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Escape from metaphase arrest by Ehrlich ascites cells grown *in vitro* during treatment with 5-chloropyrimidin-2-one

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Previous work has shown that 5-fluoro- and 5-chloropyrimidin-2-one exhibit metaphase arrest on human cells grown *in vitro* [1, 2], similar to that of colcemid and vincristine although at a higher concentration.

To study this group of inhibitors further, the effect of 5-chloropyrimidin-2-one on another mammalian cell line has been investigated. The cell line used originates from an Ehrlich ascites tumor cell line in mice and was grown in suspension culture. The inhibitor was found to exhibit an unexpected effect in this cell line. The cells overcame the metaphase arrest and returned to interphase without the appearance of telo- or anaphases or any change in cell number. Experiments describing this effect are reported.

5-Chloropyrimidin-2-one was synthesized by the method of Crosley and Berthold [3]. Colcemid and puromycin were commerical products.

The mouse cell line was obtained from Professor H. Klenow, University of Copenhagen, and was established as an *in vitro* culture by J. Allunans at the Public Health Laboratory in Oslo. The mean chromosomal number was 42. This cell line was grown with a doubling time of 15 hr at 37° in Eagle's Minimal Essential Spinner Medium (Gibco F 13) supplemented with 10% foetal calf serum, HEPES buffer (pH 7.3) at a final concentration of 15 mM, 1 ml of Flow 100× non-essential amino acids to 100 ml medium benzylpenicillin 100 u/ml and streptomycin 100 μg/ml.

The percentage of cells in prophase, metaphase, anaphase, telophase and interphase was determined by counting 1000 cells in smears of cells after fixation in Carnoy fixative (acetic acid–ethanol, 1:3, v/v) and staining with a 2% aceto-orcein solution.

The cell number was counted in a Bürker haemacytometer. The number of dead cells was determined after staining with 0.4% Trypan blue.

DNA histograms showing the distribution of cells among various phases of the cell cycle were obtained after staining with mithramycin [4] using a laboratory-built flow cytometer as described previously [5, 6]. The relative number of cells in G1, S and G2+M determined from the DNA histograms [7, 8] of untreated exponentially growing cultures were about 15 per cent in G1, 55 per cent in S and 30 per cent in G2+M. The mean phase durations calculated from six control histograms assuming cell cycle duration of 15 hr, were G1 = 1.6 ± 0.2 hr; S = 7.9 ± 0.6 hr and G2+M = 5.5 ± 0.7 hr.

The concentration of puromycin at which cell protein synthesis was inhibited was determined by studying the incoroporation of L-[^{14}C]leucine (0.007 μC/ml cell suspension sp.act. 588 Ci/mol) into the protein fraction after incubation for 30 min at 37° in the presence of puromycin. The cell pellet was treated with ice-cold 5% trichloroacetic acid for 30 min, washed with ethanol–ether (3:1 v/v), dissolved in soluene and counted in toluene containing 5 g/l PPO, 0.05 g POPOP and 30% Triton X-100.

The effect of 5-chloro-pyridin-2-one on the accumulation of cells in metaphase in exponentially growing cells is shown in Fig. 1. The concentration used was the lowest which would prevent the appearance of any ana- and telophase. The percentage of cells in metaphase increased for about 8 hr after drug administration, then declined and approached the control at the end of the experiment. The maximum value for the percentage of cells in metaphase and the time elapsed before reaching this value varied somewhat from experiment to experiment. Cells in prophase remained at approximately 1 per cent for 11 hr and subsequently no prophases were seen. The percentage of dead cells (judged from the uptake of Trypan blue) remained at 1 per cent for 21 hr which was the same as in the control. After 33 hr the figure rose to 3 per cent. When the percentage of cells in metaphase declined, the metaphase arrested cells presumably returned to interphase without dividing, since no ana- or telophases were seen during the whole experiment and the cell number remained constant (see lower part of Fig. 1).

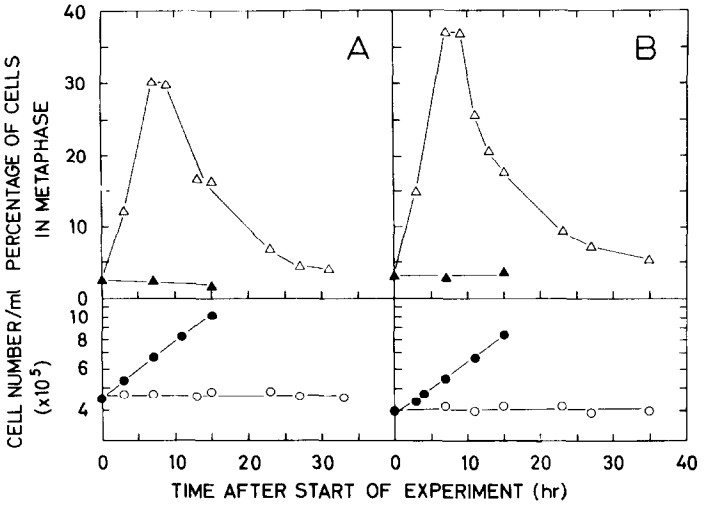

Fig. 1. Effect of 3 mM of 5-chloropyrimidin-2-one (A) and 0.1 μM of colcemid (B) on metaphase arrest and cell number in Ehrlich ascites cells. △ and ▲ (upper part) indicate percentage of cells in metaphase in drug-treated and control cells respectively. ○ and ● (lower part, logarithmic scale) indicate number of cells/ml in drug-treated and control populations.

Since 5-chloropyrimidin-2-one belongs to a new group of metaphase inhibitors, the effect observed could be specific for this inhibitor. Therefore, the effect on these cells of the well known metaphase inhibitor colcemid was examined. When colcemid was used at the lowest concentration which prevents the appearance of any ana- and telophases, the percentage of cells in metaphase increased and then declined without any change in cell number as was the case with 5-chloropyrimidin-2-one (Fig. 1). Prophases remained at about 1 per cent for 13 hr and subsequently no prophases were seen. The percentage of dead cells was as in the experiment with 5-chloropyrimidin-2-one. Hence the effect of 5-chloropyrimidin-2-one and colcemid, at the concentrations used, were qualitatively similar.

The distribution of cellular DNA content at different intervals after the addition of the inhibitors is seen in Fig. 2. From the histograms recorded at 13 and 21 hr it appears that the cells which had overcome the metaphase block and escaped into interphase with a DNA content of 4C, start doubling their DNA content to 8C. After 33 hr the peak in the histogram for cells with a DNA content of 8C is pronounced in both cases but larger in the colcemid treated cells.

After 33 hr about 60 per cent of the cells (1000 cells counted) having overcome metaphase arrest were multinucleated as compared to 3.3 per cent in the control culture. Upon prolonged incubation with the inhibitors, the cells began to disintegrate.

The effect of 5-chloropyrimidin-2-one and colcemid on the cells at the concentration used were also qualitatively similar in that concomitant protein synthesis was not required for the arrested cells to overcome the block (Fig. 3). This was shown by using the incorporation of L-[^{14}C]leucine into the protein fraction as a measure of protein synthesis. A concentration of 50 μg/ml of puromycin was sufficient to inhibit L-[^{14}C]leucine incorporation. After a period of 4 hr with colcemid or 5-chloropyrimidin-2-one respectively, 50 μg/ml of puromycin was added (Fig. 3).

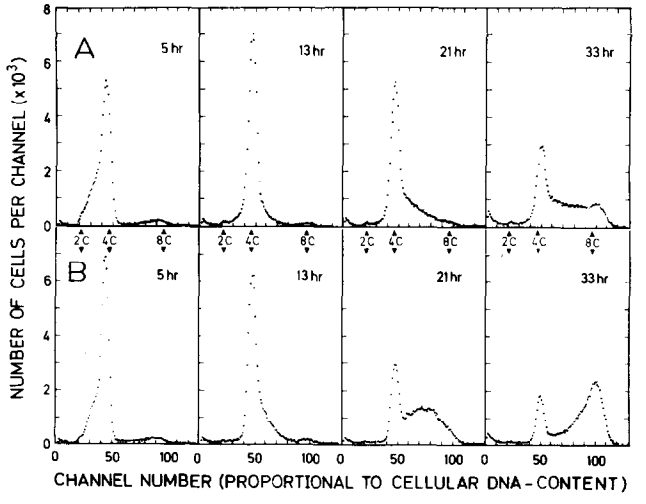

Fig. 2. DNA histograms of populations in Ehrlich ascites cells treated with 3 mM of 5-chloropyrimidin-2-one (A) and 0.1 μM of colcemid (B). Time elapsed after addition of inhibitor is indicated in panel. 80,000 cells were registered for each histogram.

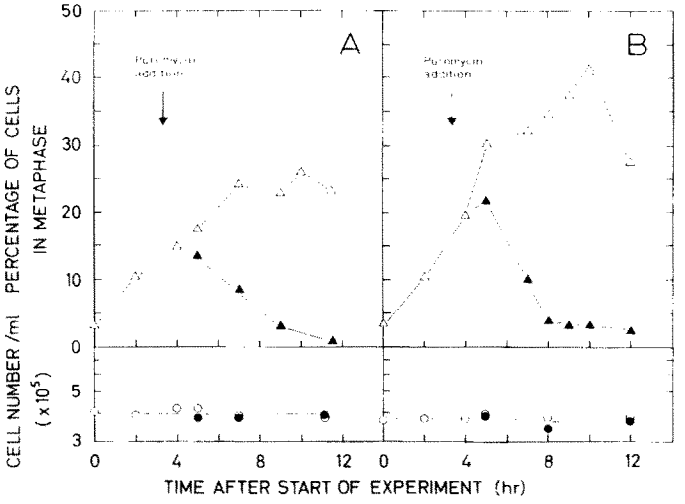

Fig. 3. The effect of puromycin on the ability of Ehrlich ascites cells blocked at metaphase to escape into interphase without dividing. 3 μM of 5-chloropyrimidin-2-one (A) and 0.1 μM of colcemid (B) were added at zero time. After 4 hr each suspension of cells was then divided in two and puromycin (50 μg/ml) added to one part. Percentage of cells in metaphase in the absence △ and presence of puromycin ▲ (upper part). Cell count in the absence ○ and presence of puromycin ● (lower part, logarithmic scale).

Using Trypan blue it could be shown that there was no increase in dying cells after the addition of puromycin. The addition of puromycin prevents cells from entering mitosis and there is, as expected, no further accumulation of metaphase cells (Fig. 3). Puromycin did not prevent the exit of cells arrested in metaphase with colcemid or 5-chloropyrimidin-2-one, since the rate of exit was about the same as for cells not treated with puromycin.

The results show that the new metaphase inhibitor 5-chloropyrimidin-2-one affects the mouse cell line in an unusual manner as the cells after a while escape metaphase arrest and return to interphase with a 4C DNA content, and then subsequently start doubling their DNA content to 8C.

In contrast to the present finding HeLa S3 cells are blocked completely in metaphase (results not shown) whereas the 'Don' strain of the Chinese Hamster cells which escape metaphase arrest go through anaphase with or without fusing [9].

The mouse cells in this work possibly overcome the metaphase arrest by dissolution of the chromosome followed by reformation of the nuclear membrane. During these experiments we have examined smears of cells (about 10,000 cells in each experiment) but have not been able to detect any distinct intermediate stages in this process. Since we have not succeeded in growing the cells in monolayer, it has not been possible to study in detail single cells to reveal intermediate stages when cells were escaping metaphase arrest.

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