EXPERIMENTAL BIOLOGY

Spatiotemporal Characteristics of Glycogen Content in Rat Liver Lobule

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We evaluated spatiotemporal characteristics of glycogen content in cells of rat liver lobule. Glycogen content in the liver lobule and its circulatory subzones underwent diurnal fluctuations with acrophase at 5.00. Spatial changes were characterized by a dependence of gradients in the lobule on the phase of diurnal rhythmic fluctuations. Total glycogen content in the peripheral subzone of the lobule 5.2-fold surpassed that in the central subzone. Our results illustrate spatiotemporal organization of glycogen content in the liver lobule.

Key Words: *liver lobule; glycogen; spatiotemporal organization*

Hepatocytes constituting the liver lobule are characterized by structural and metabolic heterogeneity [2]. However, there is no general agreement regarding glycogen localization. Some authors reported that this polysaccharide occupies mainly peripheral hepatocytes of the lobule [6], while others believe that it is localized in hepatocytes of the central zone. Glycogen content in the liver is maximum at night and in early morning [1]. No relationships were found between topographic and rhythmic changes in glycogen content in the liver lobule. Published data show that glycogen is regularly distributed in the glycogen-rich liver lobule. When the total content of glycogen decreases, it mainly occupies peripheral cells of the liver lobule. Other experiments demonstrated that under these conditions hepatocytes of the central zone in the lobule contain the maximum amount of glycogen [1].

Specific features and relationship between spatiotemporal changes in glycogen content in the liver lobule remain unknown. These problems are of importance for understanding functions of the liver lobule as a biological structural and functional system.

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MATERIALS AND METHODS

Experiments were performed on 60 intact male outbred albino rats weighing 180-200 g and kept under the 12:12-h light/dark conditions (daytime 9.00-21.00). The intensity of illumination was 300-400 lx. Liver samples were taken at 4-h intervals (9.00, 13.00, 17.00, 21.00, 1.00, 5.00). Glycogen content in hepatocytes was measured by the method of McManus—Hotchkiss. Liver sections were stained with Schiff reagent. Polysaccharide concentration was estimated on a TsIMF-2 cytospectrophotometer.

The study was performed by the chronotopohistochemical method [3]. The experiment was conducted in several stages. The presence of glycogen or enzyme activity in hepatocytes was determined histochemically. Glycogen content and enzyme activity were measured by cytospectrophotometry. Spatial changes in the amount or activity of the enzyme were estimated in standard transverse sections of liver lobules. Eighteen cells were localized on a line connecting the central vein with portal vessels (radius of the lobule, topohistochemical assay). These lobules correspond to the microcirculatory hepatic moiety (acinus) [7]. The radius was divided into 3 equal parts consisting of 6 cells and corresponding to circulatory zones 3, 2, and

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1 of the liver acinus in a direction from the center to the periphery of the lobule.

Each zone was divided into 2 equal subzones that included 3 cells lying along the radius (subzones 3a, 3b, 2a, 2b, 1a, and 1b). The average content of glycogen or enzyme activity in the lobule and circulatory zones and subzones was determined after cytophotometry of each hepatocyte (S_H). The total content of glycogen or enzyme activity in the subzone was obtained by multiplying these indexes for the individual hepatocyte by the number of cells in each circulatory subzone of the histological section. It reflected the value of test parameters in the cell population of the subzone (S_{HP}).

Subzones of rat liver lobules (from 3a to 1b) contained 65, 153, 219, 261, 318, and 363 hepatocytes. The topographic (spatial) distribution gradient for S_H was estimated by calculating the percentage changes in position 2-18 cells relative to position 1 cells. The spatial distribution gradient for S_{HP} was estimated by calculating the percentage changes in subzones 3b-1b relative to subzone 3a. Both gradients were presented graphically. In the next stage we studied temporal changes in glycogen content or enzyme activity in the lobule, subzones, and zones (chronohistochemical assay). Topohistochemical indexes were recorded 6 times a day. The data were analyzed by the graphic and parametric method for studying biological rhythms [4,5]. We determined the midline estimating statistic of rhythm (MESOR), acrophase, active (AP) and passive phase (PP), length and midpoint of AP, absolute (AA) and relative amplitude (RA), and rhythm synchronization index. Structural characteristics of the biological rhythm were represented in a phasogram. Spatial and temporal organization of glycogen content or enzyme activity was determined in the liver lobule (chronotopohistochemical assay). We determined spatial changes in test parameters over 24 h (in time) and changes in biological rhythms of parameters for topographically different zones and subzones of the lobule (in space).

The relationships between spatial and temporal changes in test parameters were evaluated by pairwise correlation analysis. We calculated coefficients of correlation (r) between 24-h changes in glycogen content or enzyme activity in various subzones of the lobule (rhythm) and spatial changes in cells of the lobule at different times of day (spatial gradient). This analysis reflected the degree of synchronism between spatial and temporal changes of the parameter in time and space, respectively. AA and RA of diurnal rhythms in various subzones of the lobule characterized the degree of synchronism between spatial and temporal changes.

The differences were significant at $p \leq 0.05$.

RESULTS

Visual examination of histological preparations showed that the degree of periodic acid—Schiff reaction in hepatocytes of various zones of the liver lobule depended on the time of day. At the beginning of the light period (9.00-13.00) cells adjacent to the central vein and localized in the central zone of the lobule (zone 3) were rich in glycogen. Cells of intermediate zone 2 contained smaller granules of glycogen whose amount was lower than in zone 3 cells. Glycogen content was lowest in hepatocytes of peripheral zone 1. The total glycogen content in cells markedly increased in the dark period (1.00-5.00). Glycogen was regularly distributed in the liver lobule. Glycogen granules were large in size. Their conglomerates occupied the cell cytoplasm.

Glycogen content in individual hepatocytes and population of cells in the liver lobule of intact rats was characterized by a monophasic 24-h rhythm. The amount of glycogen was maximum at 5.00 and minimum at 13.00 (Fig. 1).

Monophasic 24-h changes in glycogen content with acrophase at 5.00 were observed in various subzones of the lobule. AP of 24-h changes in glycogen content in individual hepatocytes and population of cells in subzones and lobule corresponded to the dark period. The length and temporal position of phases were practically similar in studied rhythms. Our results indicate that these rhythms were synchronous in various subzones of the lobule. It was confirmed by the coefficient of correlation between changes in glycogen content in individual hepatocytes of various lobular subzones over a 24-h period (r=0.94±0.03).

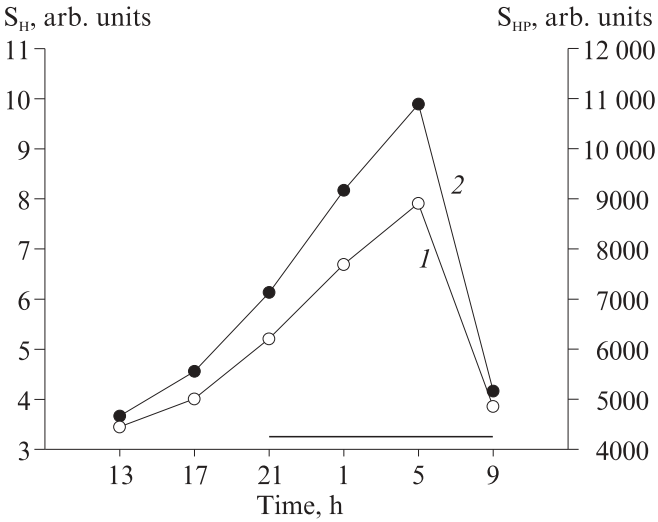

Fig. 1. Diurnal changes in glycogen content in the liver lobule of intact rats. S_H, glycogen content in the hepatocyte. S_{HP}, glycogen content in the population of hepatocytes in the lobule. Horizontal line: dark period.

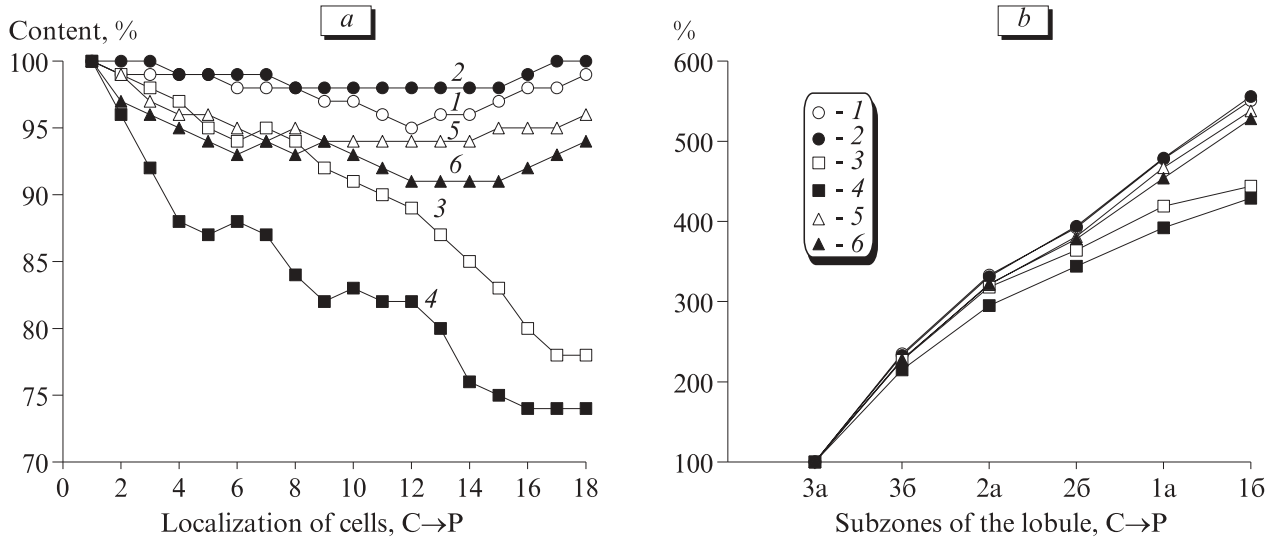

Fig. 2. Spatial gradients of glycogen content in the liver lobule of intact rats at different times of day. Changes in glycogen content in cells of different localization (%, *a*) and population of hepatocytes in various subzones of the lobule (%, *b*). C and P: central and peripheral regions of the lobule, respectively. 1.00 (*1*), 5.00 (*2*), 9.00 (*3*), 13.00 (*4*), 17.00 (*5*), 21.00 (*6*).

The correlation coefficient for the population of hepatocytes in various subzones of the lobule was r=0.93±0.03.

MESOR for glycogen content in individual hepatocytes progressively decreased in a direction from subzone 3a to subzone 1b (by 7%, statistically insignificant). However, this index increased in the population of cells in the subzone. MESOR for glycogen content in subzone 1b was 417% higher than in subzone 3a (p<0.001). Most pronounced spatial differences were observed in subzones 3a and 3b (increase by 128%, p=0.02). S_{HP} in subzone 2a was 41% higher than in subzone 3b (p=0.05). The differences in S_{HP} in other subzones were 14-20% (Table 1).

These data indicate that peripheral cells in the liver lobule possess higher biological activity (power) in accumulating a specified amount of glycogen compared to central and intermediate cells.

Glycogen content in individual hepatocytes localized in various zones of the lobule was practically similar at 17.00, 21.00, 1.00, and 5.00, but decreased in a direction from the center to the periphery at 9.00 and 13.00 (by 22 and 26%, respectively, Fig. 2, *a*). These differences in spatial changes of S_H affected the coefficient of correlation between the spatial distribution of glycogen in individual hepatocytes of the lobule at different times of day. It was practically similar to the distribution of glycogen at 1.00, 5.00, 17.00, and 21.00 (0.65±0.03). However, the distribution of glycogen at 1.00, 5.00, 17.00, and 21.00 differed from that at 9.00 and 13.00 (0.44±0.03 and 0.57±0.03, respectively).

Changes in glycogen content in cell populations (S_{HP}) were similar in various subzones of the lobule. The amount of glycogen progressively increased from subzone 3a to subzone 1b (Fig. 2, *b*). Glycogen con-

TABLE 1. Diurnal Rhythms of Glycogen Content in the Hepatocyte and Population of Hepatocytes in the Liver Lobule and Its Subzones in Intact Rats (arb. units)

Morphological structure	Hepatocyte			Hepatocyte population		
	MESOR	AA	RA	MESOR	AA	RA
Lobule	5.20	4.46	2.29	7096	6225	2.22
Subzones						
3a	5.45	4.03	2.02	354	262	2.02
3b	5.28	4.30	2.18	808	656	2.18
2a	5.21	4.39	2.26	1141	961	2.26
2b	5.14	4.45	2.31	1341	1162	2.31
1a	5.05	4.66	2.46	1608	1482	2.47
1b	5.06	4.92	2.61	1831	1786	2.61

tent at 9.00 and 13.00 increased by 344 and 329%, respectively, compared to that observed at 17.00, 21.00, 1.00, and 5.00 (by 438, 429, 451, and 456%, respectively). The average correlation coefficient for spatial changes in S_{HP} was high over a 24-h period (0.83±0.12) and surpassed that for spatial changes in S_H at different times of day (0.59±0.16).

Despite different relationships between spatial gradients of S_H and S_{HP} at different times of day, RA of their diurnal rhythms was similar in various subzones of the lobule. RA of S_H and S_{HP} in subzone 1b was 29% higher than in subzone 3a (Table 1).

Diurnal fluctuations in glycogen content in individual hepatocytes localized in various subzones of the liver lobule in intact rats were characterized by high degree of synchronism. However, spatial changes in glycogen content depended on the time of day and, therefore, on the phase of its diurnal rhythm. Glycogen content in individual hepatocytes differed little in various subzones of the lobule during AP of rhythm. The gradient of spatial changes was revealed during PP of rhythm: glycogen content decreased in a direction from the center to the periphery. The dependence of spatial changes on the phase of rhythm was revealed in studying the topographic distribution of S_{HP} in subzones of the lobule. During PP the increase of this parameter in the spatial gradient was less pronounced than during AP. Independently on the phase of rhythm the total glycogen content in the peripheral subzone of the lobule 5.2 times surpassed that in the central subzone.

We showed that spatial changes in glycogen content in the liver lobule depend on temporal changes over a 24-h period. These data reflect metabolic heterogeneity of hepatocytes and illustrate spatiotemporal organization of this polysaccharide.

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