BINUCLEAR AND POLYPLOID HEPATOCYTES IN DIFFERENT STRAINS OF MICE

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A comparative cytological study was made of the liver in mice of strains A/He, CBA, and C57BL/6. The number of binuclear and polyploid hepatocytes differs in the liver of mice of the different strains. Binuclear cells are more common in A/He mice, where they account for 34% of all hepatocytes, and least common (19% of all hepatocytes) in C57BL/6 mice. Cytophotometric analysis of mononuclear and binuclear hepatocytes showed that polyploidy is more marked in the liver of A/He mice than in the other two strains. Consequently, the binuclear and polyploid character of the intact mouse liver may be largely determined by the genotype of the strain concerned.

In recent years there has been a steady increase in research into the comparative study of internal organs in animals belonging to different strains. For example, considerable differences in the number of nucleoli in the lymphocytes and hepatocytes have been found in pure-line mice [3]; interlinear polymorphism of certain proteins has been detected [1]; significant differences have been found in the number of ovulations and in the pre- and postimplantation mortality of embryos of female mice of different strains [4]; unequal mitotic activity and an unequal rate of regeneration of the liver after resection, which is associated with it, have been demonstrated in mice of strains C3H, C57BL, CC57Br, and CBA [2].

However, there is little information in the literature on the relationship between the cytological features of the internal organs of mammals and their genotype. Nevertheless, the study of the cytological mechanisms concerned in normal and reparative growth of the internal organs of different strains of animals could be very important not only as means of improving the selection of experimental animals but also for establishing certain biological principles.

The object of this investigation was to study binuclear and polyploid hepatocytes in mice of different strains.

EXPERIMENTAL METHOD

Sexually mature male mice of three strains were used in the experiments A/He, C57BL/6, and CBA; the weight of the mice varied from 23 to 27 g. Each group of animals comprised from 14 to 16 mice. The animals were killed at 10-11 a.m. The liver was weighed, and pieces of it were fixed in Carnoy's fluid. The material was embedded in the usual way in paraffin wax, and histological sections, 7 μ in thickness, were stained by Feulgen's method and counterstained with light green. The number of mononuclear and binuclear hepatocytes was counted in these preparations and the percentage of the latter calculated. Preparations of isolated cells were obtained by the writer's own method, stained by Feulgen's method, and counterstained weakly with light green, and then examined photometrically on the MUF-5 apparatus. Photometry was carried out separately on the nuclei of the mononuclear hepatocytes and on the two nuclei of the binuclear hepatocytes. From 70 to 100 nuclei of cells of both types were so examined in each liver. For this purpose, 7 to 10 animals were taken from all three groups after preliminary perfusion of their liver with cold physiological saline. The numerical results were subjected to statistical analysis by the Fisher–Student method.

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TABLE 1. Percentage of Binuclear Hepatocytes in Liver of Different Strains of Mice

Strain of mice	No. of animals	Body wt. (g)	Wt. of liver		Mean number of cells counted in one liver		% of binuclear cells
			absolute (g)	relative (%)	mononuclear	binuclear	
A/He	14	25	1,50	6,0	10 520	3530	34
CBA	16	26	1,35	5,2	12 355	3891	24
C57BL/6	16	25	1,37	5,3	11 213	2337	19

TABLE 2. Percentage Distribution of Mononuclear and Binuclear Hepatocytes by Ploidy Classes in Liver of Different Strains of Mice

Strain of mice	Ploidy hepatocytes							
	mononuclear					binuclear		
	2n	4n	8n	16n	32n	2n × 2	4n × 2	8n × 2
A/He	12	62	26	0,3	—	20	56	24
CBA	30	64	6	—	0,3	36	63	—
C57BL/6	11	78	11	0,2	—	33	63	4

EXPERIMENTAL RESULTS

The results of counting the binuclear hepatocytes in the liver of mice of the different strains are given in Table 1. They show that the number of binuclear hepatocytes in the intact liver differs in mice of different strains. Binculear cells are more common in A/He mice in which they account for 34% of all hepatocytes, and least common (19% of all hepatocytes) in C57BL/6 mice; the difference between these two figures is significant ($P = 0.001$). Binuclear hepatocytes account for 24% of the total population of hepatocytes in the CBA mice; i.e., their number was intermediate between the values obtained for A/He and C57BL/6 mice.

The cytophotometric analysis showed that most of the mononuclear hepatocytes in the intact liver of mice of all these three strains are tetraploid cells: their percentage varied from 62 (A/He) to 78 (C57BL/6). The number of cells with diploid nuclei was greater (up to 30%) in the CBA mice, while in the rest it was 11-12%. Meanwhile octaploid cells were more common (up to 26%) in the A/He mice; in the CBA mice they accounted for only 6% of the total number of mononuclear hepatocytes. Differences between the numbers of octaploid mononuclear hepatocytes in strains A/He and CBA and strains A/He and C57BL/6 were not significant ($P = 0.001$; Table 2).

To simplify the comparative cytological analysis of polyploidy of the intact liver in the different strains of mice, the distribution of binuclear hepatocytes by classes of ploidy was based on the DNA content in only one of the two nuclei, although the total DNA content in each cell was twice as high. For example, a binuclear cell conventionally described as diploid (containing two diploid nuclei) was equal in its total DNA content to a mononuclear tetraploid cell, a binuclear tetraploid hepatocyte was equal to a mononuclear octaploid cell, and so on.

Just as in the mononuclear hepatocytes, polyploidy in the binuclear cells was most marked in the liver of the A/He mice. In this case cells with two tetraploid nuclei accounted for 56%, and cells with octaploid cells for 24% of the total, whereas in the CBA mice only hepatocytes with tetraploid nuclei (63%) were found, and there were no cells with octaploid nuclei. The number of binuclear cells with polyploid nuclei in the liver of the C56BL/6 mice occupied an intermediate position between their numbers in A/He and CBA mice: there were 63% of cells with tetraploid and 4% with octaploid nuclei. The number of hepatocytes with two diploid nuclei was 20-37% of the total number of binuclear cells in all strains (Table 2). Differences between strains A/He and CBA and A/He and C57BL/6 as regards the diploid nuclei of the binuclear hepatocytes were close to significant ($P = 0.06$ and 0.02, respectively).

No strict correlation was thus observed between the numbers of binuclear and polyploid mononuclear hepatocytes in the liver of the three strains of mice (A/He, CBA, C57BL/6) studied. The relative percentages of the ploidy classes of mononuclear hepatocytes were similar to those for binuclear cells in the mice of that strain. Comparison of the ploidy of the hepatocyte nuclei in mice of different strains shows that polyploidy is most marked in A/He mice and least marked in CBA mice; C57BL/6 mice occupy an intermediate position between them. Consequently, the binuclear and polyploid character of the intact mouse liver may be largely determined by the genotype of the particular strain concerned.

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