# EFFECT OF THYROXINE ON AGE CHANGES IN DIURNAL RHYTHM OF MITOSES IN RAT LIVER PARENCHYMATOUS CELLS

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UDC 612.35.014.3:612.6].014.46:
615.357.441

Changes in the diurnal rhythm of mitoses were investigated in the liver of rats aged 2, 7, 15, 22, 45, and 90 days. In the period of early postnatal development (2-15 days) the rhythm of mitosis is biphasic with peaks of mitotic activity (MA) in the morning and evening (between 4 and 10 P.M.). At the age of 22 days, although the rhythm remains biphasic, a high proportion of the cell divisions is found in the morning (10 A.M.). By the age of 45 days the rhythm of of mitosis becomes monophasic, and MA reaches its maximum at 4 A.M. and its minimum during the afternoon and evening, corresponding to the rhythm in adult animals. At no stage of the investigation was a significant increase in the number of mitoses found 1 h after intraperitoneal injection of thyroxine. Conversely, at certain times of day in the animals of some age groups MA was significantly lower than normally.

Despite many investigations of the diurnal rhythm of mitotic cell division in mammalian tissues, the question of age changes in the character of the rhythm, particularly in the early stages of postnatal development, has been inadequately studied. An important investigation in this field was that conducted by Bogatova [1] on the corneal epithelium and adrenal gland of rats, in which it was shown that the definitive rhythm of mitosis becomes established in the course of postnatal development. Meanwhile, data of this type are essential for an explanation of the age changes in the level of cell proliferation in different tissues and for evaluation of changes in mitotic activity (MA) of the tissues after exposure to certain factors [2].

The object of the present investigation was to study age changes in the diurnal rhythm of mitoses in the rat liver. The possibility of changes in MA taking place in the liver cells after administration of thyroxine to the animals was studied at the same time. The starting point for this series of experiments was the results obtained by Romanov et al. [5, 6], showing that MA rises sharply (by 3-4 times) in certain organs, including the liver, at short time intervals after injection of thyroxine. This worker called this phenomenon the "$G_2$-effect of thyroid hormones." It was decided to study to what extent this effect is manifested in animals of different ages and whether it depends on the phase of the diurnal rhythm of mitosis.

## EXPERIMENTAL METHOD

Rats of six age groups (2, 7, 15, 22, 45, and 90 days) were used. The animals were kept under natural conditions of lighting and with free access to food. The rats were sacrificed in groups of four or five at a time at 3-hourly intervals during the 24-h period. Mitoses were counted in the sections on the average among 20,000-30,000 cells for each animal. The mitotic index (MI) was expressed per thousand cells. In the experiments to study the effect of thyroxine, the technique suggested by Romanov et al. [5, 6] was used. The experimental animals aged 7 days or more received an intraperitoneal injection of thyroxine 1 h before sacrifice in a dose of 10 $\mu$g/100 g body weight. The numerical results were subjected to statistical analysis by the Fisher–Student method.

Department of General Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 88-91, April, 1973. Original article submitted May 16, 1972.

TABLE 1. Diurnal changes in MI (in ‰) in Rats of Different Ages under Normal Conditions and after Injection of Thyroxine

| Time of day | Age of animals (in days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 7–8 | | | | 15 | | | |
| | normal | | normal | | injection of thyroxine | | normal | | injection of thyroxine | |
| | MI | P | MI | P | MI | P | MI | P | MI | P |
| 10 A.M. | 10,2 | 0,015 | 3,5 | 0,07 | 2,5 | 0,27 | 4,0 | 0,000 | 3,9 | 0,004 |
| 1 P.M. | 5,5 | 0,148 | 3,1 | 0,24 | 2,9 | 0,204 | 2,7 | 0,003 | 2,7 | 0,047 |
| 4 P.M. | 3,6 | 0,05 | 4,0 | 0,09 | 2,4 | 0,403 | 2,5 | 0,351 | 3,1 | 0,444 |
| 7 P.M. | 6,8 | 0,17 | 3,2 | 0,003 | 2,7 | 0,235 | 3,9 | 0,192 | 3,4 | 0,01 |
| 10 P.M. | 6,0 | 0,07 | 4,4 | 0,053 | 2,6 | | 3,4 | | 2,8 | |
| 1 A. M. | 4,0 | 0,001 | 3,0 | | 3,8 | | 3,5 | | 2,8 | |
| 4 A. M. | 3,6 | | 3,1 | | 2,3 | | 3,6 | | 2,6 | |
| 7 A. M. | 1,5 | | 2,3 | | 1,7 | | 3,6 | | 3,5 | |
| Mean for 24-h period | 5,1 | | 3,3 | | 2,6 | | 3,4 | | 3,1 | |

| Time of day | Age of animals (in days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 22 | | | | 45 | | | | 90 | | | |
| | normal | | injection of thyroxine | | normal | | injection of thyroxine | | normal | | injection of thyroxine | |
| | MI | P | MI | P | MI | P | MI | P | MI | P | MI | P |
| 10 A. M. | 10,8 | 0,001 | 9,8 | 0,009 | 0,9 | 0,081 | 0,5 | 0,081 | 0,03 | | 0,09 | 0,27 |
| 1 P.M. | 4,4 | 0,20 | 3,1 | 0,10 | 0,3 | | 0,1 | | 0,03 | | 0,04 | |
| 4 P.M. | 2,8 | 0,5 | 1,4 | | 0,5 | | 0,2 | | 0,05 | 0,17 | 0,00 | |
| 7 P.M. | 2,2 | 0,007 | 1,6 | 0,05 | 0,4 | 0,128 | 0,2 | | 0,11 | | 0,04 | |
| 10 P.M. | 2,9 | 0,001 | 1,9 | 0,30 | 1,1 | 0,631 | 0,1 | 0,15 | 0,08 | 0,26 | 0,04 | |
| 1 A. M. | 7,2 | 0,07 | 5,1 | | 1,6 | 0,03 | 1,9 | 0,18 | 0,02 | 0,003 | 0,05 | |
| 4 A. M. | 2,2 | 0,037 | 3,4 | 0,06 | 12,6 | 0,27 | 8,4 | | 0,19 | 0,003 | 0,14 | |
| 7 A. M. | 6,0 | | 3,8 | | 7,1 | 0,017 | 7,0 | 0,02 | 0,02 | | 0,00 | |
| Mean for 24-h period | 4,8 | | 3,8 | | 3,0 | | 2,3 | | 0,07 | | 0,05 | |

## EXPERIMENTAL RESULTS

The results are given in Table 1. On the 2nd day after birth the diurnal rhythm of mitoses was biphasic with a significant increase in MA at 10 A.M. and at 7-10 P.M., with a minimal level of cell division at 7 A.M. ($P < 0.003$). The evening maximum of the number of mitoses was lower than the morning maximum.

On the 7th-8th day of postnatal life the diurnal changes in MA were expressed as small but significant differences between the maximal (at 4 and 10 P.M.) and minimal (at 7 A.M.) values of cell proliferation. Injection of thyroxine led to a decrease in MI at nearly all times of the investigation in the course of the 24-h period, and a significant decrease was obtained at the times of the maxima of cell division in the normal animals (at 4 and 10 P.M.). The small increase in MI at 1 A.M. is not significant.

At the age 15 days a tendency toward an increase in the number of cell divisions during the morning (7-10 A.M.) began to appear. At the same time, however, the evening maximum of MA continued at 7 P.M. Both increases in MA were significant relative to the minimal level of cell division at 1-4 P.M. The rhythm was biphasic in character. After injection of thyroxine MI was invariably a little below normal although the decrease was close to significant only in the period from 10 P.M. to 1 A. M. The small increase in MA at 4 P.M. is not significant.

The diurnal rhythm of mitoses in the liver of the 22-day rats was distinctly biphasic in character with the maximal number of mitoses at 10 and 1 A.M. and the minimal number at 7 P.M. and 4 A.M. (differences

significant, P = 0.001). The level of cell proliferation observed during the morning was much higher than its level in the evening and at night. The amplitude of the fluctuations in MA increased sharply, mainly on account of an increase in the number of mitoses at the times of the maximum. The diurnal changes in MI after injection of thyroxine coincided to a large extent with the changes in the intact rats. It must be noted, however, that at all periods of the investigation MI in the course of the 24-h period was a little below normal after injection of thyroxine, although the differences were not significant. The increase in MI at 4 A.M. is not significant.

In rats aged 45 days the diurnal changes in cell proliferation had the character of a monophasic rhythm, similar to the rhythm in adult animals, with a maximum of MA at 4 A.M. and a minimum in the afternoon and evening. The differences are significant ($P \leq 0.01$). The high level of MA at 4 A.M. (12.6 $^0/_{00}$) was evidently due to the beginning of mitosis of a large number of binuclear cells. Injection of thyroxine led to a decrease in MI compared with normal, especially at 1, 4, and 10 P.M. (P = 0.011, 0.017, and 0.037, respectively). The character of the change in the number of mitoses was very similar in the two groups of rats.

In the liver of the rats aged 90 days the diurnal rhythm of mitoses was biphasic in character with maxima of MA at 7 P.M. and 4 A.M. and minima at 1 and 7 A.M., but the maximum at 4 A.M. was significant (P = 0.003) whereas the increase in MA at 7 P.M. was not quite significant (P = 0.06). Nevertheless, the evening rise in the number of mitoses was not accidental, for it was very similar to the evening maximum in MA characteristic of the rhythm of mitosis during early postnatal development, and it can evidently be observed under certain conditions in adult animals also. Injection of thyroxine led to a decrease in the level of MA at most times of investigation throughout the 24-h period. However, the decrease was significant only at 4 P.M. (P = 0.037). Twice in the course of the 24-h period (at 10 and 1 A.M.) MI was a little nigher in the animals receiving thyroxine than in the intact rats, but this increase was not significant (P = 0.08 and 0.17, respectively). The character of the mitotic rhythm changes slightly after the injections of thyroxine, and a significant increase in the number of cell divisions was obtained at 10 A.M. (P = 0.01).

During the early postnatal development of rats the diurnal rhythm of cell division is thus distinguished by a general biphasic course with a characteristic maximum of the number of mitoses at 7-10 P.M. The morning maximum of MA observed in the liver of rats aged 2 days is possibly a reflection of the maternal rhythm. Starting about from the age of 3 weeks, a large number of cell divisions is observed in the morning, and by the age of 45 days a distinct monophasic rhythm of mitoses, characteristic of the adult animal, is established.

So far as the rhythm of mitoses in rats aged 90 days is concerned, it is worth noting that a high level of MA in the liver of adult rats and mice during the morning has been reported by several writers [2-4]. However, it is known that under certain experimental conditions a second evening maximum of MA can arise in the liver at 6-8 P.M., i.e., at a characteristic time of high MA in young animals.

It is interesting to note that the decrease in the mean MA for the 24-h period appeared for the first time after the age of 45 days.

After injection of thyroxine no significant increase in the number of mitoses was found at any period of the investigation. Meanwhile there was a slight tendency for the number of mitoses to diminish, as shown by a significant decrease in MA after injection of the compound at certain times of day in animals of different ages. The results probably confirm the views expressed [7, 8] regarding the accelerating action of thyroxine on mitosis but they do not confirm the concept of the "$G_2$-effect of thyroid hormones."

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