EFFECT OF PROGESTERONE ON MITOTIC CYCLE OF EPITHELIUM OF THE REPRODUCTIVE ORGANS

N. A. Roslyakova

UDC 612.61./62.014.3:612.6].014.46:615.256.52

Following a single injection of progesterone, the duration of the S-period in the epithelium of the reproductive organs of mice, determined from the curve of labeled mitoses after administration of thymidine-H^3, was 7.5-8 h. The duration of the mitotic cycle as a whole was 13-16 h. The use of a double label showed that after administration of progesterone to mice the duration of the S-period in the reproductive organs is reduced on the average by 2 h.

Administration of progesterone to ovariectomized animals is known to evoke active DNA synthesis and proliferation in the epithelium of the reproductive organs [2, 3]. The effect of progesterone on individual parameters of the mitotic cycle has not hitherto been investigated.

The effect of other ovarian hormones (estrogens) on the mitotic cycle has been extensively studied. However, data in the literature on this matter are contradictory [1, 4, 5, 6].

The duration of individual phases of the mitotic cycle in the epithelium of the uterine cavity, uterine glands, and vagina was determined in ovariectomized mice during stimulation by progesterone. The "curve of labeled mitoses" [7] and the double label method [4] were used and the results for the epithelium of the reproductive organs obtained by these two methods were compared.

MATERIAL AND METHOD

Experiments of Series I (using the curve of labeled mitoses). The experiments of this series were carried out on 108 C57 mice with a mean weight of 20 g. Ovariectomy was performed 28 days before the experiment began. The animals were divided into three groups: 1) 33 mice receiving a single dose of 0.5 mg progesterone; 2) 33 mice receiving 1 mg progesterone; 3) 42 mice receiving 0.1 mg oil.

The hormone was injected at 4 P.M. Thymidine (6 Ci/mmole, England) was injected into the animals at 9:30 A.M. in a dose of 0.7 μCi/g body weight. The animals were sacrificed 30 min, 1.5, 3.5, 8.5, 12.5, 14.5, 18.5, 21.5, and 24.5 h after injection of thymidine: at each time three mice of the experimental group and three controls were sacrificed. The period of exposure was 2 weeks. In every case 100 mitoses were counted.

Experiments of Series II (double label method). In this series of experiments 22 mice weighing 20 g were used. Ovariectomy was carried out 6-7 days before the beginning of the experiment. Each of 12 mice received 0.5 mg progesterone, and each of 10 control mice 0.1 mg oil. The hormone was injected at 10 A.M.; the first dose of thymidine-H^3 (0.5 μCi) after 4 h; the second dose (5 μCi) after 5 h.

Two mice received a single large dose of thymidine-H^3 5 h after progesterone (thymidine of Soviet manufacture, progesterone batch 30368, emulsion type M, exposure 16 days). In the experimental group, 1000 labeled cells were counted in each animal.

Laboratory of Cytology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 10, pp. 92-94, October, 1970. Original article submitted April 16, 1970.

TABLE 1. Duration of S-Period in Epithelium of Reproductive Organs of Ovariectomized Mice and Mice Receiving 0.5 mg Progesterone Determined by the Double Label Method

	Epithelium of					
	uterine cavity		uterine glands		vagina	
	S	S with correction	S	S with correction	S	S with correction
Control	5.7 ±0.27	6	5.5 ±0.63	5.9	5.7 ±0.16	6.1
0.5 mg progesterone	3.8 ±0.16	4.1	3.3 ±0.2	3.5	4.2 ±0.06	4.4
P (control compared with experiment)	0.000		0.012		0.03	

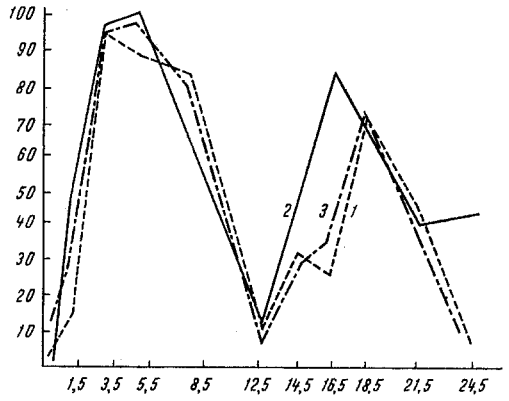

Fig. 1. Change in number of labeled mitoses in epithelium of uterine cavity (1), uterine glands (2), and vagina (3) at different times after injection of thymidine-H^3. Abscissa, hours after injection of thymidine; ordinate, percentage of labeled mitoses.

RESULTS

Series I. Determination of the duration of individual phases of the mitotic cycle from the curve of labeled mitoses.

In the epithelium of the uterine cavity several labeled early prophases were observed 30 min after injection of thymidine in one of the three animals. This indicates that the minimal G-period is 30 min. After 2.5 h, 50% of mitoses were labeled. It is generally accepted that this period is equal to 1/2 M+G_2. After 3.5 h, the number of labeled mitoses reached a maximum (Fig. 1), after which it remained high until 8.5 h, and subsequently it fell at 12.5 h. The second maximum of mitoses was observed at 18.5 h, after which their number fell at 24.5 h. The interval between the point on the curve corresponding to the time when the number of labeled mitoses was 50% and the point on the curve when it had fallen again to 50% after the maximum gives the mean S time. In the present experiment this was 7.5 h. The duration of the mitotic cycle, determined from the distance between two consecutive 50% levels of the increase in number of mitoses, was 15 h. In the epithelium of the uterine glands (Fig. 1, 2), the S-period also was 7.5 h, while the G_2 and T period was somewhat shorter: 1/2 M+G_2 =2 h and T=13 h. In the vaginal epithelium (Fig. 1, 3), S=8 h, 1/2 M+G_2 =2 h, and T=15 h. Doubling the dose of progesterone produced no significant changes.

It was impossible to plot a reliable curve of the mitotic cycle for the control animals, because very few mitoses were found (from 0 to 7) in all investigated organs. The S time in the actively dividing population, stimulated by progesterone, was thus 7.5-8 h when determined by this method. The time chosen for determination of the S-period covered the period of active DNA synthesis and of rapid increase in the number of mitoses. The method can give correct results if the duration of the S-period remains unchanged during this time interval (18-24 h after a single injection of the hormone). It therefore was decided to use the double label method to determine the S-period under these conditions, because the time required to determine the S-period by this method is much shorter. In addition, the double lable method enables the duration of the S-period to be determined in the presence of a minimal number of mitoses, since the number of synthesizing cells has been shown to be on the average 10 times greater than the number of mitoses. Another advantage of this method is that the duration of the S-period can be determined individually for each animal.

Series II. Determination of the duration of the S-phase by the double lable method.

In this series of experiments the time from the beginning of the experiment to ovariectomy was reduced to 7 days, because results have shown that a certain number of mitoses take place at this time, and accordingly synthesizing cells can also be found, whereas at later stages after ovariectomy, virtually no

mitoses are observed. An adequate number of synthesizing cells was found in most ovariectomized animals. However, at this period the data for the number of synthesizing cells and for the morphological picture of the uterine epithelium showed considerable scatter. Preparations without a background were chosen for counting. Nuclei were counted only if cut regularly. To begin with, labeled nuclei were counted in two animals which received only a large dose of thymidine (5 μCi). Besides strongly labeled nuclei in which the number of grains could not be counted, there were also on the average 12% of weakly labeled nuclei. These were probably nuclei incorporating label at the end of the S-period or nuclei commencing synthesis when the action of thymidine-H^3 was at an end.

In sections obtained from animals receiving two different doses of thymidine, the number of strongly labeled and weakly labeled nuclei was counted. Weakly labeled nuclei contained from eight to 16 grains per nucleus. The duration of the S-phase was calculated by the adopted formula (Table 1). It is clear from Table 1 that after injection of 0.5 mg progesterone, the duration of the S-phase in the epithelium of the uterine cavity was shortened by 2 h, in the epithelium of the uterine glands by 2.5 h, and in the vaginal epithelium by 1.7 h, compared with the control. A correction can be introduced into these calculations. Since no data are available concerning the uniformity of DNA synthesis in the investigated tissue, the 12% of weakly labeled cells obtained after injection of only 5 μCi thymidine-H^3 can be divided conventionally into 6% of cells which have passed through the S-period and 6% of cells which are starting DNA synthesis when the action of thymidine-H^3 comes to an end. In the present experiment the first 6% of cells were labeled by the preceding small dose of thymidine. The second 6% of cells, on the other hand, which had just started synthesis at the time when the action of thymidine was ending, can probably be subtracted from the total number of weakly labeled cells. After subtraction of these 6%, the duration of the S-phase is correspondingly increased (Table 1).

Because of the insufficient number of mitoses, it was impossible to determine the duration of individual phases of the mitotic cycle in the ovariectomized mice. Following a single injection of progesterone, the duration of the S-period in the epithelium of the reproductive organs, determined by the curve of labeled mitoses method, was 8-7.5 h. The time T=13-16 h.

It was found by the double label method that the S-period in mice receiving progesterone was shortened on the average by 2 h. However, this method gives underestimated values by comparison with the curve of labeled mitoses method. It should also be noted that the duration of the S-period, calculated by the curve of labeled mitoses method, was closer to results obtained by other workers.

LITERATURE CITED

1. O. I. Epifanova, Hormones and Cell Multiplication [in Russian], Moscow (1965).
2. N. A. Roslyakova, Byull. Éksperim. Biol. i Med., No. 5, 93 (1970).
3. H. Beato, B. Lederer, E. Boanol, et al., Exp. Cell Res., 52, 173 (1968).
4. P. Galand, F. Rodesch, F. Leroy, et al., Exp. Cell Res., 48, 595 (1967).
5. B. Peckham and W. Kiekhofer, Am. J. Obstet. Gynec., 83, 1021 (1962).
6. H. Quastler and F. G. Sherman, Exp. Cell Res., 17, 420 (1959).
7. S. D. Trasher, F. S. Clark, and D. R. Clarke, Exp. Cell Res., 45, 232 (1967).