CHARACTERISTICS OF THE MITOTIC CYCLE OF ASCITES HEPATOMA 22A CELLS IN THE TERMINAL STAGE OF DEVELOPMENT

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The characteristics of growth of ascites heptoma 22A in the terminal stage of development were studied. It was shown by an autoradiographic method that some cells may be in the G_1- or R_1-period for 2 or even 4 days. The mean duration of the G_2-period was 16 h. Of the total number of cells 55-60% were in the greatly lengthened G_1- and R_1-period, 7% in the S-period, and 9% in the G_2-period. The remaining 25-30% of cells evidently had irreversibly left the mitotic cycle, for they did not participate in proliferation in response to stimulation of cell division.

KEY WORDS: ascites hepatoma 22A; mitotic cycle; resting state.

Considerable attention is currently being paid to the mechanisms of subsidence of division in ascites tumors and the possibility of departure of cells from the mitotic cycle [1-11]. Because of the contradictory nature of evidence in the literature and the absence of data on these matters for ascites hepatomas it was decided to undertake the present investigation, which was a continuation of the study of proliferation of ascites hepatoma 22A (AH22A) cells [1-3]. Attention was concentrated on the following problems: determination of the duration of the separate periods of the mitotic cycle of cells in the terminal stage, the character of completion of the mitotic cycle during stimulation of division in such a tumor, and determination of the relative number of cells in the various periods of the mitotic cycle in the terminal stage of development of AH22A.

EXPERIMENTAL METHOD

An AH22A maintained by transplantation of 0.25 cm^3 ascites fluid (about 40×10^6 cells) at intervals of 2 weeks in C3HA mice weighing 20-24 g was used for the experiments. The maximal life span of the animals was 14-15 days, and the last few days constituted the terminal stage of development of the tumor. The mitotic index (MI) was determined by counting 2000 cells and the index of labeled nuclei (ILN) was studied in 1000 cells in each animal and expressed in promille. Thymidine-^3H was injected intraperitoneally in a dose of 0.3 μCi/g in the terminal stage and 0.1 μCi/g during the first 24 h after transplantation. Thymidine-^{14}C in a dose of 0.2 μCi/g was used as the second label and the relative number of cells with either label was determined. Films of ascites fluid were coated with type M liquid emulsion and exposed for 10-20 days at 4°C. In the experiments with colchicine the drug was injected intraperitoneally in a dose of 0.25 μg/g.

EXPERIMENTAL RESULTS

In the terminal stage of development of AH22A the values of MI and ILN were determined: They were 2.5 ± 1.4 and 70.0 ± 1.4‰ respectively (mean for 4 animals), about one-tenth of the corresponding indices in tumors in the early stages of growth. One cause of the subsidence of division in ascites tumors is an increase in the duration of the mitotic cycle [7, 9-11, 14]. From the curve of labeled mitoses at this stage only the mean duration of the postsynthetic (G_2) period can be judged. The initial segment of such a curve is shown in Fig. 1: The mean duration of the G_2-period was about 16 h (in tumors on the fifth day of development 6 h). To form some idea of the maximal duration of the G_2- and G_1 (presynthetic)-periods and on the rates of their completion in response to stimulation of division, the

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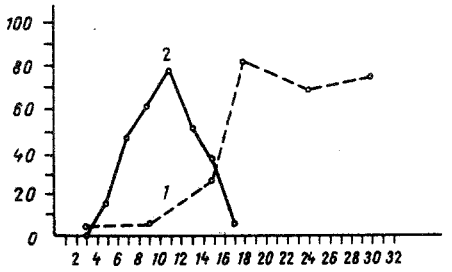

Fig. 1

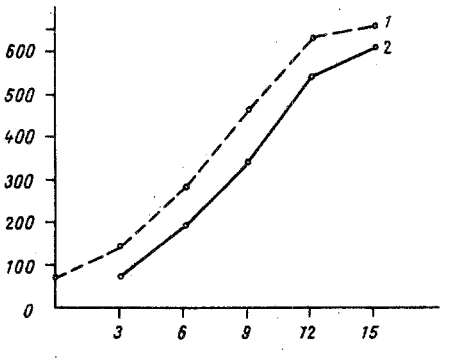

Fig. 2

Fig. 1. Changes in percentage of labeled mitoses at different periods after administration of thymidine-^{3}H. Abscissa, time (in h) after injection of thymidine-^{3}H; ordinate, percent of labeled mitoses (each point on curve represents mean for 4-6 animals). 1) Without transplantation, in 14-day AH22A; 2) after transplantation of 14-day AH22A.

Fig. 2. Changes in ILN at different times after transplantation of 14-day AH22A. Abscissa, time (in h) after transplantation of tumor; ordinate, ILN (in $^0/_{00}$); each point on curve is mean for 3-4 animals). 1) Change in total ILN; 2) change in number of cells with ^{3}H label.

following experiment was carried out: Mice were given injections of thymidine-^{3}H, after the 10th-14th days of development of AH22A, during the last two or four days at intervals of 12 h [this was evidently shorter than the duration of the synthetic (S) period in the terminal tumor, for the duration of the S-period was about 20 h even on the fifth day of development], after which the ascites fluid was injected into intact animals. These animals were killed at different times after the injection and the time of appearance of unlabeled mitoses and their number were recorded. Unlabeled mitoses represented division of cells which did not synthesize DNA throughout the period of administration of the label, and which were evidently in the G_1- and G_2-period. The experimental results are given in Table 1 and they show that in the early periods after transplantation there were virtually no unlabeled mitoses. Since it is at this time that cells must leave the G_2-period to begin mitosis, it can be assumed that in the stage of terminal ascites, first, there were no cells for which the duration of the G_2 period exceeded two, let alone four days, and second, there were no cells in the resting state (entered from the G_2-period of the mitotic cycle). However, the possibility cannot be ruled out that in the terminal stage of growth of AH22A there were cells which had left the mitotic cycle in the G_2-period irreversibly. A considerable number of unlabeled mitoses appeared 12 h after transplantation, and by 15-18 h their proportion had increased to 20-40%. After supplementary administration of thymidine-^{3}H 6 h before sacrifice all mitoses were labeled. Hence it follows that after 12 h cells which had been in the G_1-period for two and four days in the stage of terminal ascites (or had left it to enter the resting stage R_1) began to divide. The proportion of cells with a G_1-period longer than two days in duration, incidentally, was about 35% of the total number of cells leaving the G_1-period. Cells whose G_1-period exceeded 4 days in duration were considerably fewer in number (only 12-15%). Consequently, shortening the duration of the G_1-period during stimulation of division by transplantation took place at about the same times in these cells, i.e., the duration of the G_1-period (or the length of stay in the resting state R_1) does not affect the degree of shortening of the G_1-period during stimulation of division. This confirms the results of the writers' previous investigation [3].

As already stated, ILN at the terminal ascites stage was 70 $^0/_{00}$ or 7%. This is evidence that this proportion of the population in the tumor was in the S-period. An attempt was made to determine the distribution of the remaining 93% of cells among other periods of the mitotic cycle. From the change in ILN after transplantation, using the double label, the relative number of cells which were in the G_1-period in the terminal ascites stage could be determined. Cells synthesizing DNA in the 14-day AH22A were labeled with thymidine-^{14}C. This ascites fluid was injected after 1 h into intact mice and further thymidine-^{3}H was injected at different times after transplantation. Cells which changed from the G_1-period into

TABLE 1. Number of Labeled Mitoses (in ‰) at Different Times after Transplantation of 14-day Ascites Hepatoma 22A

	Time after transplantation, h														
	1	2	3	6	9	12		15		18		21		24	
Injection of thymidine-^{3}H for 2 days	100	100	—	98	100	90	100*	68	99*	64	98*	66	—*	76	—*
	100	100	98	100	100	82	100	64	100	52	—	66	—	50	—
	100	96	100	100	100	74	100	62	—	64	100	64	—	54	—
Mean						82		65		60		65		60	
Injection of thymidine-^{3}H for 4 days	100	100	99	100	99	94	100	86	100	80	100	86	100	74	100
	100	100	100	100	100	92	100	85	100	94	100	80	100	84	100
	100	100	100	100	100	100	100	56	100	80	100	90	100	84	100
			100	100	100	96	100	90	100	76	100	96	—	92	100
Mean						95,5		80		83		88		83	

Legend. *) Number of labeled mitoses (in %) during supplementary administration of thymidine-^{3}H after transplantation, 6 h before sacrifice.

the S-period after transplantation had the ^{3}H label. The results are illustrated in Fig. 2. After 3 h, ILN was already twice its original level (i.e., ILN at the terminal ascites stage). After 12-15 h ILN reached a plateau (600-650‰), and evidently it was at this time that the maximal number of cells starts from the G_1-period into the S-period (550-600‰). During stimulation of division by transplantation, about 55-60% of their total number in the terminal stage starts from the G_1-period into the S-period. This took place both as a result of a sharp decrease in the duration of this period and also, perhaps, as a result of return from the resting state R_1.

The number of cells in the terminal ascites stage in the G_2-period was determined by using colchicine to collect mitoses in the early stages after stimulation of division. After transplantation of cells from a terminal tumor, labeled 1 h beforehand with thymidine-^{3}H, labeled mitoses appeared after 3 h and reached 80% by 10-11 h after transplantation (Fig. 1). Hence it follows that the cells had in fact completely ceased to enter mitosis from the G_2-period at that time. During the first and subsequent 6-h periods after transplantation colchicine collected about 8.8% of unlabeled mitoses (7.5 ± 1.1 and 1.3 ± 0.2% respectively — mean for 3 animals, label injected in this experiment immediately after transplantation). This indicates that in the terminal ascites stage about 9% of the cells were in the G_2-period, and with a sharp decrease in its duration, they reached mitosis in the early periods after transplantation.

The total number of cells capable of passing into active proliferation after stimulation of division was approximately 70-76% (in the terminal ascites stage 55-60% of cells were in the prolonged G_1- and R_1-periods, 7% in the S-period, and 9% in the G_2-period). After administration of thymidine-^{3}H at intervals of 12 h during the last four days of growth of AH22A, ILN was 580‰ (58%). In the course of this period, all the cells remaining in the cycle evidently incoprorated thymidine-^{3}H, and some of them, passing through the G_2-period, divided so that the label was diluted. It can tentatively be suggested that the true size of the proliferative pool (the number of cells in the mitotic cycle) in the late stages of tumor development was less than the value of ILN obtained in this particular experiment.

Comparison of the above data leads to the following conclusions: 1) During stimulation of division more cells become involved in active proliferation than remain in the mitotic cycle at the terminal stage of development of the tumor. This takes place evidently through the emergence of cells from the resting state R_1; 2) in the terminal stage the AH22A contains a fairly large number (about 25% of the total volume of the population) of cells which have irreversibly left the mitotic cycle and do not participate in the restoration of proliferation in response to stimulation of division. The results confirm basically the data in the literature [4, 7, 9-11]. However, there are differences: 1) The return of cells from the resting R_2 to the mitotic cycle in response to stimulation of division was not observed, as

it has been for certain tumors [4-8]; 2) irreversible departure from the mitotic cycle has been described in two ascites tumors, but only for a very small part of the population [5, 8, 11], whereas in the terminal stage of development of AH22A there were about 25% of such cells. These differences can evidently be attributed to differences in the age of the tumors or (less probably) differences in the pattern of development of ascites tumors of different origin.

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