COMPARATIVE STUDY OF THE MITOTIC CYCLE IN PRIMARY AND TRANSFORMED CULTURES OF GREEN GUENON KIDNEY CELLS

Z. A. Dzhemilev and F. I. Adzhigitov

UDC 612.35.014.2:612.6.03]-085

The method of autoradiography with thymidine-H^3 was used to plot the curve of labeled mitoses and hence to determine the duration of individual phases of the mitotic cycle for primary and transformed cultures of green guenon kidney cells. They were found to be identical for both types of cultures.

* * *

No information concerning the duration of the mitotic cycle and stage of interphase for cells or primary kidney cultures from the green guenon could be found in the accessible literature. However, these normal values are essential for correct interpretation of facts obtained during cytogenetic investigation of normal and transformed cells of kidney cultures from this species of monkey.

EXPERIMENTAL METHOD

Primary and transformed kidney cell cultures from green guenons (Cercopithecus ethiops) were used as test objects. Primary cultures were made from kidney tissue from a monkey aged 3 years. A transformed culture (PZM-7) was obtained by treatment of primary cultures of green guenon kidney cells with material from a Burkitt's lymphoma. At the time of the investigation described below, the PZM-7 culture had been subcultured more than 40 times in the course of 12 months. To label the cells in the S-period, thymidine-H^3 was used in a concentration of 1-1.2 μCi/ml (specific activity 1.2 Ci/mmole). For radioactive labeling, the primary culture was used at the age of 72-96 h and the PZM-7 culture 72-96 h after the last subculture. Both cultures were grown under adequate conditions (38°, medium No. 199 with 20% calf serum, cell concentration $3 \cdot 10^5$/ml). Cover slips with a monolayer of cells were immersed in heated medium No. 199 with the isotope, poured into a glass bath, and incubated for 10-15 min. The cover slips with the cell monolayers were washed 3 times with medium No. 199 to remove thymidine-H^3. The cultures were then fixed 2, 3, 5, 6, 8, 10, 12, 14, 16, 22, 26, and 30 h after labeling with a mixture of glacial acetic acid and methyl alcohol (1:3). Some cultures were treated with a hypotonic solution (0.8% solution of trisubstituted sodium citrate) before fixation in order to obtain metaphase plates. The preparations were stained by Feulgen's method, coated with type M photographic emulsion, and exposed in a refrigerator for 8-10 days. After development of the autoradiographs, the preparations were counterstained by Unna's method [2]. The background of these autoradiographs was negligible (approximately 1 grain per interphase cell). Cells were regarded as labeled if they had more than 5 grains per nucleus. Not less than 500 mitoses were counted at each period of fixation.

EXPERIMENTAL RESULTS

Analysis of the results showed that the number of labeled mitoses in primary and transformed cultures of green guenon kidney cells after cultivation for different periods reached a maximum 8 h after labeling. The number of labeled mitoses then fell to reach a minimum by 22 h. The percentage of labeled mitoses

Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 12, pp. 83-85, December, 1969. Original article submitted April 28, 1969.

TABLE 1. Duration of Mitotic Cyle and Its Phases in Normal and Transformed Cells of Green Guenon Kidney Culture

Phase of mitotic cycle	Duration of mitotic cycle and its phases (in h)	
	primary culture	transformed culture
$M+G_2$	5	5
S	8—9	8—9
I	0,37	0,36
T	17,8—20	18,3—20,6
G_1	4,8—6	5,3—6,6

again began to rise 26 h after labeling, reflecting the onset of the second mitotic cycle.

Analysis of the number of labeled mitoses at each time of fixation of the cultures enabled the duration of the mitotic cycle (T) and of its individual phases to be determined. The $M+G_2$ period measures the time from labeling until large-scale entry of labeled cells into mitosis, when more than 50% of mitoses contain label. Consequently, the mean duration of the $M+G_2$ period was 5 h. The mean duration of the S stage was taken to be the time during which 50% or more of mitoses in the first cycle were labeled. The duration of the S stage was 8-9 h. The duration of the mitotic cycle (T) for an asynchronously growing cell population was determined, in the same way as by other investigators [4, 5], by the formula

$$T = 0.825\left(\frac{t_s}{I}\right),$$

where 0.825 is a coefficient introduced for cultures in the stage of logarithmic growth, t_S the duration of the S stage, and I the labeled fraction of the population (the labeling index). Most workers define the labeling index as the ratio between the number of labeled interphase cells and the total cell population. In this case, the proliferative pool (P_C) must be taken into consideration [1, 3]. In the present case the labeling index was defined as the ratio between the number of labeled mitoses and the total number of dividing cells. There was thus no need to introduce a correction into this formula for the proliferative pool. The duration of the presynthetic period G_1 was defined as the difference $G_1 = T-(M+G_2+S)$.

The results of a comparative investigation of the duration of the mitotic cycle and its phases in the primary and transformed cultures are given in Table 1, showing that the duration of the mitotic cycle for cells of a primary green guenon kidney culture varies between 17.8 and 20 h; the mean $M+G_2$ period is 5 h, and the S stage 8.9 h; the G_1 stage lasts 4.8-6 h. No significant difference was found between these indices for the normal and transformed culture.

LITERATURE CITED

1. A. I. Zosimovskaya and N. A. Lyapunova, Tsitologiya, No. 2, 208 (1966).
2. S. I. Slezinger and A. A. Prokof'eva-Bel'govskaya, Tsitologiya, No. 2, 187 (1966).
3. M. G. Chumak, Tsitologiya, No. 1, 24 (1963).
4. H. A. Johnson, Cytologia (Tokyo), 26, 32 (1961).
5. W. E. Kisieleski, R. Baserga, and H. Lisco, Atompraxis, 7, No. 3, 81 (1961).