DIURNAL RHYTHM OF CELL DIVISION IN TISSUE CULTURES

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Most animals and plants are characterized by a diurnal periodicity of their physiological processes, reflecting the complex ecological relationships between the organism and the external environment [9, 12]. One manifestation of this phenomenon is the diurnal periodicity of the mitotic activity of the cells, which has been established in the case of various tissues and organs [1, 3, 7, 10, 11, 13, 14, 19].

The question arises whether this diurnal rhythm of mitosis is maintained in tissues cultivated in vitro. Olivo and Delorenzi [18], who were among the first workers to study the time of appearance and the distribution of mitoses in cultures of fibroblasts from the heart of the chick embryo, showed that the distribution of mitoses in the cultures in time is random in character. According to Hupe and Gropp [16], the diurnal variations in the number of mitoses in cultures of fibroblasts from the heart of the chick embryo are small and not statistically significant. However, in cultures of osteogenic fibroblasts of the mouse they found a regular increase in the number of mitoses in the course of the 24-hour period, with two maxima – at about 8 h and 20 h. They emphasize that the pattern of cell division which they found was not dependent on changing the nutrient medium, and bore the character of a diurnal rhythm reflecting the endogenous rhythm of growth of this tissue.

The problem of the presence of a diurnal rhythm of mitotic activity in tissue cultures is particularly important at the present time in connection with the development of the technique of monolayer cultures from tissues treated with trypsin [15], and its wide application in virology, toxicology, and radiobiology. Monolayer trypsinized cultures are particularly suitable for establishing the principles governing cell division in vitro, for their zone of growth is formed very quickly and it is more uniform in character than during growth of fragments of tissue in Carrel flasks of rotating tubes.

In the present paper we describe the results of the study of the mitotic rhythm in trypsinized (monolayer) cultures of cells from the cortical layer of the kidney of the monkey Macacus rhesus and of HeLa cells.

EXPERIMENTAL METHOD

Cells were cultivated in the following nutrient media: monkey's kidney cells in a 0.5% hydrolyzate of lactalbumin in Hanks's solution with the addition of 10% calf serum; HeLa cells in a 0.5% hydrolyzate of lactalbumin in Hanks's solution with the addition of equal percentages of horse and calf serum (7.5%). A suspension of trypsinized cells (monkey's kidney cells in their initial concentration – 300,000 units/ml and HeLa cells in a concentration of 80,000 units/ml) was poured in doses of 2 ml into test tubes, into which had previously been inserted glass disks measuring 0.5 × 2.5 cm, on which a zone of growth was formed. According to our own observations and to reports in the literature [6], the mitotic activity of cells reaches a maximum in cultures of monkey's kidney cells 4-5 days after seeding, and in cultures of HeLa cells 3-4 days after seeding. These times were selected for the study of the diurnal rhythm of mitosis. The disks with the zone of growth were extracted from the tubes at intervals of 3 h for a period of 24 h, fixed with Carnoy's mixture (in the first series of experiments fixation was carried out at intervals of 6 h), and stained with Carazzi's hematoxylin and by the Feulgen method.

Particular difficulty was experienced when the number of mitoses present in 1000 cells was counted. Although the zone of growth in the trypsinized cultures is comparatively uniform in its distribution, its outlines are usually irregular; in some cases, moreover, it is broken up into areas of different sizes and of irregular shape. It must also be

TABLE 1. Mitotic Activity of the Cells of Culture of a Monkey's Kidney at Different Times of Day

Series of experiment	Time of day (in hours)							
	12	15	18	21	24	3	6	9
First	3 8083/167 20,6		3 7088/204 28,7		3 8949/213 24,1		4 10511/216 21,5	
Second	1 1565/48 30,7		1 1828/64 35,0		3 5048/131 26,0	2 3775/113 30,0	2 2951/88 30,0	
Third	3 4216/66 15,6	3 5003/82 16,3	3 5084/72 14,1	3 5609/111 19,8	3 4902/67 13,6	3 4628/81 17,5	3 4487/62 13,8	4 5733/69 12,0
Fourth	5 34943/742 21,2	5 32796/472 14,2	5 33616/488 14,5	5 24686/399 16,0	5 35146/435 12,2	5 36123/444 12,2	5 37494/509 13,5	5 35015/604 17,2
Mean mitotic coefficient	22,0	15,1	23,0	17,9	19,0	20,0	19,7	15,0
P	0,924	0,296	—	0,469	0,510	0,706	0,635	0,333

Note. The upper row represents the number of cultures, the middle row the number of cells (numerator) and the number of mitoses (denominator), and the lower row the mitotic coefficient (in ‰). The significance of the differences was calculated in relation to the value obtained for 18.00 h.

TABLE 2. Mitotic Activity of the Cells of Cultures of Strain HeLa at Different Times of Day

Series of experiment	Time of day (in hours)							
	12	15	18	21	24	3	6	9
First	1 2129/75 35,7		2 4151/133 32,0				2 4725/161 34,0	
Second	3 6015/213 35,4	3 7714/291 37,6	3 9497/315 33,1	3 8619/240 28,0	3 7406/231 31,2	3 5545/191 34,4	3 6295/189 30,0	3 4476/158 35,3
Third	3 17755/541 30,5	3 5375/51 9,5	3 4777/46 9,6	3 10008/234 23,3	3 11776/312 26,0	3 9658/242 25,0	3 8388/165 19,6	3 7574/88 11,6
Mean mitotic coefficient	33,9	23,5	25,0	25,6	29,0	29,7	28,0	23,4
P	—	0,391	0,333	0,063	0,188	0,391	0,263	0,352

Note. The upper row represents the number of cultures, the middle row the number of cells (numerator) and the number of mitoses (denominator), and the lower row, the mitotic coefficient (in ‰). The significance of the differences was calculated in relation to the value obtained for 12.00 h).

remembered that the cells of the zone of growth differ in size, as a result of which different numbers of them are present in unit area. The cells of cultures of the monkey's kidney are distinguished by their particular polymorphism [5]. The cells in HeLa cultures are usually at a distance from each other. These factors interfere with the precise counting of mitoses per unit area, containing a constant number of cells. We therefore decided to resort to the laborious but more accurate method of direct counting of all the cells in the zones of growth, including the cells undergoing mitotic division. The numerical results were treated by the methods of variational statistics, the degree of significance being determined by the Fisher – Student method.

EXPERIMENTAL RESULTS

The changes in the diurnal mitotic activity of the cells of the renal cortex of rats have been investigated by several workers [2, 4, 8]. Their findings were essentially the same: the maximal number of mitoses was observed during the morning and early afternoon.

Analysis of our results shows that no regular changes could be observed in the mitotic activity of the cells in cultures of the cortical layer of the monkey's kidney (Table 1). When different tissues are cultivated in identical conditions the intensity of their growth and proliferation usually differs. The mitotic coefficient varied in individual experiments from 12 ‰ (third and fourth series) to 35 ‰ (second series). The maximal increase in mitotic activity in the first two series of experiments occurred at 18.00 h, in the third at 21.00 h, and in the fourth at 09.00 - 12.00 h. In other words, these maxima were not regular in character and were not associated with any definite time of day, as confirmed by statistical analysis. Comparison of the mean data obtained for each time of day showed that the differences were not statistically significant: in relation to the highest value of the mitotic coefficient (23‰ at 18.00 h) P varied from 0.296 to 0.924.

The same remarks also applied to the diurnal mitotic activity of the cells of the HeLa strain (Table 2). The total number of cultures investigated, like the total number of cells counted, was slightly smaller in this case. Naturally, the variations in the mean indices of the HeLa cultures were slightly larger (in individual experiments the mitotic coefficient varied from 9.5 to 35.3 ‰). Nevertheless, here, too, the random character of the diurnal changes in mitotic activity were quite clear. As with the cultures of the monkey's kidney, the maximal increase of mitotic activity did not coincide in successive series of experiments. For instance, in the first and third series, the highest value of the mitotic coefficient was observed at 12.00 h (35.7-30.5 ‰), and in the second series at 15.00 h (37.6 ‰). Statistical analysis of the mean values obtained at each time of day, in relation to the highest value of the mitotic coefficient at 12.00 h (33.9 ‰) showed that the differences were not statistically significant (P varied from 0.063 to 0.391).

Hence, analysis of the mitotic activity of the cells at different times of day in cultures of monkey's kidney cells and HeLa cells failed to reveal any endogenous diurnal mitotic rhythm. Such a rhythm would be more likely to be expected in the cultures of the monkey's kidney, extracted from the host animal immediately before seeding, than in the HeLa cells, which had grown in vitro for several years. For this reason particular attention was paid to the cultures of the monkey's kidney. The absence of a regular diurnal rhythm of division of cells growing in vitro is perfectly comprehensible from the biological point of view. Naturally, this does not exclude the possibility of changes in the rhythm of cell division in tissue cultures being brought about by various other factors (changes in temperature, the action of embryonic extract, etc.). The problem of the experimental synchronization of cell division [17] is one of undoubted interest to research using the tissue culture method.

SUMMARY

Mitotic activity of cells was studied in five 24-hour cultures of monkey kidney and in three 24-hour cultures of HeLa strains. The cultures were fixated at an interval of 3 hours (in some experimental series - 6 hours) for 24 hours. There were no changes of a regular nature indicative of the presence of the 24-hour mitotic rhythm in the mentioned cultures.

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