EFFECT OF GROWTH HORMONE ON DURATION OF INDIVIDUAL PERIODS OF THE MITOTIC CYCLE IN STRATUM BASALE CELLS OF THE RAT ESOPHAGEAL EPITHELIUM

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The method of autoradiography using thymidine-H^3 with plotting of the "tagged mitoses" curve was used to determine the duration of periods of the mitotic cycle and the daily number of cells synthesizing DNA in the esophageal epithelium of intact rats and experimental rats preliminarily injected with growth hormone.

Administration of growth hormone shortened the periods of the mitotic cycle but did not affect the number of cells participating in reproduction during the 24-h period.

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The study of the effect of hormones on the mitotic cycle as a whole and its individual phases is of considerable interest because hormones are natural regulators of proliferative processes. So far, however, only the effect of estrogens on the mitotic cycle of cells in target organs has been studied [1, 6, 8, 9], and the action of other growth hormones on the mitotic cycle has not yet been investigated.

The object of these investigations was to study the effect of an excess of growth hormone (STH) on some parameters of the mitotic cycle of stratum basale cells in the esophageal epithelium of albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on 56 noninbred male albino rats with a mean body weight of 150 g. The experiments were carried out both with a single injection of thymidine-H^3 to determine the individual periods of the mitotic cycle from the curve showing changes in the percentage of labeled mitoses at various time intervals after injection of thymidine-H^3 [13], and with repeated injections to determine the total number of cells synthesizing DNA in the 24-h period.

Four days before injection of thymidine-H^3 all the experimental animals received STH in a dose of 1 mg in 0.5 ml physiological saline per rat daily for 4 days.

STH was obtained from the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR (the hormone was generously presented by E. A. Kolli) by Raben's method [14]. Contamination with biologically active thyrotropic hormone is excluded by this method. The activity was 1.2 units/mg.

On the day after the last injection of hormone the experimental group of animals received thymidine-H^3. Intact rats also receiving thymidine-H^3 acted as controls.

In experiments in which a single injection of isotope was given to all the animals at 6 a. m., thymidine-H^3 was injected intraperitoneally in a dose of 0.6 μCi/g body weight (Soviet thymidine-H^3, specific activity 1.4 Ci/mmole). The animals were sacrificed 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 25 h after injection of the isotope. Two control and two experimental animals were taken at each time.

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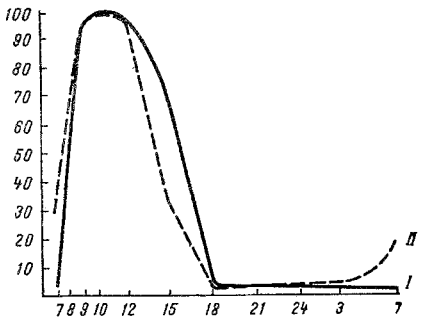

Fig. 1. Changes in percentage of tagged mitoses in esophageal epithelium of rats under normal conditions (I) and receiving STH (II). Abscissa, time of sacrifice; ordinate, percentage of tagged mitoses.

In the experiments with repeated injections, thymidine-H^3 was injected 5 times in the course of the 24-h period at intervals of 5 h. The animals were decapitated 1 h after the last injection. Each group contained 6 animals.

A piece of esophagus taken 1 cm from its junction with the stomach was fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5 μ in thickness were coated with type R (NIKFI) liquid nuclear emulsion (exposure 12-18 days). The emulsion was developed and the sections stained with Carazzi's and Mayer's hematoxylin. The duration of periods of the mitotic cycle was determined from the curve of "tagged mitoses" [13]. The number of tagged and untagged mitoses (as a rule 100) and the number of tagged nuclei were counted in the sections. The nucleus was regarded as tagged if at least 4 or 5 grains of silver were present above it.

To determine the index of tagged nuclei and the mitotic index, 3000 cells were examined. Statistical analysis of the results was carried out by the Fisher – Student method.

EXPERIMENTAL RESULTS

The first tagged mitoses in the stratum basale of the esophageal epithelium could be seen 1 h after injection of thymidine-H^3, corresponding to the minimum duration of the G_2 period. As the graph (Fig. 1) shows, the number of tagged mitoses in the esophageal epithelium of the control series was 50% 2 h after injection of thymidine-H^3. After reaching a peak, the number fell to 50% 10 h after injection of thymidine-H^3. This time interval lying at the 50% level between the ascending and descending parts of the curve measured 8 h, corresponding to the mean duration of the period of DNA synthesis.

The results are in general agreement with those obtained by other workers. According to Frankfurt [4], Cameron [7], and Pilgrim and Maurer [11], the duration of the period of DNA synthesis in the stratified squamous epithelium of the esophagus and proventriculus of mice is 7 h, and according to Wolfsberg [15] 10-11 h. The corresponding figures for rats are unknown.

Somewhat different results were obtained in the experimental groups of animals. A slightly larger number of tagged mitoses (30%) was observed 1 h after injection of thymidine-H^3. The number of tagged mitoses in the experimental group of animals 2 h after injection reached a mean level of 65%, evidence of a somewhat increased rate of passage of the cells through the G_2 period. It is clear from the graph that the duration of the G_2 period in the experimental animals was shortened to 1.5 h, while the S period was shortened from 8 h in the control to 6.5 h in the experimental series.

Under the influence of STH some shortening of the S period thus took place, i.e., DNA synthesis was shortened by 1.6 h or 20% of the total period of DNA synthesis.

The index of tagged nuclei (ITN) and the mitotic index (MI) were determined in a separate experiment. The mean value of ITN for the control mice was 8.3% and the mitotic index 11.9%. ITN for the experimental animals was 23.7%, i.e., almost 2.5 times higher than in the control. MI was 13.2%.

Investigations have recently been published showing that the number of cells synthesizing DNA fluctuates during the 24-h period [2, 3, 5, 10, 12], and it may thus be postulated that the observed changes in ITN in the experimental group were due to a shift in the time of the maximum of the number of cells synthesizing DNA.

To clarify the meaning of these results an additional experiment was carried out in which repeated injections of thymidine-H^3 were given in the course of the 24-h period in order to determine the daily number of cells synthesizing DNA in the experimental and control series. ITN for the group of control animals was 65.9%, and for the group of experimental animals 61.2%. The differences are not statistically significant.

It can thus be concluded from these results that in the presence of an excess of STH the G_2 period is shortened from 2 to 1.5 h and DNA synthesis is speeded up by 1.6 h (on the average by 20%). This change

TABLE 1. Various Parameters for the Experimental and Control Groups

Group	Period S	Period G_2	INT (in %)	MI (in %)	Diurnal INT (in %)
Control	8	2	8.3	11.94	65.9
Experimental	6.4	1.6	23.7	13.7	61.2

in the duration of the period of DNA synthesis in turn changes the diurnal periodicity of the number of cells synthesizing DNA, as is confirmed by the different values of the tagging index in the experimental and control series.

It can also be concluded from the results of the experiments with repeated injections of thymidine-H^3 that, under the influence of STH, no increase is found in the number of cells synthesizing DNA in the course of the 24-h period, i.e., there is no increase in the size of the proliferative pool.

The increase in proliferative activity in organs is ultimately determined by two mechanisms: the number of cells participating in the mitotic cycle and the duration of the mitotic cycle. Under the influence of STH, only the passage of the cells through the mitotic cycle is speeded up.

It has thus been shown that, besides estrogens, STH can also stimulate the proliferative activity of cells, and not only in the target organs.

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