CIRCADIAN RHYTHM OF TOPOGRAPHIC DISTRIBUTION OF PROLIFERATING HEPATOCYTES IN THE LIVER LOBULE OF INTACT RATS

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KEY WORDS: liver lobule; dividing cells; DNA-synthesizing hepatocytes.

Analysis of parameters of cellular kinetics that depend on the intralobular localization of hepatocytes has been undertaken by several workers mainly after partial resection of the liver [3-7]. Very few investigations have been conducted on the intact liver, and they have demonstrated the random character of distribution of proliferating hepatocytes in the lobule [3, 4].

We have studied the topographic distribution of DNA-synthesizing and dividing hepatocytes in the liver lobule of intact rats during the 24-h period.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 140 g, kept on a schedule of 12 h of daylight (from 6 a.m. to 6 p.m.) and 12 h of darkness. The animals were killed over a period of 28 h at intervals of 2-4 h. All animals were given an injection of ^{3}H-thymidine in a dose of 1.0 mCi/g body eight 1 h before sacrifice. Part of the left lobe of the liver was taken for investigation, fixed in Carnoy's solution, and embedded in paraffin wax; sections were cut to a thickness of 4 μm. Histoautoradiographs were stained with hematoxylin and eosin. The following parameters of hepatocyte proliferation were determined: general mitotic index (GMI) and general index of number of DNA-synthesizing cells (GRI), for which 10,000 parenchymatous cells were examined, and also the mitotic indices (MI_1, MI_2, MI_3) and indices of the number of DNA-synthesizing cells (RI_1, RI_2, RI_3) in different zones of the lobule, for which purpose 15-20 lobules with a radius of 18 cells were analyzed in preparations from each animal. The lobule was divided into three zones (1 – periportal, 2 – middle, 3 – central), so that the radii of the zones were equal to one another, and in each zone values of the mitotic and radioactive indices were determined for a certain number of fields of vision. Parameters of circadian rhythms of dividing and DNA-synthesizing cells were determined by the method in [1, 8]. The numerical results were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

Data on parameters of circadian rhythms of dividing and DNA-synthesizing liver cells are given in Table 1 and Fig. 1.

The results show that the maximal number of proliferating hepatocytes was observed during the morning, and that the time shift of the acrophase of the rhythm of GMI relative to the rhythm of GRI was 0.5-3 h. Higher values of RN and CS in the rhythm of dividing hepatocytes will be noted. The 24-hourly pool of DNA-synthesizing cells was about 1.5 times greater than the 24-hourly pool of dividing cells. At the same time, it must be pointed out that the relative percentages of dividing and DNA-synthesizing cells during the active phase of the circadian rhythms of these cells were about equal, and amounted to about 3/4 of the total number of dividing and DNA-synthesizing cells in the 24-h period.

In the period of higher values of GRI in the rhythm (2.30 a.m. on the 1st day and 3.30 a.m. on the 2nd day) the frequency of observation of labeled hepatocytes was increased in

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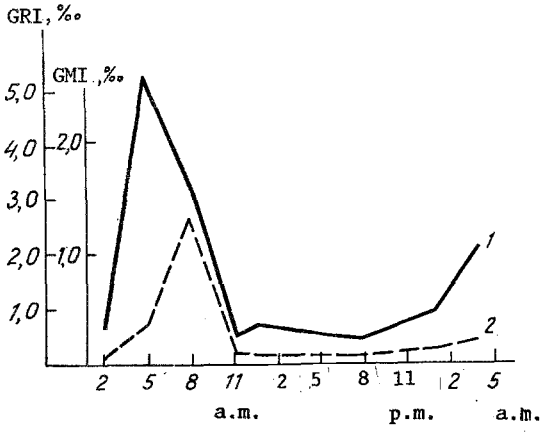

Fig. 1

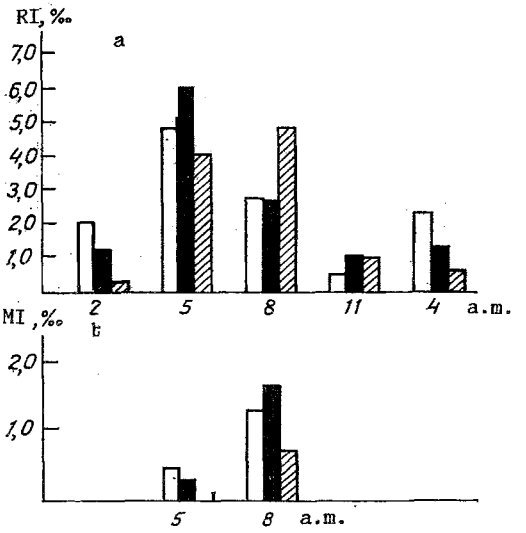

Fig. 2

Fig. 1. Dynamics of dividing (1) and DNA-synthesizing (2) cells in liver of intact rats during 24-h period. Here and in Fig. 2, clock time plotted along abscissa.

Fig. 2. Distribution of DNA-synthesizing (a) and dividing (b) hepatocytes in liver lobule of intact rats during period of their higher values in the rhythm. Unshaded columns – zone 1 of the liver, black columns – zone 2, obliquely shaded columns – zone 3.

TABLE 1. Parameters of Circadian Rhythms of Dividing and DNA-Synthesizing Cells

Parameter	AA, ‰	RA	CS	Mesor, ‰	AP, h	Act / 1/2 AP	P_{24h}, ‰	P_{AP}, ‰	P_{AP}, %	Shift of MI relative to RI	
										Arc, h	1/2 AP, h
GRI	4,95	18,68	2,07	1,435	3,0—10,0 / 7,0	5,0 / 6,5	4,16	2,95	70,9	3,00	0,5
GMI	1,30	130,0	8,67	0,22	4,0—10,5 / 7,0	8,0 / 7,0	2,84	2,21	77,87		

zones 1 and 2 of the lobule, more especially in zone 1 ($p < 0.05$). The value of RI_3 at this time was significantly lower than RI_1 and RI_2 ($p < 0.05$). At the peak of the number of DNA-synthesizing cells in the course of 24 h, the value of RI_2 was highest, and RI_1 and RI_3 were closely similar to each other (Fig. 2a). By 8 a.m. the value of MRI was lower and RI_1 and RI_2 were simultaneously reduced, whereas RI_3 remained at its previous level. During the period of minimal values of GRI the topographic distribution of DNA-synthesizing cells in the liver lobule became random.

During the period of increased mitotic activity, dividing hepatocytes, and also DNA-synthesizing cells, were observed mainly in zones 1 and 2, but in zone 3 no dividing hepatocytes were found. At the peak of GMI mitoses were recorded in all zones of the lobule, but in zones 1 and 2 they were regularly more numerous than in zone 3.

Previously investigations on the intact liver [3, 4] showed a random distribution of DNA-synthesizing cells in the liver lobule during the 24-h period. The present investigation confirmed the existence of circadian rhythms of the number of both DNA-synthesizing and dividing hepatocytes, and revealed a regular topographic distribution of proliferating cells in the period of their higher values in the rhythm. The process of activation of proliferation evidently begins in the periportal zone, then spreads to the rest of the lobule, but predominating in the middle zone in the period of maximal values of cell proliferation parameters. This is evidence, first, that cells of the liver parenchyma of intact animals are potentially capable of dividing whatever their position in the lobule, second, that the cells of all zones of the lobule are involved in the formulation of acrophases of rhythms of

DNA-synthesizing and dividing hepatocytes, but third, the degree of their involvement in this process differs. It was suggested previously that hepatocytes are equal in their proliferative potential, on the basis of their random distribution in the liver lobule [3].

Data on the higher mitotic activity of hepatocytes in zones 1 and 2 of the lobule at the time of its maximum in the circadian rhythm are in agreement with results obtained during the study of the regenerating liver in the period of maximal mitotic activity, when MI_3 was lower 24, 28, and 32 h after hepatectomy, than MI_1 and MI_2 [2].

It is also an interesting fact that in the period of decline of GRI in the rhythm, the number of DNA-syntheizing cells in the central zone remained just as high as during acrophase of the rhythm, but the number of mitoses fell equally sharply in all zones of the lobule. It can be tentatively suggested that a raised value of RI_3 is connected with the need to make good the number of polyploid cells, which are most numerous in the central zone [9].

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EFFECT OF ANTIOXIDANTS ON FREE-RADICAL OXIDATION OF LIPIDS IN TESTS OF RATS OF DIFFERENT AGES AND ON REPRODUCTIVE CAPACITY IN CHRONIC POLYANTIOXIDANT INSUFFICIENCY

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The development of ideas on the role of free radicals in aging has led to the enunciation of the free-radical theory of aging [6-9]. Keeping animals on a diet deficient in bioantioxidants caused the development of changes similar to those observed during aging: lipofuscin accumulates, differentiation of the spermatogenic epithelium is disturbed, interstitial connective tissue proliferates in the tests, and atherosclerotic changes appear in the blood vessel walls [3].

In the investigation described below biochemical parameters of free-radical oxidation (FRO) of lipids were studied in the tests of rats of different ages and with chronic polyantioxidant insufficiency, the reproductive capacity of rats was investigated under conditions of excessive FRO of lipids, and the protective action of a combination of antioxidants (AO) was examined.

EXPERIMENTAL METHOD

Wistar rats of three age groups were used: sexually mature (6.5 months), elderly (18.5 months), and old (28.5 months). Animals in each age group were divided into three series:

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