CELL PROLIFERATION IN THE BASAL LAYER OF THE STRATIFIED SQUAMOUS EPITHELIUM OF THE ESOPHAGUS IN HYPOPHYSECTOMIZED RATS

Yu. V. Bardik

UDC 612.315.014.2:612.6]-06:612.432-089

The method of autoradiography using thymidine-H_3 was used to determine the duration of periods of the mitotic cycle and the number of cells synthesizing DNA during the 24-h period in intact and hypophysectomized rats. As a result of hypophysectomy the various periods of the mitotic cycle are lengthened and the number of cells taking part in reproduction is reduced.

* * *

Several studies of the effect of hypophysectomy on mitotic activity have been published but their results are not always consistent [4, 6, 9]. The most interesting results in this respect were obtained by Liozner and co-workers [1], who investigated mitotic activity in various organs of rats two weeks after hypophysectomy and found a reduction in mitotic activity in the epithelium of the liver, salivary glands, and esophagus. With the development of the study of mitotic cycles [5] resulting from the use of autoradiography, processes preceding mitosis can now be studied.

An interesting subject for investigation is thus the effect of hypophysectomy on the individual periods of the mitotic cycle and analysis of the number of cells synthesizing DNA, reflecting proliferative activity in hypophysectomized rats.

EXPERIMENTAL METHOD

Experiments were carried out on 56 noninbred male rats with a mean body weight of 50-60 g. Hypophysectomy was performed by the usual parapharyngeal method. The rats of the experimental and control groups received thymidine-H^3 18 days after the operation. The controls were intact rats of the same breed, attaining a body weight of 150 g during the experiments.

Thymidine-H^3 was injected either once, to determine the individual periods of the mitotic cycle from a curve of "tagged mitoses" [8] or repeatedly, to determine the total number of cells synthesizing DNA in the course of the 24-h period (thymidine-H^3 of Soviet manufacture, with specific activity 1.4 Ci/mmole, was used). In the experiments with a single injection, all the animals of the experimental and control groups received thymidine-H^3 intraperitoneally in a dose of 0.6 μCi/g body weight at 6 a.m. The animals were sacrificed 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 25 h after injection of thymidine-H^3. Two control and two experimental rats were taken at each time. In the experiments in which repeated injections of thymidine-H^3 were given, 5 injections were given at intervals of 5 h in the course of the 24-h period. The animals were sacrificed by decapitation 1 h after the last injection. Each group consisted of 6 rats. After decapitation, the body weight of the animals and the weight of their testes and adrenals were determined, demonstrating the completeness of hypophysectomy. Only those animals from which the pituitary had been completely removed were used in the experiments.

A piece of esophagus taken 1 cm from its entrance into the stomach was fixed in Carnoy's fluid and embedded in paraffin wax. Sections, 5 μ in thickness, were coated with type R liquid nuclear emuslion (NIKFI) and exposed for 12-18 days. The emulsion was developed and the sections stained with Carazzi's and Mayer's hematoxylin. To determine the duration of the periods of the mitotic cycle from the curve showing the change in percentage of "tagged mitoses" [8], the "tagged and untagged mitoses" (as a rule

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 N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 118-120, September, 1969. Original article submitted November 12, 1968.

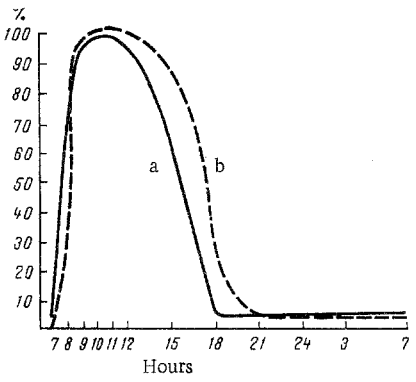

Fig. 1. Curves showing changes in percentage of "tagged mitoses" in esophageal epithelium of intact (a) and hypophysectomized (b) rats. Abscissa, time of decapitation of animals; ordinate, percentage of "tagged mitoses."

100) were counted in the sections. To determine the index of nuclear tagging (INT) and the mitotic index (MI), 3000 cells were examined. The nucleus was regarded as tagged if 3 or 4 silver grains were present above it.

EXPERIMENTAL RESULTS

The first "tagged mitoses" were found in the basal layer of the esophageal epithelium 1 h after injection of thymidine-H^3, corresponding to the minimal duration of the G_2 period. The number of "tagged mitoses" in the esophageal epithelium of the control animals was 50% 2 h after injection of thymidine-H^3 (Fig. 1). After reaching this peak, the number fell to 50% 10 h after injection of thymidine-H^3. The time interval corresponding to the level of 50% of the peak value of the curve was 8 h, corresponding to the mean duration of the period of DNA synthesis [8].

These results agree on the whole with those obtained by other workers. According to Frankfurt [2], Cameron [3], and Pilgrim and Maurer [7], the duration of the period of DNA synthesis for stratified squamous epithelium of the esophagus and forestomach of mice is 7 h, while according to Wolfsberg [10], it is 10-11 h. No corresponding data for rats could be found in the literature.

In hypophysectomized animals solitary "tagged mitoses" also were found 1 h after injection of thymidine-H^3. Their mean number 2 h after injection was 30%. This indicated some increase in duration of the G_2 period. The number of "tagged mitoses" increased to a maximum at 9 a.m., i.e., 3 h after injection of thymidine-H^3. A decrease in the height of the curve to 50% was observed 12 h after injection of thymidine-H^3, i.e., the period of DNA synthesis was increased on the average to 9.5 h.

The values of INT and MI were determined in the same sections. To do this, the rats sacrificed at the first 5 periods were combined into groups of 8-10 animals each. INT for the control animals was 37% and MI 5.4%, while the corresponding values for the hypophysectomized animals were 135.8% and 5.4%.

To shed light on these apparently paradoxical results, a further experiment was carried out with repeated injection of thymidine-H^3 during the 24-h period in order to determine the number of cells synthesizing DNA during this period in experimental and control animals. INT for the control animals was 65.9%, compared with 37.4% for the hypophysectomized rats. The differences are statistically significant.

It can thus be concluded from these results that, following hypophysectomy there is an increase in the G_2 period and also in the duration of the period of DNA synthesis, on the average by 20% (1.5 h). The fact that this does not agree with the values of the tagging index determined in animals of the control and experimental groups during the morning after a single injection of thymidine-H^3 is presumably evidence that the diurnal rhythm of INT does not coincide in the animals of these groups.

It can be concluded from the results of the experiments with repeated injections of thymidine-H^3 that, as a result of hypophysectomy, there is a significant decrease in the number of cells synthesizing DNA during the 24-h period, or in other words, the proliferative pool is reduced.

Proliferative activity in organs is determined by two mechanisms: the number of cells taking part in reproduction and the duration of the mitotic cycle. After hypophysectomy there is a marked change in proliferative activity as a result both of lengthening of the periods of the mitotic cycle and, probably, of the mitotic cycle as a whole, and also of a decrease in the number of cells participating in reproduction.

The results of this investigation agree with those obtained by Liozner and co-workers [1], who showed in particular that the mitotic activity in the esophageal epithelium of hypophysectomized rats falls by 45%.

LITERATURE CITED

1. L. D. Liozner, N. S. Artem'eva, A. G. Babaeva, et al., Byull. Éksperim. Biol. i Med., No. 8, 77 (1962).
2. O. S. Frankfurt, Tsitologiya, No. 2, 175 (1967).

3. J. L. Cameron and R. C. Greulich, J. Cell. Biol., 18, 3 (1963).
4. F. J. Ebling, J. Endocrinol., 9, No. 3, 31 (1953).
5. A. Howard and S. R. Pelc, Heredity, 6, Suppl. 261 (1953).
6. C. P. Leblond and R. Carriere, Endocrinology, 56, 261 (1955).
7. C. Pilgrim and W. Maurer, Exp. Cell Res., 37, 183 (1965).
8. H. Quastler and F. G. Sherman, Exp. Cell Res., 17, 420 (1959).
9. H. Teir and I. Carpen, Acta Path. Microbiol. Scand., 47, 291 (1959).
10. M. F. Wolfsberg, Exp. Cell Res., 35, 119 (1964).