EFFECT OF COLCEMID ON THE RATE OF CELL PROLIFERATION IN ANIMAL TISSUES

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To determine the duration of mitosis and the rate of cell proliferation in animal tissues the colchicine method is widely used. Colchicine and its derivatives, it is considered, only block cell division without affecting the rate of entry of cells into mitosis. However, the question of the preprophase action of colchicine has not been adequately studied. In can be elucidated by comparing the number of prophases in tissues of control animals and of animals treated with colchicine. Only if the number of prophases is the same can it be concluded that colchicine does not affect the rate of cell proliferation.

It was shown previously [1, 2] that colcemid inhibits the entry of cells into mitosis in adult mouse tissues.

The object of the present investigation was to study the action of colcemid on the rate of cell proliferation in the tissues of young animals.

EXPERIMENTAL METHOD

Rats aged 7 days were used. The action of colcemid (1.5 mg/kg) was studied on cells of the exocrine part of the pancreas and on parenchymatous cells of the liver. The experimental animals were killed (4 or 5 rats at each time) at intervals of 4 h during the 24-h period, and control animals were killed at intervals of 2 h during the 24-h period (3 rats at each time). Mitoses were counted by phases in the pancreas on average in 9000 cells, and in the liver in 10,000 cells from each animal.

The mitotic index (MI), the blocked metaphase index (MI_c), and the prophase index (PI) were calculated for control and experimental animals in promille. The results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

The rate of accumulation of blocked mitoses (MI_c) during the 24-h period differed in pancreatic cells (Table 1). For instance, during the day period (10 a.m.-6 p.m.) the number of c-mitoses was 2.9 times greater than the mean number of mitoses in the control animals, whereas during the night and early morning period (6 p.m-10 a.m.) the number was 1.9 times greater.

At all times of the investigation PI was higher in the control than in the experimental animals. Whereas these differences were not significant between 10 a.m. and 2 p.m., at all subsequent times they were significant. The nonsignificance of differences between 2 and 6 a.m. was accidental, for at this time histological investigation of the pancreas was possible in only two rats.

The lower value of PI in the experimental than in the control rats during the day (10 a.m.-6 p.m), incidentally, was less marked ($P = 0.048$) than at night and in the early morning ($P = \infty$).

These results show that colcemid, especially at night, lowers the rate of entry of cells into mitosis, i.e., it has an undoubted inhibitory preprophase action.

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TABLE 1. Changes in Mitotic and Prophase Indices (in %) in Pancreatic Exocrine Cells of 7-Day-Old Rats during the 24-h Period

Time of day	MI	MI_c	PI_{cont}	PI_{colc}	*P*	PI_{colc}/PI_{cont}, percent
10 a.m.-2 p.m.	5,6	20,0	1,32	1,14	0,562	86,4
2-6 p.m.	4,3	10,7	0,95	0,53	0,035	55,8
6-10 p.m.	7,0	12,6	1,76	0,43	0,002	24,4
10 p.m.-2 a.m.	7,7	15,0	1,74	0,45	0,007	25,9
2-6 a.m.	8,4	15,5	1,61	1,05	0,220	65,2
6-10 a.m.	7,4	16,1	1,79	0,39	0,003	21,8
10 a.m.-6 p.m.	5,1	14,7	1,19	0,79	0,048	66,4
6 p.m.-10 a.m.	7,7	14,9	1,72	0,52	≤	30,2
Mean value for 24-h period	6,6	14,8	1,49	0,61	≤	40,9

TABLE 2. Changes in Mitotic and Prophase Indices (in %) in Parenchymatous Cells of Liver of 7-Day-Old Rats during the 24-h Period

Time of day	MI	MI_c	PI_{cont}	PI_{colc}	*P*	PI_{colc}/PI_{cont}, percent
10 a.m.-6 p.m.	4,1	7,2	0,12	0,39	0,021	325,0
6-10 p.m.	4,0	10,9	0,22	0,25	0,922	113,6
10 p.m.-2 a.m.	5,6	4,4	0,27	0,51	0,113	188,8
2-10 a.m.	6,10	9,2	0,22	0,31	0,484	140,9
Mean value for 24-h period	5,0	7,8	0,20	0,37	0,014	185,0

The degree of the inhibitory action of colcemid was clearly revealed by calculating the ratio PI_{colc}/PI_{cont} (in percent). During the day (10 a.m-6 p.m.) the ratio is 66.4%. Consequently, during this period entry of the cells into mitosis was reduced by 33.6%, but at night (6 p.m.-10 a.m.) it was reduced by almost 70%, with an average for the 24-h period of 60% (Table 1).

When the liver was investigated the number of early prophases also was counted and the early prophase index (EPI) calculated.

Since at certain times too few cases were analyzed, the data for some periods were pooled: 10 a.m.-2 p.m. and 2-6 p.m. into one group (10 a.m.-6 p.m.), and 2-6 a.m. and 6-10 a.m. into another group (2-10 a.m.). In the liver cells (Table 2) the number of c-mitoses during the day (10 a.m.-10 p.m.) was significantly greater than the number of mitoses in the control animals. During the first half of the night (10 p.m.-2 a.m.) these differences were not significant on account of a sharp fall in the rate of accumulation of c-mitoses in the experimental animals.

Unexpected results were obtained when the values of PI were compared: They were higher in the experimental than in the control animals. These excesses, except between 10 a.m. and 6 p.m., were not significant, but if mean values for the 24-h period were compared, the differences were significant ($P = 0.014$). Higher values of PI in the experimental animals also were confirmed by changes in the PI_{colc}/PI_{cont} ratios (Table 2).

TABLE 3. Changes in EPI (in %) in Parenchymatous Cells of Liver of 7-Day-Old Rats during the 24-h Period

Time of day	EPI_{cont}	EPI_{colc}	P	EPI_{colc}/EPI_{cont}, percent
10 a.m.-6 p.m.	0,33	0,018	$\leq$	5,45
6-10 p.m.	0,26	0,045	0,027	17,30
10 p.m.-2 a.m.	0,30	0,048	0,008	16,00
2-10 a.m.	0,24	0,034	0,007	14,16
Mean value for 24-h period	0,28	0,030	$\leq$	10,71

It might be concluded from these data that colcemid not only does not inhibit the entry of cells into division in the liver of young rats, but actually stimulates this process. However, a different conclusion could be drawn from analysis of the data concerning changes in EPI.

It follows from Table 3 that the values of EPI in all intervals of the 24-h period were significantly lower in the epxerimental animals than in the controls. This points to an undoubted inhibitory action of colcemid on the rate of entry of cells into mitosis. The degree of such inhibition can be judged from changes in the EPI_{colc}/EPI_{cont} ratio (in percent). During the day (10 a.m.-6 p.m.), for instance, the number of cells in the liver of the experimental rats commencing division was almost 95% lower than the number in the control rats, and if mean data for the 24-h period were used it was lower by almost 90%.

Since the frequency with which each phase of mitosis is found is proportional to its duration, the increase in the values of PI may be attributed to the fact that colcemid delays passage through this stage in the liver of young rats. This hypothesis is supported by the data showing very low rates of cumulation of c-mitoses at all times of the 24-h period, but between 10 p.m. and 2 a.m. the number of c-mitoses was actually lower than the mean number of mitoses during this period in the control.

Colcemid thus not only inhibits the entry of cells into mitosis, but it may delay passage through prophase.

These results indicate that the colchicine method cannot be used to determine the rates of cell proliferation and, in particular, the duration of mitosis in animal tissues.

LITERATURE CITED

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