RATE OF PASSAGE THROUGH THE FIRST MITOTIC CYCLE BY ASCITES HEPATOMA 22A CELLS OF DIFFERENT AGES AFTER STIMULATION OF DIVISION

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The rate of passage through the first mitotic cycle by ascites hepatoma 22A cells of different ages after the stimulation of division was studied. "Aging" (11 days old), terminal (14 days), and "delayed" (4 days older than terminal) ascites fluid was used. The highest values of the index of labeled nuclei (due mainly to the transition from the resting state of the cells into the S period) were found to occur toward 9-12 h, and of the mitotic index toward 18-21 h after transplantation regardless of the age of the tumor. It follows from these results that the duration both of the prereplicative (G_1) period and of the whole of the first mitotic cycle after stimulation of division is independent of the duration of stay of the ascites hepatoma 22A cells in the resting state (or the very protracted G_1 period).

KEY WORDS: *ascites hepatoma 22A; phases of the mitotic cycle; age of tumor.*

Several papers have recently been published on the subject of the connection between the duration of stay of normal cells in the resting state and the character of their return to the mitotic cycle after stimulation of division. These investigations have shown that: 1) cells left for a long time in a resting state change to DNA replication only after repeated stimulation of division [10]; 2) with an increase in the duration of stay in the resting state the duration of the prereplicative (G_1) period of cells stimulated to divide is increased [3, 12].

There is no information in the literature to show whether a similar relationship exists between these phenomena in tumor cells.

The object of this investigation was to examine the relationship between age of an ascites hepatoma 22A and the rate of passage of its cells through the first mitotic cycle after stimulation of division.

Proliferative activity in ascites tumor (including mouse ascites hepatoma 22A) is known to become very low as their age increases. This is due to a marked increase in the duration of all phases of the mitotic cycle and, possibily, to the transition of the cells into a resting state [1, 2, 5, 7-9]. During aging of the tumor not only the total number of resting cells, but also the duration of their stay in the resting state increases. Some workers [4, 6, 11], however, consider that tumor cells cannot pass into a resting state analogous to that of normal cells, but they remain in a very protracted G_1 period.

By transplanting ascites tumors of different ages into a new host the writers were able to study how the duration of stay in a resting state (or in a very protracted G_1 period) affects the rate of passage by the cells through the first mitotic cycle after stimulation of division.

EXPERIMENTAL METHOD

Ascites hepatoma 22A Gel'shtein was used. The tumor was obtained among a group of strains from the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, and maintained by transplanting 0.25 cm^3 of ascites fluid (about 40×10^6 cells)

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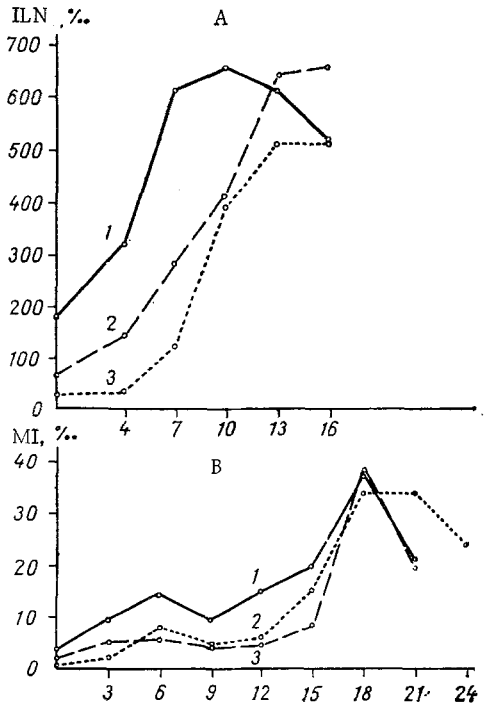

Fig. 1. Changes in index of labeled nuclei (ILN) and mitotic index (MI) at various times after transplantation of tumor. A) Change in ILN at various times after transplantation of "aging" (1), "terminal" (2), and "delayed" (3) ascites fluids; abscissa, time (in h) after transplantation of tumor; ordinate, ILN (in °/₀₀) (each point on the curve is the mean for 3-4 cases); B) change in MI at various times after transplantation of same asites fluids; abscissa, time (in h) after transplantation of tumor; ordinate, MI (in °/₀₀) (each point on curve is mean for 3-7 animals).

once every 2 weeks into C3HA mice. Ascites fluids of the following ages were taken for the experiments: 1) an 11-day tumor, described as "aging," 2) a 14-day tumor, described as terminal (the longest period of survival of mice into which the tumor was transplanted in this way, namely 14-15 days), 3) tumors 4 days older than the latter. The fluid was obtained by transferring the terminal ascites fluid in almost its entire volume (10-14 cm^3) into a new host, in which it survived for this period without any signs of activation (it was injected into the peritoneal cavity of an intact mouse by a fine needle through the thigh muscles, which prevented it from leaking). This ascites fluid will be described as "delayed." Clearly the age of the "delayed" ascites fluid was 18 days and it was 7 days older than the "aging" fluid (11 days).

The number of labeled nuclei (cells) and the number of mitoses in the ascites fluid were determined during the first 24 h after transplantation. To determine the index of labeled nuclei (ILN) the mice were given an injection of ^{14}C-thymidine 1 h before transplantation in a dose of 0.2-0.3 μCi/g. At various times after transplantation (3, 6, 9, 12 and 15 h) an injection of ^{3}H-thymidine was given to the animals in a dose of 0.1-0.15 μCi/g and they were killed 1 h later; double labeling enabled cells synthesizing DNA at these times after transplantation (labeling with ^{3}H) to be distinguished from cells which had been in the S period before transplantation (labeling with ^{14}C) or which continued DNA synthesis after transplantation (labeling with ^{3}H and ^{14}C). Films of ascites fluid were covered with type M liquid emulsion and exposed for 10-15 days at 4°C. ILN was determined by counting 1000 cells from each mouse, noting separately cells labeled with ^{3}H or ^{14}C only and cells with the double label.

The mitotic index (MI) for mice killed at various times after transplantation was determined by counting 2000 cells. These indices were expressed in promille. In all cases films

of ascites fluid were made at the time of its injection into a new host. The values of ILN and MI for such ascites fluids are plotted at the origins of the graphs in Fig. 1.

EXPERIMENTAL RESULTS

Data showing changes in ILN at different times after transplantation of the "aging," "terminal," and "delayed" ascites fluids are given in Fig. 1A. The increase in ILN after transplantation of the tumor into a new host was due mainly to two factors: 1) the transition of cells still remaining in the cycle into the S period as a result of a sharp decrease in the duration of their G_1 period. The number of these cells was relatively small, especially in the terminal and delayed ascites fluids. They contained the ^3H label and evidently were the first cells to start DNA synthesis after the stimulation of division; 2) the transition of cells from the resting state into the S period. The greater part of the ILN at the end of the period under consideration was probably accounted for by these cells (their nuclei naturally were labeled with ^3H also). As Fig. 1 shows, the maximal values of ILN (500-600‰) were reached at about the same times after transplantation of tumors of different ages, namely 9-12 h after transplantation. This is evidence that the resting cells of all the tumors passed through the prereplicative G_1 period in similar lengths of time.

Values of MI at various times after transplantation of the tumor into a new host are given in Fig. 1B. Clearly in every case the maximal values of MI (35-38‰) were found at the same time, toward 18-21 h after transplantation. Consequently, the whole mitotic cycle is completed in about the same time in tumors of different ages stimulated to proliferate.

It is impossible at present to say what the resting state of ascites hepatoma 22A cells is: whether it is in fact a state of rest (G_0; outside the cycle) or a very protracted G_1 period of the mitotic cycle. The data described above are evidence merely that the duration of the stay in the resting state (or in the protracted G_1 period) of ascites hepatoma 22A cells has no effect on the rate of passage through the first mitotic cycle after stimulation of division. In this respect cells of ascites hepatoma 22A differ from the normal cells described earlier.

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