A RADIOAUTOGRAPHIC STUDY OF THE INHIBITORY INFLUENCE OF REPEATED INJECTIONS OF ESTRONE ON CELL DIVISION OF THE UTERINE EPITHELIUM OF MICE

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Previously [1, 2] it has been shown that two injections of 2.5 μg of estrone raised the mitotic index in mouse uterine epithelium. A further six or more injections lead to a reduction in the number of cell divisions followed by a renewed increase when the hormone injections are discontinued. Published reports [12, 13] as well as our own observations suggest that this effect is not directly related to the amount of estrone injected. We have put forward the idea [3] that the adrenal complex (adrenaline – ACTH – adrenal cortex) is concerned in the regulation of the uterine epithelium when repeated estrone injections blocking one of the stages of interkenesis are given. Cessation of the estrone injections removes this block, and mitosis starts again.

The comparative rapidity with which high mitotic activity is reached after the hormone action has ceased suggested that the block occurs immediately before mitosis starts, i.e., in the premitotic period G_2. However, radioautographic study [4] has compelled us to change this idea. It was found that the mitotic cycle of the uterine epithelium is much shorter than would be supposed from experiments with cochichine [3], the whole period of DNA synthesis together with the premitotic period ($S + G_2$) is no more than a few hours. This suggests that the reduction in the number of mitoses of the uterine epithelium brought on by repeated estrone injections may result from the arrest of cells in one of the earlier stages of the mitotic cycle.

In the present work we have attempted to determine which stage of mitotic cycle of the uterine epithelium is most sensitive to the inhibitory reaction of repeated estrone injections.

EXPERIMENTAL METHOD

We used radioautography with thymidine-H^3.

Mice weighing 20-25 g.were castrated 20 days before the start of the experiment. The experimental animals received 2.5 μg estrone in 0.1 ml peach oil daily. The mice were divided into five groups of 3-4 animals each; the first group received only oil, the second, third and fourth groups were given 2,6 and 8 estrone injections, respectively. In the animals of the fifth group no estrone was given after the injections. Mice of groups 1-4 were killed 24 h after the last injection, and the fifth group were killed on the third day after the injections had been completed. Four h before they were killed all the mice received 15 μ curies of thymidine-H^3 in 0.1 ml physiological saline given intraperitoneally. At various times two experiments were carried out; one on females of the line CBA and the other on mice of an impure strain; also in the first experiment we used thymidine-H^3 of English origin, having a specific activity of 2.5 C/mM; in the second experiment we used Belgian thymidine (3C/mM).

The whole uterus was fixed in Carnoy's fluid. Autographs of the uterus were made on a liquid emulsion type M (NIKFI – motion picture and photography scientific research institute) by a method described previously [4]. On the autographs counts were made of the number of labeled and unlabeled mitoses (in 6,000 cells or more), the number of labeled and unlabeled cells (per 2,000 cells) of the epithelium lining the cavity of the uterus. The mitotic index was taken as the ratio of the number of mitoses to the total number of cells, and expressed as the number per thousand. The index of labeling was taken as the percentage of cells which were labeled. As a rule the percentage mitoses was calculated from a count 100 mitoses.

EXPERIMENTAL RESULTS

The results are shown in the figure of the form of three graphs which indicate the change of a number of indices in the epithelial uterine cells in relation to the measures taken. Graph A indicates the results of the first test which was more or less a pilot experiment; for technical reasons no results were obtained for animals of the fourth group (eight injections of estrone). Graphs B and C indicate the results of the second experiment carried out on a large number of animals. The initial values of the mean quantities used to construct the graphs B and C are given in the table.

From the results given in the figure and table it follows that the number of mitoses in the uterine epithelium increases considerably after two injections of estrone, falls after six injections, and remains at the same level when the number of injections is increased to eight. (See Fig., B); it rises once more after the injections have been discontinued (see Fig., A and C). These changes are associated with parallel and analogous alterations in the number of labeled cells, while the percentage of labeled mitoses and the number of silver granules per labeled cell shows practically no change throughout the whole experiment. The small increase of the number of silver granules after two injections of estrone is shown in Graph A is not significant.

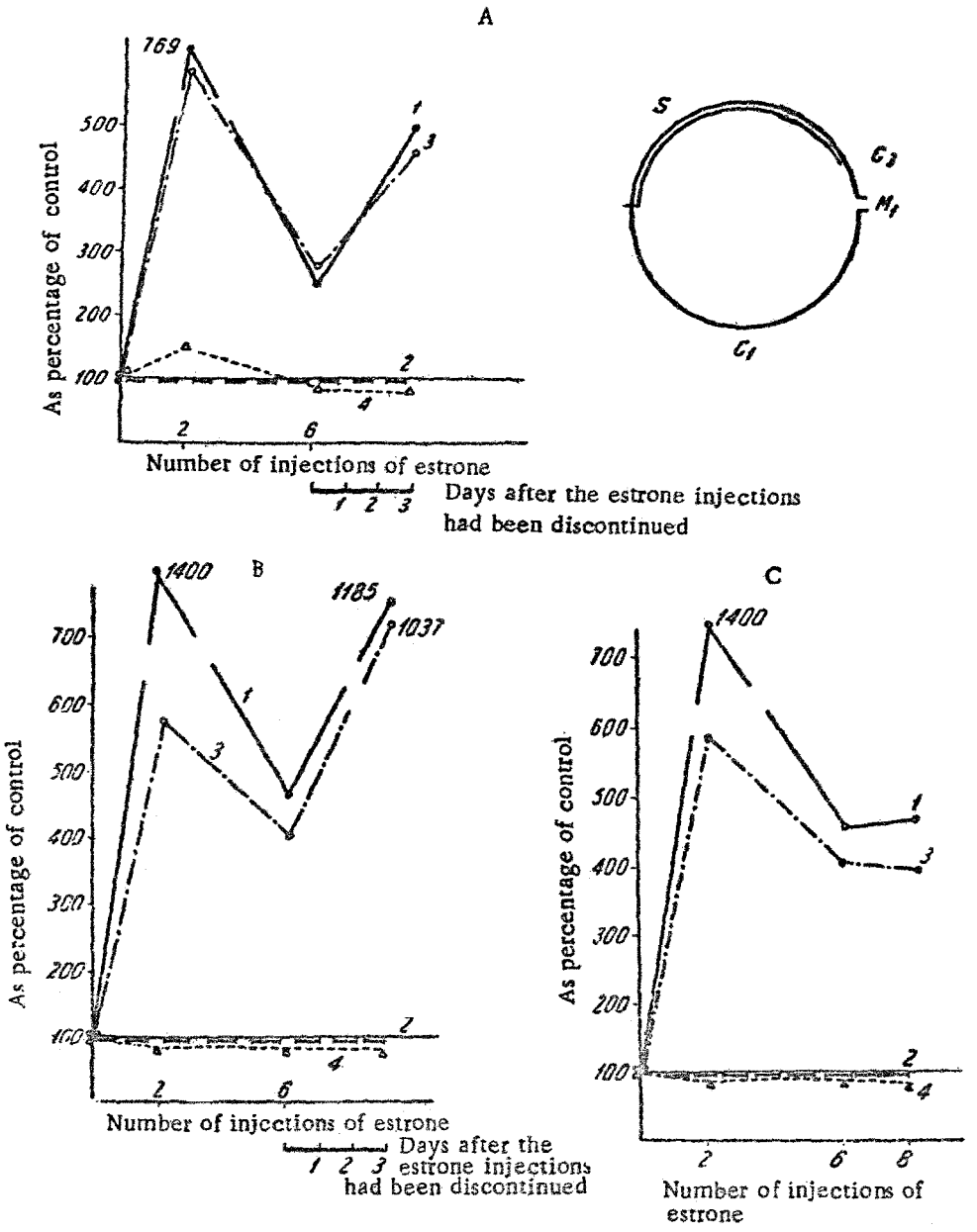
The absence of any change in the number of silver granules per labeled cell with repeated injections indicates that the intensity of DNA synthesis is then not reduced, because, if it were, there should be a fall in the number of silver granules. Consequently, there is no disturbance to the cells passing through the Period S. The fact that at all times there were practically no unlabeled mitoses in the uterine epithelium indicated that there was no block to prevent the cell emerging from period G_2. Were such a block to occur with repeated estrone injections, cells would accumulate in the uterine epithelium which had completed DNA synthesis, (because there is no fall in its rate) and which were ready to undergo division. If, after the end of the estrone injections the mitotic index had been restored, chiefly through the accumulation of such cells which had completed the period G_2, then removal of the block would increase the number of labeled mitoses in the uterine epithelium; however, no such event occurred. Cells which divide at the period of maximum reduction of the mitotic index begin mitosis after having completed the period S, and do so apparently without any delay in period G_2, because all of them contain the labels which were taken up before the onset of division.

There is, therefore, no disturbance of the range of DNA synthesis or of the passage of the cells through the post-synthetic period when repeated estrone injections are given. At the same time similar changes in the number of labeled cells, i.e., of cells synthesizing the DNA correspond to an increase or to a decrease in the number of mitoses throughout the experiment. Because, as we have said already, the rate of synthesis itself shows no change, the reduction of uterine epithelium mitotic activity occurring during repeated estrone injections can be related only to a reduction in the number of cells capable of undertaking DNA synthesis; in other words to a block of the transition of the cells from the presynthetic period D_1 to the S-period. We must note that this block is incomplete (see Fig., A and C, 3), because some of the cells continue to synthesize DNA, to pass through G_2 and to start mitosis, with the result that the mitotic index is not reduced to the original level. The cessation of the estrone injections removes the block G_1 – S, and the number of labeled cells again increases.

The results are in line with those obtained previously [4], where it was shown that the stimulus to cell division in the uterine epithelium brought about by two estrone injections is related chiefly to an increase in the number of

Mitotic Index (MI) Index of Labeling (I). Percentage of labeled mitoses and the mean number of silver granules per labeled cell in the uterine epithelium 4 h after the injection of thymidine-H^3 to estrone-treated mice.

Group of mice	Conditions of experiment	MI (per cent)	I (per cent)	Labeled mitoses (percentage)	Number of silver granules per cell
I	Controls	0.2 ± 0.02	0.8 ± 0.1	100	22.5 ± 4.5
II	Two injections of estrone	28.0 ± 7.9	4.7 ± 1.2	99	21.2 ± 1.5
III	Six injections of estrone	9.3 ± 3.3	3.3 ± 1.1	97	22.0 ± 1.1
IV	Eight injections of estrone . . .	9.8 ± 5.7	3.0 ± 1.4	100	20.3 ± 0.5
V	Estrone injections discontinued after the sixth	23.7 ± 3.4	8.3 ± 0.0	100	20.6 ± 0.7



Rates of mitotic division and DNA synthesis in the mouse uterine epithelium and the effect of repeated injections of estrones. A) First experiment; B) second experiment; injection of estrone throughout the experiment; C) second experiment: administration of estrone. After the sixth injection: 1) mitoses; 2) labeled mitoses; 3) labeled cells; 4) silver granules. The diagram in the upper right-hand corner illustrates the amount of each cycle; M_1) Mitoses, G_1) postmitotic (presynthetic). S) period of DNA synthesis, G_2) postsynthetic (premitotic), time medium) H^3 was injected four hours before the animals were killed.

cells capable of synthesizing DNA, whereas the rate of synthesis itself and the duration of the period G_2 do not change. Consequently estrone is able to induce profound reorganization of cells of the uterine epithelium without acting directly on the ultimate stages of interkinesis. The conditions of our experiments practically exclude the possibility of a direct suppression of cell division in the uterine epithelium by adrenaline secreted by the adrenals during the repeated estrone injections, because adrenaline is known to be a rapidly acting preparation inhibitor of mitotic division.

Nevertheless the results obtained do not eliminate the possibility that adrenaline might act indirectly, by suppressing the mitotic activity through activation of other components of the adrenal complex, for example ACTH [5], and consequently liberating hormones of the adrenal cortex able to suppress the activity of the estrone itself. Edgren and Calhoun [7, 8] quote abundant evidence of the inhibitory influence of many steroid hormones, including cortical steroids, on the growth of the uterus in rats and mice during the action of estrone. They therefore advanced the hypothesis that there is in the organism a system of "estrogenic buffers" of a steroid nature. In our case it was less likely that there was any indirect transformation of estrone which is an active estrogen, into an inactive form such as, for example, 17 alpha-estradiol which inhibits the ability of 17 beta-estradiol to activate the dehydrogenase of isocitric acid in the endometrium [14]. As other investigators have shown [9], in the endometrium and in many tissues estrone is able to transform only 17 beta-estradiol, and conversely, neither the one compound nor the other can be transformed either into estradiol or into 17 alpha-estradiol.

Finally there remains the important possibility of a direct action of estrone on many mechanisms regulating the several stages of formation of the precursors of DNA by a kind of negative feed-back [6]. Recently [10, 11] a considerable part has been assigned to estrogens as activators of the transdehydrogenases responsible for the transfer of hydrogen to the system of di- and tri-phosphopyridine nucleotides. The reduced trip-phosphopyridine nucleotide is in turn able to catalyze the reduction of ribose to desoxyribose, which in turn leads to an increase in the intracellular pool of DNA precursors; however, the large amount of triphosphates of certain other nucleosides formed suppress this reaction, and DNA synthesis ceases [6]. Here it is not only the absolute concentration of estrogens that is important, but rather the number of times a dose within a certain range is given [7, 8].

Therefore, the suppression of cell division in mouse uterine epithelium brought about by repeated injections of oestrones is due chiefly to failure of the cells to make the transition to DNA synthesis. If, however, the DNA synthesis has already begun, it continues at the normal rate and there is no hindrance to the cells starting mitosis.

SUMMARY

Spayed female mice received a daily injection of 2.3 γg of esterone. Intraperitoneal injection of 13 γ CH^3 thymidine was given to mice after a various number of estrone injections. The mice were killed 4 h later. The mitotic index of the uterine epithelium increased after two estrone injections, fell after six and rose again after the injections had been discontinued. The changes of mitotic activity were accompanied by similar changes in the number of labeled cells, whereas the percentage of labeled mitosis and the number of silver grains per labeled cell remained constant. These results indicate that depression of mitotic activity after prolonged estrone administration was due to inhibition of transition of the cells from the period G_1 to the S-period. At the same time the rate of DNA synthesis and the capacity of the cells to pass through the G_2 period to start mitosis remained unchanged.

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