DYNAMICS OF PHASES OF MITOSIS AND RELATIVE DURATION OF CELL DIVISION

V. N. Dobrokhotov and V. S. Valvas

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No satisfactory methods of determining the duration of mitosis in animal tissues have yet been suggested. Nevertheless such data are essential for detection of the mitotic activity (MA) of tissues, i.e., the rate of formation of new cells per unit time, an important factor when the level of cell renewal or physiological regeneration of animal tissues has to be judged.

Determination of changes in the duration of mitosis in organisms exposed to the influence of physical, chemical, hormonal, and other factors is no less important. Judgment on results obtained in experiments after exposure to such factors are usually based on values of mitotic indices (MI) found after an arbitrarily chosen time: A significant increase in the values of MI as regarded as evidence of stimulation of cell proliferation, a decrease as a manifestation of its inhibition. However, it is not permissible to assess changes in MA purely on the basis of values of MI, for MA depends not only on the rate at which the cells enter mitosis, but also on possible changes in the duration of mitosis as a whole or of its individual phases.

The object of this investigation was to establish a basis for a method of determining changes in the relative duration of mitosis, using as the example the effect of adrenalin on cell proliferation in the corneal epithelium of rats of different ages.

EXPERIMENTAL METHOD

It was shown previously [1] that after subcutaneous injection of adrenalin into rat fetuses 1-2 days before birth in a dose of 2 μg/g body weight, a significant increase in the values of MI is observed after 45 min in the corneal epithelium. The same phenomenon also was observed under similar experimental conditions in young rats aged 3 and 4 days. Starting with the age of 7 days, a gradual decrease is observed in differences in MI values in the control and experimental animals, with the development of the ordinary response of the epithelial cells to adrenalin, namely sharp inhibition of entry of the cells into mitosis, as many workers have described. This considerable increase in MI in fetuses and in very young rats in the experimental animals compared with the control was accompanied by well-marked changes in the number of individual phases of mitosis. The following considerations were used as the starting point for further analysis of these data in the investigation described below.

The frequency of occurrence of each phase of mitosis is known to be proportional to its duration. Consequently, with an increase or decrease in the number of mitoses in each phase in the experimental animals compared with their number in the controls, it would be valid to conclude that there is a corresponding increase or decrease in the duration of that phase and of mitosis as a whole.

For the aims of the present investigation, values obtained for changes in the frequency of each phase had to be represented in the form of the absolute number of mitoses in each phase or the ratio of that number to the total number of interkinetic cells (in promille). The use of data for phases of mitosis in the form of percentages of the total number of all mitoses is inadmissible, because a change in the percentage of any phase must inevitably lead to a corresponding change in the percentage of the other phases. This makes it possible to discover what actual absolute changes took place in the number of mitoses in each stage.

Finally, we had to make sure that the factor tested does not change the rate at which the cells enter into mitosis. This could be verified by comparing the number of early prophases and of prophases in the experimental and control animals. In the absence of significant differences in the number of these phases it could be concluded that the factor concerned does not disturb the rate of entry of the cells into division.

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TABLE 1. Changes in Relative Duration of Mitosis and Its Phases in Corneal Epithelium of Rats of Different Ages after Injection of Adrenalin

Age of animals, days	Group of animals	Mean number of mitoses	MI, ‰	P	Mean number of phases of mitosis and their relative duration					Relative duration of mitosis, percent
					EP	P	M	A+T	RN	
Fetuses	Control	20,9	2,3	0,001	3,0 14,3	0,7 3,4	4,5 21,5	7,5 35,9	5,2 24,9	100,0
	Experiment	80,9	10,8		2,0 9,5	3,2 15,5	30,4 145,2	33,5 160,3	11,8 56,5	387,0
3	Control	34,8	4,1	0,002	1,5 4,3	0,8 2,3	5,0 14,4	12,8 36,8	14,7 42,2	100,0
	Experiment	84,4	9,6		1,4 4,0	1,6 4,6	24,2 69,6	36,2 104,0	21,0 60,3	242,5
4	Control	45,4	5,3	0,001	2,0 4,4	0,6 1,3	6,6 14,5	19,4 42,8	16,8 37,0	100,0
	Experiment	93,8	11,1		2,8 6,2	3,2 6,9	31,4 70,5	42,4 93,1	14,0 30,8	207,5
10	Control	40,5	3,8		2,2 5,4	5,5 13,6	7,3 18,0	16,0 39,5	9,5 23,5	100,0
	Experiment	41,8	4,0	0,80	1,0 2,4	1,2 2,9	11,0 27,1	19,8 48,9	8,8 21,8	103,1

Legend. EP) Early prophases; P) prophases; M) metaphases; A + T) anaphases and telophases; RN) reconstructions of nuclei.

EXPERIMENTAL RESULTS

As Table 1 shows, MI in the corneal epithelium of fetuses of the experimental animals was significantly higher (P = 0.001) than in the control.

Comparison of the frequencies of occurrence of each of the phases of mitosis in these two groups of animals showed that the mean number of early prophases and prophases did not differ significantly (P = 0.1-0.5). Consequently, adrenalin has no effect on the rate of entry of the cells into division. The mean number of metaphases, ana-telophases, and reconstructions of the nulcei was significantly higher in the cornea of the experimental fetuses. This excess for each phase was absolutely significant (P = 0.001-0.0001). It follows from these data that the duration of mitosis in the corneas of fetuses of the experimental rats was longer than in the corneas of the control animals.

Since the real duration of mitosis in the test tissue under normal conditions is unknown, the next step was to study changes in the relative duration of mitosis in the experimental animals compared with the control.

If the duration of mitosis in the cornea of the control animals was taken as 100%, the duration of each phase of mitosis would be proportional to the frequency of occurrence of that phase. The greatest duration was found for ana-telophases and nuclear reconstructions (35.9 and 24.9% of the total duration of mitosis).

In the experimental animals the number of metaphases, ana-telophases, and reconstructions was significantly greater than in the control animals. This excess was absolutely significant for each phase (P = 0.001-0.0001). Hence it can be concluded that the duration of these phases and of mitosis as a whole in the corneas of fetuses of the experimental rats was longer than in the corneas of the control animals. It is easy to show that the duration of metaphase in the experimental series would be 145.2% of the duration of that phase in the control, the duration of ana-telophase would correspondingly be 160.3%, and the duration of the reconstruction phase 56.5%. The duration of mitosis as a whole was increased by 3.87 times.

A similar picture was found when the corneas of young rats aged 3 and 4 days were studied. At these times the mean MI in the experimental animals was significantly higher than for the controls. In both groups the number of early phases of mitosis did not differ statistically significantly (P = 0.1-0.5), but the number of metaphases and ana-telophases was significantly greater in the experimental animals (P = 0.001-0.0001). Consequently, the duration of these stages was sharply increased under the influence of adrenalin. The duration of mitosis as a whole in the 3-day-old experimental rats was increased by 2.42 times, and in the 4-day-old animals by 2.07 times.

Adrenalin was thus found to have a unique kind of action on cell division in the corneas of rat fetuses and also of young rats aged 3 and 4 days. Adrenalin did not inhibit entry of the cells into mitosis but it sharply disturbed the rate of passage through mitosis. By the 10th day of postnatal life a gradual decrease took place in the differences in MI, the numbers of individual phases, and their duration, and of the duration of mitosis as a whole.

If MA of the tissues was judged from the value of MI, it would have to be concluded that cell proliferation is stimulated in the corneas of young rats under the influence of adrenalin. However, a comparative study of the frequency of occurrence of the phases of mitosis in animals of the control and experimental groups showed that the increase in the

mean MI in the experimental series was due to a sharp delay in the course of mitosis itself.

This approach to the evaluation of changes in the duration of mitosis based on analysis of the frequency of occurrence of its phases can be called the "temporal dynamics of the phases of mitosis." This concept emphasizes the importance of the study of temporal relations arising in the course of mitosis.

It can be tentatively suggested that the study of the temporal dynamics of the phases of mitosis will shed a clearer light on many other problems connected with the evaluation of cell proliferation during regeneration and malignant growth.

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POLYCLONAL TOLERANCE IN NONIBRED MICE

K. L. Chimishkyan, L. N. Buachidze, S. I. Shereshkov, and D. M. Mkheidze

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KEY WORDS: irradiation; fetal liver; tolerance.

This paper gives the results of a study of the possibility of inducing polyclonal tolerance in noninbred mice to skin allografts after lethal irradiation of the recipients and their protection by allogeneic fetal liver cells obtained from many donors.

EXPERIMENTAL METHOD

Noninbred mice weighing 25-30 g at the stage of 16-19 days of pregnancy, bred at the Kryukovo Nursery, and C57BL/6j mice from the Stolbovaya Nursery were used.

The mice were irradiated on a ^{137}Cs γ-ray source, with a dose rate of 25 rads/min and in a dose of 1300 rads.

The fetal liver was minced in medium No. 199 with 10% bovine fetal serum. The resulting cell suspension was filtered through two layers of gauze and, on the day of irradiation, it was injected intravenously into mice in a dose of $(15\text{-}20)\cdot10^6$ cells/ml.

Transplantation of the caudal skin and chromosome analysis of bone marrow cells were carried out by the usual method.

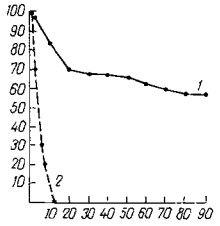

Fig. 1. Survival rate of mice after lethal irradiation and protection by allogeneic fetal liver cells. Abscissa, days of observation; ordinate, survival rate of mice (in percent). 1) Experimental mice after irradiation and protection, 2) mice irradiated in a dose of 1300 rad.

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