PROLIFERATIVE POOL IN THE EPITHELIUM OF REPRODUCTIVE ORGANS OF OVARIECTOMIZED MICE AND MICE RECEIVING PROGESTERONE

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The proliferative pool was determined in the epithelium of the reproductive organs of mice. Among mice receiving a single injection of progesterone, a 100% proliferative pool was found in animals injected with thymidine-H^3 every 3 h for 27 h.

The term proliferative pool is used to describe the ratio between the number of proliferating cells and the total cell population. The method most commonly used to determine the proliferative pool is to inject thymidine-H^3 at shorter time intervals than the mean duration of the S-period for a total period of time which exceeds the mean duration of the total mitotic cycle for cells of the given population [1, 5]. Under these conditions all the proliferating cells of the population must be labeled. The reason why these conditions must be observed is that the duration of the individual phases of the mitotic cycle in some cells of the proliferating part of the population may differ very considerably from the mean time characteristic of most proliferating cells.

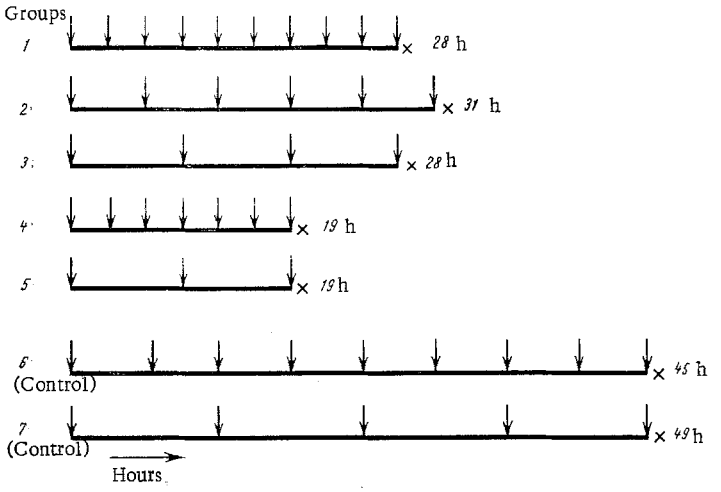

Fig. 1. Scheme of experiment to determine the proliferative pool. Groups 1 and 4) thymidine-H^3 injected every 3 h; groups 2 and 6) every 6 h; groups 3 and 5) every 9 h; group 7) every 12 h. Arrows pointing downward denote injections of thymidine-H^3; cross shows sacrifice of animals.

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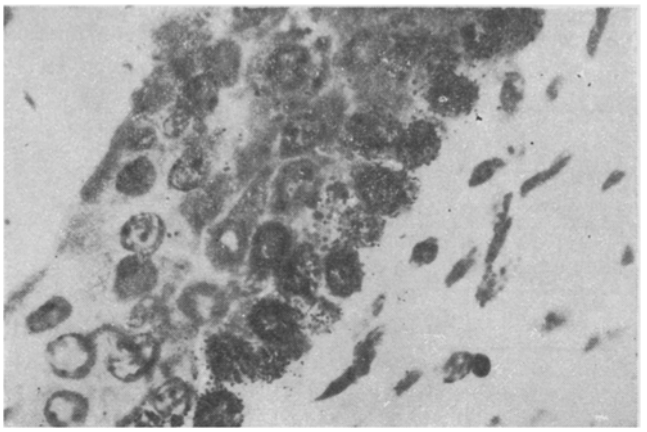

Fig. 2. Vaginal epithelium after injection of thymidine-H^3 every 3 h for 27 h.

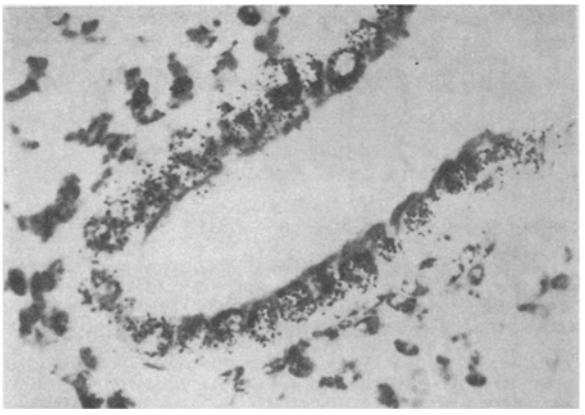

Fig. 3. Uterine epithelium after injection of thymidine-H^3 every 3 h for 27 h.

When determining the proliferative pool of the uterine epithelium of ovariectomized mice and mice receiving estrone, Epifanova [2] chose intervals between thymidine-H^3 injections and a total duration of the experiment which corresponded approximately to the duration of the S-period and the duration of the mitotic cycle. She obtained low values of the proliferative pool.

In a later investigation [4], thymidine-H^3 was injected at hourly intervals for a much longer period than the total duration of the mitotic cycle. In this way a 100% pool was obtained in the vaginal epithelium of rats receiving estrogen. Under the same experimental conditions a 100% pool was also obtained in the epithelium of the reproductive organs of ovariectomized mice [5].

All the investigations mentioned above were undertaken with estrogens. The effect of progesterone on the proliferative pool of the epithelium of reproductive organs remained uninvestigated. It likewise was not known whether such short intervals are necessary between the injections of thymidine-H^3, for oversaturation of the organism with thymidine could distort the results [1].

The object of the present investigation was to study these problems. In addition, an attempt was made, by choosing groups with different intervals between injections of thymidine and different total durations of the experiment, to obtain results enabling the duration of the S-period to be compared with the duration of the mitotic cycle in the epithelium of the reproductive organs of ovariectomized mice and of mice receiving progesterone.

EXPERIMENTAL METHOD

Experiments were carried out on female C57B1 mice. All the animals were ovariectomized 5 days before the beginning of the experiment and then divided into 7 groups. Group 1 included 8 mice, and all the other groups 6 mice. The animals of the first 5 groups received 1 mg progesterone, and 18 h later the mice of all groups started to receive thymidine-H^3 injections as follows: group 1 at intervals of 3 h for 27, group 2 at intervals of 6 h for 30 h, group 3 at intervals of 9 h for 27 h, group 4 at intervals of 3 h for 18 h, group 5 at intervals of 9 h for 18 h, group 6 (control) at intervals of 6 h for 48 h, and group 7 (control) at intervals of 12 h for 48 h (Fig. 1). The scheme of the experiment was chosen on the basis of the results of an earlier investigation to study the duration of phases of the mitotic cycle in mice receiving progesterone [3].

Thymidine-H^3 was injected in doses of 0.7 μCi/g body weight. The duration of exposure was 12 days. In the epithelium of the uterine cavity and in the stratum basale of the vagina 3000 cells were counted, and in the epithelium of the uterine glands 1000 cells.

TABLE 1. Determination of Proliferative Pool in Epithelium of Reproductive Organs of Mice

Group of mice	Duration of administration of thymidine-H^3 (in h)	Interval bet. thymidine-H^3 injections (in h)	No. of animals in group	Mean number of labeled cells (in percent)		
				epithelium of uterine cavity	uterine crypts	vaginal epithelium
1	28	3	8	100 (99—100)	78 (71—90)	100 (98—100)
2	31	6	6	85 (72—97)	71 (68—76)	93 (82—100)
3	28	9	6	44 (28—50)	32 (26—42)	67 (54—84)
4	18	3	8	70 (55—86)	34 (28—42)	77 (64—88)
5	18	9	6	30 (24—38)	25 (16—42)	58 (28—80)
6 (Control)	48	6	6	20 (10—51)	56 (39—80)	40 (18—62)
7 (Control)	48	12	6	11 (6—22)	21 (12—35)	16 (10—24)

Note. Minimal and maximal values for the group are given in parentheses.

EXPERIMENTAL RESULTS

In the animals of experimental group 1 (thymidine-H^3 was injected every 3 h for 27 h) a 100% proliferative pool was found in the epithelium of the uterine cavity and vagina (Figs. 2 and 3), while in the mice of group 2 (thymidine-H^3 was injected for about the same total period of time but at intervals of 6 h) the proliferative pool did not reach 100% but was still fairly high (85% in the epithelium of the uterine cavity and 93% in the vaginal epithelium). In the mice of group 3 (the intervals were chosen so as to be definitely longer than the S-period) the proliferative pool in the epithelium of the uterine cavity was 44%, and in the vagina 67%. In the animals of group 4 (thymidine-H^3 was injected, as in group 1, at intervals of 3 h but for a total period of 18 h) the proliferative pool in the uterine epithelium was 70% and in the vaginal epithelium 77%. In group 5 (intervals between injections of thymidine-H^3 were 9 h and the total period of administration was 18 h) the lowest value of the proliferative pool was obtained (Table 1).

The following conclusions can thus be drawn from the results of the experiments on the animals of group 1: in mice receiving 1 mg progesterone 5 days after ovariectomy, all cells in the epithelium of the uterine cavity and vagina took part in proliferation, and passed through the phase of DNA synthesis in the course of not more than 46 h (18 h from the time of injection of progesterone until the beginning of the experiment + 28 h of thymidine-H^3 administration). In addition, if the results are correlated with the conditions of administration of thymidine-H^3 in this group, it can be deduced that the duration of the S-period for all cells of the population was not less than 3 h, and the total duration of the mitotic cycle was less than 28 h.

Comparison of the results of the experiments on the mice of groups 1 and 2, for which the duration of thymidine-H^3 administration was the same, but the intervals between the injections were different, suggests that if thymidine-H^3 is injected at intervals of 6 h the proliferative pool does not reach 100% because a small proportion of cells in the population has a shorter S-period than 6 h.

In group 4 the intervals between injections were equal to the intervals in group 1, but the duration of thymidine-H^3 administration was 18 h. In this group 100% labeling was not achieved, probably for two reasons. First, some of the cells at the end of the S-period and beginning of the G_2-period at the time of the first injection of thymidine-H^3 did not start on the next period of synthesis during this time, and in that case the total duration of $G_2 + M$ of the previous cycle + G_1 of the new cycle was greater than 18 h. Second, some cells in the population have a long latent period after administration of progesterone, and after this time they still have not started on the phase of DNA synthesis.

In the epithelium of the uterine crypts a 100% proliferative pool was not obtained in any single group. The dose of progesterone chosen probably has an inhibitory action on certain epithelial cells of the uterine crypts. In all groups, an irregular distribution of labeled nuclei was observed in different sections through the crypts. In some sections all nuclei were labeled while in others only a few nuclei were labeled.

In the two control groups the proliferative pool was low. This may probably be because the duration of the mitotic cycle of this particular population was greater than 48 h, or that under the experimental conditions used (ovariectomy 5 days before the experiment) only part of the population passed through the mitotic cycle.

Comparison of the results obtained in the two control groups, in one of which (6) the time between the injections was 6 h, while in the other (7) it was 12 h, shows that in the first group the proliferative pool was twice as high as in the second. This may indicate that the duration of the phase of DNA synthesis in mice 5 days after ovariectomy is shorter than 12 h.

It follows from these results that after administration of progesterone to ovariectomized mice the duration of the mitotic cycle of the epithelial cells in the reproductive organs was significantly reduced.

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