Postimplantation development of CB-induced triploid mouse embryos

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SUMMARY

By subjecting A strain eggs at the time of fertilization and polar body extrusion to 5 µg/ml cytochalasin B, digynic triploidy was produced in 80% fertilized eggs. Tripronucleate eggs were transplanted to recipients and examined between 9-11th day of pregnancy. Development of triploid mouse embryos up to day 7 is normal and most embryos form early egg cylinder. At day 8 the embryonic part of the cylinders is under-developed and later development fails to form an embryo. Development of foetal membranes is much less affected, CB-induced triploids survive to 10th-day of pregnancy.

INTRODUCTION

Mammalian embryos of abnormal ploidy have been used in developmental genetics research. This approach involves genome manipulation and experiments on effect of various types of heteroploidy on embryonic development. Efficient methods of obtaining mono- and trisomic mouse embryos (Gropp, 1975, 1976, 1978; White, Tijo, Van der Water & Crandall, 1974a, b), gynogenetic hypohaploid, haploid and diploid embryos (Kaufman & Sachs, 1975; and literature review: Graham, 1974; Kaufman, 1975, 1978; Tarkowski, 1975; Bałakier & Tarkowski, 1976) and haploid embryos from fertilized eggs (Modliński, 1975; Tarkowski, 1977) have been worked out.

Cytochalasin B turned out to be a useful tool in producing polyploidy in the mouse; it proved to be superior to all the other methods employed in production of triploidy (Niemierko, 1975; Niemierko & Komar, 1977) and tetraploid embryos (Snow, 1973; 1975; 1976; Tarkowski, Witkowska & Opas, 1977). The other methods for inducing triploidy and tetraploidy in mice are described by Niemierko & Opas (1978).

The attempts to induce triploidy in mammals were carried out in rat, rabbit and mouse (see Discussion). Most of experiments on triploidy in mouse dealt with preimplantation period (for references see Niemierko, 1975). The viability

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Table 1. Postimplantation development of CB-induced triploid mouse embryos

<table>
<thead>
<tr>
<th>Ploidy of eggs</th>
<th>3n</th>
<th>Total 3n</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of embryos (day)</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>No. of eggs</td>
<td>13</td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>No. of implantation (%)</td>
<td>5</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>(38,5) (37,8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of implantation without embryo</td>
<td>1</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>with embryo</td>
<td>4</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Embryos examined histologically</td>
<td>—</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>karyologically</td>
<td>4</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Ploidy of embryos</td>
<td>4 × n.d</td>
<td>5 × 3n</td>
<td>—</td>
</tr>
<tr>
<td>1 × n.d.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

n.d.: not detected, not analysable metaphase plates.
* 65 embryos examined morphologically.

of embryos carrying this aberration was only estimated in postimplantation period (see Discussion). Morphological expression of spontaneous triploidy in mouse was rarely analysed in detail (Wróblewska, 1971; Baranov, 1976). The present study describes postimplantation development of cytochalasin B-induced triploid embryos in the mouse.

MATERIALS AND METHODS

Triploid dygynic mouse oocytes were obtained by inhibiting cytokinesis of the 2nd maturation division in fertilized oocytes by means of cytochalasin B (Niemierko, 1975). Oocytes of A strain from spontaneous ovulation, fertilized in delayed, controlled matings, were used. Three hours after the vaginal plug was found, eggs were released from oviduct, cleaned of follicular cells and put...
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in drops of medium with cytochalasin B (5 µg/ml) under liquid paraffin. After culture eggs were examined under the inverted microscope for the presence of three pronuclei and triploid ones were transplanted to recipients in first day of pseudopregnancy. Control experiments involved transplantation of diploid eggs in which cytochalasin B did not block the 2nd polar body extrusion. Post-implantation development was examined on 9th, 10th, and 11th day. Implanted triploids and some diploids embryos were fixed in Bouin fixative, cut in 6 µm section and stained with haematoxylin and eosin or, after being dissected from uterus, karyologically. Chromosomal preparations from some triploid and control egg cylinders were made using the technique of Evans, Burtenshaw & Ford (1972). Remaining control embryos were studied morphologically. Their development was assessed by comparison of morphology with the embryos of equivalent post-coital age and on the basis of data given by Snell & Stevens (1966), and Theiler (1972).

RESULTS

Sixty-five triploid and 92 diploid eggs were transferred to oviducts (Table 1), both kinds of eggs being transferred separately to different recipient females.

After transplanting triploid eggs five implantations, one lacking an embryo, were found on the 9th day of development. They contained four embryos at an early egg-cylinder stage. The embryos looked normal although they were smaller than controls of the same age. Their ploidy was not assessed, but they can be classified as triploids, because block of 2nd polar body is irreversible (Niemierko, 1975). Moreover, selection of eggs for transplantation was very rigorous.

Fifteen implantations were found on the 10th day of development, but only 10 of them contained egg cylinders. Among six embryos studied karyologically in all but one case mitotic plates with 60 chromosomes were found. The genetic sex of those embryos was not determined.

Four egg cylinders examined histologically were different from each other. One of them was an empty yolk sac with no embryo inside (Fig. 3). The remaining three egg cylinders contained embryos in a pre-somite stage (Fig. 2, 5, 7–11). Egg cylinder No. 1. (Fig. 2) was at the stage of amnion formation with a distinctly shortened embryonic part. The embryo had a shortened ectoplacental cone in form of loose group of cells. Its stage corresponded to the early 8-day A strain diploid embryo. Egg cylinder No. 3 (Fig. 5, 6) was the most advanced in that stage.
organogenesis found in present study; it contained an incomplete embryo. Only the head part of this embryo, in form of headfold primordia, was developed. Also the foregut pocket could be clearly seen (Fig. 5). The embryo had regular membranes: two-layered amnion and allantois separated from ectoplacental cone and contained rudimental heart mesoderm. Dividing cells were visible in neural ectoderm and allantois (Fig. 6). Egg cylinder No. 4. contained no developed embryo. Scanty embryonic ectoderm in a neural plate stage (Fig. 7-11) and scanty mesoderm adhering to it could be seen. The cylinder contained two-layered amnion, rudimentary allantois and poorly developed ectoplacental cone (Fig. 7).

After transplanting control CB-treated eggs 75 implantation (85% of transplanted eggs) were found on day 10 of development. Diploid cylinders were retarded by 0-5-1 day in their development. An embryo was lacking only in two cases and in one case implantation in form of trophoblastic giant cells was found (2% of transplanted eggs). Each of six karyologically studied control embryos was diploid. Embryos studied histologically were normal: they were either in process of turning (Fig. 1) or prior to turning (Fig. 4).

On day 11 of development no implantation or resorption after transplanting triploid eggs was obtained.

**DISCUSSION**

Triploid embryos produced by means of cytochalasin B are capable of implanting. The yield of triploid implantations found in this study (37%) is similar to the yield of triploid blastocysts developed from CB-produced triploids eggs (Niemierko, 1975). This allows one to suppose that irrespective of the further course of development, all triploid 5-day embryos have the capacity to implant.

Up to date studies on triploidy in mouse indicate that this aberration may express itself in various ways show a whole range of anomalies: from egg cylinders as vesicles composed of foetal membranes, to a very few with embryonic structures at different stages of development.

Anomalies of triploid embryos become distinct only after 8th-9th day of development. The early triploid embryos, i.e. 6.5-7.5 days old, showed slight retardation, with no manifestation of anomalies in organogenesis of ectodermal and endodermal structures and therefore they were classified as normal (Fischberg & Beatty, 1951; Takagi, 1970; Takagi & Oshimura, 1973; Takagi & Sasaki, 1976; Baranov, 1976). Also, in the present study 9-day triploid embryos were morphologically normal although smaller.

Histologically studied, 10-day-old A strain triploid egg cylinders are comparable in size to early 8-day diploid egg cylinders of this strain. Triploid egg cylinders display characteristic developmental anomalies, comprising total lack or poor development of embryonic structures, with mesodermal derivatives being affected to a large extent. Foetal membranes of those egg cylinders are
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developed, although in size and proportion they seldom correspond with the embryos age. In none of the embryos examined was the allantois fused with ectoplacental cone. A properly developed ectoplacental cone was found only in one case (Fig. 3).

Similarly affected is the development of triploids of CBA strain (Wróblewska, 1971) and tetraploid CB-induced tetraploids described by Tarkowski et al. (1977).

Postimplantation mortality of triploid embryos in the mouse occurs in two specific periods. The majority of triploid ceases to develop around the 8th day and soon degenerate. In those few strains where triploids achieve relatively advanced organogenesis, they do not survive after the 12th day of pregnancy (Vickers, 1969; Takagi & Oshimura, 1973; Wróblewska, 1971, 1978; Baranov, 1976; Opas, 1977). Triploid embryos of other laboratory animals also survive only until mid-pregnancy. Triploid embryos of the rabbit survive until the 17th day (Bomsel-Helmreich, 1965, 1971), those of rat – until the 12 day (Piko & Bomsel-Helmreich, 1960).


Disorganized growth and limited life span of triploids reflects abnormalities in their cell properties, which affect cellular proliferation, differentiation, and cellular interactions.

Studies by Wróblewska (1978) suggest the influence of genotype on development of mouse triploids. Among spontaneous triploids derived from 16 genetic combinations, the most advanced organogenesis and highest viability was achieved by triploids of 129/Sv strain and its crosses. Morphologically variable expression of triploidy in different mouse strains (CBA, A–cylinders with no embryos; 129/Sv and its crosses–cylinders with embryos) support the latter conclusion i.e. that developmental capacities of embryos with that type of aberration are dependent on genetic background. This is most likely also the reason for differences in developmental potencies of tetraploid embryos obtained by Snow (1973, 1976), and Tarkowski et al. (1977).

Influence of genotype on development of triploids even before implantation was suggested by Braden (1957) who observed lower survival rate and high mortality of extensively homozygotic triploids in silver-strain mice. Also McGrath & Hillman (1981) found that dispermic triploids +/+/t12 and +/t12/t12 developed normally to the blastocyst stage, but triploid t12/t12/t12 are inviable.

Experimentally induced triploids described in the present study show phenotypic anomalies similar to those found in spontaneously arising embryos. The method described yields about 40% of implanted triploid embryos, which is
10 times the rate of spontaneous appearance of such embryos. It offers possibility of mass production of triploids for experiments on preimplantation embryos which may be crucial importance in solving the question of their abnormal development.

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REFERENCES


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