in the early stages of ontogeny the response of the animals to different types of stimuli (hurmoral—adrenalin and reflex—pain) may differ in character.

LITERATURE CITED

1. I. A. Alov, G. Ya. Pavlenko, and M. V. Sukhinina, Byull. Éksp. Biol. Med., No. 4, 63 (1955).
2. V. N. Dobrokhotov and V. S. Valvas, Byull. Éksp. Biol. Med., No. 4, 102 (1974).
3. V. V. Kozlov, "Reactive changes in proliferative processes under normal and pathological conditions," Author's Abstract of Doctoral Dissertation, Leningrad (1969).
4. I. A. Morozova, in: Experimental Investigations of Pain [in Russian], Lugansk (1968), p. 73.
5. O-Cheng, "Reactive changes in processes of cell division in different tissues of rats exposed to unconditioned and conditioned stimuli," Author's Abstract of Candidate's Dissertation, Leningrad (1955).
6. A. K. Ryabukha, "Reactive inhibition of cell division and its dependence on neurohumoral influences," Author's Abstract of Doctoral Dissertation, Leningrad (1974).
7. G. S. Strelin, I. B. Bychkovskaya, and V. V. Kozlov, Dokl. Akad. Nauk SSSR, 99, 165 (1954).
8. G. S. Strelin and V. V. Kozlov, Arkh. Anat., No. 2, 3 (1959).
9. L. V. Suvorova, "Reactive inhibition of cell division in animals and development of their capacity for such inhibition in ontogeny," Candidate's Dissertation, Leningrad (1955).
10. W. S. Bullough and E. B. Laurence, Proc. Roy. Soc. B, 154, 540 (1961).
11. I. S. Friedenwald and W. Buschke, Am. J. Physiol., 141, 689 (1944).

DIURNAL CHANGES IN THE NUMBER OF MITOSES AND OF DNA-SYNTHESIZING CELLS IN TISSUES OF YOUNG RATS

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Regular diurnal changes in the number of mitoses (MI) and the number of DNA-synthesizing cells (ILN) were demonstrated in the liver, epidermis, and exogenous part of the pancreas of rats aged 7 days. The character of these changes differed in the various tissues. No regular correlation was found between diurnal changes in MI or ILN.

KEY WORDS: *Mitotic index (MI); index of labeled nuclei (ILN); diurnal changes in MI and ILN; liver; epidermis; pancreas.*

The study of the character of the diurnal rhythm of DNA-synthesizing cells is important in order to determine the time of most effective administration of inhibitors or stimulators of cell division which act mainly on the premitotic stage of DNA synthesis [2]. This study is also interesting as a means of shedding light on age changes in the character of the rhythm of cell division.

As yet this problem has been studied virtually entirely in tissues of adult animals [4, 5, 6-11], whereas in young animals, distinguished by both the pattern of their diurnal rhythm of mitosis and the level of cell proliferation, it remains almost completely unstudied. The investigation described below was accordingly carried out.

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TABLE 1. Diurnal Changes in Number of Mitoses (MI) and Cells Synthesizing DNA (ILN) in Different Tissues of Rats Aged 7 days

Time of day or night	Liver			Epidermis			Pancreas		
	mean value of MI, ‰	mean value of ILN, ‰	ILN/MI	mean value of MI, ‰	mean value of ILN, ‰	ILN/MI	mean value of MI, ‰	mean value of ILN, ‰	ILN/MI
10 a.m.	1,9	43,3	22,7	3,5	22,4	6,4	2,3	57,9	25,1
1 p.m.	1,8	20,0	11,1	6,6	33,5	5,0	3,0	47,2	12,7
4 "	0,9	16,4	18,2	7,4	39,0	5,2	3,8	61,9	16,2
7 "	1,4	17,2	12,2	6,9	26,6	3,8	4,4	20,4	4,6
10 "	1,2	23,6	19,6	6,9	35,6	5,1	2,6	58,1	22,3
1 a.m.	1,0	14,2	14,2	3,0	24,4	8,1	4,7	60,7	12,9
4 "	1,0	13,1	13,1	6,8	38,2	5,6	2,3	50,9	22,1
7 "	0,7	12,4	17,7	5,0	29,3	5,8	1,3	49,3	37,9
Mean diurnal values	1,2	20,0		5,7	31,1		3,1	50,0	

EXPERIMENTAL METHOD

In experiments on 48 young rats aged 7 days six rats were killed at each of the eight times of investigation during the 24-h period. ^{3}H-thymidine (specific activity 4.6 Ci/mmole) was injected intraperitoneally in a dose of 1 μCi/ g body weight 1 h before the animals were killed. The number of mitoses and of cells with labeled nuclei was counted in autoradiographs prepared in the usual way. The number of mitoses (MI) and the number of cells synthesizing DNA (ILN) were expressed in promille after examination of 10,000-15,000 hepatocytes, 3000-4000 cells from the basal layer of the epidermis, and 8000-12,000 cells from the pancreatic acini.

EXPERIMENTAL RESULTS

Liver. Determination of MI in the hepatocytes revealed a biphasic diurnal rhythm of the number of mitoses (Table 1). Higher values of MI were observed at 10 a.m.-1 p.m. and 7-10 p.m. At night and in the early morning the number of dividing cells was small [$P_{1-4\ p.m.} = 0.001$; $P_{4\ p.m.-(7-10\ p.m.)} = 0.01$]. The lowest values of MI were observed at 7 a.m. ($P_{7\ p.m.-7\ a.m.} = 0.006$).

Diurnal changes in the number of labeled cells, as also in MI, were characterized by a bimodal increase in the values of ILN, at 10 a.m. and 10 p.m. ($P_{10\ a.m.-4\ p.m.} = 0.005$; $P_{4\ p.m.-10\ p.m.} = 0.01$). The lowest values of both indices were found at 1-7 a.m.

It could be postulated that the maximal values of ILN at 10 a.m. caused the increase in MI at 7-10 p.m. and that the increase in ILN at 10 p.m. caused the increase in MI at 10 a.m.-1 p.m. On this assumption the interval between the maxima of ILN and MI was 9-15 h. It could also be postulated that not all cells completing DNA synthesis in the morning had begun to divide at 7-10 p.m., whereas the relatively smaller increase in ILN at 10 p.m. explained the larger increase in MI in the morning. However, for this conclusion additional data on the duration of DNA synthesis at different times of the 24-h period would be necessary. The ILN/MI ratio fluctuated considerably during the 24-h period, evidently on account of phase differences in the diurnal changes in ILN and MI.

Epidermis. High values of MI were observed for a long time in the epidermis. They were found both in the afternoon and in the evening, from 1 p.m. to 10 p.m. ($P_{10\ a.m.-4\ p.m.} = 0.003$; $P_{10\ p.m.-1\ a.m.} = 0.004$). From 1 to 10 a.m. the number of dividing cells was relatively small and its changes were not significant.

Comparison of these results with those of previous investigations [1, 3] showed that in both the liver and the epidermis of young rats of this age there are certain differences in the character of the diurnal rhythm of mitosis, for maximal values of MI observed in the

present investigation were timed to take place earlier, thus emphasizing the instability of the rhythm of mitosis in the tissues of animals of this age.

The highest value of ILN was observed at 4 p.m. ($P_{10\ a.m.-4\ p.m.} = 0.003$). The decrease in ILN between 4 and 7 p.m. was significant ($P = 0.015$). Changes in ILN at other times were not significant. The highest values of ILN in the epidermis coincided in time with the period of the greatest number of dividing cells (4 p.m.). Changes in the ILN/MI ratio during the 24-h period were not significant, probably because the rhythms of these processes coincided in phase.

Examination of histological preparations at all times of the investigation (except 7 and 10 a.m.) revealed labeled early prophases. This suggests that the duration of the G_2 period in some of the epidermal cells was short, approximately 1 h.

Pancreas. A diurnal rhythm of MI was observed in the cells of the pancreatic acini with a maximum in the evening or night (4 p.m.-1 a.m.). The decrease in MI at 10 p.m. was not significant. The minimal number of cell divisions was observed at 7 a.m. ($P_{1-7\ a.m.} = 0.04$).

The value of ILN was considerable at nearly all times of the investigation. Only at 7 p.m. was there a sharp decrease in ILN ($P_{4-7\ p.m.} = 0.001$; $P_{7-10\ p.m.} = 0.002$).

The high values of ILN over a long period of time, combined with the relatively short period of the MI maximum could indicate heterogeneity of the exocrine cell population of the pancreas. Possibly not all cells synthesizing DNA start mitosis at the same time. For this reason, evidently, the ILN/MI ratio changed considerably during the 24-h period.

In all organs investigated significant diurnal changes in MI and ILN were thus found.

The dynamics of the diurnal changes in ILN differed in the different tissues of the young rats. In the liver the highest values of ILN were found at 10 a.m. and 10 p.m., whereas in the epidermis and pancreas there was only partial coincidence of the maxima of ILN and MI.

LITERATURE CITED

1. N. G. Bystrenina, in: Diurnal Rhythms of Physiological Processes of the Organism [in Russian], Moscow (1972), p. 14.
2. V. N. Dobrokhotov, I. V. Markelova, L. V. Sokolova, et al., in: Regeneration and Cell Division in Animals [in Russian], Moscow (1964), pp. 165-185.
3. V. N. Dobrokhotov, N. G. Bystrenina, A. S. Kudryavtseva, et al., in: Mechanisms of Regeneration and Cell Division (Proceedings of the Sixth Conference on Results of Recent Research into Processes of Regeneration and Cell Division) [in Russian], Moscow (1971), p. 43.
4. I. V. Markelova, Tsitologiya, No. 3, 318 (1967).
5. I. V. Markelova, Tsitologiya, No. 10, 1269 (1968).
6. Yu. A. Romanov, I. K. Rakhmatullina, and M. N. Zaikina, in: The Biology of Cell Reproduction [in Russian], Moscow (1972), p. 7.
7. I. Brown and R. Berry, Cell Tissue Kinet., 1, 23 (1968).
8. S. Gelfant, Exp. Cell Res., 32, 521 (1963).
9. E. Llanos, M. Aloisso, M. Souto, et al., Virchows Arch. B, 8, 309 (1971).
10. C. Pilgrim, K. Lennartz, K. Wegener, et al., Z. Zellforsch., 68, 138 (1965).
11. E. Tvermyr, Virchows Arch. B, 11, 43 (1972).