# EFFECT OF COLCEMID ON EHRLICH'S ASCITES CARCINOMA CELLS DURING SYNCHRONIZED CELL DIVISION

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Diurnal changes in the number of normal and colcemid mitoses were studied in Ehrlich's ascites carcinoma cells in mice receiving colcemid after preliminary partial synchronization of cell division produced by injection of dibutyryl cyclic AMP and also in animals after injection of colcemid or of dibutyryl cyclic AMP alone. Synchronization of cell division in the tumor was shown to increase by 2.6 times the number of tumor cells blocked by colcemid and to lead to more rapid arrest of colcemid mitoses.

KEY WORDS: colcemid; Ehrlich's ascites carcinoma; synchronization of cell division.

Synchronization of cell division in tumors is a possible way of increasing the effectiveness of cytostatic therapy [5, 6, 8]. Synchronizers of cell kinetics usually used, such as inhibitors of DNA synthesis or mitotic poisons in small doses [5, 8, 9], are able to synchronize the passage of the cells through the mitotic cycle to a considerable degree not only in tumors, but also in normal tissues [7]. The writer showed previously that a fairly high degree of synchronization of cell division can be obtained in Ehrlich's ascites carcinoma without any significant changes in the mitotic regime in the epithelium of the esophageal mucosa after a single injection of dibutyryl-cyclic 3',5'-adenosine monophosphate (DB-cyclic-AMP) into animals.

The object of this investigation was to study the action of the mitotic poison colcemid on a population of synchronized Ehrlich's ascites carcinoma cells during the period of minimal synchronization of mitoses in the esophageal mucosa.

## EXPERIMENTAL METHOD

Experiments were carried out on 192 male noninbred albino mice into which $1 \times 10^6$ cells of a diploid strain of Ehrlich's ascites carcinoma were injected intraperitoneally 4 days before the experiment. DB-cyclic-AMP was injected into the group of experimental animals in a dose of 20 mg/kg at 10 a.m., and colcemid was injected in a dose of 5 mg/kg at 6 p.m., at the time of expected commencement of division of many of the tumor cells and a sharp decrease in mitotic activity in the esophagus [2]. The mice were killed in groups of eight animals at intervals of 2 h throughout the day and night. Mice receiving DB-cyclic-AMP at 10 a.m. only or colcemid at 6 p.m. only served as the control. They also were killed in groups at intervals of 3 h throughout the day and night. Films were made from the ascites fluid, fixed with methanol, hydrolyzed in 1 N HCl at 56°C for 4 min, and then stained with methylene blue. The number of mitoses in the various phases was counted in the films in 4000 cells in each case. The mitotic index (MI) was expressed in pro mille.

## EXPERIMENTAL RESULTS

Diurnal changes in the values of MI in the tumors of mice treated with DB-cyclic-AMP at 10 a.m. are shown in Table 1. The value of MI 3 h after injection of the compound were low (0.08 $^0/_{00}$), they reached a maximum after 9-12 h (44.9-50.3 $^0/_{00}$), and subsequently fell again. These results were close to those obtained previously for synchronization of cell division in Ehrlich's ascites carcinoma following injection of DB-cyclic-AMP into mice, based either on the time of appearance of the wave of synchronized mitoses or on the maximal values of MI in the period of greatest synchronization [3].

In the next two groups (Table 1), throughout the first 3-4 h of exposure to colcemid, exclusively colcemid mitoses (C mitoses) were found, with chromosomes scattered through the cytoplasm, metaphase "stars," and

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TABLE 1. Diurnal Changes in Values of MI, CI, and DI (in ‰) in Ehrlich's Ascites Carcinoma

| Time of day | Injection of DB-cyclic-AMP at 10 a.m. | | Injection of colcemid at 6 p.m. (b) | | | | Injection of DB-cyclic-AMP at 10 a.m. and colcemid at 6 p.m. (c) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MI | P | MI | CI | P | DI | MI | CI | P | DI |
| 1 p.m. | 0,08 | | | | | | | | | |
| | | 0,0001 | | | | | | | | |
| 4 p.m. | 10,6 | | | | | | | | | |
| | | 0,0001 | | | | | | | | |
| 7 p.m. | 50,3 | | | | | | | | | |
| 8 p.m. | | | | | | | 101,3 | 91,9 | | 0,0 |
| 9 p.m. | | — | 28,1 | 20,4 | | 0,0 | | | 0,05 | |
| 10 p.m. | 44,9 | | | | 0,7 | | 59,3 | 48,7 | 0,08 | 0,6 |
| midnight | | 0,001 | 30,9 | 25,0 | | 0,8 | 41,7 | 28,2 | | 17,5 |
| 1 | 15,1 | | | | 0,13 | | | | 0,013 | |
| 2 | | | | | | | 79,0 | 64,4 | | 18,6 |
| 3 | | — | 30,6 | 16,0 | | 4,4 | | | — | |
| 4 | 10,3 | | | | 0,0001 | | 77,0 | 48,6 | 0,0001 | 6,4 |
| 6 | | — | 26,0 | 5,2 | | 6,8 | 24,9 | 10,2 | | 4,4 |
| 7 | 17,6 | | | | — | | | | 0,0001 | |
| 8 | | — | | | | | 32,9 | 3,4 | | 2,1 |
| 9 | | | 32,9 | 5,2 | | 7,3 | | | 0,1 | |
| 10 | 11,4 | | | | 0,0001 | | 16,8 | 1,2 | — | 1,7 |
| 12 | | | 22,9 | 0,6 | | 4,1 | 7,9 | 0,0 | — | 0,6 |
| 2 p.m. | | | | | — | | 11,5 | 0,2 | — | 0,0 |
| 3 p.m. | | | 27,0 | 0,0 | | 2,9 | | | — | |
| 4 p.m. | | | | | — | | 9,2 | 0,0 | | 0,0 |
| 6 p.m. | | | 14,9 | 0,0 | | 0,0 | 8,6 | 0,0 | — | 0,0 |

Legend. MI) Combined value of indices of prophases, normal metaphases and telophases (a, b), and also colcemid mitoses (b, c).

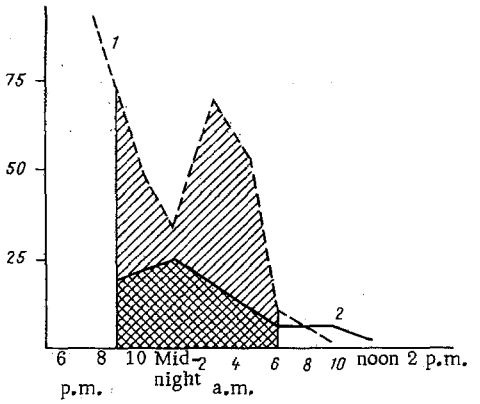


Fig. 1. Dynamics of index of colcemid mitoses (CI) after injection of colcemid into animals preceded by synchronization of cell division by DB-cyclic-AMP (1) and after injection of colcemid alone at 6 p.m. (2). Abscissa, time of day; ordinate, CI (in ‰). Zone bounded by curve 1 is proportional in area to number of cells delayed by colcemid during synchronization of mitoses. Zone bounded by curve 2 is proportional in area to number of cells delayed by injection of colcemid alone.

hollow mitoses. The number of these cells was counted to determine the index of C mitoses (CI). Mitoses with signs of destruction of the chromosomes and with vacuolated and fragmented nuclear material appeared later. Cells of this type were counted separately and the index of degenerating C mitoses (DI) was calculated.

In the mice with tumors, after injection of colcemid alone only a low level of accumulation of C mitoses was observed (Table 1); meanwhile, parallel with the decrease in the values of CI from 25‰

at midnight to 5.2‰ at 9 a.m. an increase in the values of DI from 0.8 to 7.3% was observed. These results agree with observations of other workers [4] who showed that in rapidly renewing tissues, starting from the 4th hour of action of the stathmokinetic agents, signs of lysis are found, followed by disappearance of C mitoses.

The values of the general MI in the mice after injection of colcemid at 6 p.m. preceded by synchronization of cell division were very high and were determined mainly by correspondingly high values of CI (Table 1). By 8 p.m., for instance, CI reached 91.9‰, it fell significantly to 28.2‰ by midnight ($P = 0.001$), and rose again to 64.4‰ at 2 a.m., followed by a gradual fall. After 10 a.m. practically no C mitoses were found in any group of animals, evidence that the stathmokinetic effect had ended by this time. The values of CI in the mice after injection of DB-cyclic-AMP and colcemid were higher than in animals receiving colcemid alone. Death of the C mitoses in these mice also was more synchronous, for the period of high values of DI in these animals lasted only 8-10 h, compared with at least 15 h in mice receiving colcemid alone.

Comparison of the areas beneath the curves reflecting the dynamics of the values of CI in the two groups of animals (Fig. 1) indicates that colcemid, if injected 8 h after DB-cyclic-AMP, within the period from 9 p.m. to 6 a.m. blocks 2.6 times more mitoses than colcemid given without preliminary synchronization of cell division. In fact, the area $S_1$, measuring 710 units and reflecting the number of C mitoses in the first case, was 2.6 times greater than the area $S_2$, measuring 275 units and reflecting the number of C mitoses in the control mice.

Preliminary synchronization of mitoses in a tumor produced by injection of DB-cyclic-AMP thus increases the cytostatic effect of colcemid considerably.

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