DNA SYNTHESIS IN CELLS OF THE UTERINE AND VAGINAL EPITHELIUM IN VARIOUS STAGES OF THE SEX CYCLE

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During the sex cycle in mice there are two periods of intensive DNA synthesis; proestrus and the first day of diestrus in the uterine epithelium, and diestrus–proestrus and the first day of diestrus in the vaginal epithelium. DNA synthesis takes place immediately before cell division in the same stage of the sex cycle. The number of cells synthesizing DNA depends on the sensitivity of the particular epithelium to estrogens.

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Data in the literature concerning changes in the index of labeled nuclei (ILN) in the epithelium of reproductive organs of ovariectomized rats [10, 11] and mice [3, 4, 9] under the influence of exogenous estrogens do not allow changes in this index to be estimated in the course of the sex cycle. Nor can changes in ILN in individual stages be estimated from the work of Walker [13], who studied physiological regeneration of the uterine and vaginal epithelium in mice during the sex cycle and found that the increase in number of mitotic divisions observed in proestrus is preceded by an increase in DNA synthesis in the nuclei of the basal layer of vaginal epithelium and in the uterine epithelium before proestrus. However, Walker injected thymidine-H^3 before proestrus (6 times, every 4 h during the 24 h period), and sacrificed the animals receiving the label only on the 1st, 2nd, 3rd, 4th, and 8th days after injection.

The work of Epifanova [4] and Laguchev [5, 7, 8] has shown that the uterine and vaginal epithelium during the sex cycle exhibits two increases in mitotic activity: the first in proestrus, when the endometrium proliferates before ovulation, the second in metestrus–diestrus, during replacement of the cells of the epithelial lining [4].

It has been conclusively shown that estrogens stimulate mitotic activity in the epithelium of the reproductive organs only in small, optimal doses, acting for short periods. If the dose exceeds the optimum or the estrogens act for long periods, inhibition of mitotic activity ensues [8].

The results of an investigation by Thrasher and co-workers [12], who showed that the highest increase in DNA synthesis occurs in proestrus (32.9%) and estrus (29.4%), and the lowest percentage of labeled cells in early diestrus, appear to conflict with this conclusion.

In a preliminary investigation [1], the writer found that the increase in mitotic index (MI) in the first day of diestrus is preceded by an increase in DNA synthesis in metestrus.

Because of this contradiction, the present investigation was carried out to determine ILN at all stages of the sex cycle in C57Bl mice.

EXPERIMENTAL METHOD

The autoradiographic method [1] was used.

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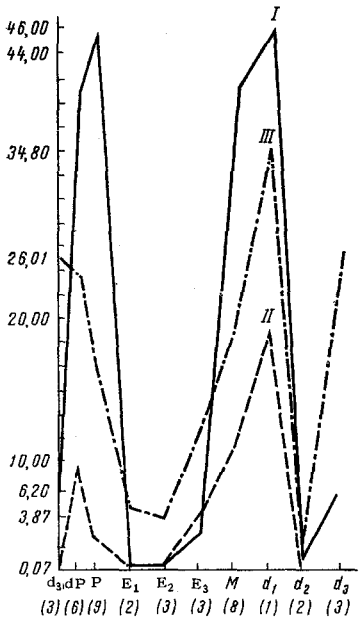

Fig. 1

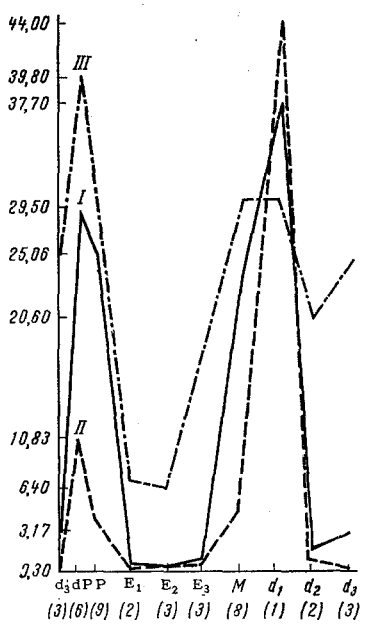

Fig. 2

Fig. 1. Changes in ILN of the sex cycle. I) Epithelium of uterine cavity; II) epithelium of uterine glands; III) vaginal epithelium. Stages of sex cycle: d_3) 3rd day of diestrus; d_p) diestrus–proestrus; P) proestrus; E_1) 1st day of estrus; E_2) 2nd day of diestrus; E_3) 3rd day of estrus; M) metestrus; d_1) 1st day of diestrus; d_2) 2nd day of diestrus. Abscissa, stages of sex cycle; number of females given in parentheses; ordinate, changes in ILN (in %).

Fig. 2. Changes in MI in epithelium of reproductive organs depending on stages of the sex cycle. Abscissa, stages of sex cycle; number of females given in parentheses; ordinate, mitotic index (in $^0/_{00}$). Remainder of legend as in Fig. 1.

By daily testing of vaginal smears from 100 sexually mature virgin female C57Bl mice (18-20 g) for 3 weeks, 37 mice in different stages of the sex cycle (9 groups) were chosen. The number of females in each experimental group is shown in parentheses on the graph.

At 10 a.m. the selected mice received a single injection of thymidine-H^3 in a dose of 0.7 μCi/g body weight (activity of thymidine-H^3 8600 mCi/mmole). At 11 a.m., the mice were sacrificed, and the uterus and vagina quickly removed and fixed by Carnoy's method. Paraffin sections 5 μ in thickness, coated with type R emulsion (NIKFI), were exposed for 10 days at 4° and then developed with amidol and stained with Ehrlich's hematoxylin. The number of labeled nuclei was counted separately in the epithelium lining the uterus, the epithelium of the uterine glands, and the basal layer of the vaginal epithelium.

In each case 3000 epithelial cells were counted. Nuclei above which there were at least 3 grains of silver were regarded as labeled.

EXPERIMENTAL RESULTS

Curves showing changes in ILN in the epithelium of the reproductive organs are given in Fig. 1. The common feature of these curves is the existence of two maxima and two sharp falls in the number of cells synthesizing DNA during the sex cycle. Changes in ILN in the left-hand half of the graph were associated with an increase in the concentration of estrogens, reaching a maximum in estrus. Later, after ovulation, the supply of estrogens stops and they are rapidly excreted from the body. Against the background of a decrease in the estrogen concentration, changes took place which are reflected in the right-hand part of the graph.

The epithelium of the uterine glands is known to be most sensitive to estrogens [7, 8]. The number of cells synthesizing DNA was found to be higher in this epithelium during early proestrus than in late proestrus and higher still on the first day of diestrus.

On the 1st and 2nd days of estrus, in connection with the prolonged and continuous action of considerable concentration of estrogens, the number of cells synthesizing DNA fell sharply (0.03-0.04%), and on the 3rd day of estrus a sharp increase in ILN (4.35%) began, reaching a maximum on the 1st day of diestrus (20.10%), associated with a sharp decrease in the concentration of estrogens to the optimum level.

Later, evidently in connection with a further decrease in the concentration of estrogens, the ILN fell in the course of 24 h to 0.01% and remained low during the 3rd day of diestrus (d_3 = 0.07%).

Similar changes in ILN were found in the epithelium of the uterine cavity. Being less sensitive to estrogens than the epithelium of the uterine glands, the epithelium lining the cavity of the uterus gave a greater increase in ILN in late proestrus (44.12%), followed by a sharp fall of the curve to 0.01% on the first day of estrus, a gradual rise from the 3rd day of estrus, since the number of cells synthesizing DNA gradually increased (E_2 = 0.28%, E_3 = 2.49%), and a sharp increase in metestrus–diestrus (M = 40.34%, d = 45.09%).

In the vaginal epithelium, ILN reached its first maximum on the 3rd day of diestrus (26.01%). On the 2nd day of estrus, ILN reached its lowest value (3.87%), but it was much higher than ILN of the epithelium of the uterine glands and epithelium of the uterine cavity. A fresh rise of the curve began on the 3rdday of estrus (10.94%), to reach a maximum on the 1st day of diestrus (34.8%).

The value of ILN depends on changes in the concentration of estrogens in the body and the duration of their action on cells of the target organs (Fig. 1).

Analysis of the course of the curve showing changes in MI in C57Bl mice shows that the course of the MI curve for the uterine gland epithelium coincides with the course of the curve showing changes in ILN in the same epithelium (Fig. 2).

It is interesting to note that on the 2nd and 3rd days of estrus MI was close to zero, while on the first day of diestrus the curve rose sharply(44.0‰),and that MI in early diestrus was 4 times higher than MI in early proestrus (11.83‰).

The values of MI in the epithelium of the uterine cavity in early(29.46‰)and late(75.70‰)proestrus were close to each other, and MI in this region was much higher than in the epithelium of the uterine glands as was found by Laguchev [5, 6, 7] when investigating the epithelium of noninbred mice. In the course of estrus the ratio tended to equalize, since MI for the epithelium of the uterine glands on these days was close to zero (10-0.2-0.3‰) as it also was in the epithelium of the uterine cavity (0.16-0-0.63‰). The value of MI in metestrus in the epithelium of the uterine cavity was 24.13‰, to reach 37.66‰ about 1-1.5 days later. However, as also in the noninbred mice, on the first day of diestrus MI in the epithelium of the uterine cavity remained lower than in the epithelium of the uterine glands (44.0‰). On the 2nd day of diestrus MI had fallen to 1.80‰, and by the 3rd day of diestrus a slight increase was observed.

During early proestrus, ME in the vaginal epithelium was 39.78‰, and in late proestrus a slight decrease to 30.45‰ was observed. On the 1st day of estrus MI was 6.95‰, and on the 2nd day 6.40‰. On the 3rd day of estrus the curve showed a sharp rise (18.06‰), while in metestrus–diestrus it formed a plateau at a height of slightly more than 30‰. On the 2nd day of diestrus, MI fell to 20.60‰, followed by a slight rise on the 3rd day. Characteristically, MI, like ILN, in the vaginal epithelium did not fall to zero at any stage of the sex cycle. This (just as in noninbred mice) is evidence that the vaginal epithelium evidently contains cells with unequal sensitivity to estrogens. The least sensitive of these cells continued to respond by DNA synthesis and division during the prolonged action of high concentrations of estrogens.

It was thus shown that during the sex cycle in mice there are two periods of intensive DNA synthesis: proestrus and the first day of estrus in the uterine epithelium, and the end of diestrus–proestrus and the first day of diestrus in the vaginal epithelium.

Estrogens thus evidenly have a synchronizing action on and stimulate DNA synthesis in epithelial cells of the uterine cavity and uterine glands of intact mice, similar to their action on the epithelium of ovariectomized rodents [4, 9, 10]. Cells synthesizing DNA during proestrus divide in the same stage of the sex cycle. The same can also be postulated for metestrus and the first day of diestrus.

The almost total absence of cells synthesizing DNA in the epithelium of the uterine cavity and uterine glands during estrus is probably due to the prolonged action of high concentrations of estrogens on DNA synthesis and mitotic activity.

The number of cells starting to synthesize DNA during the sex cycle, like fluctuations in MI, is evidently due to differences in the sensitivity of cells belonging to these epithelia to estrogens [2, 8]. The concentration and duration of action of estrogens influence the level of ILN.

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