MITOTIC CYCLE IN ASCITES HEPATOMA 22A CELLS UNDER ARTIFICIALLY CREATED UNFAVORABLE CONDITIONS

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The decline of proliferation and changes in the character of the mitotic cycle in the course of normal development of ascites tumors are evidnetly basically connected with worsening of the conditions of existence of the cells of a given population.

The object of this investigation was to study changes in the character of the mitotic cycle in ascites hepatoma 22A cells under artificially created unfavorable conditions, namely a sharp reduction in food and oxygen supply, an increasing immune reaction, and so on, i.e., during the changes which gradually arise aging of tumors [5, 15].

EXPERIMENTAL METHOD

Experiments were carried out on ascites hepatoma 22A Gel'shtein (AH 22A), the principal characteristics of whose proliferation were described previously [1-4]. An AH 22A at 5.5-6.5 days of development was used, when proliferation of its cells was sufficiently intensive. A tumor of this age was taken (into a syringe) from three mice (total volume 8-9 ml), treated with 2-3 ml of cell-free ascites fluid (to prevent an increase in the cell density through absorption of some of the fluid), and transferred into the peritoneal cavity of an intact mouse (with a fine needle through the thigh muscle. The ascites tumor remained in this united state for 24 h. The large volume of ascites fluid, namely 10-12 ml, adversely affected the conditions of existence of cells of the united tumor stepwise [^{3}H]thymidine in a dose of 0.1-0.3 μCi/g, [^{14}C]thymidine in a dose of 0.05 μCi/g, and colchicine in a dose of 0.25-0.5 μg/g were injected intraperitoneally. Films of ascites fluid were covered with type M liquid emulsion and exposed for 2-4 weeks at 4°C. The mitotic index (MI) and index of labeled nuclei (ILN) were determined by counting 2000 and 1000 cells, respectively and were expressed in promille. In the experiments with double labeling the relative number of labeled cells was determined by counting 1000 cells.

EXPERIMENTAL RESULTS

MI was determined in ascites fluid taken for pooling the tumors and was 12.3 + 0.6% (mean for six mice). Changes in MI at different times after pooling the AH 22A were determined in small samples (withdrawn through the thigh muscle) of ascites fluid. MI 2 h after pooling was 12.2 ± 0.5% after 4 h it was 7.8 ± 1.0%, after 6 h 5.0 ± 0.5%, after 8 h 2.5 ± 0.9%, after 10 h 1.5 + 0.4%, and after 24 h 2.1 + 0.7% (mean for 3-8 mice). Consequently, a significant decrease in MI took place until 4 h after pooling, minimal values were observed 10 h after pooling, and they remained at this same level until the end of the experiment (24 h after pooling). ILN during the 24 h of existence of the AH 22A in a united state decreased from 335 + 11.3% (mean for four mice) to 28 + 10.7% (mean for five animals). These data indicate that in the early stages after pooling of the tumor the transition from the G_2-period into mitosis (a fall in MI) is disturbed, but at the same time, during the period of pooling most cells which, at the time of pooling were in the S-period, were able to complete it (a fall in ILN).

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To determine how a change in the conditions of existence affects the transition of cells from the G_1-period into the S period (completion of the G_1-period), double-labeling experiments were carried out. At the moment of pooling (zero time) or 3 or 6 h thereafter, the animals were given an injection of [^{14}C]thymidine, 3 h later they received an injection of [^{3}H]thimidine, and 1 h later they were sacrificed. The relative number of cells with the ^{3}H-label only (these cells had switched during the 3-h interval from the G_1-period into the S-period) was determined in the autoradiographs. The number of cells with the ^{3}H-label 0.3 h after pooling the AH 22A was 34.0%, 3-6 h after pooling it was 10.0%, and 6-9 h after pooling 7.0% (mean for 2-3 mice), the control value was 50.3 ± 7.0%. Consequently, within the first 3 h after pooling the flow of cells from the G_1-period into the S-period decreased, and it fell even more between the 3rd and 6th and between the 6th and 9th hours after pooling (transition from the G_1-period into the S-period was determined in the control over a period of 3 h in a tumor of the 6th day of development).

In the experiments with transplantation of the pooled AH 22A (after existing for 24 h in the united state) into intact mice, the possibility that cells of individual subpopulations (in a certain period of the mitotic cycle) continued to proliferate during stimulation of cell division was studied. To determine the size of the G_2-subpopulation at the time of inoculation of the pooled AH 22A, an injection of [^{3}H]thymidine was given to the intact mice and mitoses were collected with colchicine over a period of 12 h (divided into 6-hourly intervals) and the relative number of unlabeled cells among them was determined. This was found to be 95% (the sum of the means for two groups, with three mice in each group). These dimensions of the G_2-subpopulations were rather larger than those for tumors at the 5th-6th day of development [1], indicating accumulation of cells in this period. In a special experiment 1 h before pooling the AH 22A [^{3}H]thymidine was injected (to label all cells in the S-period), the tumor was transplanted after a stay of 1 day in the united state, and in the early periods after transplantation the relative number of labeled mitoses was determined. The values obtained 2, 4, and 6 h after transplantation were 32, 31 + 4.4, and 40 + 6.0%, respectively (mean for 2-3 mice at each time). Consequently, 29-38% of cells emerge in the composition of the G_2-subpopulation in the form of labeled mitoses (31-50% of 95%).

The experiments showed that during 24 h in the united state about 300% of cells switch from the S-period into the G_2-period. Comparison of these data with the number of cells entering mitosis from the G_2-period in the early stage after transplantation (yield of the G_2-subpopulation) indicates that most cells, during pooling of those which have switched from the S-period into the G_2-period, later emerge into an irreversible resting state and are eliminated from the composition of the population. The number of those cells is quite large — rather more than 250%, i.e., about one-quarter of the cell population. The rest of the G_2-subpopulation (in the experiment it completed the mitotic cycle after transplantation in the form of unlabeled mitoses) evidently consisted mainly of cells which switched from the G_1-period into the S-period in the early stages after pooling, completed it, and emerged into the G_2-period.

The size of the G_1-subpopulation evidently changed very little during pooling, for both emergence from the G_1-period into the S-period and entry into the G_1-period were disturbed (inhibition of mitosis). The values of MI were determined 15, 18, and 21 h (the time of emergence of cells of the G_1-subpopulation into mitosis) after transplantation of the pooled AH 22A. It was found to be 23.2 ± 2.0, 22.1 ± 2.4, and 23.0 ± 0.5% respectively, in the control (AH 22A was transplanted at the 6th day of development) the values of MI at these same times were 25.0 ± 1.3, 29.0 ± 0.3, and 21.5 ± 1.5% (mean values for 3-4 mice), respectively. The close values of MI thus obtained indicate that the size of the G_1-subpopulation in the pooled AH 22A evidently remained close to the control.

Cells of an AH 22A at the 5th-6th days of development thus undergo the following changes in the character of their progress through the mitotic cycle if the conditions of existence deteriorate sharply. In the early stage after pooling the transition of cells from the G_1-period into the S-period and from the G_2-period into mitosis is blocked. Cells which at the time of pooling are in the S-period complete it and move into the G_2-period. A very small proportion of them remain capable of returning into the mitotic cycle, whereas most switch into an irreversible resting state and are evidently eliminated from the population. Cells of the G_1-subpopulation remain in the G_1-period during the period of pooling (some, perhaps, in a reversible resting state), and complete the mitotic cycle after transplantation of the tumor.

Disturbances in the mitotic cycle thus affect cells in both G_1 — and G_2-periods. However, delay in the transition from the G_1-period into the S-period (no longer so considerable for that period) does not prevent normal completion of the mitotic cycle by cells of the G_1-subpopulation when the unfavorable conditions are removed. Delay of cells in the G_2-period (essential for this subpopulation, since the duration of the G_2-period is minimal) leads to the conversion of most of them into an irreversible resting state and, evidently, to subsequent death.

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