (obtained from Simonsen Laboratories, Gilroy, California) (see Fig. 1 for diagram of breeding scheme). Standard procedures were then used to flush embryos from oviducts or uteri with modified Whitten's medium (Epstein, Wegienka & Smith, 1969; Golbus & Epstein, 1974). The day the fertilization plug is observed is designated as day 0.

B. Karyotyping of preimplantation embryos. Embryos were first incubated for 3–6 h in medium containing 0.07 μg/ml vinblastine (Velban, Gibco). After incubation, the embryos were placed in 0.75% sodium citrate for 2 min and then transferred to a 3:1 methanol:acetic acid fixative for an additional 2 min. Each fixed embryo was then transferred to a pre-cleaned microscope slide, and
Fig. 3. Preparation and characterization of aneuploidy 19→ normal chimaera.

The zona pellucida was removed from 2-cell embryos with 0.5% pronase (Calbiochem) in phosphate-buffered saline (Mintz, 1962). The embryos were then washed five times in medium. The two blastomeres of an individual embryo were separated from one another by gentle aspiration in a micropipette (Epstein, Smith, Travis & Tucker, 1978). The pairs of blastomeres were then transferred to wells of a Terasaki microtest tissue culture plate (Falcon 3034) and cultured at 37 °C in 5% CO₂ in air under oil (10 ml Fisher light paraffin oil per plate). On the morning of the fourth day of culture, one twin was karyotyped (Fig. 2a) while the other twin (Fig. 2b) was used for experimental purposes.

D. Two-dimensional (2D) electrophoresis. Monosomic, trisomic or diploid twin embryos at the early to mid blastocyst stage were grouped into pools of 6 and were then incubated for 4 h in medium containing approximately 2 mCi/ml [³⁵S]methionine (specific activity of 900–1100 Ci/mmole, Amersham) (Magnuson & Epstein, 1981b). After incubation the embryos were washed three times with phosphate-buffered saline and transferred to a glass test tube. The excess phosphate-buffered saline was removed with a micropipette and the embryos were solubilized in 20 µl of O'Farrell's (1975) isoelectric focusing
Aneuploid mouse embryos

Table 1. Survival of early mouse embryos aneuploid for chromosome 19*
Cross: ICR × Rb(9.19)163H/Rb(5.19)1Wh

<table>
<thead>
<tr>
<th>Stage</th>
<th>Sample size</th>
<th>Monosomic</th>
<th>Trisomic</th>
<th>Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>8- to 16-cell</td>
<td>132</td>
<td>16</td>
<td>21</td>
<td>63</td>
</tr>
<tr>
<td>Morula</td>
<td>102</td>
<td>11</td>
<td>13</td>
<td>76</td>
</tr>
<tr>
<td>Early blastocyst</td>
<td>122</td>
<td>17</td>
<td>17</td>
<td>56</td>
</tr>
<tr>
<td>Late blastocyst</td>
<td>77</td>
<td>3</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>Day 3 to day 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(in vitro)</td>
<td>161</td>
<td>7</td>
<td>22</td>
<td>71</td>
</tr>
<tr>
<td>Day 3 ICM† to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 5 ICM (in vitro)</td>
<td>58</td>
<td>3</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>Day 9‡</td>
<td>61</td>
<td>0</td>
<td>21</td>
<td>79</td>
</tr>
</tbody>
</table>

* Embryos were flushed at appropriate stage and then karyotyped.
† ICM = inner cell mass, isolated by immunosurgery (Solter & Knowles, 1975).
‡ Cross: C57BL/6 × Rb(9.19)163H/Rb(5.19)1Wh.

sample buffer. Two-dimensional gel electrophoresis was performed as outlined by O’Farrell (1975) with a 4.5% stacking gel and a 10% separating gel being used in the second dimension. Kodak NS-2T film was used for autoradiography of the dried gels.

E. Electron microscopy. Twin embryos at the early to mid-blastocyst stage were prepared for thin-section electron microscopy as previously described (Magnuson, Demsey & Stackpole, 1977). The stained sections were examined with a Hitachi HS-8 electron microscope.

F. Preparation of aneuploidy 19 chimaeras. Aggregation chimaeras were made by the general method described by Mintz (1971) (see Fig. 3 for diagram). Briefly, superovulated C57BL/6 females were mated with Rb(9.19)163H/Rb(5.19)1Wh males to produce monosomic, trisomic or diploid embryo litter-mates that were glucose phosphate isomerase-1 type b (GPIb). The embryos were removed from the mother at the 2-cell stage and cultured overnight until the 4-cell stage, at which point the zona pellucida was removed. These embryos were then aggregated with 8- to 16-cell BALB/c embryos (GPIa) in a 1:100 dilution of phytohaemagglutinin (PHA-P, Difco) in phosphate-buffered saline. The aggregates were washed and cultured overnight until the late morula to early blastocyst stage. Then, approximately 10–12 embryos were implanted with a micropipette into each uterine horn of pseudopregnant ICR females. The pregnant females were killed 7 days later and the embryos dissected out.

G. Determination of chimaerism. The existence of chimaerism was assessed by analysis of the GPI pattern. Portions of the head and tail and a portion of
Table 2. Mean cell number per embryo*

<table>
<thead>
<tr>
<th>Stage</th>
<th>Monosomic</th>
<th>Trisomic</th>
<th>Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>8- to 16-cell</td>
<td>13±2†</td>
<td>13±2</td>
<td>12±2</td>
</tr>
<tr>
<td>Morula</td>
<td>15±2‡</td>
<td>12±2</td>
<td>22±2</td>
</tr>
<tr>
<td>Early blastocyst</td>
<td>46±3‡</td>
<td>60±3</td>
<td>56±2</td>
</tr>
</tbody>
</table>

* Cell counts were done on the same population of embryos used in Table 1.
† ± SEM.
‡ Significantly different from normal and trisomic littermates, \( P < 0.005 \).

The extraembryonic membranes of each embryo were placed in separate aliquots of 0.04 M-Tris-glycine, pH 8.6, and vigorously aspirated. After freeze-thawing three times, 3–5 \( \mu l \) of the extract was applied to a cellulose acetate (Cellogel, Chemetron) sheet and electrophoresed in the Tris-glycine buffer at 250 V (constant current) for 3.5 h. The GPI bands were stained as described by Van Someren et al. (1974).

For karyotyping, the remaining portions of each embryo and membranes were incubated for 6 h in Dulbecco's modified Eagle's medium with 10% foetal calf serum, penicillin and streptomycin, and vinblastine (0.07 \( \mu g/ml \)). After the incubation, each sample was treated with 0.56% KCl for 15 min at 37 °C, fixed in 1 ml of 3:1 methanol:acetic acid for 15 min and then transferred to 0.1 ml 60% acetic acid. After 5 min, the acetic acid solution was transferred to a slide pre-warmed to 50 °C. After drying, the slides were stained with Giemsa.

RESULTS

A. Survival of embryos aneuploid for chromosome 19

Approximately equal frequencies of monosomic and trisomic embryos were found at the 8- to 16-cell, morula and early blastocyst (day 3) stages (Table 1, lines 1–3). However, at the late blastocyst stage (day 4), a considerable drop in the percentage of embryos that were monosomic was found (Table 1, line 4). In contrast, the percentage of trisomic embryos remained the same. At day 9, while the expected frequency of trisomic embryos was present, no monosomic
Aneuploid mouse embryos
Aneuploid mouse embryos

Fig. 6. Glucose phosphate isomerase (GPI) patterns of monosomy ↔ diploid (M-D) and diploid ↔ diploid (D-D) litter-mate chimaeras. The monosomic or diploid metacentric component of the chimera is marked by GPI\(^b\) and the normal diploid by GPI\(^a\). C, control mixture of GPI\(^a\) and GPI\(^b\).

embryos were found (Table 1, line 7). This loss of monosomic embryos occurred both \textit{in vivo} (Table 1, line 4) and \textit{in vitro} (Table 1, line 5) and with both intact blastocysts and isolated inner cell masses (Table 1, line 6). Since the chromosome 19 which is present in monosomic embryos comes from the mother (see Fig. 1), experiments with C57BL/6 mothers show that this loss of monosomy 19 embryos is not due to the expression of recessive lethal genes (Table 1, line 7; see also data from Epstein & Travis, 1979).

While the ability to form blastocysts is not interfered with and, on a gross morphological level, monosomy 19 embryos appear normal until the time of death, we have found that, beginning at the morula stage, monosomic embryos have fewer cells than normal and trisomic litter-mates (Table 2). This decrease in cell number was also found in early blastocysts.

B. \textit{2D gel electrophoresis}

A comparison of about 500 proteins synthesized by monosomic, trisomic or diploid twin embryos was made using 2D gel electrophoresis and autoradiography. Approximately equal numbers of radioactive counts were loaded on to each gel which was then exposed to X-ray film for about the same amount of time. When the autoradiograms were examined, no consistent difference was found in the proteins synthesized by the three types of embryos (Fig. 4).

Fig. 5. Thin-section electron micrographs of inner-cell-mass cells from a monosomic (A) and diploid (B) embryo. Arrows, degradative bodies; arrowheads, mitochondria; L, lipid droplets; G, Golgi apparatus; E, endoplasmic reticulum. Magnification: A, 44 370×, B, 47 430×.
Fig. 7. Light micrographs of a monosomy<->diploid (A) and a diploid<->diploid (D) chimaera. For the monosomy<->diploid chimaera, the monosomic karyotype (39 chromosomes) is shown in B and the diploid karyotype (40 chromosomes) is shown in C. For the diploid<->diploid chimaera, the diploid metacentric karyotype is shown in E (38 acrocentric and 1 metacentric chromosome, the metacentric being indicated by the arrow) and the diploid normal karyotype (40 chromosomes) is shown in F. Magnification, A and B: 60 x.

C. Cellular ultrastructure

When examined by thin-section electron microscopy, monosomic twin embryos appeared to be at the same developmental stage as their diploid litter-mates (Fig. 5). No difference in the ultrastructure of the nucleus, nucleoli, mitochondria, endoplasmic reticulum, Golgi apparatus, polysomes or inter-
Table 3. Composition of chimaeric embryos*

<table>
<thead>
<tr>
<th>Rbl Wh/Rbl63H input</th>
<th>Number of chimaeric embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M19→2N</td>
</tr>
<tr>
<td>&lt;50%</td>
<td>5</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>0</td>
</tr>
</tbody>
</table>

* Determined either by counting chromosome spreads or by estimating relative intensities of GPI bands. These determinations were not made on all of the chimaeras.

cellular junctions was found when three monosomic and three diploid embryos were analysed. The ultrastructure of these organelles appeared similar to that described by Calarco & Brown (1969) as being normal for blastocysts. The only monosomy 19-associated defect observed was an increase in the number of degradative bodies in the cytoplasm of monosomic embryos when compared to diploid embryos. When 21 random sections from three monosomic embryos were examined and compared to an equal number of sections from three normal embryos, 3.18 ± 0.69 (SEM) degradative bodies per section were found in the monosomic embryos and 1.85 ± 0.4 per section were found in normal embryos ($P < 0.05$).

D. Analysis of aneuploidy 19 chimaeras

A total of 744 aggregates were made and transferred into foster mothers. Of these, 90 (12%) implanted and grew. When dissected at day 9, 74 (82%) of the implants were chimaeric as determined by GPI analysis (Fig. 6). Ten were chimaeric in the extraembryonic membranes only while 34 were chimaeric in both membranes and embryos. The remaining 30 were chimaeric but embryos and membranes were not separated. Of the 74 implants, 69 were successfully karyotyped. Five (7%) of the chimaeras were monosomy 19 ↔ diploid. Of these, three were chimaeric in both embryo and extraembryonic membranes and two were chimaeric but embryo and membranes were not separated. Nine (13%) of the chimaeras were trisomy 19 ↔ diploid. Of these, four were chimaeric in both embryo and membranes, two were chimaeric in membranes only and three were chimaeric but embryo and membranes were not separated. All of the monosomy 19 ↔ diploid chimaeras appeared as healthy day-9 embryos (Fig. 7). The aneuploid contribution in the chimaeric embryo was always less than 50% for the monosomy 19 ↔ diploid chimaeras (Table 3). In contrast, the aneuploid or metacentric contribution in the trisomy ↔ diploid and diploid ↔ diploid chimaeras was usually greater than 50%.
DISCUSSION

In an earlier study (Epstein & Travis, 1979), we found that embryos monosomic for chromosome 19 appear to die between days 3 and 4 of in vivo or in vitro development. We have repeated these studies using a metacentric combination which is different from the combination used in our previous study and have obtained similar results. In addition to the intact embryo dying, we have now observed that isolated inner cell masses also die at this time, indicating that the defect cannot be attributed to the trophectoderm alone. Of the other aneuploids so far examined, only monosomy 17 embryos die earlier than the monosomy 19 embryos (Baranov, Dyban & Chebotar, 1980; Magnuson & Epstein, unpublished results). The others (monosomy 1, 12 or 15, and possibly 5, 9 or 14) die later than day 4 but the exact time of death is not yet known (Dyban & Baranov, 1978; Epstein & Travis, 1979).

The earliest manifestation of monosomy 19 is retarded cell growth. Beginning at the early morula stage, the monosomy 19 embryos lag behind in the number of cells per embryo. No other specific gross or ultrastructural abnormality was observed except for an increase in the number of degradative bodies found in the cytoplasm of monosomic embryos. This increase is more likely a manifestation of a dying embryo rather than a specific defect associated with monosomy 19. When two-dimensional gel protein patterns of monosomy 19, trisomy 19 and diploid embryos were examined, no consistent difference was found. Thus, the cause of death of monosomy 19 embryos remains unknown.

The ability to rescue monosomy 19 embryos in aggregation chimaeras suggests that the diploid cells are able, for at least some period of time, to provide whatever is lacking so that aneuploid cells can participate in normal development. This implies that monosomy 19 is not a cell lethal but rather that specific correctable defect(s) may be associated with this aneuploid condition. These results, together with our two-dimensional gel data, are compatible with the idea that monosomy 19 does not result in general genomic disturbances. Instead, the defect is likely to be due to a 50% dosage deficiency in chromosome 19 gene products needed at that time in development.

Previous work indicates that autosomal trisomy also does not result in widespread alterations in the synthesis, degradation or modification of gene products coded for by genes not located on the aneuploid chromosome (Weil & Epstein, 1979; J. Sawicki & C. J. Epstein, unpublished results). A number of studies do, however, indicate that aneuploidy does result in primary dosage effects for genes located on the chromosome in question (Krone & Wolf, 1977; Epstein, Tucker, Travis & Gropp, 1977; Feaster, Kwok & Epstein, 1977; Kurnit, 1979). Thus, the exact mechanisms by which aneuploidy results in death or abnormal development are probably due to alterations in specific cellular processes or structures directly affected by the aneuploid genes. The important difference between monosomy and trisomy is that monosomy results
Aneuploid mouse embryos

in an obligatory state of dosage deficiencies which might then have more serious consequences on concentration-dependent cellular processes than trisomy (Epstein, Epstein, Cox & Weil, 1981).

In all cases, the monosomy 19 contribution to the aggregation chimaeras was less than 50%, while the trisomic or diploid metacentric component was usually much greater than 50%. The decreased monosomic component could be due to the inability of a chimaera to survive if a majority of the cells are monosomic. If this were the case, one would expect to find a significant decrease in the proportion of monosomy 19 ↔ diploid chimaeras when compared to the proportion of trisomy 19 ↔ diploid chimaeras recovered. While a difference was observed, the numbers are too small to determine if it is significant. An alternative explanation for the decreased monosomic component may be that while monosomy 19 cells are able to survive in a chimaera, they continue to divide at a slower rate than the diploid cells and, consequently, are eventually diluted out. If this were true, the monosomy 19 contribution should be inversely proportional to the age of the embryo. We are presently testing to see if this is in fact the case.

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REFERENCES


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