EFFECT OF L-THYROXINE ON DURATION OF PERIODS OF THE MITOTIC CYCLE

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The duration of the G_2- and S-periods of the mitotic cycle of hepatocytes and of the corneal epithelial cells of male rats and the index of labeled nuclei were studied autoradiographically by means of thymidine-H^3 before (control) and after administration of L-thyroxine. Administration of L-thyroxine for 2 days led to a substantial increase in the number of cells synthesizing DNA and shortened the duration of the synthetic period in both organs studied. The duration of the postsynthetic period was unchanged by the action of the hormone.

Investigations have shown that thyroid hormones, when administered in certain doses, can stimulate mitotic activity [1, 9].

Romanov et al. [2] found a highly significant increase in the mitotic index of the corneal epithelium, the bone marrow and liver 1 h after a single injection of L-thyroxine into rats. They concluded that this action of the hormone was explained by large numbers of cells of the G_2-population starting to undergo mitosis.

The objects of the present investigation were: 1) to show by the use of thymidine-H^3 whether, if mitotic activity is increased by administration of L-thyroxine, the mitoses are labeled or whether most of them do not contain label. This would confirm the existence of a G_2-population in the liver; 2) to examine the effect of L-thyroxine on the duration of certain periods of the mitotic cycle of cells of the liver parenchyma and corneal epithelium.

EXPERIMENTAL METHOD

The experiments of series I conformed to the basic conditions of the experiment of Romanov et al. [2]. Male rats (150 g in weight) received a single dose of 10 or 100 μg L-thyroxine (Gedeon-Richter, Hungary) at 8 A.M. Intact rats served as the control. Five rats from the experimental and control series were sacrificed 15, 30, and 45 min after the injection. Unlike in Romanov's experiments, all the rats received an intraperitoneal injection of thymidine-H^3 (225 μCi per animal) on two occasions, 9 and 4 h before injection of the hormone. The mitotic index and percentage of labeled mitoses were determined in paraffin sections (5 μ) of the liver and cornea, stained with Carazzi's hematoxylin and treated by the usual histoautoradiographic method.

The experiments of series II were carried out on 140 male rats weighing 150 g. Since the action of thyroid hormones becomes apparent after a long latent period, L-thyroxine was injected subcutaneously into the experimental rats once daily in a dose of 100 μg per animal for 2 days. Intact rats were used as the control (the thyroxine was dissolved before use in 0.001 N KOH solution).

At 7 A.M. on the 3rd-4th day, the experimental and control rats were given a single injection of thymidine-H^3 in a dose of 250 μCi per animal. The control and experimental rats were then sacrificed in

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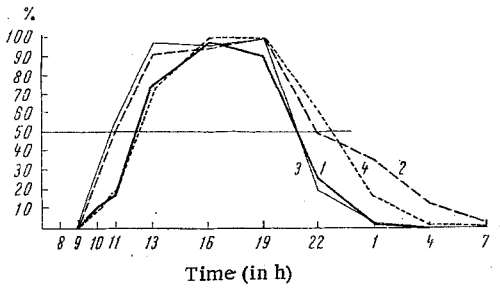

Fig. 1. Changes in percentage of labeled mitoses in corneal epithelium and liver of rats under normal conditions and after administration of L-thyroxine. Abscissa, time of sacrifice of animals (in h); ordinate, percent of labeled mitoses; 3) cornea (L-thyroxine); 2) cornea (control); 1) liver (L-thyroxine); 4) liver (control).

groups of 3 at intervals of 30 min and 1, 2, 4, 6, 9, 12, 15, and 18 h. The eyes and pieces of liver were fixed in Carnoy's fluid and the material was embedded in paraffin wax. Sections were cut to a thickness of 5 μ from the cornea and liver, stained with Carazzi's hematoxylin, coated with type R liquid radiosensitive emulsion (NIIKHIMFOTO), and kept in darkness for 2 weeks.

For each time in the control and experimental series the mean percentage of labeled mitoses was determined, and the durations of the G_2- and S-periods were measured by the method of Quastler and Sherman [7].

EXPERIMENTAL RESULTS

Contrary to expectations, in the experiments of series I no increase in the mitotic index was observed in the liver and corneal epithelium 15, 30, or 45 min after injection of either the small or the large dose of L-thyroxine. Only single mitoses were unlabeled in the control and experimental series. One possibility was that the activity of the hormone was low. However, tests by various methods confirmed that its activity was sufficiently high.

In the experiments of series II injection of L-thyroxine for 2 days caused a significant increase in the index of labeled nuclei of the hepatocytes and cells of the basal layer of the corneal epithelium compared with the control. The mean index of labeled nuclei for the liver was 2.5% in the control and 5.3% in the experimental group, while the corresponding figures for the corneal epithelium were 3 and 6.5%. The change in percentage of labeled mitoses is shown in Fig. 1. L-Thyroxine evidently had no effect on the G_2-period in the organs examined. In both organs, in both experimental and control series, the first labeled mitoses were observed 3 h after injection of thymidine-H^3. The mean duration of the premitotic period in the control and experimental groups was 5 h for the liver and 4 h for the cornea. The rise in the curve of labeled mitoses was less steep for the liver than for the cornea, so that the variations in the duration of the G_2-period for individual liver cells were greater.

As Fig. 1 shows, the duration of DNA synthesis was very similar in both the cornea and liver of the intact animals, i.e., about 11 h, but after administration of L-thyroxine it was shortened to 8.75 h (liver) and 10 h (cornea).

The results for the duration of DNA synthesis in the hepatocytes and corneal epithelial cells of normal rats are of interest on their own account, for little information of this sort is available in the literature. Post and Hoffman [5] estimated that the duration of the S-period for hepatocytes in rats is 16 h. According to Stöcker and Heine [7], the mean duration of DNA synthesis in the rat liver is 18 h. The present experiments showed that the mean duration of the synthetic period for hepatocytes of normal rats is 11 h. This difference is evidently significant, and hard to explain, so that a further investigation of this problem is desirable.

In the present experiments the duration of the S-period for corneal epithelial cells under normal conditions is 11 h. This is slightly longer than the values obtained by other workers [3, 4], who found that the duration of the S-period of corneal epithelial cells of mice and rats is 8-9 h. The values obtained for the duration of the G_2-period in normal animals for the liver and cornea agrees with data in the literature [3, 4].

It can be concluded from these results that L-thyroxine stimulates the initiation of DNA synthesis in the hepatocytes and corneal epithelial cells, and also shortens the duration of the period of synthesis to some extent. At the same time, no change could be detected in the duration of the G_2-period under the influence of L-thyroxine. These experiments did not confirm the view that L-thyroxine has a rapid action on mitotic activity of hepatocytes or that this action of the hormone is associated with mobilization of the G_2-population.

LITERATURE CITED

1. I. A. Alov, Outlines of the Physiology of Mitotic Cell Division [in Russian], Moscow (1964).
2. Yu. A. Romanov, B. S. Kasavina, A. N. Blokhina, et al., in: Regeneration and Cell Division [in Russian], Moscow (1968), p. 355.
3. M. G. Chumak, Dokl. Akad. Nauk SSSR, 149, No. 4, 960 (1963).
4. C. Pilgrim and W. Maurer, Naturwissenschaften, 49, 544 (1962).
5. J. Post and J. Hoffman, Exp. Cell Res., 36, 111 (1964).
6. H. Quastler and E. Sherman, Exp. Cell Res., 17, 420 (1959).
7. E. Stöcker and W. D. Heine, Beitr. Path. Anat., 131, 410 (1965).