AUTORADIOGRAPHIC STUDY OF THE MITOTIC CYCLE OF HARDING-PASSY MELANOMA IN CULTURE

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The mitotic cycle and its parameters in Harding-Passy melanoma were studied during growth in culture. The duration of the mitotic cycle of the melanoma cells was 40.3 h, the S-period 10.2 h, the G_2-period 5 h, and G_1 + M 25.1 h.

Establishment of the correct frequency of administration of antitumor preparations on the basis of studies of the duration of mitotic cycles and their individual phases in normal and tumor tissues increases the effectiveness of the procedure by producing maximum injury to the tumor cells.

Cultures of tumors are usually used to study the duration of the mitotic cycle and its parameters, because such investigations using labeling techniques are difficult in vivo. The fact that such a system can be used is demonstrated by observations showing that the normal duration of the mitotic cycle is maintained by a tumor during prolonged cultivation [8, 11, 12].

The use of primary cultures of tumors is particularly promising in this direction, because as several workers have shown [3, 7], the cells of primary cultures retain not only the histotypical, but also the functional characteristics of the original tissue (enzymic and hormonal activity).

This paper describes the results of a study of the parameters of the mitotic cycle of a Harding-Passy melanoma by an autoradiographic method.

EXPERIMENTAL METHOD

The source of the tumor for culture was a Harding-Passy melanoma obtained from a C57B1 mouse 2 weeks after implantation. Explants on the 7th-9th day of growth were taken for the experiments, because at this period the principal types of cells characterizing this tumor can be differentiated in the zone of growth [9].

To determine the proliferative pool and the dynamics of accumulation of labeled cells, the cultures were incubated with thymidine-H^3 in a dose of 0.3 μCi/ml medium (specific activity 3 μCi/ml).

To study the dynamics of the increase in number of labeled mitoses in the culture, thymidine-H^3 was added in a dose of 1 μCi/ml for 15 min and then removed by washing with medium No. 199 and replacement with fresh medium (37°C).

The period of observation after radioactive labeling was 1-13 h. During continuous saturation with thymidine-H^3 the material was fixed at various times after addition of the isotope for 5 days. Cultures were fixed with a 3:1 mixture of acetic acid and ethanol and stained with Mayer's hematoxylin. Autoradiographs were obtained by means of type M(NIIKhFP) liquid nuclear emulsion. Cells were regarded as labeled if at least 5 grains of silver were found above the nucleus against a generally low background. At each time of investigation 2-3 cultures were examined, and at least a 1000 cells were counted in each. Two

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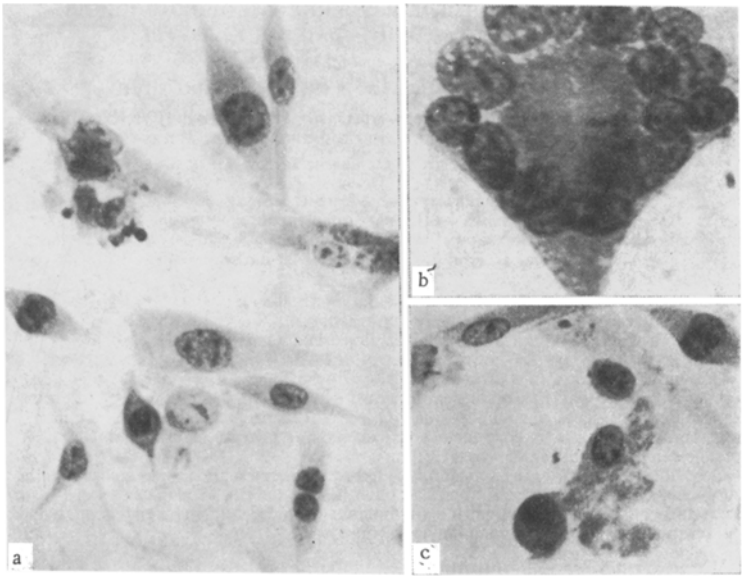

Fig. 1. Cells of Harding-Passy melanoma (7-day culture). Hematoxylin-eosin: a, c) 520 ×; b) 1360 ×.

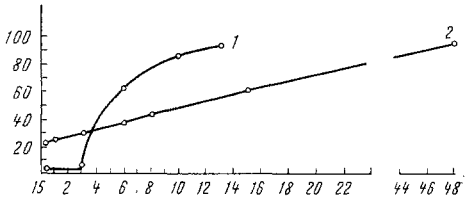

Fig. 2. Change in number of labeled mitoses in culture of Harding-Passy melanoma after short (1) and long (2) incubation with thymidine-H^3. Ordinate, labeled cells and labeled mitoses (in %); abscissa, time after addition of isotope (in h).

parameters were used to analyze the autographs: the index of labeled cells and the increase in number of labeled mitoses per 1000 culture cells.

With the appropriate calculations [2, 5], this method enabled the duration of the mitotic cycle and its various phases to be determined for the given culture.

EXPERIMENTAL RESULTS

Two types of cells were clearly distinguishable in the cultures of Harding-Passy melanoma. The cells of the first type were large, bipolar and multipolar cells, giving off processes (Fig. 1a). The nucleus was round or oval in shape with 1 or 2 nucleoli. The pigment was pale brown, amorphous, and located around the nucleus or at the periphery of the cell. Multinuclear cells with large quantities of melanin concentrated in the center also were seen (Fig. 1b). The type 1 cells accounted for 73-82% of the total cell population.

In accordance with existing definitions, melanophages, fibrocytes, and melanocytes (11, 7, and 5% respectively) were included in this type.

Against this background it was easy to distinguish the cells of type 2, with a round, compact nucleus (Fig. 1c). As a rule the narrow zone of cytoplasm contained large amounts of pigment. These cells were relatively less numerous (18-27%). By their characteristics these cells were identified as melanoblasts [5].

The mean mitotic activity of the cell population varied between 8 and 12%.

PARAMETERS OF THE MITOTIC CYCLE

The results of determination of the percentage of labeled cells and labeled mitoses after long- and short-term incubation with thymidine-H^3 are given in Fig. 2. The first labeled mitoses were found in the culture 3 h after addition of the isotope, their number after 6 h was 63%, and it reached a maximum (90%) after 9 h. The mean duration of the postmitotic (G_2) period, defined as the interval between the time of addition of thymidine-H^3 and the appearance of 50% of labeled mitoses, was 5 h.

A study of the index of labeling of the whole complex of cells showed that 15 min after addition of thymidine-H^3 23% of the cells were labeled.

In the experiment with continuous contact between the cells and thymidine-H^3, the index of labeled nuclei rose during the first day as a linear function of time, to reach 92% by the end of the second day. The duration of the S period, determined by calculation from the accumulation of labeled cells, was 10.2 h.

From the duration of the period of DNA synthesis, it was possible to calculate the total [1] duration of the mitotic cycle, allowing for the proliferative pool of the culture, which was 92%.

In this case the duration of the mitotic cycle was 40.3 h. The duration of the presynthetic period could thus be determined, knowing the duration of mitosis. Its value was 25.1 h.

This investigation showed that cells of a primary culture of Harding-Passy melanoma exhibit intensive incorporation of thymidine-H^3, the radioactive precursor of DNA.

A study of the intensity of distribution of the label in the cell population showed inequality in the character of incorporation of thymidine-H^3 into different types of cells, and also into different cells of the same type, indicating the heterogeneity of the culture. Because of the small number of cells of type 2, it was impossible to calculate the parameters of the mitotic cycle for the different types of cells separately, and for this reason,when a scheme of treatment is being chosen to correspond to the stages of the mitotic cycle, data for the total population of tumor cells must be used as the starting point.

It is interesting to compare these results with the durations of the parameters of the mitotic cycle as determined by administration of radioactive DOPA, tyrosine, and thymidine to mice with Harding-Passy melanoma: the duration of the mitotic cycle was 26-36 h, of G_2 1.5-4 h, of S 8.2 h, S + G_2 11 h, and G_1 15-25 h [10].

The results of the present investigation give somewhat longer parameters for the cycle of Harding-Passy melanoma in primary culture compared with the conditions appertaining in vivo.

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