

EFFECT OF 5-AZACYTIDINE ON FREQUENCY OF POLYCENTRIC CHROMOSOMES INDUCED  
IN CELLS WITH MICRONUCLEI BY 5-BROMODEOXYURIDINE

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During long-term culture of transplantable Chinese hamster cells with colcemid multinuclear cells containing micronuclei are formed. If 5-bromodeoxyuridine (BUdR) is added to the culture simultaneously with colcemid, many dicentrics and even tracentrics are found in the first division metaphases of cells with micronuclei. It has been shown that these polycentric chromosomes are formed by telomeric fusion [2]. Since polycentric chromosomes are not formed in cells with micronuclei without BUdR, it is suggested that the state of the DNA affects the formation of telomeric links of metaphase chromosomes.

The regulation of gene activity is nowadays connected with methylation of the cytosine bases of DNA [5]. By the use of 5-azacytidine (5-AC), an agent whose addition to a cell culture leads to hypomethylation of DNA, inactive gene loci have been successfully activated and the phenotype of some cell lines has been changed [3, 4].

The aim of this investigation was to study hypomethylation of DNA induced by 5-AC in relation to manifestation of the phenomenon of telomeric fusion of metaphase chromosomes.

## EXPERIMENTAL METHOD

Clone 237S of Chinese hamster line Bddd-II-FAF28 was used. The cells were cultured on Eagle's medium with 10% bovine serum in rectangular flasks 0.5 liter in volume. Chromosome preparations were obtained by the standard air-drying method.

The following versions of the experiment were carried out: 1) culture of intact cells; 2) cells in culture treated with BUdR; 3) cells in culture treated with colcemid; 4) cells in culture treated with 5-AC; 5) cells in culture treated with BUdR and 5-AC; 6) cells in culture treated with colcemid and 5-AC; 7) cells in culture treated with colcemid and BUdR; 8) cells in culture treated with colcemid, BUdR, and 5-AC.

Colcemid, BUdR, and 5-AC were added to the actively growing culture for 42 h. In versions 1, 2, 4, and 5 of the experiment the cells were treated with colcemid during the last 2 h of culture. The concentrations of the substances were: colcemid 0.1 µg/ml (Calbiochem), BUdR 20 µg/ml (Serva), 5-AC 1 µg/ml (Serva). In each version 100 metaphases were analyzed.

## EXPERIMENTAL RESULTS

The results of the experiments to induce polycentric chromosomes are given in Table 1.

In versions 1-6 of the experiment the number of cells with dicentrics and the total number of dicentrics were low and differed appreciably from those in versions 7 and 8. In this case no statistical analysis was undertaken.

Comparison of versions 7 and 8 of the experiment revealed no statistically significant difference after analysis of 100 metaphases in each case. Accordingly the experiment was repeated and another 100 metaphases counted in these versions of the experiment. Thus the total number of cells counted was 400.

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TABLE 1. Induction of Polycentric Chromosomes on Exposure to Colcemid, BUdR, and 5-AC under Different Conditions\*

Version of expt. **	No. of cells with dicentrics	Total number of dicentrics
1-	1	1
2-	5	5
3-	7	7
4-	3	4
5-	1	1
6-	7	7
7-	38/32	55/45
8-	48/39***	76/60

\*One hundred metaphases were counted in each version of the experiment.

\*\*Versions 7 and 8 of the experiment were carried out twice.

\*\*\*In two independent experiments five tracentrics and one quadricentric were found.

Since in radiation cytogenetics the probability of appearance of a tracentric is equal to the probability of appearance of two dicentrics, and that of a quadricentric equal to that of three dicentrics, the results of version 8 of the experiment were recalculated. As a result the number of cells with dicentrics was 89 and the total number of dicentrics was 149.

Comparison of versions 7 and 8 of the experiment was made by means of the chi-square test with null hypothesis  $n_1 = n_2$  [1], where  $n_1 = 100$  (number of dicentrics in version 7),  $n_2 = 149$  (number of dicentrics in version 8), and  $n_1 + n_2 = n$  (total number of dicentrics in the two version).

The calculations showed that the null hypothesis is rejected with a level of significance of  $p = 0.0019$ ,  $\chi^2 = 9.64$ , with one degree of freedom.

It can thus be concluded that 5-AC increases the intensity of formation of polycentric chromosomes. It also follows from the experimental data that hypomethylation of the cytosine bases of DNA facilitates manifestation of the phenomenon of telomeric fusion of metaphase chromosomes in cells with micronuclei exposed to the action of BUdR.

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