

2. The absence of fundamental differences in the chromatin melting profiles and also in  $p_I$  of the proteins of granulocyte and lymphocyte nuclei of the same individual suggests that the specific character of the supramolecular organization of the cell DNP complex is determined by the charge of the proteins contained in the chromatin.

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#### 5-IODODEOXYURIDINE, LIKE 5-BROMODEOXYURIDINE, INDUCES SPECIFIC DICENTRIC CHROMOSOME FORMATION IN CELLS WITH MICRONUCLEI

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Colcemid, in a dose blocking mitosis, if added to a culture of Chinese hamster cells of the B1ld-ii-FAF28 line for 30-40 h, induces the formation of cells consisting of micronuclei. If simultaneous treatment with colcemid and 5-bromodeoxyuridine (BUdR) is given, many dicentric chromosomes are found in first division metaphases of the polykaryocytes. It was shown previously [3] that dicentric formation is connected with delayed disruption of telomeric links in the interphase nucleus. Besides in B1ld-ii-FAF28 cells, this phenomenon has also been reproduced in hybrid human  $\times$  Chinese hamster somatic cells [2].

The writers have studied the possibility of formation of specific dicentric chromosomes by the use of another halogen analog of thymidine instead of BUdR, namely 5-iododeoxyuridine (IUdR).

#### EXPERIMENTAL METHOD

Clone 237 of line B1ld-ii-FAF28 was used. The cell line was generously provided by A. N. Chebotarev (Institute of Medical Genetics, Academy of Medical Sciences of the USSR). The cells were cultured on Eagle's medium with 10% bovine serum in rectangular flasks with a capacity of 0.5 liter. Chromosome preparations were made by the standard air-dried method.

The following variants of the experiment were set up: 1) intact cell culture; 2) cells in culture treated with BUdR (20  $\mu$ g/ml, from Serva) for 42 h; 3) cells in culture treated with IUdR (20  $\mu$ g/ml, Serva); 4) cells in culture treated with colcemid (0.1  $\mu$ g/ml) for 42 h;

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TABLE 1. Results of Experiments to Induce Dicentric Chromosomes in Cells with Micro-nuclei, Using BUdR and IUdR

Experimental conditions	Number of metaphases analyzed	Cells with dicentrics	Total number of dicentrics	Cells with chromosomal aberrations	Total number of chromosomal aberrations
1. Intact culture	100	0	0	0	0
2. Culture with BUdR for 42 h	100	0	0	4	4
3. Culture with IUdR for 42 h	100	0	0	22	32
4. Culture with colcemid for 42 h	100	3	3	6	6
5. Culture with BUdR and colcemid for 42 h	98	52	93	3	3
6. Culture with IUdR and colcemid for 42 h	100	27	29	7	9

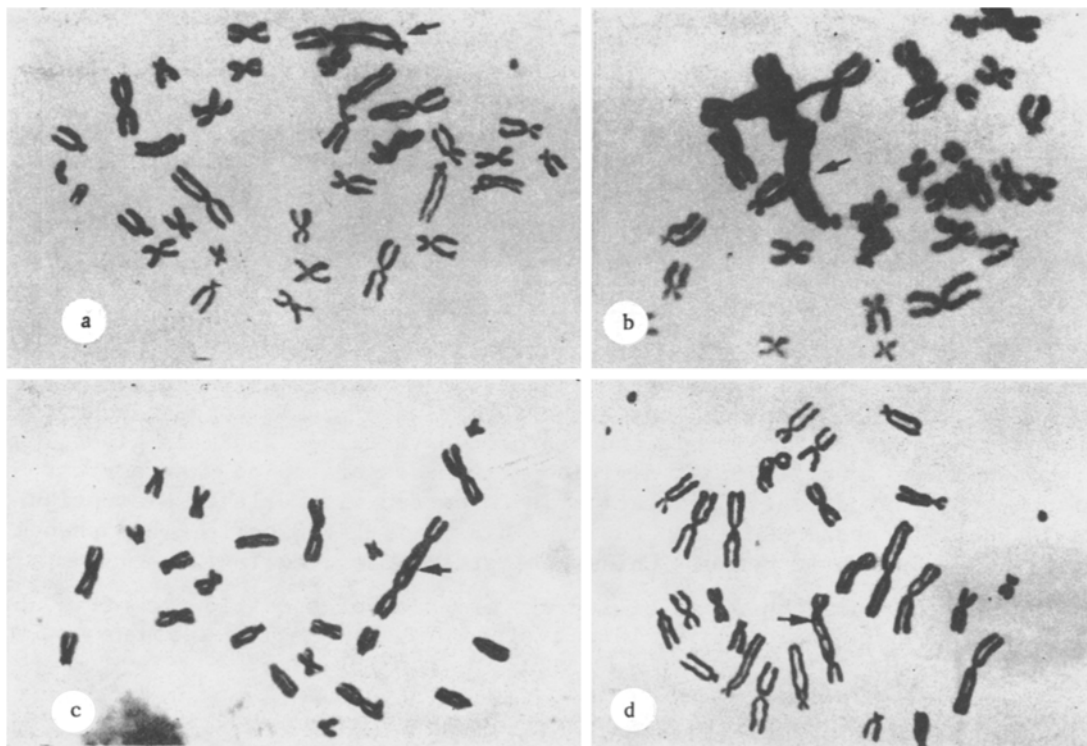


Fig. 1. Dicentric chromosomes in cells treated with IUdR and colcemid. Magnification: objective 100 $\times$ , ocular 7.7 $\times$ . Stained with azure-eosin.

5) cells in culture treated with colcemid (0.1  $\mu\text{g/ml}$ ) and BUdR (20  $\mu\text{g/ml}$ ) for 42 h; 6) cells in culture treated with colcemid (0.1  $\mu\text{g/ml}$ ) and IUdR (10  $\mu\text{g/ml}$ ) for 42 h. Colcemid (Serva) was added for the last 2 h of the culture in variants 1, 2, and 3.

In each variant of the experiment 100 metaphases were analyzed.

#### EXPERIMENTAL RESULTS

The results of the experiments to induce dicentrics are given in Table 1. In the case of an intact culture no dicentrics or chromosomal aberrations were found. When the cells were cultured with BUdR for 42 h, no dicentric chromosomes likewise were found. Four aberrant metaphases were recorded, in which chromosomes with breaks were present. In cells

treated with IUdR for 42 h, many chromosomal aberrations were observed. Altogether 22 aberrant cells were found, and there were 32 aberrant chromosomes. No dicentrics were found in this variant of the experiment. When the cells were cultured with colcemid alone for 42 h three cells with dicentrics were found. Only one dicentric was present in each cell. The number of metaphases with chromosomal breaks was six and the number of aberrant chromosomes also was six. When the cells were cultured with colcemid and BUdR, chromosomes with two or more centromeres were found in 54 cells. Tricentrics were present in three metaphases. The number of cells with dicentric chromosomes was 52. The total number of dicentrics was 93. The maximal number of dicentrics in a cell was four. There were four such metaphases. There were three cells with chromosomal breaks and three cells also with aberrant chromosomes. In the "colcemid + IUdR" variant of the experiment 27 cells with dicentrics were recorded; 25 metaphases contained one dicentric each, and two dicentrics were present in only two metaphases. The length of the dicentric chromosomes and the position of the centromeres varied as in the "colcemid + BUdR" experiment, evidence of the involvement of different chromosomes of the set in telomeric fusion (Fig. 1). In the "colcemid + IUdR" variant, lowering of the mitotic index was observed compared with the "colcemid + BUdR" experiment. The number of aberrant metaphases in the "colcemid + IUdR" experiment was seven, and there were nine chromosomes with breaks.

The results of the experiment with IUdR were used to determine the character of distribution of the cells by number of dicentrics. This distribution was found to obey Poisson's law. To verify agreement between the character of the distribution, the chi-square test was used. The mean number of dicentrics per cell in experiments 5 and 6 was 0.95 and 0.29 respectively. The mean number of chromosomal aberrations per cell also was determined. This was 0.32 in experiment 3 and 0.09 in experiment 6. The mean values were compared by normal approximation of the Poisson distribution [1]. In both cases significant differences were obtained ( $p < 0.01$ ).

In all variants of the experiment in which colcemid was present in the culture for 42 h, "premature condensation" of individual chromosomes and chromosomes with regions of delayed spiralization were found.

On the basis of these experiments the following conclusions can be drawn. IUdR, like BUdR, can induce specific dicentric chromosomes in cells with micronuclei. The effectiveness of IUdR as an inducer of dicentrics is less than that of BUdR. IUdR disturbs mitotic condensation of chromosomes in colcemid-induced polykaryocytes, carrying chromosomal aberrations in interphase, for the level of aberrations in this case is lower than in cells cultured with IUdR alone.

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