In vitro activation of mouse eggs

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SUMMARY

Unfertilized mouse eggs were activated in vitro with hyaluronidase. Subsequently they were exposed to culture medium at different osmolarities. In full strength White's culture medium they tended to form one pronucleus and a second polar body. The majority of these eggs were haploid. In 4/5 and 3/5 dilutions of this medium, the second polar body formation was suppressed and eggs tended to form with one or two pronuclei. Those with one pronucleus were diploid and those with two pronuclei could either form a diploid or form a haploid mosaic. Old eggs tended to immediately cleave and form haploid mosaics.

DNA synthesis was studied in activated eggs using tritiated thymidine and autoradiography. DNA synthesis occurred at a similar time in fertilized and activated eggs.

INTRODUCTION

The aim of this research was to find a method of activating unfertilized mouse eggs so that they subsequently developed as heterozygous diploid embryos. Parthenogenetic frog, chicken, and turkey embryos of this type will develop into adults (e.g. Olsen & Marsden, 1953; Olsen, Marks & Wilson, 1968; Volpe, 1970; Olsen & Buss, 1972). In vitro activation of mouse eggs was used in these experiments (Graham, 1970) because this technique allows the eggs to be kept under observation.

Unfertilized mouse eggs can be activated in vivo and in vitro, and they may develop into haploid, diploid or aneuploid embryos (reviewed by Beatty, 1957; Tarkowski, 1971). There are many methods of classifying and numbering the developmental routes which the parthenogenones may take (Beatty, 1957; Austin, 1961; Beatty, 1967; Graham, 1971b; Tarkowski, 1971; Kaufman, 1973c). These can be confusing and so here the routes of development are described in words (Fig. 1).

The mouse egg is usually in metaphase II after ovulation (Donahue, 1972); activation and culture may cause it to develop along the following routes (Fig. 1):

One pronucleus, second polar body. The female chromosomes divide as if the egg had been fertilized; a second polar body and a female pronucleus are formed. The embryo is a presumptive haploid.

Immediate cleavage. The egg cleaves in half, instead of forming a second polar

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body, and the female pronucleus forms in one cell and the second polar body nucleus forms a pronucleus in the other cell. If there has been recombination at meiosis, then the embryo is a presumptive haploid mosaic.

**Two pronuclei, no second polar body.** Second polar body formation is suppressed and the egg contains two pronuclei (the female pronucleus and the second polar body nucleus). At more than 10 h after activation, the pronuclei in these eggs may move apart and the cytoplasm cleave between them; this is described by the paradoxical phrase ‘delayed immediate cleavage’ (Graham, 1971b). Alternatively the pronuclei may come together at the first cleavage division and form a presumptive diploid (potentially heterozygous if there has been crossing over at meiosis).

**One pronucleus, no second polar body.** A single pronucleus develops from metaphase II, and the female pronucleus and second polar body nucleus are never clearly separate. Under the dissecting microscope, the pronucleus appears to contain one nucleolus and can be distinguished from two closely opposed pronuclei in which two nucleoli are visible. These eggs form presumptive diploids (potentially heterozygous if there has been recombination in meiosis).

It is possible for all the presumptively haploid embryos to form diploids by the failure of cytokinesis; segregation at metaphase II may be unequal and other abnormalities can be introduced during development. A study of the chromo-
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somes of eggs developing along these routes is presented in this paper. All karyotypes were prepared at the first cleavage division.

The route of development of these parthenogenetic eggs may be controlled in vitro by either altering the osmolarity of the culture medium after activation (Graham, 1972) or by altering the time between ovulation and activation (Kaufman, 1973a). It is also known that the route of development of in vivo activated embryos depends on the strain of mouse (Tarkowski, Witkowska & Nowicka, 1970; Witkowska, 1973a). This paper is a study of the effect of all these conditions on the route of development and it complements similar work by Kaufman (1973a; Kaufman and Surani, 1974).

MATERIALS AND METHODS

(1) Supply of eggs

Female mice were from inbred strains (AKR, CBA, C3H, C57BL, ICR/A). Six-week to 5-month-old virgin females were superovulated (Runner & Palm, 1953). Eight to 16 i.u. each of PMSG (pregnant mare serum gonadotrophin) followed by HCG (human chorionic gonadotrophin) (Gestyl & Pregnyl: Organon Laboratories, U.K.) were injected i.p. between 40 and 52 h apart.

(2) Activation of the eggs

At various times after the HCG injection, the eggs were dissected out of the oviduct into the medium described by Whitten (1971), at room temperature. They were next exposed to hyaluronidase; in some cases hyaluronidase crystals were shaken over the eggs at room temperature to give a final concentration of about 100 i.u./ml of hyaluronidase in the medium (crystalline hyaluronidase method, Graham 1970), and in other cases the eggs were exposed to hyaluronidase made up in solution in Whitten’s medium at 37 °C (hyaluronidase solution method, Kaufman, 1973a, c). In each case the eggs were exposed to hyaluronidase for 10 to 15 min. The eggs were either immediately cultured at 37 °C or the zona pellucida was removed with pronase (Mintz, 1964) before culture. In rare cases eggs were exposed to beta propiolactone inactivated Sendai virus to promote parthenogenesis. The conditions were exactly the same as those required for blastomere fusion except that the eggs were prevented from touching each other (Graham, 1971a).

(3) Culture of the eggs

The eggs were cultured in different dilutions of either White’s medium (Graham, 1970; Knowland & Graham, 1972) or Whitten’s medium (Whitten, 1971) in 35 mm Falcon plastic Petri dishes under 90 % N₂, 5 % CO₂, 5 % O₂, for varying lengths of time. They were then transferred to 0.05 ml drops of Whitten’s medium on glass Petri dishes covered with liquid paraffin exactly as previously described (Graham, 1971a). The development of pronuclei in these embryos was observed
with a Wild M 5 dissecting microscope and they were classified and placed in separate drops at 6 to 10 h post-activation.

(4) Preparation of chromosomes and whole mounts

At between 10 and 16 h post-activation the embryos were transferred to 0.05 ml of Whitten's 1971 medium containing 1 μg/ml colcemid (CIBA). The eggs were left in this solution for between 4 and 12 h and chromosome preparations were made by the method of Tarkowski (1966). Alternatively chromosome preparations were made without colcemid using the technique of Kaufman (1973c).

The chromosomes were counted under phase, post-fixed for 2 h in acetic acid: ethanol (1:3), counted under phase again, stained with either Giemsa or lactic orcein and counted again. In no case were chromosomes lost during this procedure. Widely spread cells were not counted because chromosomes might be missed in such karyotypes.

Whole mounts of eggs were fixed in Heidenhain's fixative and stained in Mayer's haemalum (Tarkowski & Wroblewska, 1967).

(5) Labelling embryos

At various times after activation the zona pellucida was removed with pronase and the eggs were incubated in [6-3H]thymidine (specific activity 26 Ci/mM; The Radiochemical Centre, Amersham, U.K.) at 20 μCi/ml in Mintz's medium, which contains 50% foetal calf serum (Mintz, 1964). After labelling, whole mounts were prepared and 5 μm sections cut as described by Hillman, Sherman & Graham (1972).

The sections were rinsed in 5% trichloracetic acid at 5 °C for 30 min and the slides dipped in Ilford K5 emulsion and exposed for 1–2 weeks. In the autoradiographs a nucleus was scored as labelled if the number of grains over it exceeded by two or more the number over an adjacent area of equal size in the cytoplasm.

RESULTS

(1) The effects of osmolarity of the culture medium

Most eggs were exposed for 2 h after activation to either full strength White's medium or White's medium: distilled water at 4:1 (4/5 White’s), or at 3:2 (3/5 White’s).

(a) On route of development

As the osmolarity was lowered so second polar body formation was suppressed and the percentage of eggs without a second polar body and with one or two pronuclei increased. This effect occurred in eggs of F₁ C57BL/CBA mothers at both 17–19 h post HCG (Expts. 1–3, Table 1) and at 21–24 h post HCG (Expts. 9 and 10, Table 1). The effect was also obvious in experiments with CBA eggs (Expts. 4–6, Table 1); in this case the percentage of eggs which did not form a
Table 1. Path of development (CBA and F₁ C57BL|CBA)

All eggs were exposed to various dilutions of White's medium for 1 h 50 min to 2 h 15 min immediately after activation unless stated otherwise (Expt. 15). The percentage of eggs developing along different routes was recorded at 6-10 h post-activation.

Between 70 and 90% of the eggs were activated amongst the eggs which did not abortively cleave or lyse, in all experiments begun at 16 h post HCG or later. In experiments at earlier times between 20 and 50% of the eggs were activated.

<table>
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<tr>
<th>Main variable</th>
<th>Expt. no.</th>
<th>Strain of mother</th>
<th>Time post HCG</th>
<th>Activation conditions</th>
<th>Culture conditions</th>
<th>Total egg no.</th>
<th>Second p.b., one pronucleus</th>
<th>Immediate cleavage</th>
<th>No polar body</th>
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<td>1</td>
<td>F₁ C57/CBA</td>
<td>17-19 h</td>
<td>Crystals</td>
<td>W</td>
<td>117</td>
<td>73-5</td>
<td>9-4</td>
<td>14-6</td>
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<td></td>
<td>2</td>
<td>F₁ C57/CBA</td>
<td>17-19 h</td>
<td>Crystals</td>
<td>4/5W</td>
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<td>58-1</td>
<td>16-2</td>
<td>24-8</td>
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<td>17-19 h</td>
<td>Crystals</td>
<td>3/5W</td>
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<td>4</td>
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<td>15-16 h</td>
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<td>15-16 h</td>
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<td></td>
<td>6</td>
<td>CBA</td>
<td>15-16 h</td>
<td>Crystals</td>
<td>3/5W</td>
<td>57</td>
<td>3-4</td>
<td>0</td>
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<td>56</td>
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<td>9</td>
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<td>11</td>
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<td>14-16 h</td>
<td>Crystals + pronase</td>
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<td>41</td>
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<td>5-0</td>
<td>15-0</td>
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<td>14-16 h</td>
<td>Crystals + pronase</td>
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<td>24-0</td>
<td>60-0</td>
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<td>15</td>
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<td>14-16 h</td>
<td>Crystals + pronase</td>
<td>(4/5W 6 hours)</td>
<td>36</td>
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<td>14-0</td>
<td>83-0</td>
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<td></td>
<td>16</td>
<td>CBA</td>
<td>16 h</td>
<td>Crystals</td>
<td>3/5 Special White's*</td>
<td>42</td>
<td>45-0</td>
<td>0</td>
<td>36-0</td>
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* 3/5 Special White's medium is full strength White's medium with Na⁺ and K⁺ at 3/5 normal concentration. Inactive, lysed, and abortively cleaved embryos were excluded from this table.
Table 2. Activation of different strains (AKR, C3H, C57BL, ICRjA)

Eggs were activated between 15½ and 17 h post HCG. All eggs were exposed to various dilutions of White's medium for 1 h 50 min to 2 h 15 min except in those experiments (19, 20, 21) which are marked by an asterisk and in which exposure was for 30–40 min. The percentage of eggs in the different categories was scored at 6–10 h post-activation; non-active eggs not only lacked pronuclei at this time but also had not divided by 24 h post-activation. Eggs scored as 'abortive cleavage' often contained pronuclei but these were contained in cytoplasmic fragments.

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Strain</th>
<th>Medium</th>
<th>Total egg no.</th>
<th>Not active (%)</th>
<th>Second p.b., one pronucleus</th>
<th>Immediate cleavage</th>
<th>No p.b., two pronuclei</th>
<th>No p.b., one pronucleus</th>
<th>Abortive cleavage</th>
<th>Lysed</th>
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<tr>
<td>17</td>
<td>C3H</td>
<td>4/5 W</td>
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<td>4-9</td>
<td>1-6</td>
<td>2-5</td>
<td>4-1</td>
<td>53-3</td>
</tr>
<tr>
<td>18</td>
<td>C3H</td>
<td>3/5 W</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34-4</td>
<td>3-1</td>
<td>0</td>
<td>62-5</td>
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<tr>
<td>19</td>
<td>C3H</td>
<td>W*</td>
<td>280</td>
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<td>2-8</td>
<td>3-2</td>
<td>2-6</td>
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<tr>
<td>20</td>
<td>C3H</td>
<td>4/5 W*</td>
<td>166</td>
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<td>63-2</td>
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<td>6-6</td>
<td>3-0</td>
<td>2-4</td>
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<td>3/5 W*</td>
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<td>24</td>
<td>ICR/A</td>
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<td>26</td>
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<td>0</td>
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<td>27</td>
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<td>4/5 W</td>
<td>120</td>
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<tr>
<td>28</td>
<td>AKR</td>
<td>3/5 W</td>
<td>98</td>
<td>84-7</td>
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<td>0</td>
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<td>29</td>
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<td>0</td>
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second polar body or undergo immediate cleavage was 20 in full-strength White’s medium and rose to 96 in 3/5 White’s medium.

The suppression of second polar body formation depended on the Na\(^+\) and K\(^+\) concentration. If these were reduced to 3/5 their normal concentration while other components of the medium were retained at full strength, then suppression of second polar body formation occurred in CBA mice (compare Expts. 6 and 16, Table 1). However, fewer eggs had suppressed second polar body formation compared to the case in which all components of the medium were at 3/5 dilution.

Low osmolarity for 6 h rather than 2 h increased the percentage of eggs in which there was no second polar body (\(F_1\) C57/CBA eggs, Expts. 14 and 15, Table 1).

(b) On activation and lysis

At 15–19 h post HCG, 70–90 % of eggs of \(F_1\) C57BL/CBA and CBA mothers were activated in all experiments. In these experiments the percentage of abor-tively cleaved and lysed eggs was not recorded. The effect of osmolarity on activation was studied in detail in strains which were generally less easy to activate.

Low osmolarity could increase the percentage of activated eggs. The percentage of activated eggs increased from 1·6 to 15 % when C57BL eggs were cultured in 4/5 rather than full strength White’s medium (Expts. 22 and 23, Table 2). And the percentage of activated eggs increased from 6 to 78 % when ICR/A eggs were cultured in 3/5 rather than 4/5 White’s medium (Expts. 24 and 25, Table 2). This trend was not obvious in C3H or AKR mice (see Discussion).

Low osmolarity increased the percentage of lysed eggs. This was shown in experiments with AKR eggs (Expts. 26–29, Table 2). The percentage lysed depended on the length of exposure to low osmolarity; C3H eggs did not lyse if they were exposed to 4/5 and 3/5 White’s medium for 30 min while over half did so if they were exposed to these solutions for 2 h (compare Expts. 17 and 18 with Expts. 19–21, Table 2).

(2) The effects of age

(a) On route of development

Eggs which were activated at a long time interval after HCG tended to cleave immediately; only 3·7 % of the eggs of \(F_1\) C57BL/CBA mothers did so at 13–16 h post HCG, while 35·5 % did so at 21–24 h post HCG. The percentage of eggs with a second polar body and one pronucleus and the percentage of eggs with one pronucleus decreased in 3/5 White’s medium (Expts. 7–9, Table 1).

(b) On activation and lysis

Eggs treated at 13 h post HCG were very difficult to activate while the majority of eggs treated at 30 h post HCG abortively cleaved or lysed. These observations were not recorded in detail.
Fig. 2. DNA synthesis post-activation. Eggs were exposed to tritiated thymidine for various times after activation; these times are in thick bars. After fixation and autoradiography the percentage of labelled nuclei was recorded and this figure and the number of eggs used in each experiment (in parentheses) is placed at the end of each thick bar.

(3) The effect of different activation techniques

A comparison was made between activation by hyaluronidase crystals and activation by hyaluronidase solution (Kaufman, 1973a). After culture in 3/5 White's medium it appeared that the method of activation did not greatly change the percentage of eggs without a second polar body (F₁ C57BL/CBA mothers Expts. 11 and 12, Table 1).

A comparison was made between eggs activated with hyaluronidase crystals and eggs activated in this way and immediately treated with pronase to remove the zona pellucida. The pronase treatment increased the percentage of eggs which either immediately cleaved or lacked a second polar body after culture in 4/5 White's medium (Expts. 2 and 14, Table 1), but had little effect after culture in full strength medium (Expts. 1 and 13, Table 1).

(4) Induction of DNA synthesis

Eggs of F₁ C57BL/CBA mothers were incubated in tritiated thymidine for various periods after activation by Sendai virus (Fig. 2). DNA synthesis was not detected after a 3½ h labelling period, but the majority of nuclei had incorporated label after 6 h incubation, and all had done so over longer labelling periods.

(5) Karyotypes of parthenogenetic embryos

The majority of parthenogenetic eggs possessed the karyotype which would be expected from observations on the behaviour of their pronuclei (Figs. 1, 3; Table 3). However, eggs cultured in 3/5 White's medium tended to have highly abnormal karyotypes. In this medium, half of the eggs with one pronucleus and a second polar body had either more or less than the count of 20 expected of a haploid mouse cell. In three cases a count of 40 was observed and the second polar body must have lacked a nucleus. Eggs which immediately cleaved also had abnormal karyotypes in this medium; over 70% did not have the 20+20 counts expected of normal segregation of chromosomes at second metaphase.
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Fig. 3. Karyotypes of embryos developing along different routes. (A) One pronucleus, second polar body. One polar body and 20 chromosomes are visible. (B) Immediate cleavage. There has been unequal segregation at second metaphase: one blastomere contains 25 chromosomes and the other 15. (C) Two pronuclei. There has been unequal segregation at second metaphase: one pronucleus contains 23 chromosomes and the other 17. Unless 'delayed immediate cleavage' occurs a normal karyotype would reform at first metaphase. (D) One pronucleus. A single metaphase plate containing 40 chromosomes and no polar body. Scale bar = 100 μm.

Abnormal chromosome counts were rare in eggs with either one or two pronuclei and no polar body. However, in one case an egg, classified as containing two pronuclei at 10 h post-activation, was observed to divide at 14 h post-activation. One of its cells divided again at 17½ h post-activation and the other was found to have a karyotype of 24 chromosomes.

DISCUSSION

The main purpose of this work was to discover a method for routinely obtaining diploid parthenogenones which would be potentially heterozygous if crossing over had occurred at meiosis.

(1) Osmolarity

The results show that culture of activated eggs in 3/5 White's medium increases the frequency of parthenogenones without a second polar body nucleus and with either one or two pronuclei. Both these routes of development are potentially diploid (Fig. 1). Second polar body formation can also be suppressed in rabbit eggs by chilling, in vitro culture, and by hyper- and hypotonic media (Pincus, 1939a, b; Pincus & Shapiro, 1940a, b; Thibault, 1949; Chang, 1954). Second polar body formation can be suppressed in amphibians by high pressure (Dasgupta, 1962).
Table 3. The karyotypes of parthenogenetic embryos

All eggs were obtained from F1 C57BL/CBA or CBA mothers. The figures refer to the results from both added together and the figures in parentheses refer to the results from CBA eggs alone. An asterisk indicates the chromosome count of a single blastomere of an egg which underwent 'delayed immediate cleavage'.

(a) One pronucleus, second polar body

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<th>Medium</th>
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(b) Immediate cleavage

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(c) Two pronuclei

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(d) One pronucleus

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(2) Karyotypes

Apart from a preliminary report (Graham, 1971b) the results presented here are the first study of the chromosomes of parthenogenones developing along each of the different routes. In most cases, karyotypes were collected with colcemid which could have several effects on the results:

(a) This treatment could increase the frequency of apparently normal karyotypes by the following mechanism. Subnuclei are known to be produced by electrical activation (Tarkowski et al 1970), and they might occur in these experiments and not be observed under the dissecting microscope; such subnuclei
might condense on the metaphase plate at a slightly different time to the majority of chromosomes and thus fail to segregate correctly at first cleavage. Colcemid arrests cytokinesis and might allow the chromosomes of the subnuclei to catch up and contribute to an apparently normal karyotype. It is probable that this effect does not occur because chromosome preparations made without colcemid did not show a significantly higher frequency of chromosomal abnormalities (Table 3).

(b) A prolonged treatment with colcemid might allow the karyotype to polyploidize. There are two reasons for thinking that this effect has not occurred in these experiments. Firstly, no presumed diploid had a tetraploid karyotype, and secondly, the time of exposure to colcemid was never greater than 12 h and all chromosome preparations were completed by 24 h post-activation (10 h before second cleavage chromosome condensation would be expected).

However, apparently normal karyotypes at first cleavage metaphase are not a guarantee that the chromosomes will segregate normally at this division. And the estimates of normal karyotypes should be treated with caution both for this reason and because individual chromosomes were not identified by current banding techniques (Buckland, Evans & Sumner, 1971). The presence of a metaphase plate also does not guarantee that cell division will occur at all.

(a) Second polar body, one pronucleus

Second polar body, one pronucleus parthenogenones usually had haploid karyotypes confirming the observations of Kaufman (1973c; Kaufman & Surani, 1974). However, eggs cultured in 3/5 White’s medium had abnormal karyotypes in over 50 % of the cases studied; these abnormal karyotypes could not have been caused by chromosome loss during the air-drying procedure since the majority contained more than 20 chromosomes (Table 3). In three cases these eggs contained 40 chromosomes; these must have been produced by the formation of a polar body without a nucleus. The conclusion is that haploid parthenogenones are best obtained by activation and culture in medium of normal osmolarity (Table 3).

(b) Immediate cleavage

Over 60 % of immediate cleavage parthenogenones had abnormal karyotypes. However, nearly all the karyotypes of embryos developing in this way were obtained from eggs cultured in 3/5 White’s medium and it is impossible to say whether it is the route of development or method of culture which causes the abnormality. It is certain that these abnormal karyotypes are not the consequence of chromosome loss during fixation because the total number of chromosomes in the two blastomeres usually equals 40; the abnormalities must be the consequence of an unequal second meiotic metaphase division. It is less easy to be certain that some chromosomes have not moved from one metaphase group to another during the air-drying procedure. However, in preparations without colcemid it was unusual to find abnormal karyotypes in which one set of
chromosomes was in metaphase and the other in anaphase, so that chromosome translocation during fixation could not have confused the groups.

There are three other reasons for believing that this unequal segregation of chromosomes is not an artifact. *(a)* Chromosome groups were only counted if they were clearly separate (Fig. 3B), *(b)* 3/5 White's medium gave a similar high incidence of abnormal segregation in one pronucleus, second polar body eggs and in this case no confusion of metaphases could occur. *(c)* In a large number of cases only one of the groups of chromosomes was spread during air-drying and counts of the chromosomes in the single spread metaphase showed a similar frequency of abnormality to counts of cases where both metaphases were spread.

*(c)* Two pronuclei

The majority of parthenogenones with two pronuclei had a total chromosome count of 40. This was true even of eggs cultured in 3/5 White's medium; presumably any irregular segregation of chromosomes at second meiotic metaphase was corrected by the re-association of the pronuclear products of that chromosome division at the first cleavage metaphase. The chromosomes were either in one or two groups; the presence of two groups does not imply that the groups do not co-operate in the first cleavage metaphase because normal fertilized eggs have separate groups of male and female chromosomes at the first cleavage division (McGaughey & Chang, 1971; Donahue, 1972; Kaufman, 1973b). Several metaphases contained less than 40 chromosomes. In these, small nuclear blobs were found, suggesting that some chromosomes had failed to condense.

However, eggs originally containing two pronuclei at 8–10 h post-activation could also undergo delayed immediate cleavage. In a previous series of experiments with eggs activated at 24–29 h post HCG and cultured in 3/5 White's medium, the majority of eggs with two pronuclei behaved in this way (Graham, 1971b). In the present series of experiments one egg activated at 24 h post HCG and cultured in 3/5 White's medium also showed delayed immediate cleavage. It was classified as 'two pronuclei' at 10 h post-activation and was observed to divide at 14 h post-activation. One of its cells divided again at 17½ h post-activation and the other was found to have a karyotype of 24 chromosomes. No colcemid was used. The conclusion is that eggs containing two pronuclei may either form a diploid as has been previously described (Kaufman, 1973c) or they may form a presumptive haploid mosaic (Graham, 1971b). Eggs activated at 24 h or later after HCG tend to form haploid mosaics but it is quite wrong to believe that most parthenogenones with two pronuclei develop in this way as had been previously supposed (Graham, 1971a).

*(d)* One pronucleus

The majority of eggs containing one pronucleus also contained 40 chromosomes. In those cases where less than 40 chromosomes were present small nuclear blobs were apparent and probably contained the uncondensed chromosomes.
Activation becomes progressively easier as the eggs age (Kaufman, 1973a). Culture in low osmolar solutions also increases the frequency of activation (Table 2), and eggs which are difficult to activate, such as AKR, may be exposed to culture medium at half its normal concentration. Sendai virus will also activate eggs and it appears that almost any stimulus which damages the cell membrane will start parthenogenetic development (Beatty, 1957; Austin, 1961).

Strain

At 15–19 h post HCG, 70–90% of eggs of \( F_1 \) C57BL/CBA and CBA mothers are activated in these experiments. Eggs of other strains could be arranged in decreasing order of ease of activation: C3H, C57BL, ICR/A, and AKR. There is no obvious explanation of this sequence.

Induction of DNA synthesis

DNA synthesis in fertilized eggs has only been accurately timed in CFI (Carworth) mice (Luthardt & Donahue, 1973), although many other studies have shown that DNA synthesis occurs (Alfert, 1950; Sirlin & Edwards, 1959; Mintz, 1965). In CFI mice, pronuclear DNA synthesis began at 21 h post HCG and the majority of pronuclei were engaged in DNA synthesis at 25 h post HCG. Sperm penetration mainly occurred at 12–14 h post HCG (Donahue, 1972) and so DNA synthesis started at 7–9 h post-fertilization and the majority of pronuclei were synthesizing DNA at 11–13 h post-fertilization. In parthenogenetically activated \( F_1 \) C57BL/CBA eggs (Fig. 2), the majority of pronuclei began to synthesize detectable amounts of DNA between 3\( \frac{1}{2} \) and 6 h post-activation and all had done so by 10 h post-activation. Compared to fertilized CFI eggs, parthenogenetic eggs of \( F_1 \) C57BL/CBA mothers started DNA synthesis early. This might be due to: (a) the method of activation, (b) the age of the egg before development began, (c) the strain of the mouse.

In these experiments the age of the egg had little effect on the results; after a 9\( \frac{1}{2} \)-10 h labelling period all the pronuclei were labelled in six eggs activated at 13\( \frac{1}{2} \) h post HCG and in eight eggs activated at 20\( \frac{1}{2} \) h post HCG. And after a 6 h labelling period DNA synthesis was detected in 6/6 eggs activated at 15 h post HCG and in 17/21 eggs activated at 18\( \frac{1}{2} \) h post HCG.

In control labelling experiments with eggs of \( F_1 \) C57BL/CBA mothers fertilized by C57BL males the pronuclei of 27 eggs were all labelled by 24 h post HCG, but this observation does not distinguish between explanations (a) and (c) of the different results obtained with parthenogenetic \( F_1 \) C57BL/CBA eggs and fertilized CFI eggs. However, the conclusion is that DNA synthesis in parthenogenetically activated eggs is not grossly different in timing from that in fertilized eggs.
(6) The production of diploid parthenogenones

Diploid parthenogenones can be readily produced by activating eggs and exposing them to low osmolarity for 2 h. However, low osmolarity is known to produce grossly abnormal segregation at second metaphase and must damage the second meiotic spindle (Graham, 1972). Such embryos therefore possess the correct karyotype but have been exposed to extreme environmental stress. After the first cleavage division, other abnormalities in cell division are known to be present in electrically activated eggs; some are haploid/diploid mosaics and others contain binucleate cells (Witkowska, 1973a).

(7) The death of parthenogenones

So far the further development of parthenogenetic embryos has only been studied in experiments in which a mixture of embryos developing along different routes has been inside a foster mother. Under the best circumstances only 9% of in vitro activated F₁ C57BL/CBA eggs reach the blastocyst stage (Graham, 1970) and there is a similar mortality in electrically activated eggs (Tarkowski et al. 1970; Witkowska, 1973a). Following implantation very few of these embryos proceed beyond the stage of development of normal embryos on the seventh day of pregnancy (Tarkowski et al. 1970; Graham, 1972; Witkowska, 1973b). The most advanced embryo contained eight somites (Tarkowski et al. 1970). However, C3H parthenogenones with one pronucleus and no second polar body develop well and over 50% reach the blastocyst stage after transfer to the oviduct (Graham, unpublished). The poor results of previous studies are probably the consequence of the rarity of such parthenogenones in experiments without the use of low osmolar solutions.

Abnormalities in karyotype and cell division must account in part for the failure of these embryos to develop into adults. It is also the case that eggs exposed to 3/5 White’s medium tend to lyse (AKR in Table 2) and tend to have irregular chromosome segregation at second metaphase; it appears that, at least in some strains, the more efficient the activating stimulus the greater the cytoplasmic damage, and Tarkowski (1971) has correctly pointed out that this may account for the loss of some of the parthenogenones.

Extensive cytoplasmic abnormalities have been observed in 2- and 4-cell ICR/A and AKR parthenogenones (Solter et al. 1974) and electrically activated eggs do not undergo the same zona pellucida alterations as do fertilized eggs (Mintz & Gearhart, 1973). It is also the case that the considerable homozygosity around the centromere of diploid parthenogenones obtained by suppression of the second polar body may expose recessive lethals. Such lethals might make a considerable contribution to the pre-implantation death of the parthenogenones (Beatty, 1967).

However, it is now known that parthenogenetic embryos activated in vitro are normal in the following respects. They synthesize DNA at approximately the
In vitro activation of mouse eggs

correct time in the first cell cycle (this study). They expose Concanavalin A binding sites as do fertilized eggs (Pienkowski & Koprowski, 1974). They synthesize A type virus particles at the same time as fertilized eggs (Biczysko, Solter, Graham & Koprowski, 1973). It is perhaps the case that additional studies on the control of egg activation will increase the success of development.

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REFERENCES


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