DNA SYNTHESIS IN THE ENDOMETRIUM OF PROGESTERONE-TREATED MICE

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SUMMARY

[3H]Thymidine autoradiography was used to study DNA synthesis in the uteri of spayed mice treated with progesterone and oestradiol. Progesterone suppressed DNA synthesis in the glandular epithelium whether oestrogen was given or not. It also suppressed DNA synthesis in the luminal epithelium. Here oestradiol produced morphological changes and eventual re-entry of some cells into DNA synthesis.

Progesterone altered the nuclear morphology of the stromal cells and increased the number synthesizing DNA. In these conditions a single injection of oestrogen was followed 10–15 h later by the synchronized entry into DNA synthesis of 30–40% of stromal cells. However, a second injection produced no further response.

It was concluded that progesterone stimulated stromal cells in the resting phase to enter the cell cycle and that oestrogen then accelerated their passage through a single round of replication and division by shortening the interval between mitosis and DNA synthesis, following which the cells withdrew from the cell cycle.

INTRODUCTION

In progesterone-treated mice uterine epithelial cell division is suppressed and oestrogen treatment is followed by increased mitosis in the uterine connective tissue stroma (Martin & Finn, 1968). Previous studies were based on the numbers of colchicine-arrested mitoses visible in a transverse section of uterus. In the experiments described here, [3H]thymidine autoradiography was used to determine the proportion of cells engaged in proliferation, and the time-course of this response.

ANIMALS AND METHODS

Mice of the strain used previously (Martin & Finn, 1968; Martin, Finn & Trinder, 1973) were ovariectomized through a dorsal incision using Avertin (tribromoethyl alcohol, Bayer Products Co.) anaesthesia. Two weeks later they were primed with three daily s.c. injections of 100 ng oestradiol in 0.1 ml arachis oil (Finn & Martin, 1970). After 1 day without treatment they were given 1 mg progesterone s.c. in 0.1 ml arachis oil at 10.00 h daily for 4 or 5 days. Groups were killed at various times

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after the 4th injection, or were given 50 ng oestradiol in 0·1 ml arachis oil with the 4th, or 4th and 5th injections of progesterone and were killed at various times after this. [3H]Thymidine (1·5 µg/g body wt. sp. act. 5 Ci/mM; The Radiochemical Centre, Amersham) was injected intraperitoneally in 0·1 ml 0·9 % NaCl solution, 1 h before the mice were killed.

The uteri were removed, fixed in Bouin’s fluid and 5 µm wax sections were prepared from the mid-regions. Autoradiographs were set up using Ilford K5 dipping emulsion and were exposed for 4 weeks at 4 °C. After development they were stained with Cole’s haematoxylin and eosin. Labelling indices were determined on 400 cells/animal from each tissue as described by Martin et al. (1973). Counts of mitoses were made as described by Martin & Finn (1968). Counts of labelled mitoses were made on 100 metaphases/animal. The following abbreviations are used: G₁ is the interval between mitosis (M) and the start of DNA synthesis. S is the period of DNA synthesis and G₂ the interval between the end of S and the beginning of M.

Table 1. DNA synthesis and mitosis in progesterone-treated mice after oestrogen injection

<table>
<thead>
<tr>
<th>Oestradiol given at (h):</th>
<th>Hours after 1st injection</th>
<th>Labelling index</th>
<th>No. of mitoses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Luminal epithelium</td>
<td>Stroma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesometrial</td>
<td>Submyometrial</td>
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<tr>
<td></td>
<td></td>
<td>Anti-mesometrial</td>
<td></td>
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<td>—</td>
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<td>0</td>
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<td>0</td>
<td>6</td>
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<td>11</td>
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<td>0</td>
<td>16</td>
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<td>0</td>
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<td>0</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.24</td>
<td>25</td>
<td>0.4 ± 0.3</td>
<td>8.0 ± 7.0</td>
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<tr>
<td>0.24</td>
<td>30</td>
<td>0.6 ± 0.5</td>
<td>25.0 ± 8.0</td>
</tr>
<tr>
<td>0.24</td>
<td>35</td>
<td>0.2 ± 0.1</td>
<td>13.4 ± 3.0</td>
</tr>
<tr>
<td>0.24</td>
<td>40</td>
<td>4.6 ± 1.5</td>
<td>32.8 ± 7.8</td>
</tr>
<tr>
<td>0.24</td>
<td>45*</td>
<td>12.0 ± 7.6</td>
<td>31.4 ± 2.8</td>
</tr>
</tbody>
</table>

* Two mitoses were observed in the glands of one animal in this group, giving a mean of 0.4 ± 0.4.

RESULTS

Mice treated with progesterone alone

No labelled cells or mitoses were present in the luminal or glandular epithelia. In both tissues the cytoplasm was pale with an eosinophilic surface, and the nuclei were shrunken with small nucleoli (Pl. 1, fig. 1). The pale cytoplasm was due in part to the presence of lipid vacuoles and in part to reduced basophilia. In the stroma the nuclei were swollen with dispersed chromatin and prominent nucleoli (Pl. 1, fig. 1). Occasional mitoses were visible and the labelling indices ranged from 5 to 7 % in the periluminal
region and from 0.7 to 1.4% in the submyometrial region (Pl. 1, fig. 3). There was little diurnal variation in the labelling indices of either region (Table 1).

**Mice treated with progesterone and oestrogen**

Glandular epithelium

No labelled cells were seen after oestrogen treatment and only two mitoses were observed - both in one animal killed 20 h after the second injection of oestrogen.

Luminal epithelium

Morphological changes were first seen 20 h after the injection of oestrogen. From this time onwards the nuclei and nucleoli enlarged and cell height decreased (Pl. 2, figs. 6, 7) but labelled cells did not appear until 25 h. In the mesometrial region a few cells were incorporating [³H]thymidine at 25, 30 and 35 h, but the labelling index did not increase significantly until 40-45 h (Table 1), after the second injection. Much greater changes occurred in the anti-mesometrial region. Here the labelling index increased significantly at 25 h, rose to a peak at 30 h, fell at 35 h and rose to still higher values at 40-45 h, after the second injection (Table 1, Pl. 2, fig. 5). Occasional mitoses were observed from 25 h onwards but the numbers did not rise significantly until 40-45 h.

The connective tissue stroma

Oestrogen produced marked oedema at 5-10 h and this was accompanied by swelling of nuclei and enlargement of nucleoli (Pl. 1, fig. 2). The labelling index first rose above control values at 10 h. It continued to rise to a peak at 15 h (Pl. 2, fig. 4) and fell rapidly thereafter (Table 1). Labelling was higher in the periluminal than in the submyometrial region at all times. By 25 h the labelling index in the periluminal region had fallen to control levels, after which it continued to decline despite the second injection of oestrogen. A similar decline was seen in the submyometrial stroma although the indices did not fall to pre-injection levels. From 25 h onwards the stromal nuclei and nucleoli decreased in size and the cells became densely-packed (Pl. 2, figs. 6, 7).

Mitotic indices were not determined, but counts of the numbers of mitoses/section (Table 1) showed that the mitotic response followed a time-course similar to the labelling response but delayed by approximately 5 h. Since mitosis did not increase until after the increase in DNA synthesis, it was concluded that there were no cells in G2 which could be stimulated by oestrogen to enter mitosis immediately.

In all these groups 5-10% of mitoses were labelled indicating that the minimum G2 was about 1 h. A separate group of five mice was given thymidine 15 h after 50 ng oestradiol (given concurrently with the 4th daily injection of progesterone) and were killed 6 h later. All stromal mitoses were labelled in all animals. It was concluded that all mitoses arose from cells synthesizing DNA in the period immediately beforehand. However, it is possible that some cells which went through synthesis remained in a prolonged G2 as suggested by Galassi (1968).
Cell death in the connective tissue stroma

In mice treated with oestrogen alone, epithelial proliferation is followed almost immediately by a rapid fall in cell numbers. During this decline dense basophilic Feulgen-positive droplets appear in the epithelium, as a result of nuclear breakdown by karyorrhexis (Martin et al. 1973). A few such droplets were observed in the stroma of progesterone-treated mice but there was no increase in their numbers after the cessation of DNA synthesis. It is concluded that newly produced stromal cells do not die immediately after the cessation of proliferation.

DISCUSSION

Progesterone completely suppressed DNA synthesis in the glands whether oestrogen was given or not. In the absence of oestrogen it also suppressed luminal epithelial DNA synthesis completely. Other evidence suggests that continuing treatment with progesterone alone will maintain this state indefinitely (Martin & Finn, 1968; Martin, Finn & Carter, 1970). In the absence of progesterone, oestradiol produces a large increase in luminal epithelial nuclear protein synthesis (Smith, Martin, King & Vertes, 1970) after which the majority of cells enter DNA synthesis (Martin et al. 1973). Oestrogen similarly increases nuclear protein synthesis in the progestational epithelium (Smith et al. 1970). Since cells do not then enter DNA synthesis, it seems likely that this increase is associated with the greatly enhanced synthesis of non-nuclear proteins which occurs in the progestational epithelium after oestrogen treatment (Smith, Martin & King, 1971). It is not clear whether the much later changes in epithelial morphology and responsiveness are a consequence of these early responses or result from changes in the underlying connective tissue stroma.

In untreated ovariectomized mice, most stromal cells appear to be in a resting (G0) state (Martin et al. 1973). The present data suggest that progesterone activates a large proportion to enter the cell cycle, and that in the absence of oestrogen they pass through it slowly. The duration of DNA synthesis in the stromal cells of progesterone-treated mice is approximately 9 h and is not shortened by oestrogen treatment (Das, 1972). It seems likely that the synchronized burst of DNA synthesis that follows oestrogen treatment, results from a shortening of the G1 phase of the cell cycle.

The failure of a second injection of oestrogen to induce a second burst of DNA synthesis accounts for the failure, in earlier experiments (Finn, Martin & Carter, 1969; Martin & Finn, 1970), to obtain repeated bursts of cell division. Together with the results of Das (1972) the present data indicate that stromal cells can only be stimulated by oestrogen to pass through one round of DNA synthesis and division. This is in contrast to the luminal epithelium in which after a single injection of oestrogen (Das, 1972; Martin et al. 1973) a large proportion of cells pass through two rounds of DNA synthesis and division.

In pregnancy, stromal cells differentiate into decidual cells (Galassi, 1968). Cell division appears to be a prerequisite for this and may function to bring the cells to a state in which they are sensitive to the transforming stimulus. Mitosis followed by withdrawal from the cell cycle, is obligatory to myoblast differentiation in culture.
Progesterone and uterine DNA synthesis

(Bischoff & Holtzer, 1969). The situation in the stroma appears to be analogous to this and it seems likely that withdrawal of stromal cells from the cell cycle is also a prerequisite to decidualization.

REFERENCES


DESCRIPTION OF PLATES

PLATE 1

Fig. 1. Material illustrated was stained with Cole's haematoxylin and eosin. (a) Low (× 75) and (b) high (× 700) power views of a transverse section from a uterus 11 h after the last of four daily injections of progesterone. The epithelium has a corrugated surface, pale cytoplasm and shrunken nuclei with small nucleoli. Stromal nuclei are densely packed but compared with those of untreated mice (Martin, Finn & Trinder, 1973) are greatly enlarged with dispersed chromatin and prominent nucleoli.

Fig. 2. As for fig. 1a and b but the mice were given 50 ng oestradiol with the last injection of progesterone. There is little change in the epithelium. The stroma is edematous and the nuclei and nucleoli have enlarged.

Fig. 3. An autoradiograph (× 200) from a uterus 15 h after the last of four daily injections of progesterone. No epithelial cells have incorporated [3H]thymidine. In the stroma [3H]thymidine incorporation is greatest in the perinuclear region.

PLATE 2

Fig. 4. As for fig. 3 but 50 ng oestradiol were given with the last injection of progesterone. No epithelial cells have incorporated [3H]thymidine. In the stroma a large proportion of nuclei are labelled.

Fig. 5. An autoradiograph (× 200) from a uterus 20 h after the second of two injections of 50 ng oestradiol given with the 4th and 5th injections of progesterone. The anti-mesometrial pole of the uterus is to the left. Many luminal epithelial cells have incorporated [3H]thymidine, particularly in the anti-mesometrial region. There is no incorporation in the glands and little in the stroma.

Fig. 6. (a) Low (× 75) and (b) high (× 700) power views of a transverse section of uterus 5 h after the second of two injections of 50 ng oestradiol given with the 4th and 5th injections of progesterone. Epithelial height is decreased and the surface is no longer corrugated. There is increased epithelial basophilia and the nuclei are swollen with prominent nucleoli. The stroma shows some oedema but the nuclei are shrunken with small nucleoli and condensed chromat.

Fig. 7. As for fig. 6 (a and b) but 20 h after the second injection of oestrogen, showing an epithelial mitosis, increased epithelial cytoplasmic basophilia and further decreases in the size of the stromal nuclei which are particularly densely packed.