Diploid parthenogenetic mouse embryos produced by heat-shock and Cytochalasin B

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SUMMARY

Swiss albino and C57BL/10 eggs from induced ovulations, and spontaneously ovulated A eggs, were activated in vitro by a heat shock of 44 °C for 5 or 7.5 min and cultured in the presence of 10 μg/ml of Cytochalasin B (CB) for 5–8 h. The activation rate was about 70% in Swiss albino, 40% in C57BL and 90% in A eggs. CB suppressed second polar body (2P.B.) formation in over 90% of activated eggs, with the majority containing two pronuclei. When eggs were placed in CB-free medium their surface became wrinkled and they formed protrusions of various sizes, which in some eggs detached to form enucleate or pronucleate cytoplasmic fragments; some eggs broke down completely into fragments. In most eggs, however, the surface smoothed out in a few hours and suppression of 2P.B. appeared to be permanent. The rate of development of these eggs after transplantation to the oviduct was delayed in terms both of cell divisions and of the time of blastocyst formation. Out of 41 implants collected on the 8th–10th day of pregnancy only two healthy looking egg-cylinders were found on the 8th and 9th day; both were retarded, at the stage characteristic for the 7th day of normal development. The reasons for delayed preimplantation development and low implantation rate are discussed. The present experiments corroborate earlier observations that parthenogenetic mouse embryos, even if diploid, rarely survive in the uterus beyond the egg-cylinder stage.

INTRODUCTION

During the last few years there have been several successful attempts to induce parthenogenetic development in the mouse (reviewed by Graham, 1974; Tarkowski, 1971, 1975). Activation in situ by electric shock (Tarkowski, Witkowska & Nowicka, 1970; Witkowska, 1973a) or in vitro by heat shock (Komar, 1973) or by hyaluronidase (Graham, 1970, 1971; Kaufman, 1973a) leads most often to the initiation of haploid parthenogenesis. In order to increase the proportion of diploid parthenogenones, Graham (1972) developed a technique of subjecting cumulus-free eggs to hypotonic shock, which gives a high yield of potentially diploid eggs due to suppression of the second polar body. This technique has been also used with success by other authors (Graham & Deussen, 1974; Kaufman & Gardner, 1974; Kaufman & Surani, 1974). Another attempt to produce diploid parthenogenones is presented in

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this paper. We have taken advantage of the observations that Cytochalasin B inhibits second meiotic division of the mouse oocyte (Niemierko, 1975) and cleavage divisions (Snow, 1973; Tarkowski, Witkowska & Opas, in preparation) and that short treatment of eggs with this drug does not impair further development. Diploid parthenogenetic embryos obtained by suppression of second polar body with Cytochalasin B can develop beyond implantation, but so far we have not observed survival beyond the early egg-cylinder stage.

MATERIAL AND METHODS

Eggs were obtained from Swiss albino females (randomly bred colony) and C57BL/10 and A females. With the exception of A females which ovulated spontaneously and mated with vasectomized males, all others were induced to ovulate (5 i.u. of PMSG and HCG given at an interval of 45–48 h) and were kept apart from males.

Eggs were activated in vitro within the excised oviducts by a heat shock of 44.0 ± 0.1 °C (Komar, 1973). Eggs from induced ovulations were shocked 12–20 h post-HCG and those from spontaneous ovulations between 8.00 and 9.00 a.m. Some eggs of A origin were heated for 7.5 min; all other eggs received a shock of 5 min duration.

After the shock eggs with the cumulus oophorus intact were placed in tubes with 0.5–1.0 ml of Whitten’s medium (Whitten, 1971) containing 10 μg of Cytochalasin B (CB)/ml. This concentration was worked out in our earlier experiments on suppression of cleavage divisions (Tarkowski et al., in preparation) and was found effective also by Snow (1973). The tubes were gassed with 5% CO₂ in air and kept at 37 °C. Observations of Niemierko (1975) and other pilot experiments carried out in this laboratory showed that when newly fertilized or activated eggs are kept in CB for less than 4 h many of them subsequently undergo fragmentation. Heat-activated eggs were therefore subjected to CB for 5–8 h. Subsequently cumulus was removed with hyaluronidase and the eggs placed in drops of CB-free Whitten’s medium under liquid paraffin for 1–4 h. During this period the eggs were examined under the inverted microscope for signs of activation and type of reaction. Some of these eggs were subsequently fixed and mounted as permanent preparations (Tarkowski & Wróblewska, 1967).

The activated eggs with second polar body (2P.B.) suppressed were transplanted to the oviducts of Swiss albino recipients on the first day of pseudopregnancy (day of vaginal plug = first day) or to sexually immature females (4–5 weeks old) using the technique of Tarkowski (1959). For transplantation we used only eggs which were collected 14–16.5 h post-HCG (in the majority of experiments, 15–16 h).

From a number of recipients the transplanted parthenogenetic eggs were recovered after 72–96 h (4th and 5th day) and cultured for another day in
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Whitten's medium under liquid paraffin. Colcemid (1 μg/ml) was added to culture drops 2–3 h before making air-dried preparations (Tarkowski, 1966). In order to evaluate whether or not the rate of preimplantation development of parthenogenotes is delayed, the stage of development and the number of cells were estimated in F1 (Swiss ♀ × A ♂) embryos obtained on the 4th day after night matings and after delayed matings (7.00–9.00 a.m.), i.e. at the time at which activation was performed.

Another group of recipients (only mature pseudopregnant females) were killed on the 8th–10th day and inspected for signs of implantation. Most implantation swellings recovered on the 9th day and all from the 10th day were torn open and inspected under the dissecting microscope. Eight-day and some of the 9-day implantations were examined in routine haematoxylin and eosin histological preparations.

As control experiments, eggs harvested 12, 16 and 20 h post-HCG were subjected to heat shock and cultured in CB-free medium, and non-heat-treated eggs were cultured in medium containing CB. The control eggs were fixed after the same period of time as the experimental ones and mounted in permanent preparations for examination.

RESULTS

Control experiments

Reaction of non-heated eggs to culture in CB

As can be seen in Tables 1 and 2, over 90% of eggs cultured in the intact cumulus oophorus remained in metaphase II. The incidence of activated eggs did not exceed 3%, but a number of eggs underwent complete fragmentation or were degenerate. Thus culture in CB has no appreciable effect per se on activation.

Reaction of eggs to heat shock alone (culture in CB-free medium)

In order to evaluate properly the effect of CB on activated eggs, over 800 Swiss albino eggs were subjected to heat shock alone and fixed for examination as permanent preparations 4–9 h later (Table 1). With the exception of eggs treated 12 h post-HCG, the overall activation rate in 15–16 and 20-h-old eggs was about 70%, which corresponded well to the activation rate in the experimental series (cf. Tables 1 and 2). Because of the low activation rate in the 12-h group, eggs used in experiments with CB were 13–16.5 h old. The present observations confirmed the fact, noted already by other authors, that with advancing age the eggs become more susceptible to activation but at the same time the incidence of ‘fragmentation’ increases. As already shown by Komar (1973), eggs subjected to heat shock react most often by extrusion of 2P.B. and formation of a haploid pronucleus and by immediate cleavage. Because immediate cleavage is sometimes irregular (the two cells are of uneven size
Table 1. Effect of heat-shock with or without CB on Swiss albino eggs, estimated on fixed and stained eggs (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age post-HCG (h)</th>
<th>Total no. of eggs</th>
<th>Non-activated</th>
<th>Degenerated</th>
<th>2P.B.* present or absent, many nuclei (sub-nuclei)</th>
<th>Immediate cleavage</th>
<th>Enucleation</th>
<th>Control</th>
<th>Total no. of eggs</th>
<th>Activated</th>
<th>Non-activated</th>
<th>Fragmenting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-shock, 4–9 h culture in CB-free medium</td>
<td>12</td>
<td>114</td>
<td>64-0</td>
<td>0</td>
<td>3-5</td>
<td>32-5</td>
<td>100-0</td>
<td>0</td>
<td>2-7</td>
<td>54-1</td>
<td>10-8</td>
<td>32-4</td>
</tr>
<tr>
<td>15–16 (Series I)†</td>
<td>191</td>
<td>23-0</td>
<td>1-0</td>
<td>9-9</td>
<td>6-6</td>
<td>100-0</td>
<td>4-0</td>
<td>3-2</td>
<td>57-1</td>
<td>7-9</td>
<td>27-8</td>
<td>-</td>
</tr>
<tr>
<td>16 (Series II)†</td>
<td>494</td>
<td>14-2</td>
<td>6-3</td>
<td>12-6</td>
<td>6-7</td>
<td>100-0</td>
<td>10-0</td>
<td>4-8</td>
<td>43-8</td>
<td>7-9</td>
<td>33-2</td>
<td>0-3</td>
</tr>
<tr>
<td>Heat-shock, 5–8 h culture in CB, 0–4 h culture in CB-free medium</td>
<td>13–16</td>
<td>320</td>
<td>6-3</td>
<td>15-3</td>
<td>6-6</td>
<td>71-9</td>
<td>100-0</td>
<td>6-4</td>
<td>0</td>
<td>47-8</td>
<td>7-6</td>
<td>29-3</td>
</tr>
</tbody>
</table>

* 2P.B. = second polar body. † PR = pronucleus. ‡ Series I and II were performed in identical manner, but several months apart.
### Table 2. Effect of heat-shock and Cytochalasin B on Swiss albino and C57BL/10 eggs, estimated on eggs in the living state 1–4 h after the shock (values in %)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Activated eggs</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2P.B.* suppressed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 PR†</td>
<td>1 PR</td>
</tr>
<tr>
<td>Strain</td>
<td>Age post-HCG (h)</td>
<td>Total no of eggs</td>
</tr>
<tr>
<td>Swiss albino</td>
<td>14–16-5</td>
<td>1031</td>
</tr>
<tr>
<td>C57BL/10</td>
<td>15</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>16–16-5</td>
<td>106</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>505</td>
</tr>
</tbody>
</table>

* 2P.B. = second polar body.
† PR = pronucleus.
and may be accompanied by enucleate cytoplasmic fragments) classification of such eggs either as undergoing immediate cleavage or fragmentation becomes somewhat arbitrary.

In Table 1 we included separately the results of two identical series of experiments carried out several months apart, in order to show that even when all the parameters under control remain constant and the number of eggs investigated is reasonably high (200 and 300) there may be significant differences in the proportions of eggs entering various routes of development. For instance, the incidence of suppression of 2P.B. varied between 19.6% and 7.2% and of extrusion of 2P.B. between 35.6% and 57.1%.

Contrary to the similar experiments by Komar (1973), subnuclei were formed in less than 10% of eggs. We are unable to explain this difference.

**Experiments with CB**

*Immediate reaction of eggs to heat shock and CB*

Eggs subjected to this double treatment were examined in two ways. First, 320 Swiss albino eggs after being transferred for 0–4 h to CB-free medium were fixed and examined as permanent preparations (Table 1). Second, 1031 Swiss and 208 C67BL/10 eggs were transferred for 1–4 h to CB-free medium and were examined in the living state only (Table 2). After examination some eggs from this group were selected for transplantation (see below). From both series of experiments it is evident that CB effectively suppresses formation of the 2P.B. (over 90%), the most common reaction being formation of two pronuclei. As mentioned above, suppression of 2P.B. can occur after heat shock alone, but the frequency of this phenomenon never exceeds 20%.

In C57BL/10 eggs examined in the living state only, the activation rate among eggs shocked 16–16.5 h post-HCG was twice as high as among eggs treated at 15 h. The significance of this difference is uncertain. As in Swiss albino eggs, in the majority of C57BL/10 eggs, 2P.B. was suppressed by CB.

Spontaneously ovulated eggs of A origin subjected to 5 min heat shock between 8.00 and 9.00 a.m. reacted only in 10% (4 out of 40). However, over 90% were activated when the duration of the shock was prolonged to 7.5 min (60 out of 65). Data concerning activation of A eggs are not included in Tables 1 and 2.

As long as the activated eggs stay in CB their surface remains completely smooth. However, when placed in CB-free medium the surface becomes wrinkled and forms protrusions of various size (Figs. 1, 2) which may – though not necessarily – eventually detach to form small cytoplasmic globules lying freely in the perivitelline space. Sometimes an indentation is formed in the presumptive region of the second polar body formation so that the egg acquires a bean-like shape (Fig. 1). In such a case the pronuclei may be lying on the top of the two protrusions which are formed on both sides of the indentation.
Fig. 1. Swiss albino eggs kept in CB for 5 h 10 min and subsequently in CB-free medium for 3 h 20 min. The surface of eggs is still wrinkled. The upper right egg shows an indentation in the presumptive region of the second polar body formation; the two protrusions are occupied by pronuclei. The egg shown in the middle has extruded several small enucleate fragments and a large one with a pronucleus. × 200.

Figs. 2, 3. Swiss albino eggs kept in CB for 6 h 10 min and in CB-free medium for 30 min.

Fig. 2 Five activated eggs and one non-activated (top middle). The surface of the activated eggs is nearly smooth and the eggs are only slightly deformed. In the middle egg of the lower row the two pronuclei have already moved to the centre. Note that the non-activated egg is round and has a completely smooth surface. × 200.

Fig. 3. Another group of eggs from the same experiment. The surface of eggs is smooth but the two pronuclei still remain close to the egg surface. The egg on the right has retained the first polar body. × 400.
(Fig. 1). If the furrow is not formed the two pronuclei very often lie side by side and stay close to the egg surface (Fig. 3). Similar surface reactions are observed when cleaving mouse eggs are subjected to CB at the time of cleavage and subsequently placed in CB-free medium (Snow, 1973; Tarkowski et al., in preparation).

With time the surface of activated eggs steadily smooths out (Fig. 2). However, a number of eggs undergo severe fragmentation or extrude cytoplasmic fragments which may even contain pronuclei (Fig. 1). This explains a small number of eggs with 2P.B. or undergoing immediate cleavage which are recorded in Tables 1 and 2. These phenomena occur only after the eggs are removed from CB.

The behaviour of eggs appeared to depend on the duration of treatment with CB. After 5-h treatment the egg surface was very wrinkled and they required a few hours to acquire normal appearance; the incidence of fragmentation was highest in this time group. After 8-h treatment the surface was smooth or nearly smooth and the eggs needed a much shorter time to regain their normal appearance. For practical reasons treatment of 6-h duration was usually applied. Whether the longer times of exposure of eggs to CB were more harmful for their survival could not be determined in the present study. To make sure that the eggs with 2P.B. suppressed would remain in this state after transplantation, the eggs were kept in CB-free medium for as long as was feasible (the idea being to complete the experiment in one day and thus to reduce the length of culture to a minimum) and only those which looked normal, i.e. which did not extrude cytoplasmic fragments and clearly contained one or two pronuclei, were selected for transfer.

Contrary to the activated eggs, the surface of the non-activated eggs remained all the time completely smooth. The different behaviour of these two types of eggs permits them to be easily segregated even if the pronuclei are not yet clearly visible (Fig. 2).

Preimplantation development of transplanted eggs

Healthy-looking Swiss albino eggs with 2P.B. suppressed and with two pronuclei (occasionally with one pronucleus) were transplanted to pseudopregnant or sexually immature recipients and recovered on the 4th or 5th day. The embryos were subsequently cultured for 24 h. On the 4th day the most advanced embryos were morulae (Fig. 4). Blastocysts were first observed on the 5th day; some of them transformed from morulae after 24 h in vitro (Fig. 5). All morulae and blastocysts containing analysable metaphase plates (15 out of 27 studied) were diploid.

The tempo of development of parthenogenetic embryos was clearly retarded: formation of blastocysts was delayed until the 5th day and the mean cell number of 4·5- and 5·5-day-old parthenogenomes corresponded to that of 3·5-day-old controls from delayed matings (Table 3). On the 4th day the mean cell number
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and the proportion of embryos that had reached the blastocyst stage was considerably lower in embryos originating from females mated in the morning following ovulation than from females mated at night (Table 3). It follows from this that the proper control material for parthenogenetic embryos are the embryos developed from eggs fertilized as a result of delayed matings which take place at the time when artificial activation is carried out. However, even if this correction is taken into account the parthenogenones still fall behind normal embryos.
Table 3. Preimplantation development of Swiss albino control fertilized eggs and of transplanted diploid parthenogenic eggs

<table>
<thead>
<tr>
<th>Material</th>
<th>Day of autopsy</th>
<th>No. of females</th>
<th>No. of transferred eggs</th>
<th>No. and % of recovered morulae (M) and blastocysts (B)</th>
<th>No. of M and B after 24 h culture in vitro</th>
<th>Cell number in M and B at the end of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Day of development</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Control embryos</td>
<td>4th</td>
<td>6</td>
<td>9</td>
<td>47</td>
<td>14 M, 33 B</td>
<td>4th</td>
</tr>
<tr>
<td>♀♀ mated at night with A♂♂</td>
<td>4th</td>
<td>7</td>
<td>9</td>
<td>42</td>
<td>27 M, 15 B</td>
<td>4th</td>
</tr>
<tr>
<td>Control embryos</td>
<td>4th</td>
<td>7</td>
<td>9</td>
<td>42</td>
<td>27 M, 15 B</td>
<td>4th</td>
</tr>
<tr>
<td>♀♀ mated with A♂♂ 7.00-9.00 a.m. (delayed matings)</td>
<td>4th</td>
<td>7</td>
<td>9</td>
<td>42</td>
<td>27 M, 15 B</td>
<td>4th</td>
</tr>
<tr>
<td>Parthenogenetic embryos</td>
<td>4th</td>
<td>5</td>
<td>9</td>
<td>48</td>
<td>16 M, 5 B</td>
<td>5th</td>
</tr>
<tr>
<td></td>
<td>5th</td>
<td>5</td>
<td>9</td>
<td>48</td>
<td>16 M, 5 B</td>
<td>5th</td>
</tr>
</tbody>
</table>

Overall Mean ± S.E. | Range | Overall Mean ± S.E. | Range | Overall Mean ± S.E. | Range | Overall Mean ± S.E. | Range |
<table>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>25.7 ± 2.4</td>
<td>8-41</td>
<td></td>
<td>22.4 ± 2.4</td>
<td>8-41</td>
<td></td>
<td>27.0 ± 5.5</td>
</tr>
</tbody>
</table>
Table 4. **Implantation of parthenogenetic embryos in Swiss albino pseudopregnant recipients**

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>No. of transferred eggs</th>
<th>No. of transfers*</th>
<th>No. of horns with implants</th>
<th>No. and % of implantations</th>
<th>No. of females autopsied on a given day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss albino</td>
<td>226</td>
<td>19</td>
<td>7</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.1%</td>
<td>16.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>48</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2%</td>
<td>14.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/10</td>
<td>70</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32.9%</td>
<td>34.8%</td>
</tr>
</tbody>
</table>

* Eggs were transferred either into one or into both oviducts.
† All embryos were retarded in development and only two looked healthy (one A embryo recovered on the 8th day and one C57BL/10 recovered on the 9th day).
Development after implantation

Altogether 41 implantation swellings were available for examination (Table 4). The implantation rate was generally very low but clearly depended on the donor strain. The highest rate was observed after transplantation of C57BL/10 eggs (32.9%); Swiss albino eggs and A eggs implanted only in 7.1% and 4.2% respectively.

However, although C57BL/10 eggs were superior to Swiss and A eggs as far as the implantation rate was concerned, their subsequent survival was equally poor (Table 4). Embryos could not be found in any swellings collected on the 9th and 10th day and inspected under the dissecting microscope. In histologically analysed implants eight embryos were found – five on the 8th day and three on the 9th day. Only two embryos – one A and one C57BL/10 from the 8th and 9th day respectively – looked normal and healthy. However, even these two embryos were retarded, as they were in the stage of an egg-cylinder typical for the 7th day of normal development (Figs. 6, 7). The remaining embryos were clearly abnormal and checked in development at the stage of an early 6-day cylinder.

DISCUSSION

Heat shock of 44.0 °C followed by short-term culture in the presence of 10 μg/ml of Cytochalasin B appears to be another effective method of producing diploid parthenogenetic mouse embryos (cf. Graham, 1972; Graham & Deussen, 1974; Kaufman & Gardner, 1974; Kaufman & Surani, 1974). CB suppresses second polar body formation in nearly 100% of activated eggs. Depending on the strain used (Swiss albino or C57BL/10) and the way of examination (fixed and stained eggs or living eggs) the percentage of eggs with two pronuclei varied between 80 and 90, and of those with one diploid pronucleus between 5 and 16. The incidence of eggs containing small subnuclei as well as one or two pronuclei was estimated to be about 10%. Since the same frequencies were observed in eggs cultured in CB-free medium, formation of subnuclei must be a result of heat shock rather than CB. These eggs are potentially hypodiploid.

We have also observed that in many eggs the two pronuclei were of different size, which suggested that at the 2nd meiotic division the chromatids were unequally distributed. This phenomenon was observed with the help of chromosomal studies of the first cleavage of eggs activated in vitro with hyaluronidase and hypotonic shock (Graham & Deussen, 1974). However, when 2P.B. is suppressed, as was the case in the present experiments, aneuploidy of pronuclei has probably no further implications, as diploidy will be restored at the first cleavage. The fact that all morulae and blastocysts containing analysable metaphase plates were diploid proves that suppression of 2P.B. due to CB was permanent and confirms earlier observations that both pronuclei contribute chromosomes to one metaphase plate of first cleavage (Kaufman, 1973b;
We believe, therefore, that in experiments aimed at inducing diploid parthenogenesis, aneuploidy—strictly speaking hypodiploidy—is much more likely to arise when subnuclei are formed than when there is uneven distribution of chromatids between the two pronuclei.

The tempo of preimplantation development of transplanted eggs (number of cleavages and the time of transformation of morulae into blastocysts) was in the present study rather low and unsatisfactory. There are four factors which might have been responsible for this state of affairs. First, damage produced to eggs by heat shock; second, harmful effect of treating the eggs with CB; third, shock imposed on eggs at the one-cell stage by handling and culturing them for several hours *in vitro*; and fourth—probably of minor importance—the effect of second handling and culturing for the last 24 h of the experiment. Although all these four factors might have been important, we believe that the second and the third factors were of major importance. Support for this view comes from the observations by Niemierko (1975) that when newly fertilized mouse eggs are subjected to CB *in vitro* for 5–6 h before being transplanted to the oviduct, the survival of embryos is affected and the rate of development is clearly delayed so that blastocysts do not form until the 5th day.

The low rate of implantation of the Swiss albino and A eggs was probably due to different causes. The A eggs were heated for 7-5 rather than 5 min, which might have had an adverse effect on their survival. As far as Swiss albino eggs are concerned their preimplantation development is generally slow, and these embryos were handicapped when transferred to the synchronized recipients of their own strain. In other words, in the combination of transfer 1 → 1, the parthenogenetic embryos might not have been able to ‘catch up’ with the uterus, the more so since their preimplantation development was retarded.

Unlike eggs of many other strains including Swiss albino, fertilized C57BL/10 eggs develop fast (cf. table 1 in McLaren & Bowman, 1973 and Table 3 in the present paper) and probably for this reason the parthenogenetic ones were more successful in implantation. Additional evidence for this supposition comes from the observation that the decidual swellings induced by C57BL/10 parthenogenetic embryos were regularly larger than those induced by Swiss albino and A embryos.

The inability of diploid parthenogenones to survive in the uterus beyond the egg-cylinder stage remains a mystery. Our present results are concordant with earlier observations on post-implantation development of experimental parthenogenones presented by Tarkowski *et al.* (1970), Graham (1972), Witkowska (1973a), Mintz & Gearhart (1973) and Kaufman & Gardner (1974) as well as of spontaneous parthenogenones (Stevens & Varnum, 1974). The most relevant to the problem under consideration are the papers by Graham and by Kaufman & Gardner, because these authors dealt exclusively with diploid eggs rather than a mixed population of haploids, diploids and \(n/2n\) mosaics, so the mortality
of embryos which they observed could not be due to haploidy. As suggested already in 1970 by Tarkowski et al., diploidy per se is not a sufficient condition for the survival of parthenogenones beyond the egg-cylinder stage. At present there is no satisfactory explanation for the death of these embryos at this particular stage (for a more detailed discussion see Graham, 1974; Tarkowski, 1975). The possibility that embryonic death is due to lethal genetic factors which at this stage start to operate at the cellular level can be rejected because both experimentally produced and spontaneously arising mouse parthenogenones when transplanted to ectopic sites can grow and give rise to various differentiated tissues (Graham, 1970; Stevens & Varnum, 1974; Iles et al. 1975).

Also, parthenogenetic origin does not affect trophoblast, in that giant cells are present in embryos growing in the uterus as well as in ectopic ‘growths’.

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