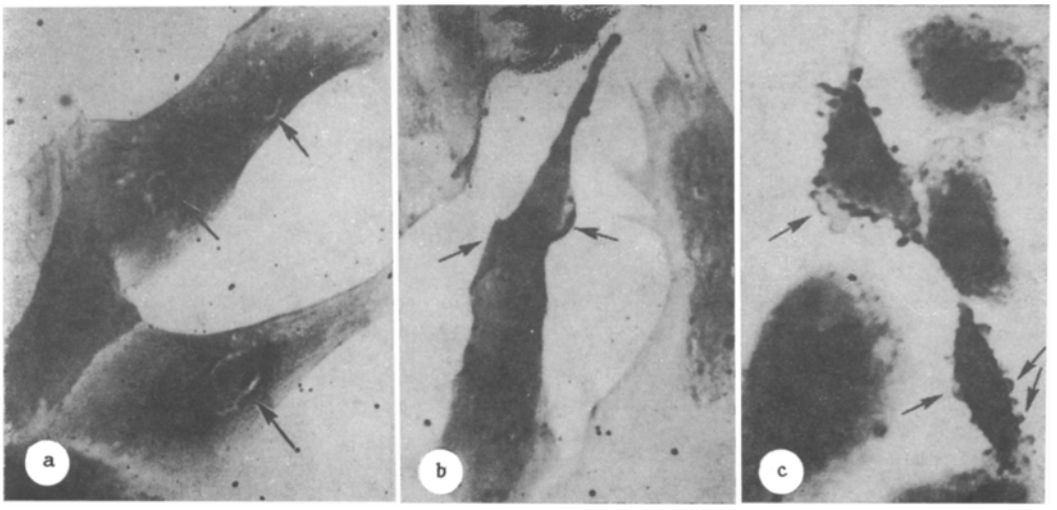

Fig. 3. Morphological changes in cardiomyocytes during experimental hypoxia. a) Gross vacuolation; b) detachment of membrane from glass; c) evaginations on cell surface and contracture of cells. Corresponding morphological changes indicated by arrows (400 ×).

LITERATURE CITED

1. D. Acosta, M. Puckett, and R. McMillan, In Vitro, 14, 728 (1978).
2. R. A. Altschuld, J. R. Hostetler, and G. P. Brierly, Circulat. Res. 49, 307 (1981).
3. E. Brussel, M. Freyss-Begun, G. Griffation, and P. Lechat, Biochem. Pharmacol., 34, 145 (1985).
4. F. H. Kasten, In Vitro, 8, 128 (1972).
5. H. M. Piper, P. Schwartz, R. Spahr, et al., J. Mol. Cell. Cardiol., 16, 385 (1984).
6. J. Rajs, J. Mol. Cell. Cardiol., 12, 1227 (1980).

EFFECT OF LONG-TERM HYPOKINESIA ON CIRCADIAN RHYTHM OF HEPATOCYTE PROLIFERATION

S. E. Li

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KEY WORDS: hypokinesia; proliferative activity; circadian rhythm.

Hypokinesia disturbs coordinated interaction between the muscular system, activity of the visceral systems of the body, and the level of their regulation [5-8, 12]. This results in changes in the temporal organization of the various physiological functions of the organs and to a disturbance of their circadian rhythms [2, 3].

The investigation described below was conducted in order to study proliferative activity of hepatocytes at different times of the 24-h period in rats kept under conditions of long-term hypokinesia.

EXPERIMENTAL METHOD

Male Wistar rats from the "Stolbovaya" nursery weighing 150-160 g were used. The total number of 38 animals was divided into two groups, one of which was kept under normal animal house conditions (control) whereas the other was exposed to hypokinesia in individual constraining

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TABLE 1. Body Weight, Weight of Liver, and Proliferative Activity of Hepatocytes in Rats at Various Times after Hypokinesia for 10 Days

Time of day, h	Number of rats	Body weight, g	Weight of liver		MI, ‰	Number of binuclear cells, %
			g	%		
7-8 a.m.	7 / 5	196,6±6,09 / 131,0±7,56	7,49±0,280 / 4,12±0,509	3,92±0,101 / 3,33±0,385	0,34±0,057 / 0,00	14,67±0,826 / 24,19±3,695
p		0,0001	0,0001	0,165	0,0001	0,001
3-4 p.m.	7 / 5	202,1±5,78 / 145,4±5,07	7,96±0,171 / 5,38±0,455	4,14±0,188 / 3,68±0,208	0,94±0,133 / 0,10±0,063	13,23±1,101 / 20,06±1,7676
p		0,0001	0,0001	0,141	0,0001	0,007
11 p.m.-midnight	8 / 6	202,1±5,69 / 130,5±6,73	8,38±0,232 / 4,30±0,214	4,16±0,124 / 3,32±0,173	0,54±0,063 / 0,10±0,052	10,79±0,968 / 18,65±1,952
p		0,0001	0,0001	0,002	0,001	0,004
Average value for 24-h period	22 / 16	200,3±5,85 / 135,6±6,45	7,95±0,227 / 4,60±0,393	4,07±0,138 / 3,72±0,255	0,58±0,069 / 0,07±0,028	12,90±1,732 / 20,96±1,704
p		0,0001	0,0001	0,2713	0,0001	0,0010

Legend. Numerator) control, denominator) experiment.

cages for 10 days (experiment). Immobilization started at various times of the 24-h period depending on the time of subsequent sacrifice. The rats were decapitated, 5-7 from each group, at 7 a.m. and 3 and 11 p.m. Material was fixed in Carnoy's mixture and paraffin sections were stained with hematoxylin and eosin. The mitotic index (MI) of the hepatocytes was determined by analysis of 10,000 cells in each preparation. The number of binuclear cells was counted and expressed in percentages after examination of 2000 cells. The results were subjected to statistical analysis by the Fisher–Student test.

EXPERIMENTAL RESULTS

After hypokinesia for 10 days the rate of increase of body weight of the rats fell by 32.3% and that of the weight of their liver by 42.1% (mean values for the 24-h period), in agreement with other data [9, 10]. MI of the hepatocytes of the intact animals changes in the course of the 24-h period (Table 1). Mitoses were most numerous in the afternoon (3 p.m.). These results differ from data in the literature [1], according to which mitoses reached a peak in the morning (8 a.m.). The fact that the largest number of dividing cells in the present investigation was observed in the afternoon can be attributed to conservatism of the biological rhythm, which was characteristic of the birth place of the rats (Moscow Region). Animals carried across seven time zones in an easterly direction were used in the experiments after 6 weeks. This period of adaptation may not be long enough for new cell rhythms to be consolidated. This phenomenon is based on mismatching between the animal organism and the requirements of its new environment [15]. Proliferative activity of the liver cells in the immobilized animals also was observed for 24 h. In the morning, for instance, no dividing hepatocytes were found. Mitoses appeared in the afternoon and evening. However, they were far less numerous than in the control. The mean value of MI for the 24-h period in the control and experimental groups differed by 8.28 times.

The number of binuclear cells in the liver of the intact rats during the morning and afternoon was stable, but at 11 p.m. it was significantly reduced (Table 1). In the experimental animals at all times of the investigation the number of binuclear cells in the course of the 24-h period exceeded the control level by 1.65, 1.52, and 1.73 times respectively. The mean values of the parameters for the 24-h period differed between the groups by 1.62 times. This is in agreement with investigations which showed that the increase in the percentage of multinuclear cells during hypokinesia is due to accumulation of a population of binuclear hepatocytes with low ploidy of the 2n × 2 type [11, 16]. Consequently, the greater part of the population in our experiments also probably consisted of diploid binuclear cells. The presence of correlation between ploidy and size of the cell is known [4]. An increased number of binuclear cells may therefore be the result of an increase in concentration of hepatocytes per unit area. However, we did not count these types of cells per standard area of the preparation, but calculated the fraction relative to a strictly determined number of hepatocytes, irrespective of their size. Thus in animals exposed to hypokinesia for 10 days variability of division of the hepatocytes with time corresponds to the circadian rhythm of mitosis of

liver cells in intact animals. These findings are in agreement with results obtained by Romanov and co-workers [13, 14], who found that the circadian rhythm of mitosis of the hepatocytes remained normal in type in rats exposed to hypokinesia for 34 days. Just as in the present experiments, they recorded a more than eightfold decrease in the mean value of MI for the 24-h period. Consequently, physiological regeneration during exposure to hypokinesia for both periods undergoes changes in the same direction. The deficit of mitoses discovered can account for delay of postnatal growth of the liver during hypokinesia. The increased number of binuclear hepatocytes, which are analogs of polyploid cells [4], under these conditions compensates for the increased demand of the body on its liver function.

LITERATURE CITED

1. I. A. Alov, Essays on the Physiology of Mitotic Cell Division [in Russian], Moscow (1964).
2. R. M. Baevskii, T. N. Krupina, and G. P. Mikhailovskii, Adaptation to Muscular Activity and Hypokinesia [in Russian], Novosibirsk (1970), pp. 19-20.
3. R. M. Baevskii, G. A. Nikulina, and T. D. Semenova, Adaptation to Muscular Activity and Hypokinesia [in Russian], Novosibirsk (1970), pp. 21-22.
4. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
5. E. A. Kovalenko and N. N. Gurovskii, Hypokinesia [in Russian], Moscow (1980).
6. A. V. Korotkov, Physiological Problems of Unfitness [in Russian], Moscow (1968), p. 74.
7. A. V. Korotkov, L. A. Ioffe, M. A. Abrikosova, et al., Kosmich. Biol., No. 3, 33 (1968).
8. T. N. Krupina, A. Ya. Tizul, N. M. Bozhevskaya, et al., Kosmich. Biol., No. 5, 61 (1967).
9. S. E. Li and O. I. Kirillov, Kosmich. Biol., No. 2, 13 (1972).
10. S. E. Li, Comparative Aspects of the Study of Regeneration and Cell Proliferation [in Russian], Moscow (1985), pp. 361-363.
11. V. F. Malyutin, V. M. Faktor, S. E. Li, and O. I. Kirillov, Proceedings of the Second All-Union Symposium on Somatic Polyploidy [in Russian], Erevan (1977), pp. 64-65.
12. N. E. Panferova, Hypodynamia and the Cardiovascular System [in Russian], Moscow (1977).
13. Yu. A. Romanov, E. A. Kovalenko, S. S. Filippovich, et al., Kosmich. Biol., No. 1, 52 (1978).
14. Ya. A. Romanov, S. S. Filippovich, É. T. Ostroushko, et al., Problems in the Temporal Organization of Living Systems [in Russian], Moscow (1979), pp. 121-135.
15. S. I. Stepanova, Biorhythmologic Aspects of the Adaptation Problem [in Russian], Moscow (1986).
16. V. M. Faktor, V. F. Malyutin, S. E. Li, and V. Ya. Brodskii, Tsitologiya, No. 4, 397 (1979).