The postimplantation development of spontaneous digynic triploid embryos in LT/Sv strain mice

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Summary

When spontaneously ovulating LT/Sv female mice are mated with fertile males, between one third and one half of the zygotes analysed at the first cleavage mitosis are found to be triploid. This is due to the fact that LT/Sv females ovulate both primary and secondary oocytes, all of which are capable of being fertilized. Fertilization of the former group results in the production of digynic triploid conceptuses, while their diploid littermates result from the fertilization of normal secondary oocytes. The present study was therefore carried out in order to investigate the 'spontaneous' level of triploidy in these mice, and to provide insight into the developmental fate of the LT/Sv triploid embryos, as previous studies had indicated that in this species triploids invariably fail to develop beyond the early postimplantation period. This study revealed that when autopsies were carried out on the 7th and 8th days of gestation, it was generally difficult to distinguish between the karyologically normal diploids and the digynic triploid conceptuses when only morphological criteria were used. However, by the 10th day of gestation, the triploid conceptuses could usually be readily distinguished from their diploid littermates by their smaller size and (occasionally) by their disorganized or abnormal morphological appearance.

Key words: mouse embryo, LT/Sv strain, digynic triploid, postimplantation.

Introduction

Various experimental techniques have been described which have allowed high rates of triploidy to be induced in the mouse (for review, see Niemierko & Opas, 1978), though the postimplantation development of somite-stage mouse, rat and rabbit embryos achieved by these means has only rarely been described (Piko & Bomsel-Helmreich, 1960; Bomsel-Helmreich & Thibault, 1962; Bomsel-Helmreich, 1965; Opas, 1977; Niemierko, 1981). In addition, since the spontaneous occurrence of this condition is so infrequently encountered in these species (Wróbleswka, 1971; Baranov, 1976), little information is available on the developmental potential of these conceptuses, though Niebuhr (1974) was able to report on the developmental fate of several hundred human triploids extracted from the world literature. The latter study would appear to indicate that this condition is probably considerably more frequently encountered in man (see also Boué, Boué & Lazar, 1975) than in the few other mammalian species that have so far been studied.

While postovulatory ageing of the oocyte within the oviduct prior to fertilization is known to be associated with a decreased efficiency in the various blocks to polyspermy, this manoeuvre does not appear to be a particularly reliable means of inducing dispermic triploid development either in vivo (Marston & Chang, 1964; Vickers, 1969; Kaufman, 1973; Beatty, 1978; Beatty & Coulter, 1978) or in vitro (Maudlin & Fraser, 1978). While diandric triploidy may also be induced by the fertilization of secondary oocytes by diploid spermatozoa, the latter appear to have a reduced level of survivability and impaired motility compared to haploid spermatozoa within the female tract (Mortimer, 1977). In addition to the production of dispermic triploids, digynic triploidy may also be induced under these conditions due to the migration of the second meiotic spindle away from the periphery of the egg, a phenomenon also known to occur in aged oocytes (Szöllősi, 1967, 1971;
Webb, Howlett & Maro, 1986), and consequent inhibition of second polar body extrusion.

Of the various experimental approaches that have been employed to induce triploid development, exposure of recently fertilized eggs to the cytokinesis-inhibiting effect of cytochalasin would appear to be one of the most efficient means available at the present time (see Niemierko, 1975; Niemierko & Opas, 1978). Exposure to phorbol ester (PMA) during fertilization in vitro also produces a high proportion of tripronucleate eggs (Niemierko & Komar, 1985). The incidence of triploidy following fertilization in vitro is in any case significantly higher than occurs following fertilization in vivo, probably due to the higher level of polyspermy (Santalo, Estop & Egozcue, 1986), and may also be related to the dose of PMSG employed (Maudlin & Fraser, 1977). Similarly, the spontaneous level of digynic triploidy encountered in vivo may also be increased following superovulation (Takagi & Sasaki, 1976).

A situation in which a much higher incidence of spontaneous digynic triploid development occurs than is normally encountered has recently been reported by O’Neill & Kaufman (1987). These authors mated spontaneously ovulating LT/Sv females with F1 hybrid males and analysed the chromosome constitution of fertilized eggs at the first cleavage mitosis, observing that about one third of the resultant zygotes were digynic triploids. This finding was not entirely unexpected, since it had previously been shown by Kaufman & Howlett (1986) that almost one half of the unfertilized eggs isolated from the oviducts of superovulated female LT/Sv strain mice had been ovulated as primary rather than secondary oocytes. The former group were ovulated at metaphase of the first meiotic division, had a 4C amount of DNA present and a 2N (diploid) number of chromosomes. However, it is possible that the use of superovulation may have precluded any meaningful estimate being made of the ‘spontaneous’ level of triploidy that it was hypothesized might arise in, and consequently be recovered from, LT/Sv strain mice shortly after conception. The present study was therefore undertaken to establish this information in the first instance. It was also hoped that it might provide insight into the developmental fate of LT/Sv triploid mouse embryos, as previous studies had indicated that the mouse triploids invariably fail to develop beyond the early postimplantation period (see Baranov, 1976).

Pregnant LT/Sv strain females were therefore autopsied between the 7-10th day of gestation, and a detailed karyological and morphological analysis of their conceptuses was undertaken.

Materials and methods

8- to 12-week-old randomly cycling female LT/Sv strain mice (MRC, Carshalton) were caged with (C57BL x CBA)F1 hybrid males. Early each morning the females were checked for the presence of a vaginal plug and the latter was taken as evidence of mating. The morning of finding a vaginal plug was considered to be the first day of pregnancy.

The females were autopsied at about midday on either the 7th, 8th or 10th day of gestation. The decidua swellings were isolated from the uterine horns and put into phosphate-buffered saline. The embryos isolated on the 10th day were then dissected free from within their extraembryonic membranes (i.e. amnion and yolk sac) with fine watchmakers’ forceps, while all the conceptuses isolated on the 7th and 8th day, and those that were developmentally retarded on the 10th day were generally left intact and treated as a single unit. The total number of resorptions present was noted. All of the morphologically normal and abnormal conceptuses were transferred into ‘199’ tissue culture medium (TC199) with added Colcemid (1 ml of a 0-1 % solution of Colcemid in 100 ml of TC199). The total number of embryos that were clearly morphologically normal or abnormal or, on the other hand, were considered to be morphologically normal but retarded in their development was recorded at this time.

The chromosome constitution of all of the conceptuses, both normal and abnormal, was then determined using a modification of the technique described by Evans, Burtschaw & Ford (1972). In the case of the developmentally more advanced embryos that had been isolated from within their extraembryonic membranes, only the latter was used to establish their chromosome constitution (i.e. ploidy). The embryos, on the other hand, were transferred to Bouin’s fixative for subsequent histological analysis after their crown–rump length and other morphological parameters had been carefully noted. In the case of the grossly abnormal conceptuses and the group of developmentally severely retarded conceptuses, both the embryo and its extraembryonic membranes had to be treated as a single unit in order to maximize the chance of determining their chromosome constitution. The chromosome preparations were stained with 5% buffered Giemsa solution (R.66, G. T. Gurr), then permanently mounted and the mitotic spreads examined under the oil-immersion objective of a Leitz photomicroscope.

Results

(A) Incidence of triploid conceptuses encountered on the 7th, 8th and 10th days of gestation

The results of the autopsies conducted on the 7th, 8th and 10th days of gestation are presented in detail in Table 1, and representative triploid mitotic spreads obtained following the disaggregation of the extraembryonic membranes of two triploid conceptuses are illustrated in Fig. 1A,B. In 18 out of the 21
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Table 1. Cytogenetic analysis of conceptuses isolated from LT/Sv females on either the 7th, 8th or 10th day of gestation

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of gestation at autopsy</th>
<th>Total females autopsied</th>
<th>Total implantations</th>
<th>Total resorptions</th>
<th>Total embryos recovered</th>
<th>Diploid (2n)</th>
<th>Triploid (3n)</th>
<th>Not analysable</th>
<th>Total embryos analysed cytogenetically (% triploid)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>7 (14-3)</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>5**</td>
<td>46</td>
<td>4</td>
<td>42</td>
<td>27</td>
<td>12</td>
<td>3</td>
<td>39 (30-8)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12**</td>
<td>119</td>
<td>11</td>
<td>108</td>
<td>81</td>
<td>25</td>
<td>2</td>
<td>106 (23-6)</td>
</tr>
</tbody>
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* One additional female had 12 implants, 11 analysed cytogenetically – all diploid.
** Two additional females had 23 implants, 20 analysed cytogenetically – all diploid.

Females autopsied, both diploid and triploid conceptuses were isolated, while in the remaining 3 females all of the embryos isolated were found to be diploid. Out of a total of 190 implantation sites isolated from females that contained both diploid and triploid conceptuses, a total of only 16 implantation sites contained resorbing embryos.

The overall incidence of triploidy encountered between the 7–10th days of gestation in this study was therefore 25% (38/152 conceptuses examined cytogenetically) which is significantly lower than the incidence of primary oocytes ovulated by LT/Sv females following exogenous hormonal stimulation (see Kaufman & Howlett, 1986). In the latter study, 47.8% out of a total of 295 recently ovulated and parthenogenetically activated eggs analysed were found to have been ovulated as primary oocytes. In a more recent study, by O’Neill & Kaufman (1987), 33.8% out of a total of 65 fertilized LT/Sv eggs analysed at the first cleavage mitosis, were assumed to have been ovulated as primary oocytes.

While the figures quoted above are clearly significantly different, it is unclear whether the reduction observed is solely due to the reduced incidence of triploid conceptuses observed at successive stages of development. It certainly seems unlikely that the detrimental effect on their development potential of possessing a triploid genome would have manifested itself as early as the first cleavage division.

A proportion of the resorptions encountered in this study might represent those triploid conceptuses that failed to develop beyond the early postimplantation period, and this might account for the higher incidence of triploid conceptuses encountered at the first

Fig. 1. Low (A) and high (B) magnification views of typical mitotic chromosome spreads from the extraembryonic membranes of two triploid LT/Sv strain embryos. Each mitotic spread illustrated in (A) contains two large metacentric chromosomes. This was the only conceptus in the present study in which such morphologically abnormal chromosomes were observed.
cleavage compared to the situation observed cytogenetically in the present study. It is of interest to note that there was no significant difference observed between the total number of conceptuses isolated per female in the present study, compared to the number recovered in previous studies shortly after ovulation in spontaneously ovulating females (present study, \( n = 21, 9.90 \pm 0.38 \) (mean \( \pm S.E.M. \)); previous studies, \( n = 7, 9.14 \pm 0.70 \) (mean \( \pm S.E.M. \); G. T. O'Neill, S. Speirs & M. H. Kaufman, unpublished observations)).

(B) Morphological appearance of triploid conceptuses encountered on the 7th, 8th or 10th day of gestation

(1) Isolation of conceptuses on the 7th day of gestation

Only one female was autopsied on the 7th day of gestation. All of the conceptuses were at the egg cylinder stage of development and were morphologically indistinguishable from each other. The one conceptus that was revealed on cytogenetic analysis to have a triploid chromosome constitution appeared in all respects to be morphologically identical to its diploid littermates. There was, in addition, no obvious difference between the extraembryonic tissues of the triploid conceptus compared to those of its diploid littermates.

(2) Isolation of conceptuses on the 8th day of gestation

Out of a total of six females autopsied on the 8th day of gestation, all but one contained a mixture of diploid and triploid conceptuses. All of the conceptuses that were morphologically abnormal or grossly retarded in development compared to their littermates proved to have a triploid chromosome constitution. However, more than one half of the triploid conceptuses were morphologically indistinguishable from their diploid littermates. No obvious differences were observed between the extraembryonic tissues of the triploids compared to their diploid littermates.

(3) Isolation of conceptuses on the 10th day of gestation

By the 10th day of gestation the conceptuses were readily distinguishable into two principal categories, namely a slightly larger group which contained morphologically normal-looking viable forelimb-bud-stage embryos with on average about 25 pairs of somites present, and a second more heterogeneous group of conceptuses. Almost without exception the conceptuses in the latter group were substantially smaller in volume and in crown–rump length than those that could be assigned to the first group. The embryos that had successfully 'turned' to adopt the fetal position occasionally showed evidence of cardiovascular stasis and morphological abnormalities involving the cephalic part of the neural axis. Several embryos were observed that were still 'unturned' while, in a few instances, partially 'turned' embryos were observed in which the appearance of the cephalic region was consistent with more advanced development than the 'unturned' or partially 'turned' state suggested. Only two examples of a completely empty embryonic sac were observed (the 'triploidy syndrome' described by Wróblewska, 1971).

Cytogenetic analysis of the extraembryonic membranes from the conceptuses isolated on the 10th day revealed that those in the first category were invariably diploid, while the great majority of those in the second category had a triploid chromosome constitution. In fact, only two of the developmentally retarded embryos had a diploid chromosome constitution. In the light of the recent observations on the relationship between the appearance of the extraembryonic membranes and the genotype of mouse conceptuses (McGrath & Solter, 1984; Surani, Barton & Norris, 1984) it is of interest to note that in the case of the triploid conceptuses the volume of extraembryonic membranes present did not appear to be in any way deficient. This may be related to the fact that the triploids possessed both paternally and maternally derived genomes. While a primitive yolk sac circulation was occasionally seen in the diploid membranes, this was never seen in the triploids. Further observations will obviously be required to establish whether this difference was significant or not.

Histological examination of ten of the advanced somite-stage triploid conceptuses all but one of which had successfully 'turned' to adopt the fetal position revealed that four were morphologically normal, though substantially smaller than their diploid littermates. As an indicator of development, three of these embryos had deeply indented otic pits, while the seven developmentally more advanced embryos had otocysts present. All six of the morphologically abnormal embryos examined had either localized or extensive neural tube defects (see Fig. 2). In one embryo, this consisted of a localized spina-bifida-like lesion in the midabdominal region of the neural axis. Five embryos had neural tube defects involving the cephalic region. These could be divided into three principal groups according to the location of the cephalic lesion present, namely (i) one 'unturned' embryo with a squirrel-type deformity in which the cephalic neural folds had failed to close in the regions overlying the fore-, mid- and hindbrain, (ii) two embryos in which the neural folds had failed to close in the regions overlying the fore- and midbrain and (iii) two embryos in which the neural folds had failed to close in the region overlying the forebrain only. In
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one of the embryos in group ii, a localized side–side duplication of the neural tube was present in the midcaudal region of the neural axis.

In those regions in which a neural tube defect was present, the edges of the neural folds were characteristically everted and the neuroepithelial cells rounded up and disorganized. Histological analyses of 12 diploid embryos isolated from the same litters was carried out. None of these embryos had any evidence of neural tube or cardiovascular abnormalities.

While no evidence of cardiovascular stasis was observed in the diploid conceptuses, this was observed in about one third of the triploids, though in all instances spontaneous contractions of the heart were seen. It is possible that the presence of cardiovascular stasis may have been indicative of the impending death of these embryos. While no specific gross cardiac abnormalities were recognized in any of the triploids, an impression was formed that the hearts were possibly somewhat larger than expected, though this might have been due to the fact that the cephalic regions were slightly smaller than normal in this group.

In only one conceptus out of all of the triploids studied, the uniform presence of two metacentric chromosomes was noted. It is unclear whether these represented de novo Robertsonian translocations. In all of the mitotic spreads examined, a minimum of five normal diploid or triploid preparations was always present with a total of either 40 or 60 chromosomes, respectively. However, in most instances, many more spreads were analysed. No examples of numerically abnormal chromosome preparations were observed except, that is, from the single conceptus with the two metacentric chromosomes indicated above (see Fig. 1A).

Discussion

In the present study in which postimplantation conceptuses from pregnant LT/Sv strain mice were examined both cytogenetically and morphologically, it was apparent that the majority of the digynic triploids induced following the fertilization of ovulated primary oocytes developed to the blastocyst stage and successfully evoked a decidual response. Furthermore, the majority of the triploids progressed to comparable developmental stages to those achieved by normal diploid embryos recovered on the 7th or 8th day of gestation. When the conceptuses were recovered on the 10th day of gestation, the triploids became increasingly more readily distinguishable from their normal diploid siblings, a finding that is in general agreement with the results of previous studies on triploidy in the mouse (recently reviewed by Niemierko, 1981).

In two of the experimental studies in each of which single somite-stage triploid mouse conceptuses had been recovered and analysed (Vickers, 1969; Opas, 1977) minimal information on their gross morphology has been provided. The embryo obtained in the former study was recovered from a pregnant female between 91–11½ days after delayed fertilization and was described in the following terms: 'structurally normal but smaller and paler than its diploid siblings, no heart beat could be detected and it seems likely that this foetus was dying'. The embryo recovered on the 12th day of gestation by Opas (1977) was of approximately the same developmental age. It had a 'beating heart, allantois, yolk sac, but no head'.

More recently, a small number of triploid conceptuses has been isolated by Kaufman & Bain (1984) following the exposure of recently mated female mice to an intragastric injection of a dilute solution of ethanol. These embryos were recovered on either the 10th or 11th day of gestation and could be divided into two easily distinguishable categories, namely those that were retarded by about 24h in development compared to their littermates, but nevertheless were morphologically normal, and a second group that were generally smaller than their normal siblings, but were in addition morphologically grossly abnormal. In the present study, an additional class of triploids has been recognized, namely those that appeared on gross inspection to be morphologically normal in appearance and at a very similar developmental stage to their diploid siblings, but had a significantly reduced crown–rump length and volume.

Despite the relatively limited number of triploids analysed in the present study, only two out of the thirty-eight triploid conceptuses recovered demonstrated any of the features of the 'triploidy syndrome' first described in detail by Wróblewska (1971; see also Niemierko, 1981). In addition, it is of interest that in the few mouse strains in which postimplantation triploid embryos have been observed, no viable conceptuses have been recovered from females beyond the 12th day of gestation (Vickers, 1969; Takagi & Oshimura, 1973; Wróblewska, 1971, 1978; Baranov, 1976; Opas, 1977; Niemierko, 1981).

In the A/He strain, high rates of triploidy were encountered following the mating of superovulated females to normal males. Many of these embryos appeared to survive into the early postimplantation period (Takagi, 1970; Takagi & Oshimura, 1973). Following this experimental treatment, the average frequency of triploidy was found to be in the region of 31% at 2.5–3.5 days after mating, 24% at 6.5 and 7.5 days, and decreased to about 15% at 10.5 and 11.5 days. In the majority of cases, the triploid conceptuses displayed all the features of the triploidy
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A genetic evidence was provided which confirmed the conceptuses were found to be triploid. Cytogenetic evidence was provided which confirmed the hypothesis that the majority of the triploids encountered were digynic in origin. In the same study, the morphology of a considerable number of the triploids that survived into the early postimplantation period was examined histologically. 10 of the 18 embryos examined on the 8th and 9th day were at the neurula stage, all appeared to be healthy, though developmentally somewhat retarded compared to their diploid littermates. The remaining embryos were smaller and morphologically abnormal. When triploid embryos were isolated on the 10th day, 25 of 31 examined were clearly being resorbed. Of the six apparently viable embryos their head end was much smoother than normally, the neural crests (sic) were flattened and asymmetrical, and the anterior and posterior invaginations of the gut were ill defined' (Baranov, 1976). The two triploid embryos isolated on the 11th day had a sluggishly beating S-shaped heart, several pairs of somites present and were clearly (from the illustrations published in this paper) at the early headfold stage of development.

The importance of genetic background on the postimplantation development of triploid mouse embryos has clearly been demonstrated by the fact that both CBA and A strain conceptuses develop the 'triploidy syndrome', whereas 129/Sv and its crosses apparently do not (Wróblewska, 1978; see also Beatty, 1957; Wróblewska, 1971). Possibly the LT/Sv strain mice used in the present study belong to this second category.

The findings reported above are clearly species specific, as triploid (? dispermic) rat embryos have been recovered on the 12th day of gestation (Piko & Bomsel-Helmreich, 1960), triploid rabbit embryos on the 17th day (Bomsel-Helmreich, 1965, 1971), while human triploid fetuses may very rarely survive to term and beyond (Niebuhr, 1974).

The fact that the triploids in the present study resulted from the fertilization of spontaneously ovulated primary oocytes which apparently were capable of developing during the preimplantation period in synchrony with their normal diploid littermates would undoubtedly have favoured their chance of survival into the postimplantation period. Whether their digynic genotype conferred any additional developmental advantage has yet to be established, but this would seem to be unlikely.

The only other mice that are apparently capable of regularly ovulating a significant number of primary oocytes are from the NMRI/Han strain (Jenderny et al. 1980; Hansmann, Jenderny & Probeck, 1983) in which up to 12 % of their eggs are ovulated as primary oocytes. However, even this relatively high level, though considerably lower than observed in the LT/Sv strain females, is only observed after the exposure of the NMRI/Han females to exogenous gonadotrophins (Bartels, Jenderny & Hansmann, 1984). However, for reasons that are not entirely clear, when Ta/O females of C3H x 101 background were mated to males of the NMRI/Han strain and the adult F1 hybrids treated with exogenous gonadotrophins, analysis of their ovulated oocytes revealed that a low level of diplomy (1-0 %) occurred in the oocytes of XX mice, but a high level of diplomy was observed (24-6 %) in oocytes from females with the XO karyotype (Beermann, Franke & Hansmann, 1986). In the LT/Sv mice, our findings would appear to indicate that significant numbers of primary oocytes are ovulated in both spontaneously ovulating

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Fig. 2. Representative intermittent serial transverse sections through three LT/Sv triploid embryos isolated at about midday on the 10th day of gestation.

(A–D) Four sections through the cephalic region of a limb-bud-stage embryo with deeply indented otic pits. Note that the neural folds overlying the entire presumptive forebrain region (arrows) have failed to fuse, whereas the hindbrain region appears to be morphologically normal. Bar, 200 µm.

(E–F) Two sections through a developmentally slightly more advanced limb-bud-stage embryo than that illustrated in Fig. 2A–D, which possessed a normally located pair of otocysts (small arrows). Note that the neural folds overlying the entire presumptive forebrain region have failed to close. The extent of eversion of the margins of the neural folds (large arrows) and associated degree of neuroepithelial cellular disorganization is clearly apparent in these sections. Bar (E), 200 µm; Bar (F), 400 µm.

(G–H) Two sections through approximately the middle of the embryonic axis of a partially 'turned' embryo which, in addition, cuts through the proximal part of the tail region. Note in particular the side–side duplication of the neural tube (arrow). These neural tubes gradually merge both cranially and caudally to give, initially, a single morphologically abnormal neural tube with a single lumen. However, more distant cranially and caudally from the duplicated segment, a single apparently morphologically normal neural tube was present along the rest of the 'spinal axis'. In the cephalic region, the neural folds overlying the presumptive fore- and midbrain had failed to fuse, whereas the neural folds overlying the presumptive hindbrain region had closed normally. Bar, 200 µm.
females and following exogenous hormonal stimulation.

The most advanced triploid mouse embryos encountered on the 10th day of gestation in the present study appeared to be developmentally substantially more advanced than those previously described by Baranov (1976). In the present study, the majority of the triploid embryos recovered on the 10th day of gestation was found to be at a similar developmental stage to their normal diploid littermates. A proportion of the morphologically abnormal embryos, however, while still viable and with a beating heart at the time of their isolation, was clearly quite unhealthy, often having areas of vascular stasis and would almost certainly have died 24-36 h later, had they been left to develop in utero. Preliminary histological analysis of the triploid conceptuses has revealed that they had several common features, in addition to their small size. More than half of the triploid conceptuses displayed anomalies of closure of the neural tube, principally involving the cephalic neural folds, the latter ranging from isolated closure defects overlying the presumptive forebrain to extensive lesions involving the entire cephalic region. While no obvious gross cardiac abnormalities were identified, in a proportion of these embryos, there was evidence of vascular stasis. It appears likely therefore that the present observations are consistent with previous observations on triploidy in the mouse (see Beatty & Fischberg, 1949, 1951; Edwards, 1954), where disturbances of organogenesis were assumed to have resulted from a reduced total embryonic cell number associated with an increase in the volume of their individual component cells. Takagi & Sasaki (1976) estimated that from their experimental findings the cell-cycle time during the preimplantation period was about 10 % longer in triploid mouse embryos than in their diploid siblings. They predicted from these values that the total cell number of triploid embryos would become half that of their diploid siblings by about the time of the 10th cleavage (about 5-5 days p.c.) and a quarter at the time of the 20th cleavage (about 10-5 days p.c.). In fact, similar findings were reported by Snow (1975, 1976) during his investigations into the postimplantation development of tetraploid mouse embryos. We hope to quantify this difference in cell number and cell volume between control (diploid) and triploid conceptuses, and describe their histological features in more detail when sufficient numbers of viable LT/Sv embryos become available to us.

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