EFFECT OF SOMATOTROPIC HORMONE ON DURATION OF DNA SYNTHESIS DURING THE MITOTIC CYCLE

Yu. V. Bardik

UDC 612.015.348:547.363.32].
019.36.014.46:612.433.65

The duration of the period of DNA synthesis in experimental and control animals was studied by an autoradiographic method using thymidine-H^3 and plotting changes in the percentage of labeled mitoses. Growth hormone was found to modify the duration of DNA synthesis in the mitotic cycle of esophageal and jejunal epithelium of albino rats.

* * *

After establishment of the periodicity of DNA synthesis and anunciation of the theory of the mitotic cell cycle [8], data have gradually accumulated in the literature concerning the duration of DNA synthesis in cells of various organs and tissues [2, 4-6, 9, 11-20]. Pilgrim and Maurer [14, 15] postulated that the duration of DNA synthesis is constant. Their work was followed by studies of the effect of estrogens on DNA synthesis in cells of hormonally dependent tissue [1, 3, 7, 10]. It was shown that estrogens significantly shorten the duration of DNA synthesis in target organs. It was therefore decided to investigate the effect of other hormones, stimulating growth and proliferation, on the duration of DNA synthesis.

The object of the present investigation was to study the action of somatotropic hormone on the duration of the period of DNA synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 88 noninbred male albino rats of the same batch. The animals were divided into four groups, 22 in each: 1) hypophysectomized animals, 2) hypophysectomized animals receiving somatotropic hormone (STH), 3) control animals, 4) intact animals receiving STH. The pituitary was removed from animals weighing 50-60 g by the ordinary parapharyngeal method. Two weeks later (when the weight of the intact animals had reached 150-160 g), the rats of groups 2 and 4 began to receive STH*. STH, produced by Raben's method [18], with an activity of 1.2 units/mg, was injected into the animal in a dose of 1 mg in 0.5 ml physiological saline daily for 4 days. On the day after the last injection, all the animals received an intraperitoneal injection of thymidine-H^3 (Soviet preparation, specific activity 1.4 Ci/mmole) at 6 A.M. in a dose of 0.6 μCi/g body weight. The rats were sacrificed 1, 2, 3, 4, 6, 9, 12, 15 and 18 h after injection of the label. Two control and two experimental animals were used at each time. At autopsy the body weight and the weight of the tests and adrenals were determined, and on this basis

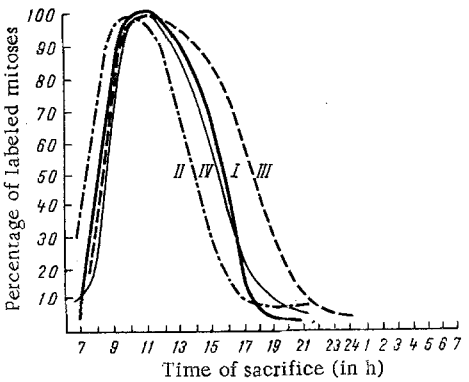

Fig. 1. Curves showing change in percentage of labeled mitoses in esophageal epithelium of rats receiving various treatments. I) Control; II) control + STH; III) hypophysectomy; IV) hypophysectomy + STH.

*STH was provided by E. A. Kolli (Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR).

Laboratory of Histophysiology, Institute of Experimental Biology, 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. 68, No. 8, pp. 106-108, August, 1969. Original article submitted July 24, 1968.

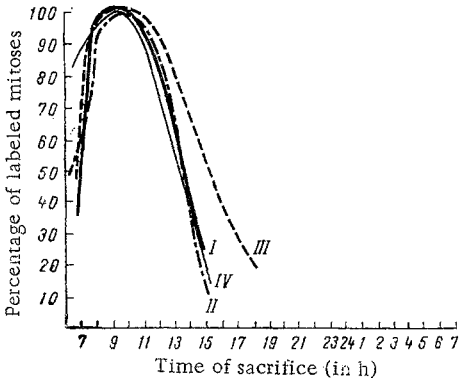

Fig. 2. Curves showing change in percentage of labeled mitoses in epithelium of jejunal crypts after various treatments. Legend as in Fig. 1.

the completeness of the hypophysectomy was estimated. Only those animals from which the pituitary was completely removed were used in the investigation. A piece of esophagus taken at a distance of 1 cm from its junction with the stomach, and a piece of jejunum taken at a distance of 10-12 cm from the pylorus, were fixed in Carnoy's fluid and embedded in paraffin wax. To obtain autoradiographs, transverse sections were cut to a thickness of 5 μ and coated with liquid type R nuclear amulsion. The sections were kept in refrigerator at 4° for 19 days, after which they were developed, fixed, stained with Carazzi's hematoxylin, and mounted in balsam. The duration of the period of DNA synthesis was determined from the change in percentage of labeled mitoses at various time intervals after a single injection of thymidine-H^3 [17]. The number of labeled and nonlabeled mitoses in the sections was counted (as a rule between 70 and 100).

EXPERIMENTAL RESULTS

The first labeled mitoses in the esophageal epithelium were observed 1 h after injection of the isotope, corresponding to the minimal duration of the G_2 period. As the graph (Fig. 1) shows, the number of labeled mitoses in the esophageal epithelium of the control series was 50% 2 h after injection of thymidine-H^3, after which it rose to a maximum and then fell again to 50% after 10 h.

The results agree in general with those obtained by other workers. According to Frankfurt [2], Cameron [5], and Pilgrim and Maurer [14, 15], the duration of DNA synthesis in the mitotic cycle for stratified squamous epithelium of the esophagus and forestomach of mice is 7 h, while according to Wolfsberg [20] it is 10-11 h.

The duration of S-period in the group of hypophysectomized animals was increased to 9.5 h, i.e., DNA synthesis was slowed by 1.5 h, amounting to 20% of the duration of synthesis. In the group of hypophysectomized animals receiving STH, the changes produced by hypophysectomy were normalized. In the group of animals with a raised body level of growth hormone (group 4) the S-period was shortened to 6.4 h.

Results of similar character were obtained in the epithelial cells of the jejunal crypts of the hypophysectomized rats (Fig. 2). The duration of the S-period in the group of control animals, determined from the curve of labeled mitoses, was 6.5 h, in agreement with data in the literature [4]. In the hypophysectomized rats the duration of the S-period was increased by 1 h. Injection of STH into hypophysectomized rats restored the normal duration of the period of DNA synthesis. In intact animals, however, this hormone did not change the duration of DNA synthesis.

It can be concluded from these results that under the influence of STH the period of DNA synthesis is shortened, and that this shortening takes place up to certain critical limits, i.e., to 6.5 h according to these observations, for the action of STH on cells in which the duration of the S-period was 6.5 h (jejunum) did not produce any change in the duration of DNA synthesis. After hypophysectomy the S-period was lengthened, presumably on account of the absence of STH, because injection of this hormone into hypophysectomized rats restored the normal duration of DNA synthesis. Lengthening and shortening of the S-period, according to Bresciana [3], are connected with the degree of synchronization of DNA synthesis in the cell genome. After hypophysectomy it is possible that DNA synthesis becomes to some extent asynchronous, and this is evidently due to the absence of STH. If the level of this hormone in the body is increased, so also is the degree of synchronization of DNA synthesis. Hence, not only estrogens, but also growth hormone are capable of modiflying the duration of DNA synthesis in cells. The view of Pilgrim and Maurer [15, 16], that the duration of DNA synthesis during the mitotic cycle is stable, is evidently relative and requires re-examination.

LITERATURE CITED

1. O. I. Epifanova, Hormones and Cell Multiplication [in Russian], Moscow (1965).
2. O. S. Frankfurt, Tsitologiya, No. 2, 175 (1967).
3. F. Bresciani, Exp. Cell Res., 38, 13 (1965).

4. A. B. Cairnie, L. F. Lamerton, and G. G. Steel, Exp. Cell Res., 39, 528 (1965).
5. I. L. Cameron and R. C. Greulich, J. Cell Biol., 18, 314 (1963).
6. S. M. Cattaneo, H. Quastler, and F. G. Sherman, Nature, 190, 923 (1961).
7. P. Galand, F. Rodesch, F. Leroy, et al., Exp. Cell Res., 48, 595 (1967).
8. A. Howard and S. R. Pelc, Heredity, 6, Suppl.,261 (1953).
9. E. Koburg and E. Maurer, Biochim. Biophys. Acta, 61, 229 (1962).
10. J. L. Ladinsky and B. M. Peckham, Exp. Cell Res., 40, 447 (1965).
11. L. G. Lajtha, in: The Kinetics of Cellular Proliferation, New York (1959), p. 173.
12. S. Lesher, R. S. Fry, and H. J. Kohn, Exp. Cell Res., 24, 334 (1961).
13. M. Lipkin and H. Quastler, J. Clin, Invest., 41, 141 (1962).
14. C. Pilgrim and W. Maurer, Naturwissenschaften, 49, 544 (1962).
15. C. Pilgrim and W. Maurer, Exp. Cell Res., 37, 183 (1965).
16. C. Pilgrim, W. Lang, and W. Maurer, Exp. Cell Res., 44, 129 (1966).
17. H. Quastler and F. C. Sherman, 17, 420 (1959).
18. M. S. Raben, Science, 125, 883 (1957).
19. F. G. Sherman, H. Quastler, and D. R. Wimber, Exp. Cell Res., 25, 114 (1961).
20. M. F. Wolfsburg, Exp. Cell Res., 35, 119 (1964).