EFFECT OF HYDROCORTISONE ON DURATION OF PERIODS OF MITOTIC CYCLE IN ESOPHAGEAL EPITHELIUM OF MICE

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The method of autoradiography with thymidine-H^3 was used to study the action of hydrocortisone on the duration of individual periods of the mitotic cycle in the mouse esophageal epithelium. A large dose of hydrocortisone caused a marked increase in the duration of DNA synthesis and prolonged the mitotic cycle as a whole. A small dose of hydrocortisone had no effect on the duration of the periods of the mitotic cycle in the stratum basale of the squamous epithelium of the mouse esophagus.

Previous work has shown that cortisone is a "less universal inhibitor" of cell proliferation than adrenalin [1, 2, 5, 11, 20]. According to Alov's findings [1], if repeated injections of cortisone are given, the rate of cell division is depressed only in the corneal epithelium, the epidermis, and the lingual epithelium of animals, and the mitotic index remains unchanged in the epithelium of the esophagus, small intestine, and other organs. The reason for this action is not yet clear.

Until now considerable attention has been given to the study of the action of hormones such as the estrogens, which shorten the mitotic cycle [6, 14, 15], whereas the study of the adrenal hormones is only just beginning [7, 13].

It was therefore interesting to study the action of hydrocortisone on the duration of individual periods of the mitotic cycle of the esophageal epithelial cells.

EXPERIMENTAL

Experimental male mice (120) of line C57B16J,* with a mean weight of 20 g, were divided into three groups: group 1 received 0.1 mg hydrocortisone per animal intramuscularly; group 2 received 3 mg hydrocortisone per animal; group 3 acted as the control.

Hydrocortisone injections were given at 5 a.m., and 1 h later (at 6 a.m.) the animals of all three groups received a single injection of thymidine-H^3 (Soviet preparation, specific activity 1.4 Ci/mmole) in a dose of 0.7 μCi/g body weight. The mice were sacrificed 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, 25, 28, and 31 h after injection of thymidine-H^3, three animals at each time. Material was fixed in Carnoy's fluid. Sections through the esophagus, 5 μ in thickness, were coated with type M emulsion (NII Khimproekt). The exposure time was 2 weeks. Autoradiographs were stained with Carazzi's hematoxylin. The percentage of labeled mitoses relative to their total number was determined in the esophageal epithelium of the mice. In addition, in animals sacrificed at the first four times after injection of thymidine-H^3, the index of labeled nuclei was calculated.

*The same animals were used in S. S. Laguchev's experiments [7].

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EXPERIMENTAL RESULTS

The first labeled mitoses in the group of control animals appeared 1 h after injection of thymidine-H^3 (Fig. 1). This time can be taken as the shortest duration of the C_2 period, and its mean duration, the time of detection of 50% of labeled mitoses, was 2 h after injection of thymidine-H^3. During the next 2 h the percentage of labeled mitoses continued to increase (84, 95.6%), after which it gradually decreased, to reach a value of 13.8% 12 h after injection of thymidine-H^3. Determination of the duration of the S period at the 50% level of the curve of labeled mitoses gave values of 8.5 or 7.5 h, if the time taken for circulation of the thymidine-H^3 in the blood is subtracted. Labeled mitoses had almost completely disappeared 27 h after injection of thymidine-H^3, but 31 h after injection a new, although small, wave of mitoses appeared (the percentage of labeled mitoses was 22). From these results a rough estimate can be obtained of the duration of the mitotic cycle of the mouse esophageal epithelial cells, namely 29-30 h. Accordingly, the duration of the C_1 period can be taken to be 19 h.

In mice receiving a small dose of hydrocortisone (0.1 mg per mouse), no appreciable changes in the percentage of labeled mitoses were found. All the values were very close to those observed in the control animals. The duration of periods of the mitotic cycle in these animals can therefore be taken as equal to that in the controls.

In animals receiving what was undoubtedly a large dose of hydrocortisone (3 mg per mouse) the results were completely different: whereas the first labeled mitoses also appeared after 1 h, their percentage after only 2 h was 88.5, and the mean duration of the C_2 period can thus be taken as 1.5 h, i.e., 30 min shorter than when the small dose was given (Fig. 1). The percentage of labeled mitoses remained high 3, 4, 6, 9, and 12 h after injection of thymidine-H^3, and not until 21 h after injection did it fall to 4.9. The duration of the period of DNA synthesis in the animals receiving the large dose of hydrocortisone can therefore be taken as 14-15 h. Consequently, injection of the large dose of hydrocortisone increased the duration of the S period by 6-7 h. Naturally because the period of DNA synthesis was lengthened in this manner, no second wave of labeled mitoses could be observed, and it was therefore impossible to calculate the duration of the other periods of the mitotic cycle in this group of animals.

The number of cells in the stage of DNA synthesis was estimated from the index of labeled nuclei (ILN). The results of calculations showed no significant differences between the mice of the control and experimental groups. For instance, the ILN of the control animals was 11.8%, compared with 10.7 and 11% respectively for animals receiving the small and large doses of hydrocortisone.

These results for the duration of the period of DNA synthesis in the esophagus of the control mice agree with those obtained by other workers, who give a figure of 7-8 h [3, 4, 9, 10, 12, 16-19]. The duration of the other periods and of the whole mitotic cycle for cells of stratified squamous epithelium also lie within the limits of the figures obtained by other workers [9].

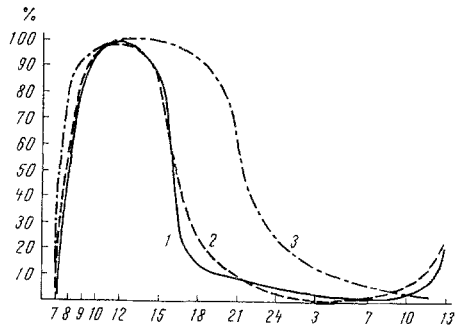

Fig. 1. Changes in percentage of labeled mitoses in esophageal epithelium of mice after injection of hydrocortisone. 1) Control; 2) small dose of hydrocortisone; 3) large dose of hydrocortisone. Abscissa, time of decapitation of animals; ordinate, percentage of labeled mitoses.

According to the present findings, a single, though definitely unphysiological, dose of hydrocortisone (3 mg) causes sharp changes in the duration of the periods of the mitotic cycle: the duration of DNA synthesis is almost doubled (from 7-8 h to 14-15 h). These findings can be compared with the results of Frankfurt's experiments [13], showing that only large doses of hydrocortisone (50 μg/g) can lead to changes in the ILN of the stratified squamous epithelium of the mouse proventriculus. The results for the action of hydrocortisone on the esophageal epithelium, described above, must be compared with results for its action on the epithelium of the jejunum of the same animals [8]. Laguchev showed that both large and small doses of hydrocortisone produce definite disturbances of the temporal parameters of the mitotic cycle in the epithelium of the jejunum. However, the more severe disturbances were observed following administration of a dose of 3 mg of the animals. The duration of the S period, for instance, was increased by approximately 2 h, while the duration of the C_1 period was almost doubled (7.5 h compared with 3.4 h).

It follows from these findings that injection of the same dose of hydrocortisone may act differently on the mitotic cycle of different organs, but there is no doubt that a large dose of hydrocortisone prolongs both the period of DNA synthesis and the duration of the mitotic cycle as a whole.

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