Differential Effects of Oestrogen on the Uptake of Nucleic Acid Precursors by Mouse Blastocysts In Vitro

J. A. Harrer* and H. H. Lee

Department of Biology, The University of Toledo,
Toledo, Ohio 43606, U.S.A.

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Autoradiographic studies indicate that mouse embryos undergoing delayed implantation show a marked increase in the incorporation of labelled amino acids or nucleic acid precursors 14 hr after oestrogen injection (Weitlauff & Greenwald, 1968) while 18 to 30 hr are needed for rats (Prasad, Dass & Mohla, 1968; Sanyal & Meyer, 1970; Jacobson, Sanyal & Meyer, 1970; Wu & Meyer, 1971). A direct effect of oestrogen on mammalian blastocysts cultured from the two-cell stage has also been observed. Although 17β-oestradiol at 25 mg/ml produces some degenerative effects in cultured mouse morulae and early blastocysts (Kirkpatrick, 1971), the hormone at 10⁻¹⁰ M, 10⁻⁹ M and 10⁻⁵ M stimulates uptake and incorporation of labelled amino acids in early mouse blastocysts cultured from the two-cell stage (Smith & Smith, 1971). These studies suggest that oestrogen has a direct effect on the blastocysts in vitro by stimulating the uptake and incorporation of amino acids in the embryo within 30 min of incubation in oestrogen.

In the present communication, evidence is presented for the hormone-induced uptake of nucleic acid precursors in cultured mouse blastocysts after incubation in 17β-oestradiol. A differential effect is observed between DNA and RNA precursors.

Two-cell embryos were collected from random-bred Swiss mice which were induced to superovulate (Fowler & Edwards, 1957) with 5 i.u. gonadotrophin (PMSG, Sigma Chemical Co.). The embryos were cultured in 35 x 10-mm tissue culture dishes (Falcon Plastics), using a bicarbonate-buffered medium containing albumin, penicillin and streptomycin, and sodium pyruvate and lactic acid as energy sources (Brinster, 1965). All cultures were maintained under paraffin oil (Fisher Chemical, viscosity 125/135) in microdrops of about 20 µl medium containing ten to twelve embryos each. All incubations were at 37°C in 5% CO₂ in air at 98% humidity.

Oestrogen-containing medium was prepared just before use from a stock made by dissolving 0.05 ml of an ethanol solution containing crystalline 17β-oestradiol (1 mg/ml, Calbiochem Inc.) in 10 ml culture medium without albumin. One ml of this solution was diluted in 4 ml of culture medium with albumin.

* Present address: Department of Anatomy, University of Colorado Medical Center, 42000 East Ninth Avenue, Denver, Colorado 80220, U.S.A.
Table 1. Effect of oestrogen on the uptake of \(^3\text{H}\)thymidine by mouse blastocysts in vitro

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration in the presence of oestrogen (1 (\mu)g/ml)</th>
<th>No. of embryos</th>
<th>Concentration (^3\text{H})thymidine ((\mu)Ci/ml)</th>
<th>% of labelled cells</th>
<th>P</th>
<th>No. of grains(\ddagger)/labelled cell</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No treatment (control)</td>
<td>7</td>
<td>10</td>
<td>49-2 (\pm) 17-0*</td>
<td>&gt;0-20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>1 hr</td>
<td>8</td>
<td>10</td>
<td>57-5 (\pm) 13-7*</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2a</td>
<td>No treatment (control)</td>
<td>14</td>
<td>10</td>
<td>28-2 (\pm) 13-7*</td>
<td>&lt;0-01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2a</td>
<td>2 hr</td>
<td>10</td>
<td>10</td>
<td>65-6 (\pm) 12-7*</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2b</td>
<td>No treatment (control)</td>
<td>4</td>
<td>10</td>
<td>31-1 (\pm) 12-9*</td>
<td>&lt;0-05</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2b</td>
<td>2 hr</td>
<td>5</td>
<td>10</td>
<td>52-6 (\pm) 8-2*</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>No treatment (control)</td>
<td>21</td>
<td>1</td>
<td>36-5(\dagger)</td>
<td>&lt;0-01</td>
<td>145-5(\dagger)</td>
<td>&lt;0-01</td>
</tr>
<tr>
<td>3</td>
<td>2 hr</td>
<td>12</td>
<td>1</td>
<td>65-8(\dagger)</td>
<td></td>
<td>313-8(\dagger)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean \(\pm\) standard deviation, Student's t test.

\(\dagger\) Non-parametric statistics applied.

\(\ddagger\) At 10 \(\mu\)Ci \(^3\text{H}\)thymidine/ml, the grains per cell are too dense to be counted.
Oestrogen effects on mouse blastocysts

Table 2. Effect of oestrogen on the uptake of $[^3H]$uridine by mouse blastocysts in vitro

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of embryos</th>
<th>% of labelled cells</th>
<th>P</th>
<th>No. of grains/cell</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no oestrogen treatment)</td>
<td>13</td>
<td>36±4±22·5†</td>
<td>&gt;0·20</td>
<td>23·0‡</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td>Oestrogen 2 hr (1 µg/ml)</td>
<td>12</td>
<td>39·8±18·0†</td>
<td></td>
<td>56·4‡</td>
<td></td>
</tr>
</tbody>
</table>

*1 µCi $[^3H]$uridine/ml.
† Mean ± standard deviation, Student’s t test.
‡ Non-parametric statistics applied.

Ethanol, 1:3) over the slide (Rafferty, 1970). The number of blastocysts analysed per group varied because of the loss of embryos in the fixation process. After drying in air, the slides were washed several times in distilled water and coated with Kodak NTB2 emulsion. The specimens were exposed for 7 days, and then developed with Kodak D-19 and acid-fixed. The specimens were stained in haematoxylin and eosin for microscopic examinations. The percentage of labelled cells/blastocyst and the average grain number above 10 grains/cell labelled in the blastocyst were determined for oestrogen-treated and control groups.

The data obtained for the embryos incubated with $[^3H]$thymidine are presented in Table 1. Oestrogen treatment for 1 hr (Exp. 1, Table 1) did not significantly increase the percentage of labelled cells above that in blastocysts not treated with oestrogen. Blastocysts exposed for 2 hr in medium containing oestrogen (Exps 2a, b and 3, Table 1) did, however, have a significantly higher percentage of labelled cells/embryo than the controls. The results are consistent at 10 µCi/ml and 1 µCi/ml $[^3H]$thymidine. The number of grains (Exp. 3, Table 1) per labelled cell in the blastocysts treated with oestrogen for 2 hr was also increased.

The results obtained for the uptake of $[^3H]$uridine (1 µCi/ml) are presented in Table 2. Oestrogen treatment of blastocysts for 2 hr did not significantly
increase the percentage of labelled cells above that in the controls. The number of grains/labelled cell in the oestrogen-treated blastocysts was, however, significantly greater than in blastocysts not incubated with oestrogen.

These results support the findings of Sanyal & Meyer (1970), indicating that oestrogen is active in stimulating the synthesis phases of nucleic acids in the cell cycle. In addition, the effect of oestrogen may be multifold: (1) oestrogen activates more cells to synthesise DNA; (2) it increases the rate of DNA synthesis in the cells that are undergoing synthesis when the hormone is added; (3) the effect is not abrupt but rather gradual over a minimum of 1 hr; (4) oestrogen increases the rate of RNA synthesis in cells that are synthesizing RNA when the hormone is added to the embryos. It appears that mouse embryos as a whole not only have a differential sensitivity toward progesterone and oestrogen as reported by Kirkpatrick (1971), but individual cells in the embryos also exhibit differential response to oestrogen treatment in vitro.

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REFERENCES


