A COMPARATIVE STUDY OF THE DURATION OF MITOSIS AND INTERKINESIS IN MOUSE TISSUES BY MEANS OF COLCHICINE AND IRRADIATION

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The investigation of mitotic cycles and their periods has undergone intensive development during recent years [10]. A more complete picture of the periods of the mitotic cycle may be obtained by using the method of autoradiography with H^3-thymidine [5, 11], although for a rapid comparison of the changes in the duration of the mitotic cycles in tissues exposed to any form of outside influence it is better to inject colchicine (statokinetic method) or to carry out whole-body irradiation of the animals.

The first method is based on the property of colchicine of causing accumulation of mitosis in the state of metaphase; their number (MI_{col}) is directly proportional to the mitotic index of the particular tissue (MI) and to the duration of the action of colchicine (A), and inversely proportional to the duration of mitosis (t). In other words,

$$MI_{col} = \frac{MIA}{t} \quad , \quad \text{whence } t = \frac{MIA}{MI_{col}} \; .$$

The duration of mitosis can also be calculated by determining the time of disappearance of all mitoses from the tissue after irradiation [9] or the time of spread of the wave of inhibition from the early phases of division to the late [6]. The ratio between the number of mitoses (n) and the number of nondividing cells (N) is known to be equal to the ratio between the duration of mitosis (t) and the duration of interkinesis (T). Hence

$$T = \frac{tN}{n} \; .$$

It should be noted that the value of T calculated in this way will reflect only the mean duration of interkinesis for the cell population as a whole. However, the cell population is most frequently heterogeneous. Therefore, in order to establish the true length of the mitotic cycle it is necessary to introduce a correction for what is called the "proliferative pool" of the tissue, i.e., to determine the percentage of cells capable of undergoing division.

Since colchicine does not usually cause arrest of 100% of metaphases, the calculated duration of the whole mitotic cycle will be slightly overestimated. The irradiation method is therefore recommended as preferable [6]. However, the irradiation method has been demonstrated to be more accurate than the colchicine method only in the case of intensively proliferating tissues such as the intestinal [1] and the corneal epithelium [6].

In the present research a comparative study was made of the duration of mitosis and interkinesis of tissues of mice with different proliferation patterns, using colchicine and whole-body irradiation of the animals.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice, 74 males and 78 females weighing 18-20 g. The females were spayed 20 days before the experiment to prevent any possible variations in the number of mitoses for the duration of the sexual cycle.

Experiments with colchicine. The experimental animals (in groups of 5 or 6), at 9 A.M. received a subcutaneous

injection of colchicine dissolved in distilled water, in a dose of 0.2 ml of a 1:5000 solution (1:2500 in the experiments with epidermis), and subsequently sacrificed at 3 P.M. The control animals (in groups of 5 or 10) were sacrificed at intervals between 9 A.M. and 3 P.M., one at a time at equal intervals in order to establish the mean diurnal variations in the number of mitoses.

Experiments with irradiation. The experiments began at 9 A.M. The experimental animals were irradiated from a cobalt γ-ray apparatus of the GUBÉ-800* type, the distance from the source of irradiation being 21 cm and the the dose rate 307 r/min. The total dose was 400 r. The experimental animals (in groups of 7-10) were sacrificed 5, 10, 15, 20, 25, 30, 45, and 60 min after irradiation; the control animals (6-10 in number) were sacrificed one at a time at equal intervals from 9 to 10 A.M.

From the females the cornea, epidermis of the ear, and uterus were taken for investigation. The material was fixed in Carnoy's fluid, embedded in paraffin wax, and cut into sections 7 μ thick, which were stained with Meyer's hematoxylin. From the males, marrow from both femora was extracted and impression films made [8]; these were fixed in Carnoy's fluid and stained by Feulgen's method.

The counting of the mitoses in the corneal epithelium, epidermis of the ear and epithelium of the uterus was done by the usual methods [2, 3]. In the marrow the number of mitoses was counted in 5000 cells of the myeloid and 5000 cells of the erythroid series. The results relating to the mitotic activity of the marrow obtained from the impression films were checked by counting mitoses in films stained by Giemsa's method.

EXPERIMENTAL RESULTS

Duration of mitosis and interkinesis in the corneal epithelium. It will be clear from Table 1 that the mitotic index (MI) of the corneal epithelium rose during the 6 hr after injection of colchicine (MI_{col}) more than four-fold ($P = 0.001$). The duration of mitosis (t) in these conditions was 86 ± 11 min, and of interkinesis (T) 296 ± 16 hr. I. A. Utkin [6], using the colchicine method, obtained figures for the corneal epithelium of mice (males) equal to 75 ± 5 min and 117 ± 8 hr, respectively. The longer duration of the interphase in our experiments was evidently associated with the spaying of the animals, for it has been shown that the administration of estrone to spayed mice almost doubles the rate of the mitotic cycle of the corneal epithelium [4]. In the same way we can account for the relatively low mitotic index of this particular tissue in the control animals, 2.6-4.8‰, compared with 9-10‰ in normal mice at the same time of day [2]. However, I. I. Utkin considers that 117 hr is an overestimate for the duration of interkinase in the corneal epithelium, since in other experiments using two different methods of determination of the duration of mitosis and interkinesis in this particular tissue (roentgen-ray irradiation and physiological action) he obtained significantly lower and, at the same time, closely similar values of T (78 ± 4 hr and 76 ± 4 hr at t = 47 ± 2 min and 46 ± 2 min).

In our experiments (Table 2) 30 min after irradiation the mitotic index in the corneal epithelium had fallen by more than half ($P = 0.008$). Carrying out the calculation by Knowlton and Widner's method [9], we obtained t = 49 ± 6 min. However, the duration of the interphase here was actually slightly greater than in the experiments with colchicine (T = 306 ± 91 hr). This gave further confirmation that the mitotic cycle in the corneal epithelium of spayed females is considerably protracted. On the other hand, at the lowered level of mitotic activity of the tissue, the results relating to the duration of interkinesis obtained by means of colchicine and irradiation came closer together (see the

TABLE 1. Mean Duration of Mitosis and Interkinesis in Mouse Tissues (Colchicine Method)

Tissue investigated	Sex of animals	MI (in ‰)	MI_{col} (in ‰)	t (in min)	T (in hr)
Corneal epithelium	Female	4,8±0,3	19,1±2,5	86±10,6	296±16
Epithelium of uterus	Female	1,7±0,6	12,6±1,1	47±7,1	452±145
Epidermis	Female	2,7±0,5	6,0±0,2	162±45	1000±268
Marrow (myeloid series)	Male	3,5±0,3	19,7±0,6	63±5,8	55±4,7
Marrow (erythroid series)	Male	5,5±0,6	30,2±0,5	65±12,1	36±6,8

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TABLE 2. Mean Duration of Mitosis and Interkinesis in Mouse Tissues (Irradiation Method)

Tissue investigated	MI in control (in ‰)	MI 30 min after irradiation (in ‰)	MI 45 min after irradiation (in ‰)	t (in min)	T (in hr)
Corneal epithelium	2,6±0,5	1,02±0,1		49±6	306±91
Epithelium of uterus	0,4±0,1	0,09±0,05	0,04±0,03	41±11	448±293
Epidermis	3,4±0,4	2,5±0,4	1,0±0,27	66±6,7	321±54
Marrow (myeloid series)	4,0±0,6	1,26±0,13		43±6	168±7,7
Marrow (erythroid series)	5,8±0,5	1,6±0,63		41±0,8	116±2,8

TABLE 3. Relative Proportions of Phases of Mitosis in Mouse Tissues 6 Hours after Injection of Colchicine

Tissue investigated	Σ of mitoses	Phases of mitosis								No. of late phases (in %)
		prophases		metaphases		anaphases		telophases		
		absolute No.	%	absolute No.	%	absolute No.	%	absolute No.	%	
Corneal epithelium	1 607	65	4,05	1 524	94,84	4	0,25	14	0,86	1,11
Epithelium of uterus	115	5	4,35	110	95,65	—	—	—	—	—
Epidermis	161	3	1,85	157	97,53	1	0,65	—	—	0,62
Marrow										
myeloid series	596	14	2,3	576	96,7	2	0,33	4	0,67	1
erythroid series	906	24	2,7	869	96	7	0,7	6	0,60	1,3

formulae cited). So far as the duration of mitosis in the corneal epithelium is concerned, it is apparent that in this case the irradiation method remained more accurate in our experiments also. After injection of colchicine, complete arrest of the metaphases was not observed in the corneal epithelium (Table 3), and although the percentage of late phases of division was low, their presence alone indicated the possibility that the results in relation to the rate of mitosis might be overestimated.

Duration of mitosis and interkinesis in the uterine epithelium. In the experiments with colchicine (see Table 1) the mitotic index in the uterine epithelium rose 7.4 times (P = 0.005). In this case late phases of division were absent (see Table 3), which suggests that this method was suitable for the subsequent determinations. The duration of mitosis under these circumstances was 47 ± 7.1 min, and of interphase 452 ± 145 hr.

In the experiments with irradiation, 30 min after exposure the mitotic index was observed to be lowered to one-quarter its original value, and after 45 min, to one-tenth (see Table 2). However, the initial number of mitoses in the control animals was so small (MI = 0.4 ± 0.1‰) that this decrease was insignificant in both cases (P = 0.336 and 0.110). The mean duration of mitosis, calculated from the fall in the number of divisions after 30 and 45 min separately, was 41 ± 11 min, and of the interphase 1448 ± 293 hr. The obvious overestimation of the duration of the interphase may be explained by the very small number of mitoses in the tissue. Hence, in order to determine the parameters of the mitotic cycle of the uterine epithelium, the colchicine method is apparently more accurate than the irradiation method.

Duration of mitosis and interkinesis in the epidermis. In contrast to the corneal epithelium, in the epidermis of the ear of spayed mice the mitotic index was almost the same (2.7 ± 0.55 and 3.4 ± 0.4) as in the epidermis of the ear of normal male mice at the same time of day (2.8‰, according to M. T. Gololobova [2]). Evidently spaying has little effect on the state of this tissue. Six hours after injection of colchicine (see Table 1) the number of mitoses in the epidermis was more than doubled (P = 0.05). The duration of mitosis was 162 ± 45 min, and of the interphase 1000 ± 268 hr. The percentage of late phases of division was very small in this case (see Table 3), but it is quite possible that these figures may be on the high side.

In experiments with irradiation (Table 2) a significant fall in the number of mitoses in the epidermis was observed only when 45 min had elapsed after exposure (P = 0.007). As in the uterine epithelium, no displacement of the

waves of mitoses in accordance with their phases could be detected. The mean duration of mitosis (from the fall in the number of divisions after 30 and 45 min) was 66 ± 6.7 min, and of the interphase 321 ± 54 hr. Widner and co-workers [12], after experiments with roentgen rays, calculated that in the epidermis of normal mice t = 30 ± 12 min and T = 670 ± 300 hr. Sherman, Quastler and Wimber [11], using H^3-thymidine, obtained t = 228 min and T = 528 hr.

Analysis of these very conflicting results shows that the mitotic cycle of the epidermis of both spayed and control mice is very prolonged, and this undoubtedly affects the accuracy of its determination. It is quite possible that the colchicine method gives results which are slightly too high, and the irradiation method too low, but a final decision on this problem must await additional experiments, possibly with fixation at shorter intervals and certainly with determination of the proliferative pool.

Duration of mitosis and interkinesis in the marrow. Six hours after the injection of colchicine (Table 1) the mitotic index in both series of marrow cells was increased approximately six-fold (in both cases $P < 0.0001$).

The duration of mitosis in these series was practically identical, but the interphase was longer in the cells of the myeloid series. A small percentage of late phases of division was observed in both series. In irradiation experiments (Table 2) a significant fall in the number of mitoses was observed 30 min after exposure ($P \leq 0.0001$). The duration of mitosis in this case was shorter than in the experiments with colchicine, but it was also equal in both series, while the duration of the interphase was longer, especially in the cells of the myeloid series. These results are in general agreement with those of Widner and co-workers [12] in experiments with roentgen-ray irradiation. Cronkite and co-workers [7], using H^3-thymidine, calculated that the mitotic cycle of the myeloid series lasts from 24 to 60 hr, and of the erythroid series from 18 to 30 hr. We see that the results of determinations using the colchicine and autoradiographic methods were closer than the results obtained by irradiation. On the whole, the two methods we have described are perfectly suitable for studying the mitotic cycles of the marrow cells.

In conclusion we may state that the choice of a particular method of determination of the duration of mitosis in a tissue must be determined by the specific features and the state of the tissue itself. In particular, if the level of mitotic activity of the tissue is low, it will obviously be preferable to judge the duration of mitosis not by the decrease, but by the increase in the number of dividing cells, i.e., to use the colchicine method (for example, for the uterine epithelium). For tissues with a high or moderately high level of mitotic activity (intestinal or corneal epithelium), in which the wave of succession of the phases of mitosis can be followed and the beginning of the decrease in the number of mitoses detected, the irradiation method gives more accurate results.

SUMMARY

A comparative study was performed on the duration of mitosis and interkinesis in the corneal epithelium, uterine epithelium, ear epidermis and bone marrow of albino mice. The colchicine technique and total irradiation were employed. Shortcomings and advantages of both methods were analyzed. It was shown that for the estimation of mitotic cycles in tissues with a low mitotic activity it is more reasonable to use the colchicine technique, whereas in the tissues with a high proliferative capacity more precise results can be obtained with irradiation.

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