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# The Influence of 17β-Estradiol on Patterns of Cell Division in the Uterus

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**ABSTRACT.** Uteri from immature (21-day-old) and adult mice show different patterns of cell division in response to a physiological dose of 17β-estradiol. In the immature mouse uterus, estradiol increased stromal and epithelial cell proliferation by shortening the cell generation time ($T_c$). The epithelial $T_c$ was reduced from 100 to 14.5 h; the stromal $T_c$ was reduced to 16 h. In both cell types, the reduction in $T_c$ was primarily due to a reduction of the $G_1$ phase of the cell cycle. In the adult mouse uterus, estradiol stimulated epithelial but not stromal cell proliferation. Here, estradiol reduced the epithelial $T_c$ from 86 to 13 h, mostly by reducing the $G_1$ phase of the cell cycle. Therefore, estrogens stimulate uterine hyperplasia by selectively decreasing the $G_1$ phase of the cell cycle in specific cell populations. At some point during reproductive tract maturation, uterine stromal cells lose their ability to divide in response to estrogen stimulation. A developmental study showed that in the intact mouse, the stromal cell population gradually lost its ability to divide in response to estrogen stimulation during days 22–52 after birth. In prepubertally ovariectomized mice, the stromal cell population showed a very low mitotic response to estrogen stimulation at all ages. Thus, an ovarian mechanism may regulate the change in stromal responsiveness to estrogen stimulation. (*Endocrinology* **114:** 694, 1984)

17β-ESTRADIOL stimulates uterine growth by inducing cellular hypertrophy and hyperplasia. The magnitude of the hyperplastic growth response is dependent upon the species and strain of animal used and its hormonal status (1–4).

Uteri from immature and mature rodents are used extensively, and often interchangeably, as model systems to evaluate estrogen-receptor interactions and tissue responsiveness to estrogen stimulation. However, important differences between the cellular responses seen in uteri from immature and mature rats have been reported. For example, in the immature rat, high doses of estrogens stimulate cell division in all uterine component tissues: epithelia, stroma, and myometria (5, 6). In contrast, in the adult castrate rat, this does not happen; only the uterine epithelial cells respond to estrogen stimulation by entering DNA synthesis and cell division (7, 8). Previous biochemical studies from this laboratory showed that in the uterus of the adult ovariectomized mouse, two peaks of DNA synthesis occur after estrogen stimulation (9). This raised the possibility that in the mouse uterus, DNA synthesis and cell division might be occurring in two different cell types at two different times after estrogen treatment.

To understand the influence of 17β-estradiol on cell division and uterine growth, it is necessary to study uterine cell kinetics. A major objective of this study was to determine whether a physiological dose of 17β-estradiol would change the cell cycle of stromal, epithelial, or myometrial cells in the adult mouse uterus. Since differences in tissue responsiveness between immature and adult rats have been reported, the influence of 17β-estradiol on cell cycle kinetics in uterine tissue from immature mice was also studied.

At some point during the development and maturation of the female reproductive tract, uterine stromal cells lose their ability to divide in response to estrogenic stimulation. An additional objective of this study was to determine at which point during uterine maturation the stromal cells loses their ability to divide after estrogen treatment and to determine whether endocrine parameters play a role in this change in responsiveness.

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## Materials and Methods

### *Animals*

Female CD-1 mice ([ICR]BR Swiss) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and maintained as previously described (10). Immature CD-1 mice

([ICR]BR Swiss) were obtained from the NIEHS animal facility and were used directly at the ages designated in the figure legends. Adult mice, 3–4 months old and approximately 30 g BW, were bilaterally ovariectomized under Nembutal anesthesia then allowed to rest for 2 weeks before use. Castrated mice were primed with three daily doses of 3.3 μg/kg 17β-estradiol, sc, in 100 μl saline vehicle (11). Adult mice were used on the fifth day after completion of priming.

*Fraction of labeled mitosis (FLM) studies*

Ovariectomized adult or immature mice received 1 ip injection of estradiol (3.3 μg/kg) in saline or saline vehicle. Twelve hours after this injection, each animal received 1 ip injection of [$^3$H]methyl thymidine (1 μCi/g BW). Groups of 3–4 animals from each treatment group were killed at various times after [$^3$H]thymidine injection. Their uteri were excised, pinned to constant tension, fixed, and rinsed in excess 10% neutral buffered formalin, then processed for autoradiography. The tissues were embedded in paraffin wax, and 25–30 serial 4-μm transverse sections were cut from the midregion of each uterine horn and mounted on light microscope slides. These slides were deparaffinized, then coated with 50% diluted Kodak NTB-3 Nuclear Track Emulsion (Eastman Kodak Co., Rochester, NY), and exposed at 4 C for 10 days. After autoradiographic development, the slides were stained with hematoxylin and eosin. The numbers of mitotic and [$^3$H]thymidine-labeled cells visible on these autoradiographs were counted using a light microscope (magnification, ×1000; oil immersion). At least 300 cells of each type were counted from each uterine tissue section to evaluate labeling indices and mitotic indices. Labeling indices obtained in this way are plotted in Fig. 1. Counts of labeled stromal or epithelial mitoses were made on 70–100 mitoses/uterus or on the total number from all available sections. Cells with more than 5 silver grains/nucleus were regarded as labeled.

The FLM method was used to estimate the duration of stages of the cell cycle. This procedure has been described in detail previously (2, 12). Briefly, the time lag between the injection of thymidine and the time at which the proportion of labeled mitoses reaches 50% approximates the mean duration of the $G_2$ phase plus prophase of mitosis. The interval between the 50% ascending and descending time points on the first peak gives an estimate of the duration of the S phase. The interval between corresponding points on the first and second peaks gives the approximate generation time, i.e. the duration of the cell cycle. The $G_1$ period can be estimated by subtracting S, $G_2$, and prophase from the generation time. In tissues that displayed only one FLM peak, the generation time was calculated using the steady state equation (13).

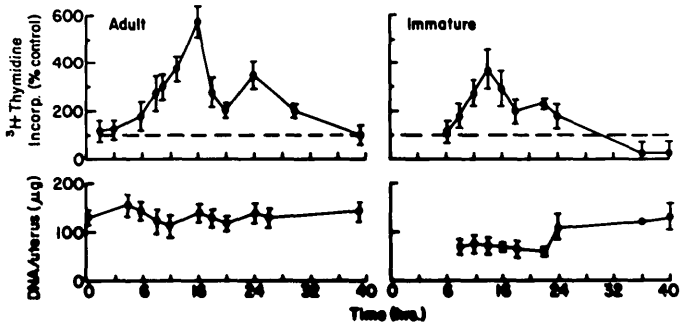


FIG. 1. Time course of DNA synthesis in adult ovariectomized or immature mice. Groups of animals were treated at zero time with a single injection of 10 μg/kg estradiol (O) or saline. *Top panel*, [$^3$H] Thymidine incorporation into uterine DNA, as previously described (9). *Bottom panel*, Total uterine DNA, determined by the Burton procedure (33). Results are expressed as the mean ± SEM from a minimum of three individual experiments. Each *time point* is composed of four animals.

*Developmental study*

Groups of immature female mice were ovariectomized or sham operated on day 15 of age. These animals are described as ovariectomized and intact, respectively. At intervals from 19–52 days of age, four animals from each group were taken to estimate [$^3$H]thymidine-labeling indices. At each time point, mice received an injection of saline or estradiol (3.3 μg/kg), followed 12 h later by an injection of [$^3$H]thymidine. Mice were killed 4 h after [$^3$H]thymidine treatment, uterine tissue was prepared for autoradiography, and labeling indices were estimated as described above.

*Reagents*

[$^3$H]Methyl thymidine (SA, 73.6 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA). Steroids were obtained from Steraloids Inc., (Wilton, NH). Kodak NTB-3 Nuclear Track Emulsion was purchased from Eastman Kodak Co. Additional reagents were obtained from commercial sources and used directly.

## Results

The time courses of uterine DNA synthesis in adult ovariectomized and 21-day-old immature mice are shown in Fig. 1. Both types of experimental animals show the same pattern; the time of maximal thymidine incorporation occurred approximately 16 h after estrogen stimulation in adult and immature animals. The level of stimulation in adults was consistently higher than that in the immature mice when data are expressed as a percentage of the control value. Figure 1 also illustrates how the total amount of uterine DNA changed after estrogen stimulation. The adult levels did not change significantly from the control values, levels in the immature mice increased at 24 h and remained elevated.

The [$^3$H]thymidine-labeling indices seen in control and estrogen-stimulated adult and immature mouse uteri are shown in Fig. 2. In the adult ovariectomized mouse uterus, control luminal epithelial cells had a low variable labeling index during the first 26 h after [$^3$H]thymidine injection (*i.e.* 12–38 h after estrogen, overall mean value = 10.7 ± 4.7%). These cells responded to estrogen stimulation by showing an increased labeling index; the labeling index rose to 75% by 2 h after [$^3$H]thymidine injection and remained above 80% up to 14 h postinjec-

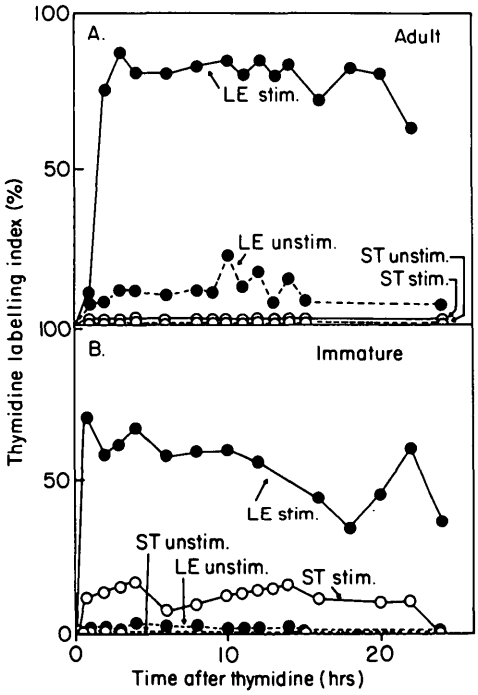


FIG. 2. Time courses of [$^3$H]thymidine incorporation into luminal epithelial (LE) and stromal (ST) cells in uteri from adult (A) and immature (B) mice. Groups of ovariectomized adult or immature mice were injected ip with estradiol (3.3 $\mu$g/kg) or saline, then injected with [$^3$H]thymidine (1 $\mu$Ci/g) 12 h later and killed at various times after [$^3$H]thymidine treatment. Results are expressed as the mean ± SEM for four individual animals and are representative of two independent experiments.

tion (overall mean value, calculated to give an index of responsiveness = 74.94 ± 20.14%). Stromal cells in control tissues had a low constant labeling index (overall mean value = <1%) and did not show a significant increase in labeling index after estrogen stimulation (overall mean value = 1% ± 0.71%).

In uteri from immature mice, control luminal epithelial cells had a low and constant labeling index (overall mean = 1.11 ± 0.88%). In estrogen-stimulated uteri, the luminal epithelial labeling index rose to 71% within 1 h of [$^3$H]thymidine injection (*i.e.* 13 h after estrogen) and then showed a gradual decrease to 33% at 18 h, followed by a second rise to 60% at 22 h, then a fall to 37% at 26 h (overall mean value = 50.6 ± 14.99%). The stromal cell population had a low labeling index in control uteri (overall mean value = 2.54 ± 1.61%). In estrogen-treated uteri, the stromal labeling index rose slowly to a peak of 16% during the first 4 h after [$^3$H]thymidine injection; an elevated labeling index was sustained in this cell type until 22 h postinjection (overall mean value = 11.2 ± 3.4%).

Light microscopic autoradiographs of [$^3$H]thymidine incorporation patterns in uteri from immature and mature mice are shown in Fig. 3. In uteri from unstimulated ovariectomized adult mice, there was little [$^3$H]thymidine incorporation into any cell type. After estradiol stimulation, [$^3$H]thymidine was incorporated into luminal and glandular epithelial cells, but not into stromal or myometrial cells. In uteri from immature, 21-day-old mice, there was little [$^3$H]thymidine incorporation apparent in any cell type. However, after estrogen stimulation, [$^3$H]thymidine incorporation occurred in three cell types: luminal epithelium, glandular epithelium, and stroma. There was little incorporation of [$^3$H]thymidine into myometrial cell DNA. The tissues shown here were taken from animals 16 h after an injection of estradiol or saline and 4 h after an injection of [$^3$H]thymidine. At this time, tissue labeling indices were maximal (see Fig. 2). However, the [$^3$H]thymidine incorporation patterns shown in this plate are representative of those seen at all times after estrogen stimulation.

FLM curves were constructed for luminal epithelial cells and stromal cells in uteri from unstimulated and stimulated adult ovariectomized mice using the injection protocol and data analysis procedures described in *Materials and Methods.* The FLM curves obtained from adult unstimulated uteri are shown in Fig. 4A. The luminal epithelial cell population had a FLM value of 22% 13 h after the injection of saline (*i.e.* 1 h after [$^3$H]thymidine injection). There was an increase in the luminal epithelial FLM value 3 h after [$^3$H]thymidine injection, and values remained elevated, ranging from 43–60% in the 3- to 10-h period after [$^3$H]thymidine injection. After this time, the luminal epithelial FLM value fell to the original value of 21%. For this tissue, the overall mean FLM value was 33.57 ± 15.38%. The broad shape of this FLM peak indicates that in the unstimulated luminal epithelium, DNA synthesis, and thus cell division, occur in an asynchronous fashion. The FLM curve obtained from stromal cells in the adult unstimulated uterus showed a flat profile, and the overall mean FLM value for this cell population was 0.92 ± 1.04%. This type of profile indicates that in the adult unstimulated uterus, stromal cells proliferate at an extremely low rate, with no indication of synchrony.

The pattern of cell turnover in the luminal epithelial cell population changed after estrogen administration (Fig. 4B). There were two FLM peaks after estrogen treatment, one 15–19 h after estrogen (*i.e.* 3–7 h after [$^3$H]thymidine) and another 26–32 h after estrogen (*i.e.* 14–20 h after [$^3$H]thymidine). The first wave of labeled mitoses occurred rapidly with a high degree of synchrony, and the maximum fraction of labeled mitosis (96%) occurred 16 h after estrogen, corresponding to 4 h after [$^3$H]thymidine injection. Thus, luminal epithelial cells go through one round of cell division in a synchronous manner after estrogen stimulation. The presence of a second FLM peak shows that some of the luminal epithelial cells then go through a second round of DNA

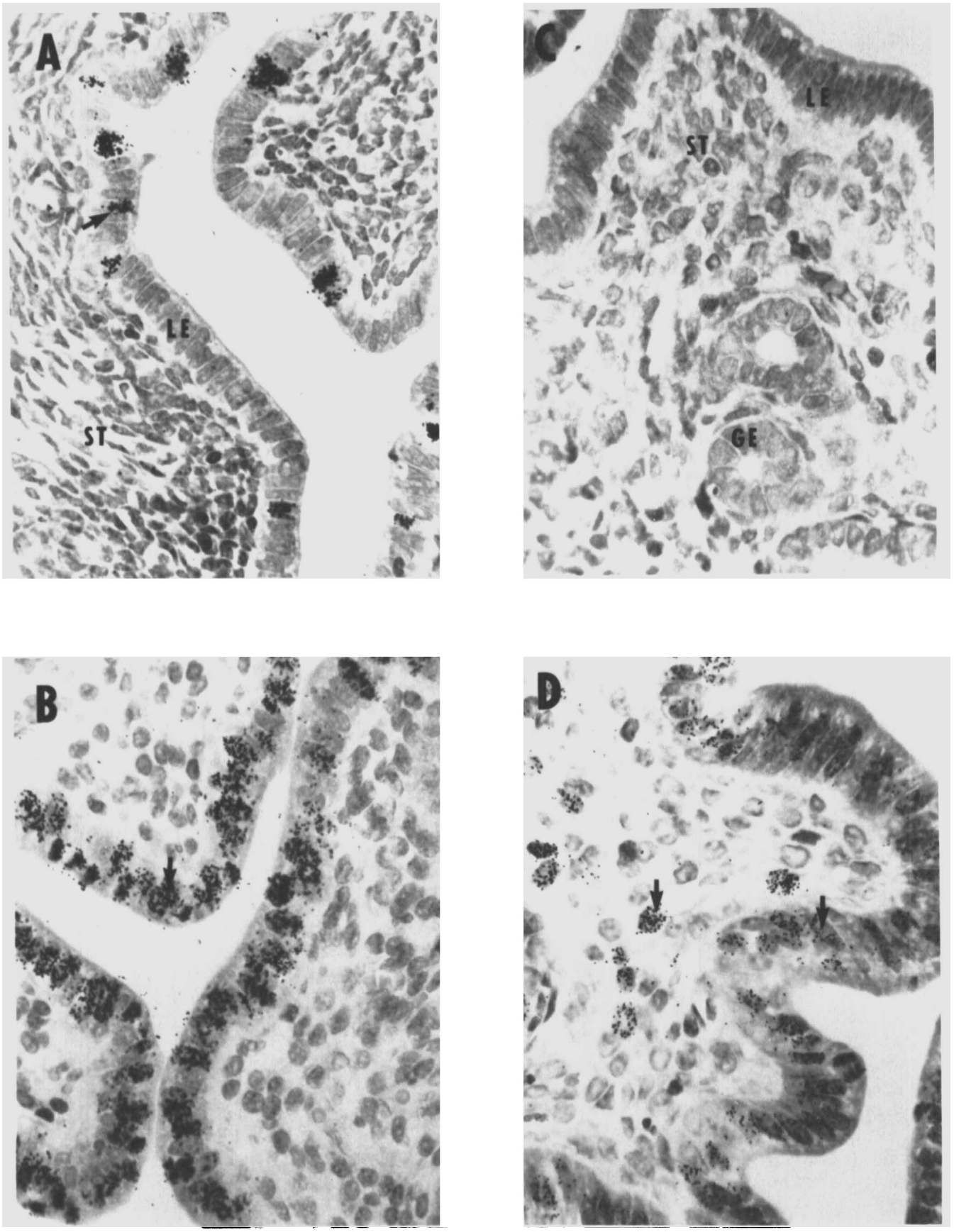


FIG. 3. Light microscopic autoradiographs (magnification ×680) from uteri 16 h after treatment with estradiol (B and D) or saline (A and C). All animals received an injection of [$^3$H]thymidine (1 $\mu$Ci/g BW) 4 h before death. Mice were ovariectomized adults (A and B) and 21-day-old immature animals (C and D). Tissue was sectioned at 4 $\mu$m, exposed for 10 days, then stained with haematoxylin and eosin. Examples of labeled nuclei are marked with *arrows* in the appropriate tissue compartments. Results are expressed as the mean ± SEM for four individual animals and are representative of two independent experiments.

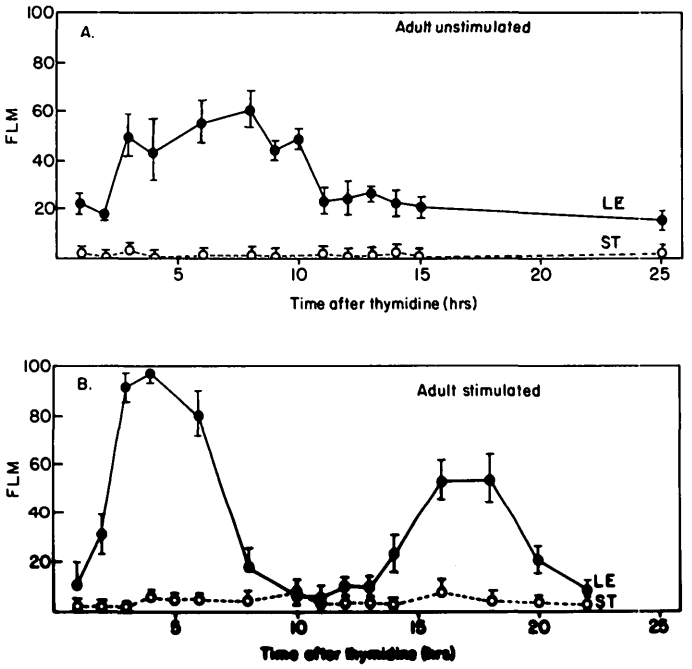


FIG. 4. FLM curves obtained from the luminal epithelial (LE; O) and stromal (ST; O) cell populations of uteri from ovariectomized adult unstimulated (A) and stimulated (B) mice. Mice received an ip injection of saline (A) or estradiol (B; 3.3 μg/kg), followed 12 h later by an injection of [$^3$H]thymidine (1 μCi/g BW). At various times after [$^3$H]thymidine injection, mice were killed, and uterine tissue was prepared for autoradiography as described in *Materials and Methods*. The percentage of labeled mitosis was then estimated by light microscopy at these times after [$^3$H]thymidine injection. Results are expressed as the mean ± SEM for four individual animals and are representative of two independent experiments.

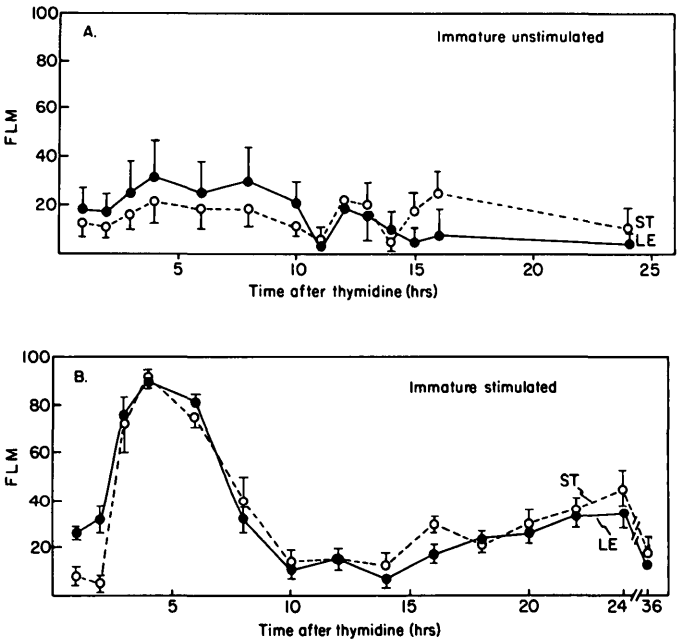


FIG. 5. FLM curves obtained from the luminal epithelial (LE; ●) and stromal (ST; O) cell populations of uteri from immature 21-day-old mice. Mice received an ip injection of saline (A) or estradiol (B; 3.3 μg/kg), followed 12 h later by an injection of [$^3$H]thymidine (1 μCi/g BW). At various times after [$^3$H]thymidine injection, mice were killed, and uterine tissue was prepared for autoradiography. The percentage of labeled mitoses was then estimated by light microscopy at these times after [$^3$H]thymidine injection. Results are expressed as the mean ± SEM for four individual animals and are representative of two independent experiments.

synthesis and cell division. By 34 h after estrogen injection (*i.e.* 22 h after [$^3$H]thymidine) the luminal epithelial FLM index was back down to its original value of 8%. In contrast, the FLM curve obtained from stromal cells in the adult stimulated uterus showed a flat profile, with no distinct peaks and a low overall mean FLM value of 3.33 ± 1.91%. The turnover rate of stromal cells appeared to be only slightly affected by estrogen stimulation, which raised the overall mean FLM value from 0.92% to 3.33%. This is a very small response compared with that seen in the luminal epithelial cell population.

The FLM curves obtained for immature mouse uteri are shown in Fig. 5. In unstimulated animals (Fig. 5A), the luminal epithelial cell population has a very broad low peak of FLM values from 15–22 h after saline injection (*i.e.* 3–10 h after [$^3$H]thymidine). During this time, the FLM values ranged from 21–32%. After a drop in FLM values to 3% 23 h after saline treatment, the values showed another low peak of 19% then declined to background values (5%) 36 h after saline treatment (*i.e.* 24 h after the injection of estrogen [$^3$H]thymidine). The overall mean FLM value for this group was 16.79%. The stromal cell population behaved in a similar way, with low broad FLM peaks and an overall FLM value of 15.38%. Thus, in the unstimulated immature mouse uterus, both the stromal and luminal epithelial cell populations appear to be turning over. After estrogen stimulation (Fig. 5B), the stromal and luminal epithelial cell populations had similar FLM profiles. Luminal epithelial cells showed a rapid increase in the FLM from an initial value of 26% to a peak value of 90% 16 h after estrogen treatment (corresponding to 4 h after [$^3$H]thymidine). The mitotic labeling index fell to the baseline level 22–26 h after estrogen injection (*i.e.* from 10–14 h after [$^3$H]thymidine) then showed a second gradual increase from 28–36 h after hormone injection. The stromal cell population also responded to estrogen stimulation and showed an almost identical biphasic FLM curve. Therefore, in the immature mouse, the luminal epithelial and stromal cell populations both respond to estrogen treatment by entry into one highly synchronous round of cell division; some cells then enter a second round of cell division, but not with a high degree of synchrony.

Using data from these and other FLM curves, the generation times and, where possible, the durations of the $G_1$, S, and $G_2$ plus M phases of the cell cycle were calculated as described in *Materials and Methods*. These

data are summarized in Table 1. In the adult uterus, estrogen treatment reduced the generation time of luminal epithelial cells from 86 to 13 h. This reduction was mainly at the expense of the $G_1$ phase, although the duration of the S phase was also reduced slightly. In the adult uterus, stromal cells had a very low turnover rate, which did not appear to be modified by estrogen stimulation.

In the uterus of the immature mouse, untreated luminal epithelial cells had a long generation time (>100 h), which was drastically reduced by estrogen treatment to a generation time of 14.5 h. Again, there was a slight reduction in the duration of the S phase and a major reduction in the duration of the $G_1$ phase. The generation times of stimulated and unstimulated luminal epithelial cells in the immature mouse uterus were similar to their respective generation tissues in the adult uterus. In addition, in the immature mouse uterus, estrogens stimulated stromal cell division. This was reflected by the change in generation times in the stromal cell population from 100 to 16.5 h. This stimulated stromal value was similar to those obtained from stimulated luminal epithelial cells in immature and mature mouse uteri in the present study.

Comparison of the labeling and FLM data from immature and mature ovariectomized mice shows that these two model systems have different patterns of cell division in response to estrogen stimulation (Figs. 3, 4B, and 5B). Therefore, at some point during maturation of the female reproductive tract, the uterine stromal cells must lose their ability to respond to estrogen stimulation with DNA synthesis and cell division. To determine when this change in responsiveness occurs, we have done a developmental study, measuring [$^3$H]thymidine labeling indices in stromal cell populations from groups of estrogen-stimulated control and ovariectomized mice at different ages. Data from this study are shown in Fig. 5. In 19-day-old intact females, the stromal labeling index was initially high (20%). This value was comparable to that shown in Fig. 1B for 21-day-old immatures. The stromal labeling index fell progressively with time, attaining a value of 9% on day 52. However, in ovariectomized females, the stromal labeling index was initially low (*i.e.* 5% 4 days after surgery) on day 19 and then gradually decreased to a value of 1% on day 52. Thus, stromal labeling indices were generally lower in the ovariectomized animals than in their intact counterparts. In the absence of the ovary, stromal cells showed the adult-type pattern and did not appear to go through a period during which they divide in response to estradiol; this observation suggests that the ovary may play some role in influencing the high level of cell division seen in the uterine stroma of immature animals.

TABLE 1. The influence of estrogen on uterine cell cycle parameters

| | $T_c$ | $G_2$ + pro | S | $G_1$ |
|---|---|---|---|---|
| Adult | | | | |
| Epithelium | | | | |
| Control | 86 h £ | 1 h, 50 min | 6 h, 30 min | — |
| Estradiol | 13 h | 1 h, 55 min | 5 h, 11 min | 6 h |
| Stroma | | | | |
| Control | — | — | — | — |
| Estradiol | — | — | — | — |
| Immature | | | | |
| Epithelium | | | | |
| Control | >100 h £ | 2 h, 50 min | 7 h, 30 min | |
| Estradiol | 14 h, 30 min | 1 h, 50 min | 4 h, 50 min | 6 h |
| Stroma | | | | |
| Control | >100 h £ | — | — | — |
| Estradiol | 16 h, 30 min | 2 h, 20 min | 6 h, 10 min | 8 h, 5 min |

$T_c$, Generation time estimated from FLM curves, or £ from the steady state equation (12); $G_2$ + pro, the duration of $G_2$ + prophase of mitosis, estimated from FLM curves; S, the duration of the S phase, estimated from FLM curves; $G_1$, the duration of the $G_1$ phase, estimated, where possible, from FLM curves; —, value cannot be estimated from the FLM curves.

## Discussion

Several studies have examined the influence of ovarian hormones on uterine DNA synthesis and cell turnover rates. During the estrous cycle, luminal and glandular epithelial cells display a cyclical pattern of proliferation and regression both in mouse (14–16) and rat (17, 18). Reports on stromal mitosis during the estrous cycle vary; stromal mitosis has been reported to occur in rat (19, 20) and mice (15) uteri, although Walker (21) did not see stromal mitosis in mouse uterine tissues during the estrous cycle. Myometrial cell division occurs at a very low level during the estrous cycle of the mouse (15) and rat (20); [$^3$H]thymidine incorporation studies confirm that very little myometrial DNA synthesis occurs during the rat estrous cycle (19).

In the present study we have examined the influence of exogenous estrogens on uterine cell cycle kinetics. Estradiol treatment greatly reduced the duration of the luminal epithelial cell cycle in uteri from adult ovariectomized mice. Estimates of cell cycle times vary according to the technique and strain of mouse used (2). However, the values obtained here from luminal epithelial cells in uteri from ovariectomized adult CD-1 mice are similar to those from Schneider mice (14) and CBA mice (22).

The present study indicates that in the adult ovariectomized mouse uterus after estrogen stimulation, a large proportion of luminal epithelial cells goes through one or two rounds of DNA synthesis. Time-course studies of DNA synthesis in the estrogen-stimulated adult mouse uterus show two peaks of [$^3$H]thymidine incorporation (9). Thus, the two peaks of [$^3$H]thymidine incorporation observed in these biochemical studies probably both arise from DNA synthesis within the uterine epithelial cell population; in the estrogen-stimulated adult mouse uterus, there is little DNA synthetic activity in any other

cell type. As little cell proliferation occurred in the stromal cells of uteri from adult ovariectomized mice, it was not possible to estimate cell cycle parameters in this cell population. Low stromal labeling and mitotic indices were also observed in uteri from adult ovariectomized mice by Lee (23) and Martin *et al.* (1). The same bimodal pattern of [$^3$H]thymidine incorporation was seen in the immature mouse, although in this case, both the stroma and epithelium were undergoing DNA synthesis. These data also show that the differences seen in stromal cell responsiveness (Fig. 6) between immature and adult mice are not a result of a change in the time course of thymidine incorporation. Total uterine DNA levels in the adult did not change significantly after estrogen stimulation. This is because only the epithelial cells (5–10% of the total uterine cell composition) are undergoing DNA synthesis. On the other hand, the immature uterus is responding with both epithelial and stromal DNA synthesis, and these two tissue types compose 35–50%. DNA synthesis from these combined compartments could demonstrate the increase above the control value seen in Fig. 1.

This is the first cell cycle kinetic study of the luminal epithelial and stromal cell populations in the immature mouse uterus. We found that estrogen has the same type of influence on the luminal epithelial cell cycle in the immature uterus as in the adult uterus, *i.e.* it greatly reduces the generation time of the cells, mainly by reducing the $G_1$ phase. In addition to this, in the immature mouse, estradiol reduces the cell cycle time of stromal cells within the uterus, thereby promoting DNA synthesis and cell division in this tissue compartment. In estradiol-stimulated stromal cells, the generation time and the durations of the cell cycle stages are similar to those seen in the luminal epithelial cell populations of immature and adult mouse uteri. Interestingly, there was evidence that some stromal cells did enter a second round of DNA synthesis and mitosis. In adult tissue, estrogen only stimulates stromal cell division after a period of progesterone pretreatment; cells enter one round of cell division and then become insensitive to further estrogenic stimulation, *i.e.* they do not divide further (14). In contrast, these data show that in the immature mouse uterus, the stromal cell population does not appear to become refractory to estrogen stimulation in this manner. Additional cellular differentiation may be necessary for development of the refractory state.

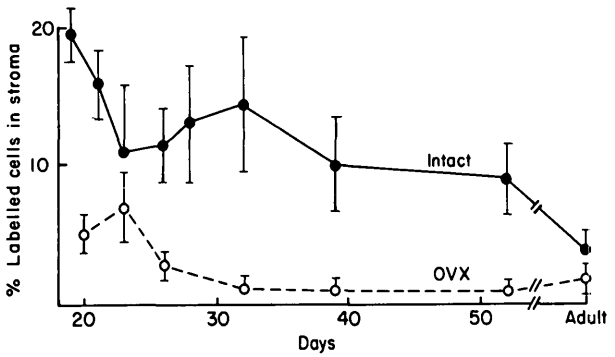


FIG. 6. Mice were left intact (●) or ovariectomized (O) on day 15 of life. At different time intervals afterward, groups of four control and four ovariectomized mice were treated with 3.3 μg/kg estradiol, then with [$^3$H]thymidine 12 h later. All animals were killed 4 h after [$^3$H]thymidine treatment, then uterine tissue was prepared for light microscopic autoradiography. The labeling indices of stromal cell populations in the uteri from these mice were measured as described in *Materials and Methods*. Results are expressed as the mean ± SEM for four individual animals and are representative of two independent experiments.

In this study there was little evidence that estradiol induced myometrial cell division in either the immature or mature mouse uterus. Similar results have been obtained by other groups, suggesting that physiological doses of estrogen induce myometrial hypertrophy but not hyperplasia (1, 23–25). However, myometrial cell division can be induced in the immature rat with supraphysiological doses of estrogens (4, 5, 26).

Estrogens are believed to stimulate uterine growth responses through an intracellular receptor-mediated mechanism (27). We have previously shown that two nuclear translocation events may be necessary for uterine growth and that these occur in the mouse uterus 1 and 8 h after estrogen treatment (28). Thus, the first nuclear estrogen peak occurs early during the $G_1$ phase of the epithelial cell cycle; the second nuclear binding peak occurs during the early S phase of the epithelial cell cycle. This second nuclear peak of estrogen binding may stimulate entry of epithelial cells into the S phase, or, alternatively, increased nuclear binding at 8 h may occur as a result of these cells already being in the S phase and having a different binding capacity. Further experiments investigating nuclear estrogen-receptor interactions in epithelial nuclei from different cell cycle phases will be necessary to more clearly determine the exact mechanism involved.

The time courses of estrogen-induced DNA synthesis and cell division in uteri from mice and rats differ considerably. Generally, these responses occur more rapidly in the mouse (Ref. 9 and the present study) than in the rat (8, 26, 29). In the ovariectomized adult mouse, two peaks of uterine DNA synthesis occur 16 and 24 h after estrogenic stimulation and are primarily due to DNA synthesis in epithelial cells (1, 9). In the ovariectomized adult rat, two waves of uterine cell division have been observed at much later times after estrogen treatment. The first, 24 h after stimulation, occurs in luminal epithelial cells; the second, 48 h after stimulation, occurs in glandular epithelial cells (8). Time courses of DNA synthesis and cell division have also been studied in the immature rat. Here again, the major peak of uterine

DNA synthesis occurs 24 h after stimulation; this appears to be due to DNA synthesis in stromal and epithelial cell populations (5, 26, 29). A smaller second wave of uterine DNA synthesis has also been reported to occur 36 h after stimulation (29).

Studies of neonatal mouse and rat uteri have shown that at this early developmental stage, stromal cells will enter DNA synthesis and mitosis in response to high doses of estrogen (30, 31). Our study shows that in the 21-day-old mouse uterus, stromal cells are still capable of entering DNA synthesis and cell division in response to estrogen stimulation. However, the stromal cell population gradually loses its ability to divide in response to estrogen stimulation during days 22–52 postnatally. In the CD-1 mouse, behavioral and morphological estrous cyclicity begins at around 28–32 days of age. During this period, the levels of circulating estrogens will, therefore, increase considerably. Prepubertal ovariectomy results in a nonresponsive stromal cell population at all ages studied. It is possible that in the immature animal, low levels of ovarian-derived estrogen are required over a long period of time to prime uterine stromal cells for division. It may be that in the immature animal some ovarian-derived endocrine factor enhances the ability of stromal cells to divide in response to estrogen stimulation. During early pregnancy, progesterone modifies the pattern of uterine DNA synthesis and cell division; progesterone, in conjunction with estrogen, promotes stromal cell division and blocks epithelial cell division (8, 25). The ratio of progesterone to estradiol is critical here; at different ratios, certain cell division will occur in the epithelial or stromal cells (25). It may be that the responsiveness of the stromal cell population seen in immature mice is due to the presence of circulating progesterone. However, this seems unlikely since there is no significant ovarian source of progesterone in the prepubertal animal. Although the adrenals do seem to be a source of progesterone and estrogens during the peripubertal period (32), adrenal output should not be influenced by prepubertal ovariectomy. It may be that immature responsive stromal cells go through a finite number of rounds of cell division in response to endogenous estrogen stimulation and then differentiate into adult nonresponsive cells which will only divide under the hormonal conditions associated with early pregnancy. However, additional developmental studies using an array of treatment schedules would be necessary to test this hypothesis.

The uterus is a complex estrogen target organ. Our data show that the three major uterine cell types differ in their abilities to respond to estrogen stimulation by entering into DNA synthesis and cell division. Additionally, different patterns of cellular responsiveness to estrogen stimulation are seen at two different developmental stages in the mouse uterus. Thus, estradiol only acts as a mitogen in specific cell populations under certain developmental and hormonal conditions.

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## Sixth Annual University of California-Riverside Nichols Institute Symposium on Cellular and Molecular Endocrinology

The sixth annual University of California-Riverside Nichols Institute Symposium on Cellular and Molecular Endocrinology will be held Friday, March 30–Saturday March 31, 1984 on the University of California-Riverside campus at the foot of the Box Springs Mountains.

The purpose of the meeting is to foster communication among scientists who are interested in the cellular, biochemical and structural aspects of endocrinology. The meeting will have four approximately three-hour sessions devoted to the topics of (a) Hormonal Control of Cellular Proliferation and Function (b) Steroid Hormone Metabolizing Enzyme Systems (c) Recombinant DNA, Growth Factors and Related Molecules and (d) Clinical Applications of "Newer" Biomedical Technology.

Thirteen internationally recognized scientists will present lectures on their work in these areas. They include the following: Alexander C. Brownie (Buffalo), Cornelia P. Channing (Baltimore), R. John Collier (Los Angeles), Achilles Dugaiczyk (Riverside), Selna L. Kaplan (San Francisco), John A. Katzenellenbogen (Urbana), Thomas C. Merigan, Jr. (Stanford), Michael G. Rosenfeld (San Diego), Russell Ross (Seattle), Judson J. Van Wyk (Chapel Hill), Michael R. Waterman (Dallas), Larry E. Vickery (Irvine), and Carl F. Ware (Riverside).

Registration fees for the meeting are $25.00 per participant ($15.00 for postdoctoral student/fellow; $10.00 for student and $125.00 for physicians wishing to enroll in the Continuing Medical Education [CME] Program [Category 1] through the University of California-Los Angeles School of Medicine).

For registration fees and further information please write: Dr. Anthony W. Norman, Program Director, Endocrinology Symposium, Department of Biochemistry, University of California, Riverside, CA 92521, (714) 787-4777.