DNA REPLICATION IN BINUCLEAR CELLS OF THE REGENERATING LIVER IN RATS AND MICE

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Both the beginning and end of DNA replication in the nuclei of binuclear cells in the regenerating liver of rats and mice are highly synchronized. At the same time, in individual stages of the S-period the nuclei of binuclear cells can synthesize different amounts of DNA. This suggests that the beginning and end of DNA replication are regulated in them at the level of the cell as a whole, whereas the amount of DNA synthesized at a given moment can be regulated at the level of each individual nucleus.

Cytological aspects of DNA synthesis in mononuclear mammalian cells, including regenerating liver cells, have been adequately discussed in the literature. Meanwhile, the character of DNA replication in binuclear cells has received relatively little study [1, 3, 5], and no information regarding DNA synthesis in binuclear cells of the regenerating liver could be found in the literature. Yet the regenerating liver is a very convenient object in which to study this problem, for it contains a large number of binuclear cells (about 30% in the liver of intact rats and about 45% in mice), and they play an equal part in the proliferative processes during regeneration as the mononuclear cells [2].

TABLE 1. Percentage of Labeled Prophases of Binuclear and Mononuclear Cells in Animals Receiving Thymidine-H^3 10-9 h (beginning of S-period) and 5-4 h (end of S-period) before Sacrifice

Time of injection of thymidine-H^3 (No. of hours before sacrifice)	Percent of labeled prophases of binuclear cells	No. of prophases of binuclear cells counted	Percent of labeled prophases of mononuclear cells
10	92	50	92
	88	50	78
	70	50	77
	88	50	84
9	98	50	
	100	25	
	99	40	
5	88	50	
	100	25	
	96	25	
4	20	100	14
	27	100	22
	20	50	17
	17	50	15

The object of the present investigation was to study the degree of synchronization of the beginning and end of DNA replication in both nuclei of the binuclear cells of the regenerating liver and to determine the degree of synchronization of DNA replication in these nuclei at individual stages of the S-period. By solving these problems it will be possible to conjecture at which level (the cell as a whole or the individual nuclei) the beginning and end of DNA replication are regulated, and at what level the quantity of DNA synthesized in each individual stage of the S-period is regulated.

EXPERIMENTAL METHOD

The work was carried out on male and female noninbred rats weighing about 180 g and on male mice (CBA×C57Bl hybrids) weighing 20-22 g. Partial hepatectomy was performed in the usual way, and the rats were sacrificed 29 h and the mice 48 h after the operation (at 8 A.M.).

Thymidine-H^3, in a dose of 0.1-0.25 μCi/g (specific activity about 800 μCi/mmole) was injected intraperitoneally into the rats, 10, 9, 7, 5, and 4 h before sacrifice (3-5 animals at each time), and into mice 9 and 4.5 h before sacrifice. Some of the animals (3 rats) were killed 20 min after receiving the isotope (21 h 20 min after the operation). The lobes

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TABLE 2. Percentage of Binuclear Cells with Equal or Unequal Incorporation of Thymidine-H^3 into Interphase Nuclei in Individual Animals

Time of sacrifice	Equal incorporation into both nuclei	Incorporation into one nucleus 1.5 times greater than into other	Incorporation into one nucleus twice or more that observed into other nucleus
20 min after injection of thymidine-H^3	55	27	18
	48	28	24
	64	13	23
Mean . . .	56	23	21
5-7 h after injection of thymidine-H^3	60	22	18
	58	20	22
	66	13	21
	65	20	15
Mean . . .	62	19	19

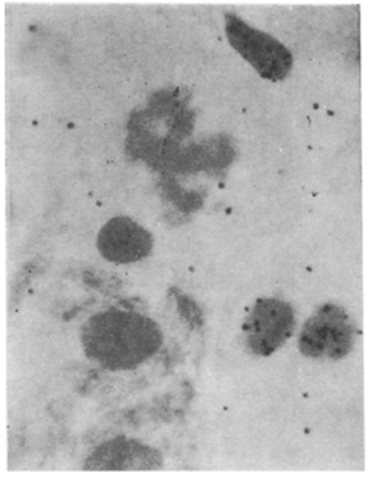

Fig. 1

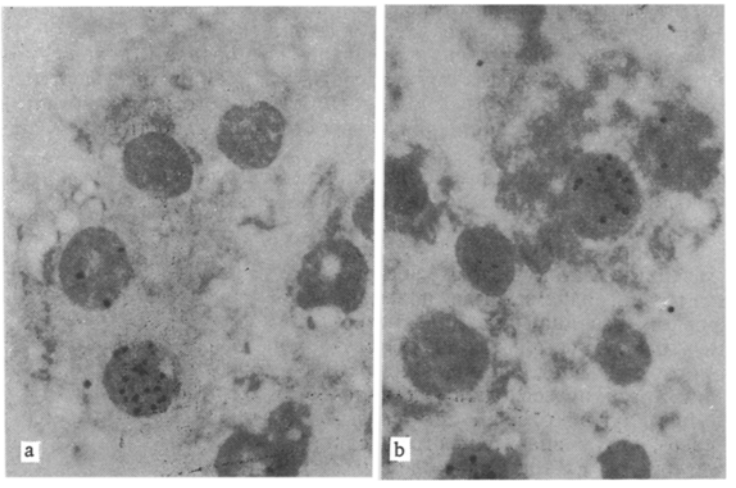

Fig. 2

Fig. 1. Late prophases of binuclear cells: both prophases are labeled in one cell and both are unlabeled in the other cell (mice receiving thymidine-H^3 4.5 h before sacrifice).

Fig. 2. Unequal (a and b) incorporation of thymidine-H^3 into interphase nuclei of binuclear cells (rats sacrificed 20 min after injection of isotope).

of the liver were perfused with warm (37°C) 0.027 M Na citrate in Locke's solution without $CaCl_2$, after which they were incubated (37°C) or left at room temperature for 10 min. Impressions of the liver sections were fixed in methanol, coated with type R liquid nuclear photographic emulsion, and exposed at 4°C for 2-4 weeks. After development, the specimens were stained with Mayer's hematoxylin and the following indices determined: 1) the percentage of labeled prophases of the binuclear and mononuclear cells and 2) the intensity of labeling, i.e., the number of silver grains above the interphase nuclei of the binuclear cells in animals sacrificed 20 min or 5-7 h after administration of the isotope.

EXPERIMENTAL RESULTS

The results of experiments in which thymidine-H^3 was injected into the rats 10-9 and 5-4 h before sacrifice, i.e., at a time when most cells reaching mitosis at the time of death had started or completed DNA replication, are given in Table 1. Determination of the label above the prophases of the binuclear cells

showed that, as a rule (with very few exceptions), either both are labeled or both are unlabeled. This indicates that the beginning and end of DNA replication, i.e., the beginning and end of the S-period, in the nuclei of binuclear cells of the regenerating rat liver are synchronized. The number of labeled prophases of the binuclear cells in each animal at all times after injection of thymidine-H^3 was about the same as the number of labeled prophases in mononuclear cells.

The results obtained for mice were absolutely analogous. When thymidine-H^3 was given 4.5 h before sacrifice (at the end of the S-period, many of the cells reaching prophase of mitosis by the time of sacrifice) both prophases of the binuclear cells were either labeled or unlabeled (Fig. 1; 100 prophases of binuclear cells counted in 2 mice), while the number of labeled prophases of binuclear cells in each animal was about the same as the number of labeled prophases of mononuclear cells. When thymidine-H^3 was given 9 h before sacrifice (the first few hours of the S-period), both prophases of the binuclear cells were labeled in 100% of cases (25 prophases of binuclear cells were counted in each of 2 mice). Consequently, in mice just as in rats marked synchronization of the beginning and end of DNA replication was found in both nuclei of the binuclear cells.

The few exceptions which were found (7 of 715 prophases of binuclear cells in rats and 2 of 250 in mice, i.e., about 1%), indicate that some degree of asynchronism is possible at the beginning and end of the S-period in a small proportion of cells.

The fact that after administration of thymidine-H^3 the number of labeled prophases of binuclear cells at the beginning and end of the S-period corresponded approximately to the number of labeled prophases of mononuclear cells of the same animal indicates equal variability in the duration of the G_1- and G_2-periods of these cells.

The results of counting the number of silver grains above the interphase nuclei of the binuclear cells of the regenerating rat liver are given in Table 2. In animals sacrificed 20 min after injection of thymidine-H^3, the number of silver grains above both nuclei was about equal in 56% of the binuclear cells (the difference was not more than 10-20%). In the remainder (44%) the number of grains over one of the nuclei was 1.5-2 times higher than the number over the other (Fig. 2). This indicates that in a large number of binuclear cells incorporation of precursors into DNA in one of the nuclei is greater at certain stages of the S-period than in the other, or in other words, that different amounts of DNA are replicated in these nuclei. If, however, the intensity of labeling was determined above interphase nuclei of binuclear cells of animals sacrificed after a longer interval (5-7 h after injection of the isotope), the number of cells with unequal incorporation of thymidine-H^3 was considerably reduced – to 38% (equal incorporation was now found in 62% of cells).

Since the beginning and end of DNA replication in both nuclei of the binuclear cells of the regenerating liver are synchronized, as was shown above, and since the nuclei of these cells are equal in ploidy [4], it can be postulated that the difference in intensity of DNA synthesis in them indicates the presence of fluctuations in the amount of DNA replicated in each nucleus. Probably during the S-period nuclei of the binuclear cells switch repeatedly from the synthesis of a certain maximal quantity of DNA to synthesis of its minimal quantity, and the time of replication of maximal and minimal quantities of DNA in one of the nuclei does not coincide, in a high proportion of cases, with the time of replication of the same quantity of DNA in the second nucleus.

The results thus indicate that both the beginning and end of DNA replication as a rule coincide strictly in time in the two nuclei of binuclear cells in the regenerating liver, i.e., they are highly synchronized. However, the amount of DNA replicated during a given stage of the S-period in one of the nuclei may differ considerably from that in the other nucleus.

It can accordingly be postulated that the beginning and end of DNA replication in the nuclei of binuclear cells of the regenerating liver in rats and mice are regulated as a rule at the level of the whole cell, whereas the amount of DNA synthesized at a given moment may be regulated at the level of each separate nucleus.

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