COLCHICINE METHOD OF DETERMINING THE DURATION OF MITOSIS IN BODY TISSUES

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To determine the rates of cell proliferation it is necessary not only to know the mean diurnal number of mitoses, but also the duration of mitosis.

Several methods have been suggested for determining the duration of mitosis, but only the stathmokinetic method, with the use of colchicine and its derivatives, has achieved wide popularity. However, some aspects of the use of this method still remain incompletely explained. Most workers consider that colchicine only blocks cell division in metaphase without disturbing the rate of entry of cells into mitosis. Meanwhile, there is evidence, although not very much, and what there is is contradictory, to show that colchicine and its derivatives can inhibit the entry of cells into mitosis [1, 4-7]. This problem can be resolved by comparing the number of prophases in the test tissues of intact animals and of animals treated with colchicine. If the number of prophases in animals receiving colchicine is statistically significantly less, it is justifiable to conclude that colchicine causes preprophase inhibition of injury of cells into mitosis.

The object of this investigation was to study whether colchamine (colcemid) in fact has the property of inducing preprophase inhibition of mitotic activity of tissues, and if so, whether it exhibits this property equally at different times of the 24-h period.

EXPERIMENTAL METHOD

Altogether, 150 control and 68 experimental mice were used; the latter were given an intraperitoneal injection of colchamine (4 mg/kg) 4 h before sacrifice. The experiments conformed to two schemes, differing only in the time of commencement: at 10 a.m. (scheme 1) and at noon (scheme 2).

Mitotic indices (MI) were determined in the tissues of the control animals, mitotic indices of the number of blocked mitoses (MI_{col}) in the experimental animals, and prophase indices (PI) in both groups. The duration of mitosis (t_m) was calculated by the usual formula [3].

The error of the duration of mitosis was calculated by the equation for error of the quotient obtained by dividing arithmetic means with their errors [2].

EXPERIMENTAL RESULTS

Diurnal changes in MI and PI in the lingual epithelium of the control mice (Table 1) showed maximal values between 10 p.m. and 2 a.m. and between 6 and 10 a.m. The changes in C-mitoses were of the same character in the experimental mice.

At all four-hourly intervals of the 24-h period a significant increase was observed in MI_{col} compared with MI in the control mice. However, whereas between 6 a.m. and 6 p.m. the level of significance was very high ($P = 0.0001$), between 6 p.m. and 6 a.m. it was more moderate.

Injection of colchamine between 10 a.m. and 10 p.m. was not followed by significant differences between PI in the control and experimental mice. Between 10 p.m. and 2 a.m. a significant decrease ($P = 0.035$) was

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TABLE 1. Diurnal Changes in Mitotic and Prophase Indices and Duration of Mitosis (t_m) in Lingual Epithelium of Mice (scheme 1)

Periods	MI ‰	MI_{col} ‰	P	t_m min	PI ‰ control	PI ‰ exper.	P
10—14	5,47	54,40	0,0001	24,1±14,9	0,99	0,32	0,258
14—18	2,50	37,83	0,0001	16,0±8,9	0,41	0,39	0,922
18—22	4,28	36,03	0,034	28,3±16,9	1,23	1,39	0,844
22—2	23,90	80,20	0,002	71,5±26,4	6,05	1,28	0,035
2—6	9,72	48,36	0,012	48,2±26,5	2,34	1,29	0,330
6—10	22,10	172,50	0,0001	31,3±5,9	6,09	1,88	0,089
10—22	4,19	42,70	0,0001	23,5±6,8	0,89	0,69	0,555
22—10	18,65	106,50	0,0001	41,9±8,6	4,81	1,52	0,014
10—10	11,69	71,70	0,0001	39,2±7,8	2,70	0,92	0,011

observed in PI in the experimental mice, but during the next two intervals, although these differences were still present they were less marked and were not significant.

Comparison of the values of PI showed that during the first half of the 24-h period (10 a.m.- 10 p.m.) colchamine caused no decrease in PI of the experimental mice (P = 0.555), whereas during the second half (10 p.m.-10 a.m.) a significant decrease in this index (P = 0.014) was found in the experimental mice. Differences were equally significant (P = 0.011) between the mean values of PI for the 24-h period.

Consequently, colchamine leads to preprophase inhibition of entry of the cells into mitosis, which is manifested more clearly at night and in the early morning.

The duration of mitosis (t_m) varied from 16 to 71.5 min. Only on comparison of these minimal and maximal values were significant differences (P = 0.028) found, namely at times when a significant decrease in PI was demonstrated in the experimental mice, when the rates of entry of the cells into mitosis were minimal.

In the pancreas (Table 2) diurnal changes in MI and PI in the control mice and MI_{col} in the experimental mice reached maximal values between 8 a.m. and noon. At nearly all times MI_{col} was significantly higher than MI in the control mice. However, between 8 a.m. and noon these differences were not significant, as was also the case at the end of the night and beginning of the day (4 a.m. to noon).

In the afternoon and evening (noon-midnight) no significant differences were found between the values of PI in the control and experimental mice (P = 0.555) during each of the time intervals studied or for this period as a whole, but at night (midnight to 4 a.m.) a significant decrease in PI was observed in the experimental mice (P = 0.003). Between 4 and 8 a.m. and between 8 a.m. and noon PI fell, but not significantly (P = 0.154 and 0.065, respectively). Corresponding comparison of PI at the end of the night and beginning of the day periods (4 am to noon) revealed a significant decrease in PI in the experimental mice (P = 0.005). The differences found on comparing PI, both during the second half of the 24-h period (midnight-noon), including the whole of the night period, and for the 24 h as a whole, likewise were significant (P = 0.14 and 0.045, respectively). Consequently, at night and in the morning, by contrast with the afternoon and evening, colchamine exhibited an inhibitory action of the entry of the cells into mitosis.

The duration of mitosis varied from 45 to 213 min. Comparison of these extreme values only gave significant differences (P = 0.028). Only between 8 a.m. and noon was a tendency for colchamine to inhibit the entry of the cells into mitosis observed.

In the parenchymatous cells of the liver, values of MI_{col} were slower than those of MI in the control mice at nearly all times of investigation. Whereas these differences during the afternoon and evening (10 a.m.-10 p.m.) were not significant (P = 0.555), at night and in the early morning (10 p.m.-10 a.m.) they were close to significant (P = 0.089). Differences between the mean diurnal values of MI (0.97‰) and MI_{col} (0.28‰) also were close to significant (P = 0.072).

Consequently, injection of colchamine not only did not lead to accumulation of blocked mitoses, but it also led to a decrease in the values of MI_{col} compared with MI in the control mice.

In the experimental mice, throughout the evening and night (from 6-10 p.m. to 2-6 a.m.) no prophases were found. This decrease in PI in the experimental animals was not significant compared with the values of PI in the control mice, for in some of the latter no prophases likewise were found. These data show that pre-

TABLE 2. Diurnal Changes in Mitotic and Prophase Indices and Duration of Mitosis (t_m) in Pancreatic Exocrine Cells of Mice (scheme 2)

Periods	MI, ‰	MI_{col} ‰	P	t_m, min	PI, ‰ control	PI, ‰ experiment	P
12—16	0,118	0,324	0,032	87,4±43,7	0,019	0,054	0,151
16—20	0,066	0,332	0,012	47,7±29,1	0,022	0,044	0,922
20—24	0,106	0,314	0,013	81,0±34,8	0,039	0,078	0,333
24—4	0,322	1,280	0,024	60,3±30,7	0,112	0,000	0,003
4—8	0,357	1,880	0,044	45,5±31,4	0,127	0,018	0,154
8—12	1,900	2,136	0,340	213,4±68,3	0,314	0,010	0,065
12—24	0,098	0,323	0,001	72,8±19,6	0,026	0,058	0,555
24—12	0,830	1,760	0,032	113,2±38,5	0,181	0,010	0,014
12—12	0,449	1,044	0,007	103,2±35,1	0,100	0,034	0,045
4—12	1,090	2,009	0,109	130,2±48,2	0,217	0,014	0,005

dominantly in the evening and night colchamine causes deep inhibition of the entry of cells into mitosis. The presence of a certain number of mitoses in the liver of the experimental animals at these times can be explained by the sharp delay in the course of mitosis itself. The absence of accumulation of C-mitoses in the liver of the experimental animals led to distinctly distorted results for the duration of mitosis: In the evening and at night the duration of mitosis (t_m) increased to 30-40 h. It is quite evident that the data obtained on the duration of mitosis do not reflect the real values of this parameter.

These results indicate that colchamine damages the whole parenchyma of the liver so severely that the colchicine technique cannot be used to investigate cell division in this organ.

It was thus shown that colchicine undoubtedly causes preprophase inhibition, by changing the rate of entry of the cells into mitosis or, as in the liver, by disturbing the whole dynamics of cell proliferation. This property of colchamine is manifested primarily in the late evening and at night. The reasons for this temporal selectivity in the sensitivity of cells to the action of colchamine are not clear. However, it may be pointed out that preprophase inhibition of mitosis, in the lingual epithelium for example, coincides approximately in time with the period of the maximal number of DNA-synthesizing cells [3].

Consequently, differences which probably exist in the duration of mitosis in the tissues cannot be revealed by the use of the colchicine method.

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