DURATION OF THE MITOTIC CYCLE OF MOUSE LIVER CELLS AT DIFFERENT STAGES OF CARCINOGENESIS INDUCED BY ORTHO-AMINOAZOTOLUENE

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Mice were given orthoaminoazotoluene for 9 months. The duration of the G_2 and S periods of the mitotic cycle was determined by an autoradiographic method and the duration of mitosis was determined by a stathmokinetic method using colcemid for hepatocytes from intact liver and for cells of adenomatous nodules and primary hepatomas. The duration of the S period for cells of the intact liver and of the adenomatous nodules was shown to be identical (13.8 and 13.9 h respectively), whereas for hepatoma cells it was reduced to 12.8 h. The duration of the G_2 period did not change substantially in the course of carcinogenesis and ranged from 2.2 to 2.7 h. The mean diurnal duration of mitosis likewise was unchanged at about 1 h. The increase in the number of mitoses and of DNA-synthesizing cells in hepatomas results from the entry of a larger number of cells into mitosis and into the S period and not from an increase in the duration of the M- and S periods of the mitotic cycle.

KEY WORDS: orthoaminoazotoluene; hepatocarcinogenesis; mitotic cycle.

It has recently been shown that during induced carcinogenesis of the liver in mice and rats the mitotic index (MI) and index of labeled nuclei (ILN) are higher than in the liver of normal animals [2, 7, 9, 12, 13]. The present writer's previous findings also indicated a marked increase in the mean 24-hourly values of MI and ILN in the liver cells of mice during carcinogenesis [5]. The increase in the two indices could be due either to an increase in the duration of the individual periods of the mitotic cycle or to an increase in the number of cells starting on these periods.

The object of the present study was to investigate the duration of some periods of the mitotic cycle of mouse liver cells at different stages of carcinogenesis induced by orthoaminoazotoluene (OAAT).

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 20-25 g were used. OAAT was given to the experimental animals for 9 months by the method described previously [5]. Control animals did not receive OAAT.

Indices of the mitotic cycle of intact hepatocytes were studied in the liver of the control mice. The same indices were studied in the experimental mice for cells of adenomatous nodules and of primary hepatomas, i.e., morphological structures corresponding to stages II and III of hepatocarcinogenesis according to Shabad's classification [6].

Experiments to determine the duration of mitosis (t_M) were performed on some of the animals of the control and experimental groups with the aid of colcemid. The compound was injected intraperitoneally in a dose of 5 mg/kg body weight 4 h before sacrifice at four times during the 24-h period. Five or six mice were used at each time. Control animals were killed on the same day, three mice at a time, 12 times during the 24-h period.

The duration of mitosis was calculated by the equation:

$$t_M = \frac{MI \cdot A}{MI_{col}},$$

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TABLE 1. Changes in MI, MI_{col}, and t_M of Liver Cells of Normal Mice and at Different Stages of Carcinogenesis Induced by OAAT during the 24-h Period

Time	MI, ‰			MI_{col}, ‰			t_M, h		
	hepatocytes of normal mice	cells of adenomatous nodules	hepatoma cells	hepatocytes of normal mice	cells of adenomatous nodules	hepatoma cells	hepatocytes of normal mice	cells of adenomatous nodules	hepatoma cells
6-10 p.m. (I)	0,33	0,56	0,84	1,03	2,14	2,28	1,28	1,05	1,47
midnight-4a.m. (II)	0,55	0,44	2,34	2,69	1,42	8,35	0,82	1,21	1,12
6-10 a.m. (III)	0,88	1,00	1,34	3,86	5,17	7,62	0,96	0,77	0,70
noon-4 p.m. (IV)	0,38	0,79	0,83	1,91	1,85	5,27	0,80	1,71	0,63
Mean values for 24-h period	0,54±0,14	0,70±0,21	1,34±0,21	2,37±0,56	2,64±0,51	5,88±0,83	0,97±0,27	1,19±0,31	0,98±0,27

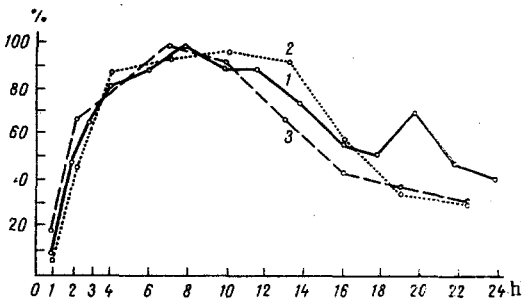

Fig. 1. Changes in percentage of labeled mitoses of liver cells of intact mice and of mice in different stages of hepatocarcinogenesis. 1) Hepatocytes of intact mice; 2) cells of adenomatous nodules; 3) cells of primary hepatomas. Abscissa, time after injection of [^{3}H] thymidine (in h); ordinate, percentage of labeled mitoses.

where t_M is the duration of mitosis (in h); A the duration of action of colcemid (4 h); MI_{col} the mitotic index in mice receiving colcemid. MI and MI_{col} were determined on the basis of examination of 10,000-20,000 cells in histological sections of the liver and hepatomas.

Other indices of the mitotic cycle were determined in some of the normal and experimental mice from the curve of labeled mitoses [11].

[^{3}H] Thymidine (USSR) with a specific activity of 19.8 Ci/mmole was injected once, intraperitoneally, at 9 a.m. into the experimental mice in a dose of 0.5 μCi/g body weight. The animals were killed 1, 2.5, and 4 h later, and subsequently at 3-hourly intervals for 24 h. [^{3}H] Thymidine was injected into normal mice at 8.30 a.m. and these animals were killed 1, 2, 3, and 4 h later and subsequently at 2-hourly intervals for 24 h.

Labeled and unlabeled mitoses were counted during examination of 50,000-60,000 cells in the autoradiographs (exposure 30-50 days). The proportion of labeled mitoses was determined in the total number of dividing hepatocytes found in all the animals (five or six mice) used at each period of the investigation. The proliferative pool (Pc) was determined by Mendelsohn's method [10] as modified by Denekamp [8].

EXPERIMENTAL RESULTS

The results in Table 1 show that the longest duration of mitosis was observed at 6-10 p.m. in the liver of normal mice (1.28 h) and in the hepatomas (1.47 h) and at noon-4 p.m. in the adenomatous nodules (1.71 h); the shortest duration of mitosis was found from midnight to 4 a.m. in the normal mice and from 6 to 10 a.m. in the adenomatous nodules and hepatomas ($P < 0.05$). It follows from Table 1 that the diurnal rhythm of mitosis was basically the same in the experimental and control mice. The highest values of MI were observed during the morning (noon-10 a.m., $P < 0.05$). These observations confirmed previous results [5] indicating the similarity of the diurnal rhythm of the liver cells in normal animals and at the successive stages of hepatocarcinogenesis. The highest values of MI correspond to the highest values of blocked metaphases. For instance, diurnal changes in MI_{col} indicate that the increase in MI was mainly due to an increase in the number of cells starting mitosis, in all the groups of animals studied. The fact that the diurnal rhythm of mitosis depends mainly on changes in the rates of entry into mitosis of the cells during the 24-h period has also been established by other workers for both normal and tumor tissues [1, 3, 4].

The mean values of MI and MI_{col} for the 24-h period for the hepatoma cells were more than twice as high as those for hepatocytes of intact mice ($P < 0.05$).

The results of the study of the duration of the periods of the mitotic cycle are illustrated in Fig. 1. They show that curves of the percentage of labeled mitoses in hepatocytes of normal mice and in cells of adenomatous nodules and primary hepatomas were very similar. However, the earlier beginning and the more gradual fall of the descending part of the curve of labeled mitoses for the hepatomas, so that it reached values below 50% fraction of labeled mitoses, will be evident. Consequently the duration of the S period for hepatoma cells (12.8 h) was rather lower than for the hepatocytes of normal liver and for the cells of adenomatous nodules (13.8 and 13.9 h respectively). The duration of $G_2 + \frac{1}{2}M$ for hepatoma cells (2.2 h) was almost the same as for normal liver cells and for cells of the adenomatous nodules (2.2 and 2.7 h respectively).

The calculated value of Pc for hepatocytes of intact mice was 1%, for cells of adenomatous nodules 1.7%, and for primary hepatoma cells 2.7%.

The process of hepatocarcinogenesis induced by OAAT is thus characterized by a short decrease in the duration of the S period. The mean values of t_M for the 24-h period (Table 1) did not change significantly during the course of carcinogenesis (in the intact and experimental mice the duration of mitosis was about 1 h).

Consequently, the results are evidence that the increase in the mean number of DNA-synthesizing and dividing cells in the 24-h period discovered by the writer previously in the course of hepatocarcinogenesis [5], and also the increase in the mean value of MI during the 24-h period for hepatoma cells observed in the present investigation are not due to an increase in the duration of mitosis or of the S period of the mitotic cycle. The increase in MI and ILN of the liver cells in the course of carcinogenesis may perhaps depend on an increase in the number of cells entering the corresponding phase of the cycle. This hypothesis is also confirmed by the increase in Pc and in the mean values of MI_{col} for the 24-h period in the late stages of hepatocarcinogenesis discovered in the present investigation.

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