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NUMBER AND SIZE OF NUCLEOLI IN BINUCLEATE LIVER CELLS

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The number of nucleoli in cells has been characterized only as being variable, encompassing the very wide range of values from 0 to 2000 in individual cells of different species (1). This variability of nucleolar number is undoubtedly determined by many genetic as well as nongenetic factors. Significant interstrain differences in the number of nucleoli were found in mouse lymphocytes and liver cells, and the inheritance of these differences was studied in hybrid animals (5, 6). Besides these genotypic differences there is a marked variation in the number of nucleoli per nucleus in different tissues of one individual and even in one cell type of one tissue. The degree of cell differentiation and the cell function itself may be responsible for this variation. Cytoplasmic regulation of RNA synthesis in the nucleus was described by Gurdon and Brown (4). However, all mentioned factors become unimportant when the numbers of nucleoli in two nuclei of one normal cell are compared. In this case, only the factors determined by the nucleus itself could be responsible for the possible difference. To provide more information about this question we studied the nucleolar number in binucleate normal liver cells from adult mice.

MATERIALS AND METHODS

Small fragments of fresh liver from adult mice 2–3 months old were scraped with a sharp scalpel in one drop of calf serum, washed with phosphate buffer saline (PBS) (2) and, after 5 min of centrifugation at 800 rpm, sediment was resuspended in one drop of calf serum. Fresh smears were stained with buffered toluidine blue to demonstrate RNA-containing structures in the cell (9, 10). In addition to naked nucleoli, the cells with well preserved cytoplasm were also present in these smears. In all, we examined 184 binucleate cells from the mice of strains A (62 cells), C3H (62 cells), and C57Bl/10 (B10, 60 cells). The microscopic image was projected on a television screen, and the outlines of the nuclei and nucleoli were traced on transparent paper (Fig. 1). The relation of the area of the nucleoli to their number and to total area of the nucleus was investigated by the method described previously (7).

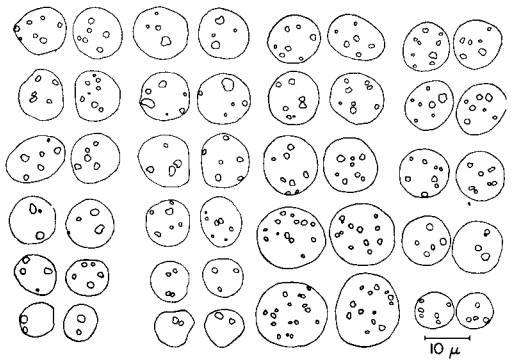
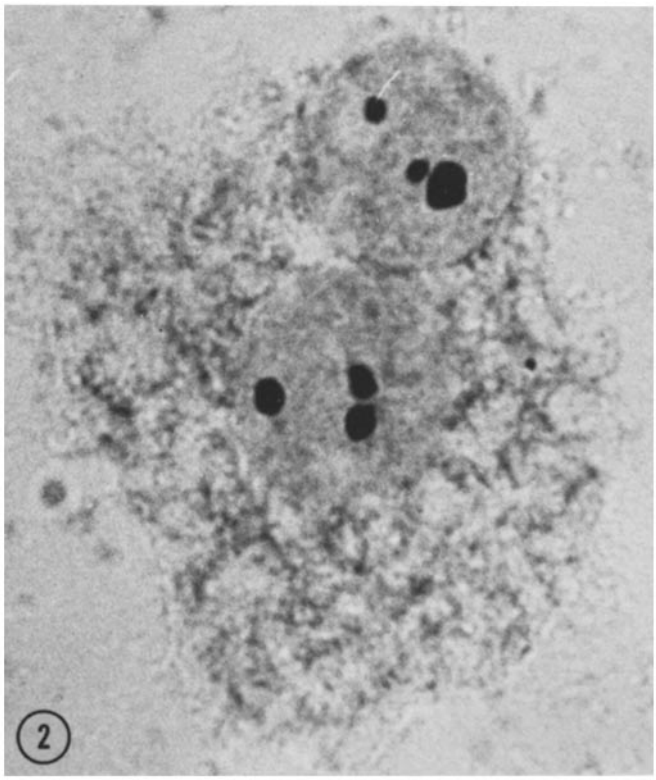
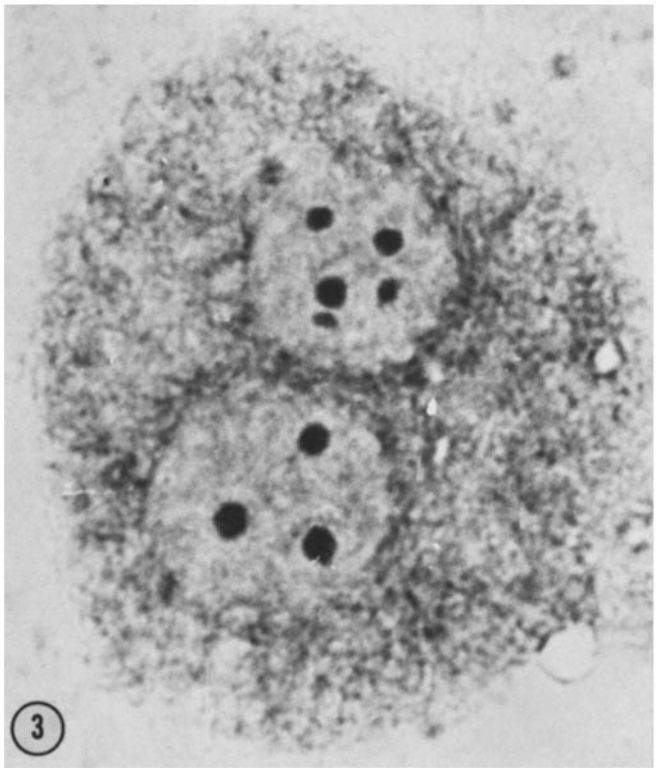


FIGURE 1 Sample of nuclei and nucleoli outlines traced from television screen.

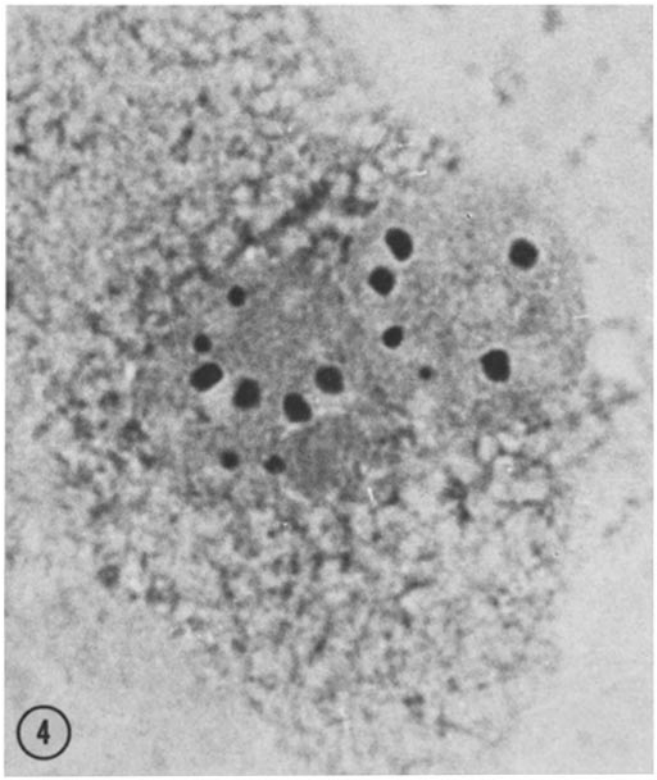
RESULTS AND DISCUSSION

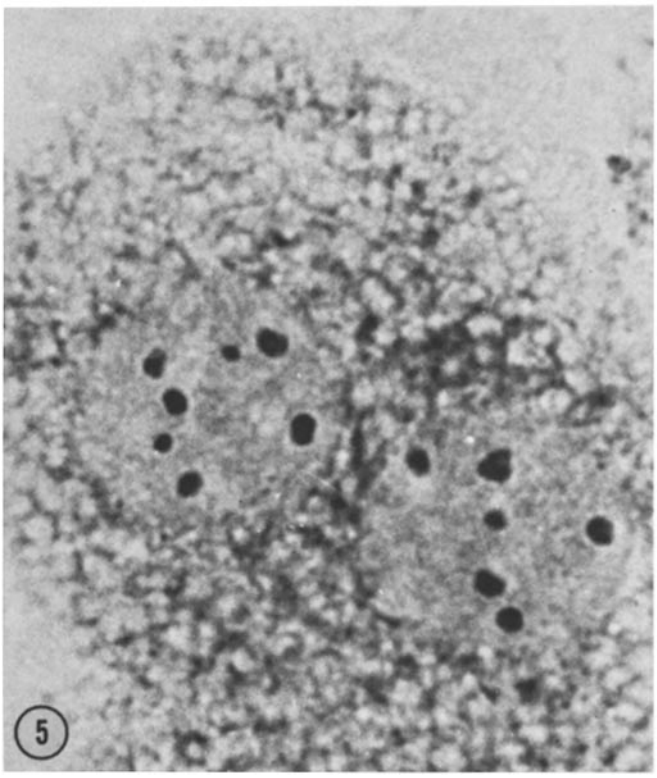
Cell nuclei were of variable size, apparently corresponding to the variable ploidy of liver cells (7, 8). However, both nuclei of one cell were almost of equal size (Figs. 2–5). The results of nucleolar





FIGURES 2–5 Binucleate mouse liver cells: (*a*) equal number of nucleoli (Fig. 2) (*b*) number of nucleoli differing by one (Fig. 5) (*c*) number of nucleoli differing by two (Figs. 3, 4).





FIGURES 4–5 See legend under Figs. 2–3.

TABLE I

Comparison of Nucleolar Number and Nucleolar Area in Relation to the Total Area of Nucleus in Both Nuclei of Binucleate Liver Cells

	A			C3H			B10			Total		
	No. of cells	% of cells	R	No. of cells	% of cells	R	No. of cells	% of cells	R	No. of cells	% of cells	R
			%			%			%			%
Equal no. of nucleoli in both nuclei	28	45	0.54	25	41	0.79	21	35	0.61	74	40	0.65
Number of nucleoli differing by one	30	49	0.44	21	34	0.86	25	42	0.68	76	41	0.66
No. of nucleoli differing by two	2	3	0.15	12	19	0.88	9	15	0.50	23	13	0.51
No. of nucleoli differing by three*	2	3	0.29	4	6	0.96	5	8	0.39	11	6	0.55
Total	62	100	0.47	62	100	0.84	60	100	0.60	184	100	0.64

* In one B10 liver cell the number of nucleoli in both nuclei differed by four.

R, the difference in the nucleolar area related to the total area of nucleus between both nuclei in binucleate liver cells.

counting are summarized in Table I. An equal number of nucleoli in both nuclei of one cell was observed in 40% of liver cells examined. In the majority of the other cells the number of nucleoli in both nuclei differed by one. A difference of two nucleoli was seen in 13% of cells, and a difference of three in only 6% of cells. The results did not differ essentially in three examined mouse strains.

In spite of the observed differences in the number of nucleoli in both nuclei of binucleate cells, the percentage of the nucleolar area in relation to the total area of nucleus was virtually identical in both nuclei of all cells examined, the difference being less than 1%. This is in agreement with our previous findings that the fraction of the nucleolar area in relation to the total area of the nucleus is approximately the same in all liver cells. This is in spite of the different size of liver cell nuclei, the different number of nucleoli in individual liver cells, and the significant interstrain differences in the number of liver cells in mice (6, 7).

In binucleate cells, besides an equal number of genes for ribosomal RNA and their equal localization on corresponding chromosomes, the cytoplasmic environment is the same for both nuclei. Therefore the possible explanations of unequal nucleolar number in both nuclei are reduced to a few hypotheses: (*a*) there may be a different expression of nucleolus organizers in both nuclei, accidental or controlled by each nucleus itself. A recently described asynchrony of nucleolar DNA synthesis in nucleoli of one nucleus, which suggests that some of the nucleolus organizing regions are involved in replication but that the others continue to synthesize RNA (3), is in good agreement with this first hypothesis; (*b*) fusion and/or fragmentation of nucleoli may be responsible, but we did not see any morphological signs in favor of this process. Cooperation of more nucleolus organizers in the formation of one nucleolus promoted by the random variability in the relative distribution of chromosomes cannot be excluded.

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