DIURNAL CHANGES IN DURATION OF S AND G_2 PERIODS OF THE MITOTIC CYCLE IN MONO- AND BINUCLEAR HEPATOCYTES OF NORMAL AND THROXINE-TREATED RATS

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The duration of the S and G_2 periods of the mitotic cycle (MC) of parenchymatous cells of the normal rat liver varies in the course of the 24-h period [3]. However, the liver is a heterogeneous cell population, of which a high percentage is accounted for by binuclear hepatocytes [1, 2, 4, 6].

The object of this investigation was to determine the durations of the S and G_2 periods of MC in mono- and binuclear parenchymatous cells of the liver at different times of the 24-h period in normal and throxine-treated rats.

EXPERIMENTAL METHOD

Experiments were carried out on 400 noninbred male rats weighing 90-100 g (age 45-55 days). The animals were kept for 2 weeks before the experiment and during its course under conditions of alternation of 12 h daylight and 12 h darkness, daylight starting at 8 a.m., and at a temperature of 22 ± 1.5°C.

There were four series of experiments. Each series included two groups of animals, with 50 rats in each group. The animals of group 1 were treated with L-thyroxine (from Reanal, Hungary) in a dose of 10 μg/100 g body weight intraperitoneally in 0.1 ml of solvent daily for 6-9 days, and the animals of group 2 received only the solvent of the hormone, namely $1.0 \cdot 10^{-5}$ N NaOH. All animals of the same series received thymidine-^{3}H simultaneously in a dose of 0.6 μCi/g body weight (specific activity 4.8 Ci/mole), and the animals (five control and five experimental at each time) were then killed after 30 min and 1, 2, 3, 4, 5, 7, 10, 13, and 16 h. The series differed in the time of administration of thymidine-^{3}H (10 a.m., 4 and 10 p.m., and 4 a.m. respectively, in series I, II, III, and IV).

The left lobe of the liver was taken for investigation, fixed in Carnoy's fluid, dehydrated, and embedded in paraffin wax. Sections 4 μ thick were coated with type M emulsion (Photographic Chemical Research Institute) and exposed for 4 weeks at 4°C, after which the developed preparations were stained with hematoxylin and eosin.

The number of labeled mitoses as a percentage of the total number of dividing cells was determined for each population, by examination of 40-50 divided mononuclear and 20-30 binuclear hepatocytes. Mitotic figures were considered to be labeled if the number of grains of reduced silver was four or more. The duration of the S period (ts) and of the G_2 period (tG_2 min; $tG_2 + m/2$) of MC was determined from the labeled mitoses curve [5].

EXPERIMENTAL RESULTS

The results of graphic analysis of curves showing the percentage of labeled mitoses in populations of mono- and binuclear parenchymatous cells from control and experimental animals are given in Tables 1 and 2. It will be clear from Table 1 that the value of $tG_2 + m/2$ in mononuclear cells of the control animals varied in the course of the 24-h period from 3.3 to 4.4 h, whereas ts varied from 8.0 to 9.8 h. The value of tG_2 min remained constant at different times of the 24-h period. In the population of binuclear hepatocytes of the con-

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TABLE 1. Duration of S and G_2 Periods of MC in Mononuclear Hepatocytes of Normal and Thyroxine-Treated Rats

Series of experiments	tG_2 min		$tG_2+m/_2$		ts	
	control	thyroxine	control	thyroxine	control	thyroxine
I	3,0	2,0	4,3	3,7	9,8	8,8
II	3,0	2,0	4,4	3,4	8,0	7,0
III	3,0	3,0	3,6	3,3	8,4	9,4
IV	3,0	2,0	3,3	2,8	9,3	11,4
Mean for 24-h period	3,0	2,3	3,9	3,3	8,9	9,15

TABLE 2. Duration of S and G_2 Periods of MC in Binuclear Hepatocytes of Normal and Throxine-Treated Rats

Series of experiments	tG_2 min		$tG_2+m/_2$		ts	
	control	thy-roxine	control	thy-roxine	control	thy-roxine
I	4,0	3,0	4,6	3,4	7,0	9,6
II	3,0	3,0	4,0	3,1	7,6	5,4
III	3,0	3,0	3,4	3,0	9,0	8,1
IV	3,0	3,0	3,0	3,0	9,1	9,6
Mean for 24-h period	3,3	3,0	3,75	3,1	8,2	8,2

trol rats (Table 2) $tG_2 + m/2$ and ts also varied in the course of the 24-h period from 3.0 to 4.6 h and from 7.0 to 9.1 h, respectively; tG_2 min varied from 3.0 to 4.0 h.

Administration of throxine altered the duration of the above-mentioned periods, but the character of action of the hormone differed at different times of day. For instance, in mononuclear cells (Table 1) throxine reduced tG_2 min by 1.0 h in series I, II, and IV, and depending on the time of day, it reduced the value ot $tG_2 + m/2$ by 0.3-1.0 h. The action of throxine on the duration of the S period also differed during the course of the 24-h period, when it either reduced ts by 1.0 h (series I and II) or increased it clearly by 1.0 and 2.1 h, respectively, in the animals of series III and IV. On average for the 24-h period tG_2 min for the experimental animals was reduced by 0.7 h and $tG_2 + m/2$ by 0.6 h, whereas ts in thyroxine-treated animals remained virtually unchanged compared with the control.

In the binuclear hepatocytes (Table 2) throxine treatment shortened tG_2 min by 1.0 h in series I and $tG_2 + m/2$ by 0.4-1.2 h in series I, II, and III; ts was reduced by 0.9 and 2.2 h, respectively, in the animals of series III and II and it was increased by 0.5 and 2.6 h in animals of series IV and I. On average for the 24-h period tG_2 min was reduced by 0.3 h and $tG_2 + m/2$ by 0.6 h; ts was virtually unchanged.

Taking the time of injection of thymidine-^{3}H to be the middle of the synthetic period of the isotope-labeled hepatocytes, time intervals in the 24-h period with minimal and maximal duration of this phase were distinguished. Minimal activity of the S period in mononuclear cells of the control rats was found to be between noon and 2 p.m. and the maximal between 11 and 3 p.m., whereas in binuclear cells they were between 7 a.m. and 8 p.m. and 6 p.m. and 8 a.m., respectively. The minimal value of ts in mononuclear hepatocytes in animals receiving throxine occurred between 12:30 p.m. and 7:30 p.m., and the maximal between 10 p.m. and 10 a.m., in the binuclear cells the corresponding times were between 1 and 7 p.m. and between 11 and 3 p.m. In both types of cells the minimal value of ts in throxine-treated animals occurred at the same times as in the control, but the value itself was significantly smaller.

In mononuclear hepatocytes in the control animals the minimal and maximal values of $tG_2 + m/2$ were observed between 10 p.m. and 7 a.m. and between 10 a.m. and 8 p.m., respectively. Under the influence of throxine the time of minimal activity of the G_2 period was shifted to between 4 and 7 a.m. and the maximal duration was observed between 10 a.m. and 2 p.m. In the binuclear hepatocytes of both control and throxine-treated animals the minimal and maximal values of $tG_2 + m/2$ were observed at the same times as in mononuclear cells, although the time during which the smallest value of $tG_2 + m/2$ was observed covered a slightly longer period: from 10 p.m. to 7 a.m.

The duration of the S and G_2 periods of MC in mono- and binuclear hepatocytes of normal animals thus varies in the course of the 24-h period. Unlike fluctuations in the duration of the G_2 period, which occur at the same time of day in the two cell populations, the rhythmic fluctuations in ts in these two populations are not synchronized. Under the influence of throxine the duration of the G_2 period is reduced in mono- and binuclear hepatocytes, but the decrease differs at different times of day. The effect of throxine on the duration of the S period in the two populations differs depending on the clock time and may be expressed either as a decrease or as an increase in ts. Treatment with throxine leads to more synchronized variations in tS during the 24-h period in mono- and binuclear hepatocytes. Incidentally throxine does not cause changes in the mean diurnal duration of the S period compared with that observed in normal animals.

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HUMAN INTERFERON-INDUCED INHIBITION OF MOUSE LIVER REGENERATION

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Proteins of the interferon family are active regulators of cell proliferation both in the intact organism and in cell culture [7]. The antiproliferative activity of interferon, combined with its activation of certain components of cell-mediated immunity, evidently constitutes the basis for the recently widely studied antitumor effect of this protein. Several aspects of the antiproliferative action of interferon can be analyzed in a purer form by studying its effect *in vivo* on regeneration.

Gresser et al. [4, 5] reported a decrease in the number of thymidine-^{3}H-labeled cells in the regenerating liver 48 h after partial hepatectomy, following injection of homologous fibroblast interferon.

The aim of the present investigation was to study the effect of both homologous (mouse) and heterologous (human) interferons on restoration of the weight of the regenerating mouse liver and also on proliferative activity of hepatocytes and Kupffer cells.

EXPERIMENTAL METHOD

Experiments were carried out on 75 noninbred mice weighing 20-26 g. Two-thirds of the liver was removed in 45 animals by the usual method [6]. Immediately after the operation and daily for the next 3 days the animals were given an intramuscular injection of 0.2 ml of partially purified and concentrated mouse fibroblast interferon (M-IFN) (group 1) [1], in a dose of $5 \cdot 10^3$ units/mouse, human leukocytic interferon (4-IFN), purified by the method in [3], in a dose of $0.5 \cdot 10^5$ unit/mouse (group 2), or a preparation of pseudointerferon from human cells (group 3). The same preparations were injected at the same intervals into control animals not subjected to hepatectomy.

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