Development of parthenogenetic and fertilized mouse embryos in the uterus and in extra-uterine sites


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SUMMARY

Mouse eggs were activated with hyaluronidase in vitro and subsequently transferred to the oviduct. In the female reproductive tract they formed morulae and blastocysts which died soon after implantation. Haploid blastocysts were transferred beneath the kidney capsule and here some formed disorganized egg-cylinder structures in a week. Morulae and blastocysts from haploid and diploid parthenogenones were also transferred beneath the testis capsule. Two to four months later the growths which had formed were sectioned. They contained neural tissue, pigment, keratinized epithelium, glandular epithelium, ciliated epithelium, cartilage, bone, muscle, adipose tissue, and haemopoietic tissue. The range of cell types was similar to that produced by fertilized control blastocysts except that the parthenogenones did not form identifiable yolk-sac carcinoma or embryonal carcinoma cells.

The growths from haploid and diploid parthenogenones in the testis were stained with Feulgen and their DNA content measured. Growths from diploid embryos contained the normal diploid amount of DNA while growths from haploid embryos contained less than this amount. Cell cultures were prepared from the growths. The cells which were investigated contained no Y chromosome, suggesting that they were derived from the embryonic cells rather than the cells of the male host. These cells contained a near diploid chromosome number, although some of them were originally derived from haploid embryos.

INTRODUCTION

After experimental activation, few unfertilized mammalian eggs develop beyond the blastocyst stage and only mouse parthenogenones have been clearly shown to develop after implantation (reviewed Beatty, 1957; Austin, 1961; Tarkowski, 1971; Graham, 1974). The majority of parthenogenetic mouse blastocysts die during the 2 days following implantation, and spontaneously activated eggs from the unusual LT strain die at a similar time in utero (Stevens & Varnum, 1974). However, the female germ cells of LT strain mice also develop spontaneously into teratomas in the ovary. These growths

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contain well-differentiated tissues and this observation suggests that the uterus may be a poor site for the extensive development of parthenogenetic mouse eggs. We have therefore compared the development of parthenogenetic mouse embryos in the uterus with their development in the kidney and testis. Our observations demonstrate that both haploid and diploid parthenogenetic blastocysts are able to form many differentiated cell types in these extra-uterine sites. We have also examined the DNA content of the cells in these growths and studied the chromosomes of cells which grew from these tumours in culture.

MATERIALS AND METHODS

1. Supply of eggs and embryos

Fertilized one-cell eggs were obtained from the oviduct of females in the afternoon of the first day of pregnancy (day of copulation plug). Fertilized blastocysts and morulae were flushed from the uterus on the fourth day of pregnancy.

Unfertilized eggs were obtained by superovulation of 6-week to 5-month-old females (Runner & Palm, 1953). Eight to 16 i.u. each of PMSG (pregnant mare's serum gonadotrophin) followed by HCG (human chorionic gonadotrophin) (Gestyl and Pregnyl, Organon Laboratories, London) were injected i.p. 40–52 h apart.

2. Activation

At various times after HCG injection, the eggs were dissected out of the oviduct into the medium described by Whitten (1971). They were transferred to hyaluronidase for 10–15 min at 37 °C (200 i.u. hyaluronidase/ml in Whitten's medium; method of Kaufman, 1973a), and rinsed three times. Next they were cultured in White's medium or dilutions of this medium for 2 h and they were then transferred to Whitten's medium (as described in Graham & Deussen, 1974).

It was necessary to distinguish haploid and diploid embryos. Five to 8 h after hyaluronidase, the eggs were sorted into five groups using a Wild M 5 microscope. Those eggs which did not contain a pronucleus were described as 'not activated'. The activated eggs either formed a pronucleus and a second polar body, or they cleaved in half so that the female pronucleus was in one cell and the second polar body nucleus was in another (immediate cleavage), or second polar body formation was suppressed and the eggs contained both the female chromosomes and the second polar body chromosomes in one or two pronuclei. It is known that eggs which have a female pronucleus and a second polar body and those which undergo immediate cleavage will form haploid blastocysts and that eggs in which second polar body formation is suppressed will form diploid blastocysts (Kaufman, 1973a, b).

The eggs of C3H, F1 C57BL/A2G, and F1 C57BL/C3H mothers were
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activated between 16 and 18 h post-HCG, and those of 129/J mothers were activated between 17 and 19 h post HCG.

3. Transfer and recovery of embryos and tumours

All transfers were effected with a glass micropipette attached by polythene tubing to a mouthpiece. The embryos were flanked by air bubbles in the micropipette and these served as visual markers of the movement of the embryos into the oviduct and under the testis and kidney capsule. The embryos were transferred into the uterus without air bubbles.

Activated and fertilized one-cell eggs were transferred under the testis capsule or into the oviduct of females on the first day of pseudopregnancy (Tarkowski, 1959). The host females were anaesthetized with 0.01 ml/g body weight of a 2.5 % (v/v) solution of tribromoethyl alcohol in phosphate buffered saline (Avertin, Winthrop Laboratories, Surbiton-on-Thames, Surrey, in solution ‘A’ of Dulbecco & Vogt, 1954). This anaesthetic is known to activate unfertilized eggs (Kaufman, 1975) and it was therefore important to show that few of the host’s own eggs developed. This was achieved by transferring the donor eggs to one oviduct only of the recipient (experimental side) so that the other side served as a control. Eggs were recovered from both tracts on the fourth day of pseudopregnancy and it was found that in the whole experimental series only one egg in the control side had developed to the morula stage. This suggests that nearly all the eggs which form morulae and blastocysts in the experimental side are derived from eggs activated in vitro.

We used the following method to estimate the percentage of transferred eggs which were recovered. The average number of eggs on the control side was calculated and this figure was doubled to give the average number of native eggs which would be expected in the total uterus. For each animal, the number of eggs in the control horn was subtracted from the average number of native eggs in both horns. This calculation is necessary because if one ovary sheds many eggs, the other ovary sheds fewer.

The morulae and blastocysts from the experimental side were cultured in Whitten’s medium for a day and were then transferred to the uterus of a random bred female or beneath the kidney or testis capsule of a syngeneic male. Transfers to the uterus were performed in the evening of the third day or morning of the fourth day of pseudopregnancy (McLaren & Michie, 1956). In addition, fertilized blastocysts were transferred beneath the capsule of the testis of a syngeneic male.

Portions of the growths which developed from embryos in the testis were transferred beneath the kidney capsule of a syngeneic host. The growths were torn apart in phosphate buffered saline and fragments about 5 x 1 x 1 mm were inserted beneath the kidney capsule using a wide flame-polished pipette. In a few instances fragments of the growths were transferred beneath the skin or to the peritoneal cavity.
4. Inspection of transfers

(a) Kidney transfers

The recipients were killed 1 week after transfer. Visible growths were dissected out and fixed, while kidneys without visible growths were fixed whole. Both were serially sectioned and we inspected every section of the growths and every second section of the whole kidneys.

(b) Testis transfers

The recipients were killed 2–4 months after transfer. About one-quarter of each tumour was fixed and serially sectioned. In growths from parthenogenetic embryos every second section was inspected while in large growths from fertilized embryos every tenth section was studied.

The growths were fixed either in Bouin's fluid or in formol saline, embedded in paraffin wax and sectioned at 8 µm. The sections were usually stained in Alcian blue at pH 2-5 followed by Masson's trichrome (Evans, 1972), but for many growths every tenth section was put aside so that special histological techniques could be used to identify particular tissues. Holmes's silver stain was used for identification of nervous tissue (Holmes, 1942), and the presence of bone was confirmed with Von Kossa's method for 'calcium' deposits (Pearse, 1972). Cell and tissue types in the growths were compared with those described in an histology text (Ham & Leeson, 1961), and with known cell types in the embryonic and newborn mouse. In these disorganized growths it is often impossible to recognize all the cell types and we have only recorded those which could be readily identified.

5. Microdensitometry

Portions (about 3 mm cube) were taken from the tumours and placed in a solid watch-glass containing 1% trisodium citrate for about 30 min. The hypotonic fluid was removed and 3–4 ml of a 3:1 ethanol:acetic acid mixture was rapidly added; 15 min to 24 h later the fixative was removed and replaced by about 0·5 ml of aqueous 60% acetic acid. After 5 min, cells began to fall away from the tumour and they were taken into a micropipette. A clean slide was placed on a hot plate at 50–70 °C. The cells were spread by two methods. Either a drop of cells in acetic acid was placed on the slide and run across the surface by tilting or a drop was expelled from the micropipette and sucked in again leaving a smear behind. This is a modification of a method used to prepare chromosome spreads (Evans, Burtenshaw & Ford, 1972). A population of predominantly G1 cells was obtained by cannulation of the thoracic duct of a rat (Bollman, Cain & Grindlay, 1948). These cells were smeared at one end of the slide containing the tumour cells and fixed in the ethanol:acetic acid mixture immediately after air-drying. The preparations were hydrolysed in
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5 N-HCl for 30 min and stained with the Feulgen reaction (method of Deitch, Wagner & Richart, 1968).

Photometric measurements were made at 565 nm over the nuclei using a Vickers model 85 scanning microdensitometer. The nuclei of the rat lymphocytes served as an internal standard of the 2 C amount of DNA; rat and mouse nuclei contain similar amounts of DNA (Mirsky & Ris, 1951). Microdensitometry of Feulgen stained nuclei is only a reliable guide to DNA amounts if the chromatin is condensed to the same extent in the experimental and standard nuclei, and apparent DNA amounts may vary greatly as chromatin condensation changes (e.g. Gledhill, Gledhill, Rigler & Ringertz, 1966; Goldstein, Bedi, 1974). The diameter of the largest nuclei from the growth was about five times that of the smallest, and to reduce variation in apparent DNA amounts we only measured nuclei in the growths which were 1–1.5 times the diameter of the standard lymphocytes. We also wished to be certain that few host cells were contaminating the cells obtained from the growths. Sperm nuclei and meiotic chromosome sets were clearly recognized after Feulgen staining and any slide which contained one of these in less than 3000 cell nuclei was discarded. About three quarters of the growths were discarded for this reason.

6. Cell culture and chromosomes

Tissue obtained from testicular growths of parthenogenetic blastocysts was chopped into lumps of about 1 mm diameter. These lumps were placed into plastic culture dishes containing Eagle’s minimal essential medium supplemented with 10 % foetal calf serum and 100 i.u./ml of penicillin and 50 μg/ml of streptomycin sulphate. Cells migrated out of these lumps and when they had grown to form a monolayer on the plastic, they were dispersed with 0.125 % trypsin and 0.02 % ethylenediaminetetraacetic acid in phosphate buffered saline for subculturing. The chromosomes were examined two to three months after the initial explant. Chromosome preparations from these cells were made following 1–3 h exposure to 0.06 μg/ml of Colcemid. The slides were air-dried and stained with Giemsa (Gurr, Searle Diagnostic, High Wycombe, Bucks, England). Some chromosome preparations were G-banded with trypsin (Seabright, 1971).

RESULTS

1. Egg activation

It was possible to distinguish haploid from diploid eggs soon after activation (see Methods). Eggs cultured in White’s medium tended to develop as haploids; the percentages developing as haploids were: C3H 89 %; 129/J 80 %; F1 C57BL/A2G 73 %. In contrast, eggs cultured in dilute White’s medium (3/5 White’s) tended to develop as diploids; the percentages developing as diploids
Figs. 1–3 Parthenogenetic embryos of 129/J mice.

Fig. 1. Group of unhatched live 129/J haploid blastocysts on the fourth day of pseudopregnancy.

Fig. 2. Hatched live 129/J diploid blastocyst on the fourth day of pseudopregnancy.

Fig. 3. Growth produced by haploid 129/J blastocyst which had been transferred beneath the kidney capsule for 7 days. The growth contained giant trophoblast cells and cells arranged as in the embryonic region of a normal eight-day-old embryo; many of these cells were in mitosis (arrows).
Table 1. Activation of unfertilized mouse eggs

<table>
<thead>
<tr>
<th>Strain of mother (total no. of eggs)</th>
<th>Culture medium</th>
<th>Not activated (%)</th>
<th>Undergoing abortive cleavage or lysis (%)</th>
<th>Activated eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H (1114)</td>
<td>White's</td>
<td>22-4</td>
<td>24-9</td>
<td>78-9 8-9 8-3 3-9</td>
</tr>
<tr>
<td>C3H (902)</td>
<td>3/5 White's</td>
<td>17-5</td>
<td>21-6</td>
<td>39-3 17-8 9-5 33-4</td>
</tr>
<tr>
<td>129/J (1194)</td>
<td>White's</td>
<td>63-4</td>
<td>15-9</td>
<td>68-6 11-9 12-7 6-8</td>
</tr>
<tr>
<td>129/J (193)</td>
<td>3/5 White's</td>
<td>10-8</td>
<td>39-3</td>
<td>11-5 22-9 44-8 20-8</td>
</tr>
<tr>
<td>F₁ C57BL/A2G (301)</td>
<td>White's</td>
<td>53-1</td>
<td>4-9</td>
<td>65-1 8-7 26-2 0</td>
</tr>
<tr>
<td>F₁ C57BL/A2G (471)</td>
<td>3/5 White's</td>
<td>20-4</td>
<td>16-6</td>
<td>8-1 28-3 35-0 28-6</td>
</tr>
<tr>
<td>F₁ C57BL/C3H (469)</td>
<td>3/5 White's</td>
<td>41-4</td>
<td>12-6</td>
<td>40-7 25-5 12-0 21-8</td>
</tr>
</tbody>
</table>

After hyaluronidase, the eggs were placed in full strength White's medium or 3/5 White's medium. Two hours later they were transferred to Whitten's medium for 3–5 h and then sorted out into the categories listed in the Table (see Materials and Methods).
were: C3H 47%; 129/J 65%; F$_1$ C57BL/A2G 64%; F$_1$ C57BL/C3H 34% (Table 1). Culture in dilute White's medium also increased the percentage of activated eggs.

2. Development of activated and fertilized one-cell eggs in the testis

We found that 10% of one-cell fertilized eggs of 129/J strain formed differentiated growths in the testis (4 growths from 39 eggs transferred), but that neither haploid nor diploid one-cell parthenogenetic eggs of this strain formed detectable growths in this site (16 eggs of each transferred). The four growths from fertilized 129/J eggs contained all the cell and tissue types listed in Table 5 (except yolk-sac carcinoma). We failed to detect any growths after transferring 30 fertilized one-cell C3H eggs and after transferring 310 haploid and 17 diploid parthenogenetic eggs from C3H.

Table 2. Development of activated eggs after transfer to the oviduct

<table>
<thead>
<tr>
<th>Developmental route</th>
<th>Strain</th>
<th>Recovered (%)</th>
<th>Recovered eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(numbers of eggs)</td>
<td></td>
<td>Morulae</td>
</tr>
<tr>
<td>Haploid</td>
<td>C3H (161)</td>
<td>96-4</td>
<td>56-3</td>
</tr>
<tr>
<td></td>
<td>129/J (188)</td>
<td>73-4</td>
<td>23-2</td>
</tr>
<tr>
<td></td>
<td>C57BL/A2G (26)</td>
<td>77-3</td>
<td>35-2</td>
</tr>
<tr>
<td>Diploid</td>
<td>C3H (96)</td>
<td>88-7</td>
<td>30-6</td>
</tr>
<tr>
<td></td>
<td>129/J (33)</td>
<td>109-0</td>
<td>34-2</td>
</tr>
<tr>
<td></td>
<td>C57BL/C3H (47)</td>
<td>72-3</td>
<td>21-8</td>
</tr>
<tr>
<td></td>
<td>C57BL/A2G (193)</td>
<td>74-6</td>
<td>58-0</td>
</tr>
</tbody>
</table>

The side to which the eggs were transferred also contains the eggs ovulated by the recipient. The percentage recovery was determined by the method described in Materials and Methods.

3. Pre- and post-implantation development of parthenogenetic embryos in the reproductive tract

The pre-implantation development of the activated eggs was studied by transferring them back to the oviduct. On the fourth day of pseudopregnancy they were flushed out, cultured in Whitten's medium for a day and sorted into blastocysts and morulae (Table 2). A small proportion of the haploid eggs from 129/J and C3H mothers formed blastocysts.

The post-implantation development of parthenogenones was studied by transferring the healthy morulae and blastocysts from the previous experiment (Figs. 1, 2) back to the uterus of a pseudopregnant female. At various times after transfer, the recipients were killed and the decidua were dissected to discover the state of the embryo. Disorganized egg-cylinders were obtained from two haploid and one diploid blastocyst (Table 3), but most decidua only
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contained fragments of trophoblast tissue. It was clear that the uterus was a poor site for the development of these embryos.

Table 3. Post-implantation development of parthogenetic and fertilized eggs in the uterus

<table>
<thead>
<tr>
<th>Ploidy</th>
<th>Day of autopsy</th>
<th>No. of pregnant recipients/number of recipients</th>
<th>No. of embryos to pregnant recipients/total no. transferred</th>
<th>No. of decidua/no. of embryos to pregnant recipients</th>
<th>No. of embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid</td>
<td>7–9th day</td>
<td>9/14</td>
<td>40/74</td>
<td>21/40</td>
<td>2</td>
</tr>
<tr>
<td>Diploid</td>
<td>7–9th day</td>
<td>6/8</td>
<td>18/26</td>
<td>11/18</td>
<td>1</td>
</tr>
<tr>
<td>Diploid</td>
<td>10–15th day</td>
<td>3/8</td>
<td>11/32</td>
<td>6/11</td>
<td>0</td>
</tr>
</tbody>
</table>

102 of the parthenogenetic morulae and blastocysts were from C3H mice and 30 were from 129/J mice.

Table 4. Transfer of blastocysts and morulae to the testis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin of embryo</th>
<th>Stage</th>
<th>No. transferred</th>
<th>No. of tumours</th>
<th>‘Take’ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/J</td>
<td>Haploid parth.</td>
<td>Morula</td>
<td>6</td>
<td>1</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>Haploid parth.</td>
<td>Blastocyst</td>
<td>83</td>
<td>10</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Diploid parth.</td>
<td>Blastocyst</td>
<td>23</td>
<td>4</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>Fertilized</td>
<td>Blastocyst</td>
<td>28</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>C3H</td>
<td>Haploid parth.</td>
<td>Morula</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Haploid parth.</td>
<td>Blastocyst</td>
<td>30</td>
<td>2</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Diploid parth.</td>
<td>Blastocyst</td>
<td>31</td>
<td>1</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Fertilized</td>
<td>Blastocyst</td>
<td>25</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>129/J/C3H F₁</td>
<td>Haploid parth.</td>
<td>Morula</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Haploid parth.</td>
<td>Blastocyst</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C3H/C57BL F₁</td>
<td>Haploid parth.</td>
<td>Blastocyst</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>A2G/C57BL F₁</td>
<td>Diploid parth.</td>
<td>Blastocyst</td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The recipients were killed 2–4 months after the transfer of an embryo to the testis of a host syngeneic with the mother which provided the eggs. Exceptionally the A2G/C57BL F₁ recipients were killed 1 month after embryo transfer.

4. Development of parthenogenetic and fertilized morulae and blastocysts in extra-uterine sites

Morulae and blastocysts were transferred beneath the testis capsule and 2–4 months later the hosts were killed. About one-quarter of each growth was serially sectioned.

We have repeated the previous observation that fertilized 129/J blastocysts can form growths in this site (Stevens, 1970). In our hands, a larger proportion
<table>
<thead>
<tr>
<th>Strain</th>
<th>Haploid</th>
<th>Diploid</th>
<th>Fertilized Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonal carcinoma</td>
<td>1?</td>
<td>1?</td>
<td>3</td>
</tr>
<tr>
<td>Neural tissue</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Pigment</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Keratin epith.</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Glandular epith.</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ciliated epith.</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cartilage</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Bone</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Muscle</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Adipose</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Haemopoietic</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yolk-sac carcinoma</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5. Differentiation shown by tumours arising from normal and parthenogenetic blastocysts

<table>
<thead>
<tr>
<th>Origin ...</th>
<th>Parthenogenetic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ploidy ...</td>
<td>Haploid</td>
<td>Diploid</td>
<td>Fertilized Diploid</td>
</tr>
<tr>
<td>Strain ...</td>
<td>129/J</td>
<td>C3H</td>
<td>F\textsubscript{1} C57/C3H</td>
</tr>
<tr>
<td>No. of tumours</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

S. A. ILES AND OTHERS
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of fertilized C3H blastocysts will form growths in the testis (Table 4). Haploid and diploid parthenogenetic morulae and blastocysts will also form growths but these are always smaller than those from fertilized embryos.

The growths obtained from parthenogenetic embryos of inbred mothers had a similar range of cell types to those obtained from fertilized embryos (Table 5, Figs. 4–8). However, the growths from parthenogenones rarely, if ever, contained cells which resembled embryonal carcinoma. The two growths obtained from embryos of $F_1$ mothers only contained one cell type. In addition to the cell and tissue types listed in Table 5, the following structures were also found in growths obtained from both fertilized and parthenogenetic embryos: sebaceous glands, hair, and a villous epithelium resembling that found in the alimentary canal.

We attempted to establish transplantable teratocarcinomas by transferring portions of the growths in the testis beneath the kidney capsule. We obtained one transplantable tumour from nine growths of fertilized C3H blastocysts but were unable to obtain any transplantable tumour from three growths of fertilized 129/J blastocysts. In some cases we were able to maintain parthenogenetic growths after they had been transferred to the kidney but in no case was it possible to obtain a transplantable tumour. Thirty fragments from six takes of haploid 129/J blastocysts in the testis were transferred and the growths were recognized in 14 kidneys. Eight fragments from three takes of 129/J diploid parthenogenetic blastocysts in the testis were transferred and the growths were recognized in one kidney. No growths were recognized after transferring ten fragments from three takes of haploid C3H blastocysts in the testis. The growths in the kidney never increased in size despite the fact that the variety of cells which were found appeared healthy.

Parthenogenetic haploid blastocysts could form growths if they were transferred directly beneath the kidney capsule; these growths were fixed one week after transfer (Table 6). They contained giant trophoblast cells, disorganized groups of cells in an extracellular matrix which were probably extra-embryonic cells, and deformed embryonic egg-cylinder structures; they resembled growths obtained from fertilized blastocysts transferred to the same site (compare Fig. 3 with illustrations in Kirby (1960) and in Billington, Graham & McLaren (1968)).

5. Ploidy of growths obtained by transferring parthenogenetic blastocysts to the testis

The parthenogenetic blastocysts formed small growths which varied in size from 1 mm diameter to a growth which occupied two thirds of the testis. Histological examination showed that in most cases the eroded remains of testis tubules ran through the growth and it was necessary to exclude microdensitometry of DNA amounts from any growth which showed signs of testis contamination. Testis contamination was identified by the presence of meiotic
Figs. 4–8. Growths produced by haploid parthenogenetic blastocysts in the testis.

Fig. 4. Bone and cartilage from a haploid 129/J blastocyst. The bone surrounded loose nucleated red cells which were probably haemopoietic tissue.

Fig. 5. Nerve and striated muscle from a haploid 129/J blastocyst. The nerve appears to have formed a motor end-plate on the muscle. Stained with Holme’s stain.

Fig. 6. Ciliated epithelium from C3H haploid blastocyst.

Fig. 7. Keratinized epithelium from C3H haploid blastocyst.

Fig. 8. Nerve net with ganglion cells from C3H haploid blastocyst.
Table 6. Development of parthenogenetic blastocysts in the kidney

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ploidy</th>
<th>No. of growths/no. transferred</th>
<th>Trophoblast</th>
<th>Disorganized small cells</th>
<th>Embryonic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/J</td>
<td>Haploid</td>
<td>5/10</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>C3H</td>
<td>Haploid</td>
<td>3/8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Kidneys were examined 7 days after blastocyst transfer.

Fig. 9. Microdensitometry of nuclei from growths obtained by transferring parthenogenetic blastocysts to the testis. The standard for the DNA amount of a G₁ diploid cell was indicated by rat lymphocytes and their absorbance is indicated by the shaded histogram; the number measured on each slide is indicated by the figure against L. The absorbance of nuclei from the growths is indicated by the open histogram and the number measured is indicated beside T. (A) Transfer of a haploid C3H blastocyst. (B) Transfer of a haploid 129/J blastocyst. (C) Transfer of a diploid 129/J blastocyst.
chromosomes or sperm heads, and only three growths were completely free of contamination.

One growth obtained after the transfer of a diploid blastocyst to the testis contained two populations of nuclei (Fig. 9C). The majority of nuclei contained DNA amounts which coincided with the 2C standard provided by rat lymphocytes. A minor population had DNA amounts which were consistent with that of diploid nuclei in the G2 phase of the cell cycle or that of tetraploid cells in the G1 phase of the cell cycle. The two growths obtained after the transfer of haploid blastocysts to the testis contained DNA amounts intermediate between those expected for G1 and G2 haploid nuclei (Fig. 9A, B).

6. Cell cultures derived from testicular growths of parthenogenetic blastocysts

Small lumps of tissue from growths derived from testicular grafts of parthenogenetic blastocysts were placed into culture and cell lines were derived from them. Seven cell lines were established from four growths of 129/J parthenogenetic blastocysts. All lines have been passaged at least five times and some more than 15 times. Line A was derived from a growth produced by a diploid parthenogenone whose microdensitometry histogram is shown in Fig. 9C. Six other lines were derived from three growths of haploid blastocysts. Lines B1 and B2 were from the growth whose microdensitometry is shown in Fig. 9B and lines C1, C2, C3 and D were from two other growths. Although cells with morphologies characteristic of various differentiated tissues initially migrated out of the cultured lumps, all but the fibroblast-like cells disappeared from the cultures after a few passages except in the case of lines B1 and C3 which consisted of small neuroepithelial-like cells.

It was of interest to determine whether the chromosome number of each cell line was the same as that of the blastocyst which was responsible for the original growth. The histogram shown in Fig. 10 shows the chromosome numbers of six of the seven cell lines. None of the cell lines derived from the presumptive haploid growths had any haploid metaphases. Lines B1, B2, C1 and C3 showed a strong mode at the diploid number of 40 while line D was primarily hypotetraploid. Line A, derived originally from a diploid blastocyst, had two modes – one at the diploid number of 40 and another at the tetraploid value of 80.

The diploid number of chromosomes in cell lines derived from presumptive haploid growths could indicate that diploidization of the haploid cells occurred either in culture or in the teratoma, or that the cell lines were derived from the diploid tissues of the host. These possibilities may be distinguished because the host cells should contain a Y chromosome as they were derived from the male testis and the graft tissue should have no Y as they were derived from females. Chromosomes from these cell lines were therefore G-banded and examined for the presence of a Y chromosome. Unfortunately a considerable amount of
Fig. 10. Frequency distribution of the chromosome number of the cell lines derived from growths initiated by parthenogenetic embryos. Chromosomes were prepared between the second and fifth passage.
chromosomal rearrangement had occurred in all cell lines. In lines B 1, B 2, and C 3, which showed the least amount of rearrangement, no Y chromosomes were found, suggesting that these cell lines were derived from the graft rather than from the host tissues. However, the possibility that the Y chromosome had been eliminated or involved in a rearrangement could not be excluded. Because of the rearrangements, it was not possible to confirm the presumptive female derivation of these cells by a demonstration of two X chromosomes.

DISCUSSION

1. Development of parthenogenetic embryos in the reproductive tract

The activated eggs developed similarly to those observed in previous studies using the in vitro activation technique (Graham, 1970; Kaufman, 1973a and b; Graham & Deussen, 1974; Kaufman & Gardner, 1974; Kaufman & Surani, 1974). Many of the activated eggs developed into morulae and blastocysts but few formed egg-cylinders following implantation. In contrast, about 10% of eggs activated in vivo by electric shock form disorganized egg-cylinders and at least one embryo has reached the 8-somite stage after this stimulus (Witkowska, 1973). Nevertheless whatever activation stimulus is used, the parthenogenetic embryos develop poorly in the female reproductive tract.

2. Development of parthenogenetic embryos in extra-uterine sites

Growths were found in many extra-uterine sites after the transfer of parthenogenetic blastocysts. There is little doubt that the majority of these were formed from the transferred embryos. The principal reasons for thinking that this is the case are:

(a) Parthenogenetic blastocysts of C3H and 129/J mothers were transferred to the kidney of syngeneic hosts. Spontaneous growths containing embryonic cells have never been reported in these sites and were not observed in the contralateral kidney. The growths that developed contained trophoblast, extra-embryonic cells, and disorganized egg-cylinders and had the characteristic morphology of fertilized blastocyst-derived growths (e.g. Billington et al. 1968).

(b) Parthenogenones from C3H, F1 129/J/C3H and F1 C3H/C57BL mothers were transferred to the testes of syngeneic hosts. Spontaneous growths containing embryonic cells have never been reported in testes of these strain combinations and were not obtained in the contralateral testes in this study. The growths that developed contained a similar variety of cell types to those produced by fertilized C3H blastocysts.

(c) Growths were also obtained after the transfer of parthenogenetic 129/J embryos beneath the testis capsule. Since it is known that spontaneous teratomas sometimes develop in the testis of this strain, it is possible that some of the growths which appeared after transfer to this site were derived from the
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host testis. However, in one batch of particularly good haploid blastocysts (Fig. 1), we obtained 8 growths after transfer of 33 blastocysts, while only one spontaneous teratoma was found in the contralateral testes of this group. We only found one other spontaneous tumour in 70 testes screened. Also, in the 129/J strain spontaneous teratomas usually develop in the left testis (Stevens & Little, 1954), and in this study the majority of parthenogenetic embryos were transferred to the right testis.

The eggs of C3H mothers tend to lyse after activation (Graham & Deussen, 1974); their development to the blastocyst stage is poor (Table 2), and their ability to form growths is limited (Tables 4, 6). However, there is no direct relationship between vigorous pre-implantation development and the ability to form growths in extra-uterine sites. Thus, the parthenogenones of 129/J mothers and the parthenogenones of F1 mothers developed well up to the blastocyst stage, but of these the 129/J parthenogenones often formed growths (15 growths after 112 transfers) while the parthenogenones of F1 mothers rarely did so (2 growths after 90 transfers).

3. Ploidy of the growths

We obtained very few mitoses from growths derived from parthenogenetic embryos and were, therefore, unable to determine the karyotype of the constituent cells. Less than 0.1% of the cells from a growth derived from a diploid parthenogenetic blastocyst were in mitosis. The DNA content of cells from this growth indicated that most nuclei contained the 2 C amount of DNA, with a few nuclei containing 4 C. We interpret this as meaning that virtually all cells were in G0 or the non-growing phase of the cell cycle, the 4 C content of some cells being due to their tetraploid nature.

The growths from haploid blastocysts had similarly low mitotic indices, but the distributions of DNA values were different from that of the diploid-derived tumour. More than 80% of the nuclei in these growths contained less than 2 C value DNA, but the majority of nuclei contained DNA amounts between 1 C and 2 C. Since most of the cells appeared to be non-growing, our failure to find a 1 C DNA content in most of the nuclei suggests that there was a large and variable amount of aneuploidy in these cells: chromosome nondisjunction may be more likely to occur in haploid than in diploid tumours.

The cell lines derived from presumptive haploid growths contained only diploid or hypotetraploid cells on subsequent chromosome analysis. The absence of a Y chromosome from those lines analysed suggested that they were derived from the female graft tissue. This female tissue was haploid at the time of the initial graft and possibly even after the growth had formed, suggesting that the diploidization may have occurred in culture. Further attempts are being made to establish haploid cells in culture.

We conclude that parthenogenetic embryos can form growths containing many differentiated cell types, but we cannot be certain that the differentiated
cells derived from haploid embryos remain haploid. The failure of partheno-
genetic embryos to develop to term is therefore not due to their inability to form the variety of embryonic and differentiated tissues observed in this study. The range of cell types produced by parthenogenetic embryos is similar to that produced by fertilized embryos and it is particularly impressive that haploid blastocysts can form giant trophoblast cells.

We believe that the failure of parthenogenetic embryos to form transplantable tumours in extra-uterine sites is due to the shortage of stem cells. We seldom found cells which resembled embryonal carcinoma in the parthenogenetic growths and we are not certain that our identification was correct when such cells were seen.

CONCLUSIONS

1. Mouse eggs activated with hyaluronidase in vitro rarely develop beyond the fifth day of pregnancy in the uterus.

2. The cells of haploid and diploid parthenogenetic embryos are able to differentiate into a wide range of cell types in the testis. The range of cell types is similar to that produced by fertilized embryos.

3. The amount of DNA in most cells from haploid embryos is less than the normal diploid value but cultures from these cells contain diploid chromosome numbers. It is probable that some haploid cells can become diploid.

4. The failure of parthenogenetic embryos to develop in the uterus cannot be due to their inability to cytodifferentiate.

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