In vitro development of haploid mouse embryos produced by bisection of one-cell fertilized eggs

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SUMMARY

F1(CBA x C57BL/10) mouse eggs originating from spontaneous or induced ovulation and fertilized by CBA-T6T6 or PO spermatozoa were bisected with a glass needle into halves each containing a pronucleus. This technique offers a unique opportunity of producing both androgenetic and gynogenetic haploid embryos from one egg.

Out of 600 operated eggs, in 406 (67.7%) both halves survived. During 96 h of culture in vitro the fragments were inspected once daily and finally examined in air-dried preparations. Eighty-seven per cent of halves underwent first cleavage but their further development was to a large extent affected by extrinsic factors connected with experimental procedure (mainly by suboptimal and variable culture conditions) and by the origin of eggs (those from spontaneous ovulation being superior). For this reason developmental capabilities of egg halves were assessed in a selected group of pairs in which at least one partner reached the stage of four or more blastomeres. The observed ratio between pairs with both or only one sister embryo developing successfully suggests that androgenetic embryos carrying Y rather than X chromosome can cleave twice but do not survive beyond 4-cell stage. None of the metaphase plates from older embryos contained a Y chromosome. These observations imply that the X chromosome is genetically active during early cleavage and that a full haploid set is required for preimplantation development to be completed.

Formation of blastocysts varied from batch to batch, with an average of 12.8% and maximal incidence of 29.5%. In 34 pairs both fragments developed beyond the 4-cell stage but in only one case did both form blastocysts. Haploid blastocysts were composed of 27 cells on average which was about a half of the number of cells in control diploid zona-free whole eggs. Ten out of 51 embryos with metaphase plates proved to be haploid/diploid mosaics.

INTRODUCTION

Haploid embryos rarely arise spontaneously in mammals. A few preimplantation embryos that have been described in the mouse were of unknown origin, i.e. it was not known whether they were parthenogenetic, gynogenetic or androgenetic (cf. Beatty, 1957). Parthenogenetic haploid blastocysts can now be easily obtained with the help of various activation techniques (reviewed by Graham, 1974; Tarkowski, 1975; Kaufman, 1975). Gynogenetic mouse embryos have been recently produced by Modliński (1975) by microsurgical removal of the male pronucleus but for unknown reasons their development into blastocysts was a

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very rare event. A different attempt to obtain haploid embryos from fertilized eggs is presented in this paper. The technique developed for this purpose consists in cutting a zona-free pronucleate egg into halves, each containing one pronucleus. The ability of such egg fragments to develop into blastocysts was proved in experiments in which they were transplanted into empty zonae pellucidae and transferred to recipient females (Tarkowski & Rossant, 1976). These encouraging results prompted me to study in more detail the development of egg fragments in the in vitro system which is technically simpler and which permits the development of the complementary sister egg halves to be followed.

The aim of this study was threefold: first, to compare the development of gynogenetic and androgenetic fragments derived from the same egg; second, to clarify the role of X chromosomes in early mammalian development by examining the fate of androgenetic Y-chromosome-bearing embryos; and third, to explore the potentialities of this approach for the production of haploid mouse embryos on a larger scale.

Haploid embryos developed from egg fragments rather than from whole eggs are defined in embryological terminology as andromerogones and gynomergones. However, since these terms have never been used as yet in mammalian embryology, they will be used in this paper interchangeably with such terms as androgenetic and gynogenetic embryos (fragments, egg halves).

MATERIALS AND METHODS

F2(CBA x C57BL/10) females 5–16 weeks old were used throughout this study. With the exception of one experiment in which PO males were used, females were mated with CBA-T6T6 males. Ovulation was either spontaneous or induced with gonadotrophins (5–10 i.u. each of PMSG and HCG ('Gestyl' and 'Pregnyl', Organon) given 45–52 h apart). With spontaneous ovulation eggs were harvested between 15.00 and 17.00 on the day of vaginal plug, and with induced ovulation 20–30 h after treatment with HCG. The eggs were released from ampullae into PBS (phosphate buffered saline) with hyaluronidase (200 i.u./ml) to remove the cumulus cells and transferred to 0.5 % pronase in PBS (Mintz, 1962) to partly dissolve the zona pellucida (about 15 mins at room temperature). Final removal of zonae was accomplished in culture medium, if necessary by pipetting. Zona-free eggs awaiting operation as well as control eggs in zonae were kept in culture medium in plastic dishes under a continuous flow of 5 % CO2, 5 % O2 and 90 % N2.

Eggs were bisected under a dissecting microscope (magnification: times 50) with a glass needle operated by hand. Operation was performed in PBS + 0·1 % crystalline bovine plasma albumin (Armour) at the temperature of ca. 5 °C on a surface of 1 % agar dissolved in 0·6 % NaCl. The low temperature was maintained with the help of a cool stage. Eggs were operated in groups of five to ten and the period in cool PBS varied between 10 and 20 min. The control eggs (in
Haploid mouse embryos were transferred to the operating dish with the last group of eggs to be bisected and were thus stored longest at room temperature. Cutting itself was accomplished by applying the needle along the plane lying between the two pronuclei (but see also the Results below) and pressing it against the surface of agar. Complete separation of the halves could be achieved in this way but often the halves remained connected by a cytoplasmic bridge and had to be separated either by repeating the operation or preferably by pipetting. The width of the glass needle was about one-third to one-quarter of the diameter of the egg.

The egg halves were transferred separately to drops of culture medium in plastic dishes or siliconized glass dishes half filled with a selected batch of liquid paraffin (Boots). The culture medium was that of Whitten (1971), containing 140 rather than 190 mg NaHCO₃ as recommended in the original formula. The cultures were kept at 37 °C under 5% CO₂, 5% O₂, 90% N₂ for 96 h and inspected every day under an inverted microscope. Colcemid was added to culture drops (final concentration about 0.2 μg/ml) a few hours before making air-dried preparations (technique of Tarkowski, 1966).

RESULTS

The cutting operation

In the course of 600 operations it was found that both halves survived in 406 cases (67.7%) and one half survived in 21 cases (overall survival rate of halves 69.4%). The survival rate varied slightly between experiments but it never dropped below 50% and in some experiments was 100%. Slightly more eggs from spontaneous ovulations survived operation than from induced ovulations (71.5% versus 65.2%) but this difference was not significant ($\chi^2 = 2.58$, $P > 0.1$). The egg halves damaged during bisection lysed either immediately or during the next hour and could be easily distinguished and discarded. The factors which seemed to increase the efficiency of bisection included low temperature, clean needle (free of cytoplasmic debris) and proper width of the needle (too thin a needle easily damages eggs).

In the majority of operated eggs the pronuclei were clearly visible and bisection usually resulted in each half containing a pronucleus (Fig. 1). However, in newly fertilized eggs the position of pronuclei often could not be determined and therefore these eggs had to be sectioned at random which sometimes resulted in one anucleate half and the other with both pronuclei (Fig. 2). In some of these eggs the position of the second polar body and of the protrusion, which forms in the place of sperm entrance, indicated the presumed localization of the oocyte chromosomes and of the sperm head and so helped in choosing the proper plane of sectioning (Fig. 3). The protrusion, which persists only for a limited time after sperm penetration, can be detached by vigorous pipetting and when examined a few hours later usually contains a pronucleus (Fig. 4).
Figures 1–4. All to same magnification (×400).

Fig. 1. Two sister egg halves, each carrying a pronucleus.

Fig. 2. Two sister egg halves: one is anucleate (left) and the other contains both pronuclei and carries 2P.B. (middle). The control whole egg is shown on the right.

Fig. 3. Two sister egg halves produced by cutting a newly fertilized egg. Fragment on the left shows a protrusion formed probably at the site of sperm entrance (presumably androgenetic); fragment on the right carries 2 P.B. (presumably gynogenetic).

Fig. 4. Whole egg from which 2 P.B. (top left) and a protrusion (like the one shown on Fig. 3) have been detached by vigorous pipetting. Photograph taken after 5 h of culture. Note one pronucleus in the egg and a nucleus in the fragment originating from the detached protrusion (top right).

The plane of cutting bore no constant relation to the polar axis marked by the position of the second polar body, because it was intended to produce two fragments of equal size each containing a pronucleus and the position of the male pronucleus was variable.
Figures 5–9. All to same magnification (×400).

Fig. 5. Control 2-cell embryo after 24 h of culture.
Fig. 6. Two haploid 2-cell embryos developed from sister egg halves after 24 h.
Fig. 7. Three egg halves which have not undergone cleavage after 24 h (presumably anucleate). Note various appearance of fragments (from left to right): intense wrinkling, moderate wrinkling, smooth surface. The fragment shown on the left was after cutting transferred to evacuated zona pellucida (operation performed by J. Rossant).
Fig. 8. 6-cell control embryo after 48 h of culture.
Fig. 9. Two 4-cell haploid sister embryos after 48 h.
Two-cell stage (24 h in culture)

Out of 712 egg halves placed in culture 620 (87.1%) underwent first cleavage (Fig. 6). Among the remaining 92 fragments eight lysed, four contained a pronucleus (sister halves cleaved) and 80 remained unchanged or showed wrinkled surface or underwent fragmentation (Fig. 7). In egg halves belonging to the latter group the nucleus could not be seen in the living state and most probably they were anucleate. Sometimes it was possible to confirm this estimate on the basis of the presence of two pronuclei in the sister half or because the embryo which developed from it proved to be diploid. It would follow therefore that the overwhelming majority of haploid egg fragments, whether gynogenetic or androgenetic, undergo first cleavage. The few undivided fragments with a pronucleus were arrested in development permanently, as they were never observed to divide during the whole culture period.

No significant difference was observed between egg halves from spontaneous and induced ovulations as regards their ability to undergo first cleavage (spontaneous: 258/303, induced: 362/409; \( \chi^2 = 1.74, P > 0.1 \)).

Four-cell stage (48 h in culture)

Only a portion of egg halves which divided once underwent second cleavage and reached the 4-cell stage (Fig. 9). It was assumed that if neither of a pair of halves reached the 4-cell stage then the egg had been damaged either by cutting or culture and an analysis was made of the development of the remaining eggs. These data are presented in Table 1, separately for eggs from spontaneous and induced ovulations. The number of pairs (halves) included in this Table is also smaller than the number of halves which underwent first cleavage (see above) because a certain number of fragments were cultured only for 24 h and then used for other purposes.

While in the first group (spontaneous ovulation) 90% of pairs reached the 4-cell stage, in the second group (induced ovulation) this figure was below 60%. Among intrinsic factors which might check development at this early stage the most obvious one is the lack of an X chromosome which is to be expected in 50% of andromeres. However, if this condition were lethal as early as at the 2-cell stage then only in 50% of pairs would both fragments cleave twice, which was not the case. Deviation from this 1:1 ratio is statistically significant for fragments originating from spontaneously ovulated eggs \( (\chi^2 = 32.0, P < 0.001) \) and for the whole material \( (\chi^2 = 18.78, P < 0.001) \) but not for fragments originating from eggs from induced ovulations \( (\chi^2 = 1.53, P > 0.1) \). The difference between the incidence of successfully developing pairs in these two groups of eggs is also significant \( (\chi^2 = 15.45, P < 0.001) \).

The different performance of egg fragments obtained from spontaneous and induced ovulation is puzzling, the more so in that control eggs from induced ovulations developed well in terms of both the number reaching the blastocyst
### Table 1. Development of sister egg halves up to the 4-cell stage

<table>
<thead>
<tr>
<th>Type of ovulation</th>
<th>No. of experiments</th>
<th>No. of pairs in which at least one egg half reached 4-cell stage</th>
<th>No. of pairs in which sister embryos were composed of the following number of blastomeres:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4:2</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>7</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Induced</td>
<td>11</td>
<td>94</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>144</td>
<td>28</td>
</tr>
</tbody>
</table>

Stage and the average cell number at the end of culture (eggs in zona: 67.5% formed blastocysts, mean cell no. 67.1 ± 2.8; zona-free eggs: 56.4% formed blastocysts, mean cell no. 51.5 ± 3.7; difference between percentages is not significant, difference between means is significant – \( d = 3.35, P < 0.001 \)). It seemed at first that this difference might have been connected simply with the age of eggs at the time of operation. Spontaneously ovulated eggs were collected within a 2 h period and were rather advanced in development, while eggs from induced ovulations were harvested within a 12 h period and varied considerably in age. However, no relationship was observed in the latter group between the age of egg halves at the time of operation and their developmental capabilities: in age groups 20–26 and 26–32 h post-HCG the proportion of pairs in which both fragments cleaved twice was 32/56 (57.1%) and 21/38 (55.2%) respectively.

In view of the results obtained with egg halves originating from spontaneously ovulated eggs it can be concluded that all or at least the overwhelming majority of androgenetic haploids carrying a \( Y \) chromosome can survive to the 4-cell stage and that the poor performance of fragments obtained from induced ovulation could not have a genetic background.

### Development of egg halves between 48 and 96 h

With progressive time of culture more and more embryos became arrested in development. The data presented in Table 2 are based on observations made after 96 h and were selected in the following way: first, only those pairs were taken into consideration in which at least one partner was composed of five or more blastomeres and, second, the pairs were selected from only those experiments in which the average cell number of ‘half-embryos’ exceeded 4.0. It was believed that such a selection would eliminate all embryos whose poor development was entirely due to extrinsic factors, mainly inadequate culture conditions. In the case of ‘half-embryos’ derived from the spontaneously ovulated eggs all pairs which were classified as developing successfully after 48 h (at least one partner composed of four cells) fulfilled these criteria and consequently the
<table>
<thead>
<tr>
<th>Type of ovulation</th>
<th>Total no.</th>
<th>Number of blastomeres</th>
<th>Pairs</th>
<th>Halves</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt; 4: ≤ 4</td>
<td>&gt; 4: &gt; 4</td>
<td>Number of cells</td>
<td></td>
</tr>
<tr>
<td>Batch no. 51</td>
<td>22</td>
<td>12</td>
<td>10</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54·5%</td>
<td>45·5%</td>
<td></td>
<td>27·3%</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>22</td>
<td>12</td>
<td>10</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Other batches</td>
<td>28</td>
<td>14</td>
<td>14</td>
<td></td>
<td>32·1%</td>
</tr>
<tr>
<td>Induced</td>
<td>48</td>
<td>38</td>
<td>10</td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79·2%</td>
<td>20·8%</td>
<td></td>
<td>42·7%</td>
</tr>
</tbody>
</table>

* These figures include also blastocysts tabulated in the next column.
Haploid mouse embryos

number of pairs presented in Tables 1 and 2 is the same. However, in the group of egg fragments from induced ovulation the number of pairs which continued development decreased from 94 after 48 h to 48 after 96 h.

The 4-cell stage is certainly critical for some embryos (Fig. 10). It appears that the embryos which are checked in development at this stage are mainly andromerosomes lacking \( X \) chromosome. This conclusion is inferred from the comparison of the number of pairs in which both embryos were composed of more than four blastomeres with the number of pairs in which only one embryo succeeded in developing beyond the 4-cell stage. Among pairs of embryos developing from spontaneously ovulated eggs the ratio between these types is close to 1:1 which is in agreement with the expected ratio of \( XX \) and \( XY \) zygotes. Development of egg halves originating from eggs obtained by induced ovulation was, however, much worse – in only 20% of pairs did both embryos cleave beyond the 4-cell stage (Table 2). These observations provide additional evidence for the inferiority of eggs from induced ovulation as material for obtaining haploid embryos by bisection.

Among embryos which were composed of more than four blastomeres most were checked in development at the 5-8-cell stage, with the exception of one batch (no. 51) in which development was in general exceptionally good. In this particular experiment, the results of which are presented separately in Table 2, the eggs were fertilized by PO rather than CBA-T6T6 spermatozoa but otherwise it did not differ from other experiments. In batch no. 51 the most numerous group of embryos were those of more than 16 cells (38.6%) while in other batches pooled together these embryos constituted only 7.1% (spontaneous ovulation) and 13.5% (induced ovulation).

The conclusive proof of the ability of haploid egg fragments to complete preimplantation development is formation of blastocysts. Blastocysts were observed to form sporadically in various experiments but the highest incidence was again observed in batch no. 51 in which as many as 29.5% of embryos underwent blastulation (Table 2). Altogether 25 blastocysts were collected; usually they were small and often not very regular and some were most probably trophoblastic vesicles rather than real blastocysts with an inner cell mass (Figs. 11, 13-15).

In very few cases did both sister halves develop to later stages (Fig. 12, 13). Altogether 34 pairs were available with both partners composed of more than four blastomeres (Table 2). However, there were only four pairs with both embryos of more than eight blastomeres and only one pair represented by blastocysts (number of cells: 20 and 29). All these five pairs were from batch no. 51.

Rate of cleavage of experimental and control eggs

In the morning of the second day of development both egg halves and control eggs were already in the 2-cell stage (Figs. 5 and 6), and in the morning of the third day in the 4-cell stage (Fig. 9). During the third day of development some
Experimental and control embryos were observed to enter 3rd cleavage (5–8 blastomeres, Fig. 8), but this was a very rare event. On the fourth day (after 72 h in culture) control embryos as well as some ‘half-embryos’ had compacted so that visual cell counts were impossible. The cell number could be precisely determined again after 96 h, from air-dried preparations. The average cell number of 10 morulae and 25 blastocysts developed from haploid egg halves was respectively 21.3 and 27.4 (variation 17–24 and 17–42) and much lower than in the control diploid zona-free embryos (see p. 7). The cell number in the three available blastocysts developed from diploid egg halves was comparable to that...
Haploid mouse embryos

of haploids (30, 30 and 8!) (Fig. 16). These observations show that while the first three cleavages of haploid embryos are not delayed, their development slows down later especially during the last 24 h. The period between 72 and 96 h was in general critical for haploid embryos since even some of those, which up to this moment developed successfully and formed compacted morulae, 24 h later presented a loose aggregation of cells, some still alive and some already degenerate.

Karyology of egg-half-derived embryos

Because of difficulties in making air-dried preparations from tiny zona-free embryos which in addition were often in the process of degeneration, many specimens were lost and the quality of metaphase plates was generally poor. Altogether 51 embryos at various stages of development contained metaphase plates (Table 3). There was no problem in estimating the ploidy, but the majority of metaphase plates were unsuitable for detecting T6 or Y chromosomes.

The majority of embryos (74.5%) contained only haploid plates. There were also ten haploid/diploid mosaics and one haploid/tetraploid mosaic. One embryo had only three diploid plates, and one a single tetraploid plate.

Among 33 embryos which developed from eggs fertilized by CBA-T6T6 sperms, the T6 marker chromosome was seen in four and was certainly absent in ten; in 19 embryos the quality of metaphase plates was not good enough to be classified in this respect. None of the four T6+ metaphase plates contained a Y chromosome. Among 18 embryos developed from eggs fertilized by PO sperms (no marker), only in two embryos was it possible to ascertain for certain that they did not carry a Y chromosome. However, because of the lack of additional chromosomal markers such information does not suffice to classify them as gynogenetic or androgenetic.

DISCUSSION

Bisection of a fertilized 1-cell egg thus separating the two pronuclei between sister halves gives rise to embryos which are haploid and carry either an egg or a sperm-derived genome, contain half of the normal amount of cytoplasm, and start development with a nucleo-cytoplasmic ratio restored to a 'normal' level. When analysing the development of egg halves one has to take into account all these intrinsic factors, each of which may have by itself important developmental implications, and also possible effects of extrinsic factors connected with experimental procedure.

The ability of egg halves, whether diploid or haploid, to develop into structurally normal blastocysts, i.e. composed of both the trophoblast and the inner cell mass (Tarkowski & Rossant, 1976, and this paper), confirms previous studies showing that removal of cytoplasm from pronucleate eggs (Opas, 1976) or separation of early blastomeres does not affect blastocyst formation, although it may lead to a tendency for trophoblastic vesicle formation (Tar-
Table 3. *Karyology of embryos developed from haploid egg halves*

<table>
<thead>
<tr>
<th>Type of ovulation</th>
<th>Male</th>
<th>Stage of embryos</th>
<th>Ploidy</th>
<th>T6 marker chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>CBA-T6T6</td>
<td>Cleaving eggs and morulae</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blastocysts</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>CBA-T6T6</td>
<td>Cleaving eggs and morulae</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blastocysts</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td>Cleaving eggs and morulae</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blastocysts</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>38</td>
<td>10</td>
</tr>
</tbody>
</table>
Haploid mouse embryos

kowskii, 1959a, b; Tarkowski & Wróblewska, 1967). In view of this tendency it is not surprising that some of the haploid egg halves developed into vesicles rather than blastocysts.

A majority of haploid embryos obtained by artificial activation and all those produced by removal of one pronucleus from the zygote start to develop from the whole egg and therefore have an abnormally low nucleo-cytoplasmic ratio. In egg halves this ratio is restored to the 'normal level' (see Discussion p. 11). In this respect they resemble those parthenogenetic embryos in which the second meiotic division takes an abnormal course and gives rise to two cells of equal size each containing a haploid pronucleus (the phenomenon often referred to as 'immediate cleavage'). Whether such a change in the nucleo-cytoplasmic ratio has a beneficial effect on haploid development remains, however, an open question. For instance, the data presented by Kaufman & Sachs (1976) on in vitro development of various types of parthenogenetic mouse eggs, show that although the immediate cleavage embryos develop into blastocysts in a much higher proportion than 'normal' haploid embryos, they have a lower rate of cleavage. Haploid embryos obtained by bisection had also a low cell number, but whether this was due to traumatic effects of operation, or to nucleo-cytoplasmic ratio, or simply to haploidy, could not be resolved because of the lack of sufficient number of embryos derived from diploid egg halves, which would serve as a control material. Inspection of egg halves and whole eggs during culture (p. 9) suggests, however, that during early cleavage neither cytoplasmic volume nor ploidy have significant effect on timing of divisions. The same appears to be true in sea urchins (Rustad, Yuyama & Rustad, 1970).

The overwhelming majority of haploid mouse embryos produced so far by various investigators were parthenogenetic and therefore carried the egg-derived genome. Although the technique of Modliński (1975) permits the removal from the egg of either of the pronuclei, all advanced preimplantation embryos obtained by him carried the maternal genome and therefore genetically resembled the parthenogenomes. The technique of bisection makes it possible to obtain both gynogenetic and androgenetic embryos from one egg which is a unique opportunity not offered by any other approach. The only serious disadvantage of this technique is that it requires removal of the zona pellucida without which early cleavage stages do not survive in vivo (Modliński, 1970; Bronson & McLaren, 1970). This implies that egg fragments have to be cultured in vitro or have to be inserted into evacuated zonae pellucidae before being transplanted into the oviduct. The latter procedure is feasible but is technically rather complicated and its efficiency in terms of recovered blastocysts is low (Tarkowski & Rossant, 1976).

Culture in vitro of complementary egg halves would be an ideal experimental system provided the culture conditions are optimal. Unfortunately, at present this is not the case. For this reason not only intrinsic but also extrinsic factors have to be considered as a possible cause of the developmental failure of egg fragments.
In order to eliminate the effect of factors connected with experimental procedure (mainly inadequate culture conditions) the developmental capabilities of egg fragments were assessed in a selected group of pairs (for criteria of selection see pp. 6 and 7). The main conclusion of this analysis is that up to the 4-cell stage all haploid egg halves, whether gynogenetic or androgenetic, are equally viable. Since half of andromerogones carries a \( Y \) chromosome, it follows that the first two cell cycles can occur in the absence of an \( X \) chromosome. Other observations presented in this paper strongly suggest, however, that these particular embryos do not survive beyond the 4-cell stage, thus confirming the suggestion made previously by Morris (1968) that lack of \( X \) chromosomes is in the mouse a lethal condition during early cleavage (according to the above author \( OY \) diploid embryos die at the 2-cell stage). Death of \( Y \) chromosome-bearing andromerogones at the 4-cell stage would reduce the group of androgenetic embryos by half and consequently the ratio between the gynogenetic and androgenetic embryos among survivors would be shifted to the advantage of the former and should approach 2:1. This might explain the rare occurrence of embryos carrying T6 marker chromosomes which was used as a label of the male-derived genome. The marker was identified in four out of 14 embryos with analysable metaphase plates (Table 3) and possibly in one of five blastocysts obtained in the \textit{in vivo} experiment (Tarkowski & Rossant, 1976).

The survival of both sister egg halves beyond the 4-cell stage was observed in over 30 pairs, but only in one case did both embryos form blastocysts. Because karyotypes with T6 chromosome were observed only in cleaving embryos this case is the only evidence for the ability of an andromerogonic mouse embryo – and for androgenetic mammalian embryos in general – to develop to the blastocyst stage. Indirect as it still is, the whole available evidence suggests, however, that androgenetic haploid development can proceed up to this stage only if the embryo carries an \( X \) and not a \( Y \) chromosome.

The lethal effect of the absence of \( X \) chromosome, which is postulated in this study, and the fact that hypohaploid parthenogenetic embryos lacking small T6 chromosome do not survive till the blastocyst stage (Kaufman & Sachs, 1975; Witkowska, personal communication), lead one to believe that the whole haploid genome may be active as early as during cleavage and may be required for the preimplantation development to be successfully completed.

Although the overall survival rate of haploid egg halves till the blastocyst stage was in the present study rather low, the results obtained in batch 51 (29-5 \% of blastocysts) show that if conditions are right this approach could be a useful way of producing haploid embryos.

Finally, attention should be drawn to the fact that a quarter of the embryos derived from haploid egg halves contained diploid or, on rare occasions, even tetraploid cells. This could not be due to inadequate culture conditions because haploid/diploid mosaicism was also observed in blastocysts developed from egg halves \textit{in vivo} (Tarkowski & Rossant, 1976). The same phenomenon occurs also
in haploid parthenogenetic embryos and irrespective of whether they develop *in vivo* (Tarkowski, Witkowska & Nowicka, 1970; Witkowska, 1973) or *in vitro* (Kaufman & Sachs, 1976). This instability of haploid state, which manifests itself as early as in the preimplantation development, may prove to be a serious obstacle in obtaining haploid cell lines from mouse embryos.

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**REFERENCES**


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