CYTOLOGICAL ORGANIZATION OF THE LIVER TRABECULA IN RATS

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The cytological organization of the liver trabecula in rats of different ages was investigated by determining frequency distributions of classes of hepatocytes, DNA-synthesizing cells, and mitoses along the liver trabecula. The arrangement of hepatocyte subpopulations in the liver trabecula has clear features of spatial organization: Most mitoses and DNA-synthesizing hepatocytes are located at the beginning of the trabecula, the first class of nuclei predominates at the beginning and end of the trabecula, and the second class in its middle. The results provide additional evidence in support of the occurrence of self-renewal processes in the liver of adult rats.

KEY WORDS: liver trabecula; localization of mitoses and labeled cells.

A distinct and irregular distribution of mitoses, DNA-synthesizing nuclei, and nuclei of different sizes has been found in specially oriented sections through the rat liver. This finding suggests that the liver is an organ capable of renewal.

Tissue reproduction requires a spatiotemporal organization for its occurrence, as is shown clearly by the example of rapidly renewed tissues and reparative regeneration [2-6]. In 1943, Sulkin [7] postulated that physiological regeneration takes place on account of cell division at the periphery of the lobule, thereby replacing cells migrating toward the center of the lobule, where they die. This hypothesis has not been put to experimental proof in later investigations. Meanwhile, experiments on reparative regeneration of the liver [1, 2, 5, 7] have shown the initial appearance of mitoses at the periphery of the lobule.

The object of this investigation was to study the topography of the cambrial zones and other elements in the cytological organization of the liver trabecula.

The unpredictability of the phase of the mitotic cycle in which the cell finds itself at each point along the liver trabecula necessitates a statistical approach to the solution of this problem, i.e., the study of the criteria chosen for investigation by determining the frequency of their occurrence at a particular point of a typical trabecula.

The distribution of frequencies of classes of nuclei belonging to hepatocytes and DNA-synthesizing cells and of mitoses in a typical straight liver trabecula was studied in rats of different ages kept on the ordinary animal house diet.

EXPERIMENTAL METHOD

The liver of male noninbred albino rats weighing 200 and 640 g was studied. The animals received an intraperitoneal injection of thymidine-H^3 dissolved in physiological saline, in a dose of 0.5 μCi/g body weight, 1 h before sacrifice. Autoradiographs were prepared by the standard method [1]. The standard method of embedding in paraffin wax was used, histological sections were cut to a thickness of 11 μ, and the nuclei were counterstained with Mayer's hematoxylin. Since the plane of the section coincided with the surface orthogonal

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TABLE 1. Mean Length of Liver Trabecula

No. of cells in trabecula	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	27
No. of trabeculae counted	1	2	5	9	13	31	35	53	107	37	11	17	6	6	2	2	1

$\Sigma_{trab.} = 338$ $\Sigma_{cells} = 5305$ $\bar{x} = 5305/338 = 15{,}69$

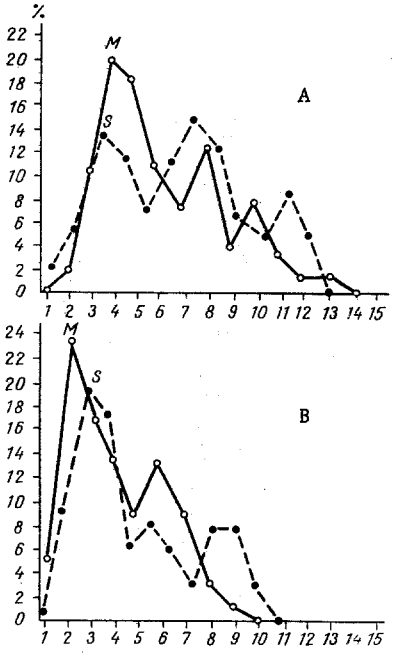

Fig. 1

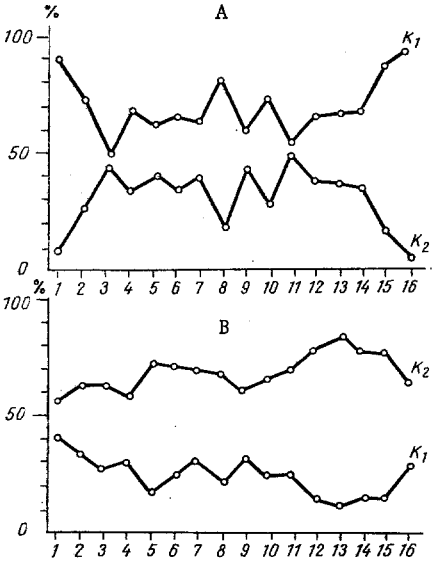

Fig. 2

Fig. 1. Distribution of mitoses (M) and DNA-synthesizing cells (S) along liver trabecula from rats weighing 200 g (A) and 640 g (B). Abscissa, serial Nos. of cells in direction from triad toward central vein; ordinate, frequency of discovery (in %).

Fig. 2. Distribution of classes of hepatocyte nuclei (K_1 and K_2) along liver trabecula of rats weighing 200 and 640 g. Remainder of legend as in Fig. 1.

with respect to the roots of the hepatic vein it contained the largest number of straight trabeculae uniting the region of the triad with the central vein of the lobule. To standardize the observations, a typical trabecula with the average number of cells determining its length was distinguished. The density of distribution of DNA-synthesizing cells and mitoses from late prophase to early telophase was studied along the liver trabecula in the direction from the triad toward the central vein in rats killed at the time of the morning peak of mitoses (6-7 a.m.).

The volume of the hepatocyte nuclei and the arrangement of the classes of nuclei along the liver trabecula were determined on photographs with a linear enlargement of 1 × 1600. To determine the distribution of the classes of nuclei, mitoses, and nuclei in the S-phase along the liver trabecula, 30-50 trabeculae, 40-45 trabeculae with mitoses, and 250-500 labeled nuclei respectively were used.

The volume of the nuclei was calculated by the equation

$$v = \frac{\pi}{6} B^2 \cdot L,$$

where L is the major diameter and B the minor diameter of the hepatocyte nucleus.

For subdivision into basic classes the volume of 520 nuclei was determined. The natural values of the volumes of the nuclei and their logarithms were distributed among 20 classes with an equal interval. The results were used to plot histograms in which classes K_1, K_2, and K_4 were distinguished. Corresponding with the maximal area for the ellipsoidal and circular forms of nuclei of each class, stencils were cut from transparent film and with their aid the class to which each particular nucleus belonged was identified on the photographs.

EXPERIMENTAL RESULTS

The typical liver trabecula was defined on the basis of the average number of cells in liver trabeculae of 15 rats weighing 160-210 g (Table 1). It will be clear from Table 1 that the typical liver trabecula can be represented as a row of 16 hepatocytes. Calculations to show the frequency of the selected features were thereafter made on typical trabeculae.

The distribution of frequencies of mitoses and DNA-synthesizing cells along the liver trabecula of rats of different ages is shown in Fig. 1. Clearly the mitoses were distributed irregularly along the trabecula: the first large maximum occurred at the 4th-5th cell in the young rat and a smaller maximum at the eighth cell of the liver trabecula. In old rats the two peaks were shifted toward the beginning of the trabecula.

The great mass of DNA-synthesizing cells was located at the beginning of the trabecula in the region of the second to eighth cell with a maximum at the third to fourth cell, followed by a second rise in the zone of the seventh to eighth cell. In old rats these two peaks were shifted toward the beginning of the trabecula.

The distribution of classes of hepatocyte nuclei along the typical trabecula in rats weighing 200 and 640 g is shown in Fig. 2. Class K_4 is included in class K_2. Plotting histograms of distributions of the classes of hepatocyte nuclei along the liver trabecula for rats of different ages showed that these distributions are irregular: Nuclei of class K_1 predominated at the beginning and end of the trabecula and nuclei of class K_2 in the middle. In old rats the number of nuclei of class K_2 was greater than in young rats, and the difference was particularly marked in the middle part of the trabecula. It will be clear from the graphs that most mitoses and DNA-synthesizing hepatocytes were located in areas of the liver trabecula where equal numbers of nuclei of classes K_2 and K_1 were found.

The arrangement of hepatocyte subpopulations in the liver trabecula thus has distinct features of spatial organization.

Since cytological transformations of hepatocytes fit into the cell cycle, the spatial structure discovered also reflects a temporal organization of the trabecula.

The facts obtained support the view that self-renewal of cells actually takes place in the adult rat liver [3, 7], for the zonal structure of the cambium can be regarded as proven, which means that cells can be said to migrate from the cambial zone.

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