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MODIFIED METHOD OF DIFFERENTIAL STAINING OF SISTER CHROMATIDS

A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova

UDC 612.014.24-086.15

A modified method of obtaining differential staining of sister chromatids is described. It is simple, quick, and highly reproducible, but at the same time is cheap and readily accessible, for the reagents used are widely available. When 5-bromodeoxyuridine was added to a Chinese hamster cell culture 24 h before fixation the proportion of metaphases with differential staining of chromatids was 95-98%, but if the substance was added 28 h before fixation to a culture of human lymphocytes the proportion varied between 75 and 90% depending on the individual. The mean number of sister chromatid exchanges in human lymphocytes was found to be independent of the fixation time.

KEY WORDS: differential staining; sister chromatids; chromatid exchanges.

The effect of 5-bromodeoxyuridine on differential staining of regions of the chromosomes was first demonstrated by Zakharov and Egolina [4]. Using this property of 5-bromodeoxyuridine, Latt [2] developed a method of differential staining of sister chromatids, which was improved by Perry and Wolff [3]. The method was based on the use of preliminary staining of chromosome preparations with the dye Hoechst 33258. Later, Korenberg and Freedlander [1] developed a method of differential staining of sister chromatids in which the preparations were heated in alkaline solutions. The final stage in these methods was staining by the Giemsa method in buffered solution. If the method of differential staining of sister chromatids in cytogenetics is to be widely used, it must be simple, cheap, and highly reproducible. A method satisfying these conditions and tested on cultures of human lymphocytes and Chinese hamster cells is described below.

EXPERIMENTAL METHOD

A transplantable culture of Chinese hamster cells of clone 237_{2a} with 18 chromosomes in the karyotype and a short-term culture of human peripheral blood lymphocytes were used. 5-Bromodeoxyuridine in a concentration of 10 μg/ml was added 24 h before fixation to the culture of Chinese hamster cells and 28 h before fixation to the lymphocyte culture. The lymphocyte culture was fixed 72 and 96 h after addition of phytohemagglutinin. Colchicine was added to the cultures 2 h before fixation in a concentration of 0.5 μg/ml. Hypotonicity was produced when Chinese hamster cells were used by means of 1% sodium citrate (20 min) and when human lymphocytes were used, by means of a 0.55% potassium chloride solution (10 min) at 37°C. The cultures were fixed in a mixture of methanol and acetic acid (3:1). The specimens were prepared by applying the cell suspension to cold wet slides which were then dried in the air. After staining, the preparations were kept in a thermostat (37°C) for not less than 24 h.

Laboratory of Mutagenesis, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR B. A. Lapin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 242-243, February, 1978. Original article submitted May 25, 1977.

0007-4888/78/8502-0252$07.50

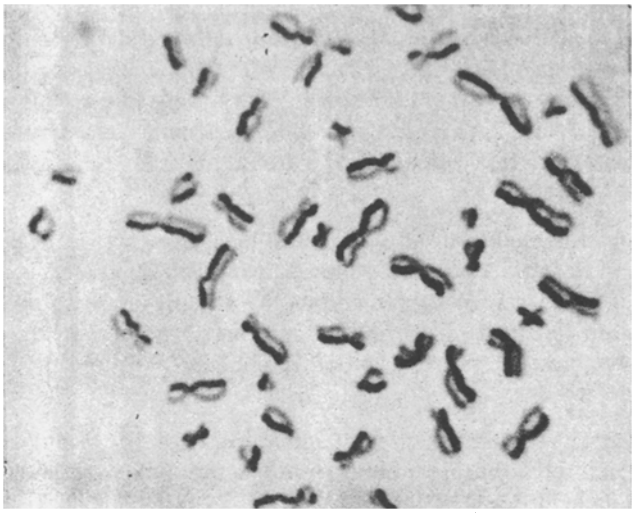

Fig. 1. Metaphase plate of culture of human lymphocytes with differential staining of sister chromatids. Here and in Fig. 2, 800×.

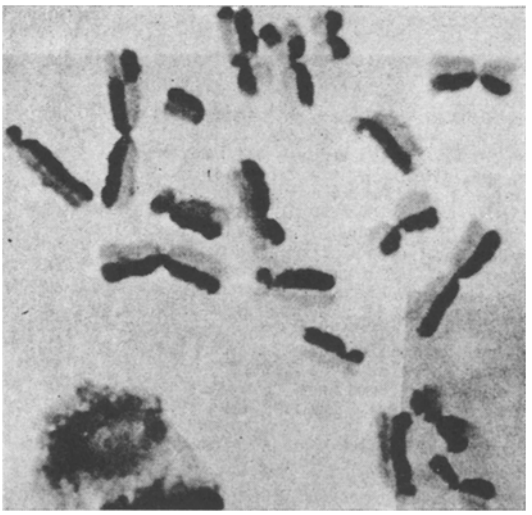

Fig. 2. Metaphase plate of culture of Chinese hamster cells with differential staining of sister chromatids.

The films were stained as follows. They were kept for 10 min in an aqueous solution of acridine orange (10^{-5} M), washed with water, and dried. They were then coated with a thin layer of 0.07 M Na_2HPO_4 solution and irradiated for 15 min by an SVD-120A ultraviolet lamp at a distance of 30-40 cm. After irradiation the films were washed in tap water and treated in a saturated solution of barium hydroxide at room temperature for 5 min in the case of human lymphocytes and 30 min in the case of Chinese hamster cells. The films were then washed and dried and then stained with 2% Giemsa solution made up in phosphate buffer (pH 6.8) for 15-20 min.

EXPERIMENTAL RESULTS

Differential staining of the sister chromatids obtained by the method described above is illustrated for human lymphocytes in Fig. 1 and for Chinese hamster cells in Fig. 2. During elaboration of the method various alternative stains were tested. It was found that without acridine orange treatment differential staining did not take place. It likewise was not obtained if the films were not irradiated with ultraviolet light and also without barium hydroxide treatment even though all other procedures of the method were carried out. It was

also noted that satisfactory results were obtained by irradiating preparations with ultraviolet light while in acridine orange solution. The Na_2HPO_4 solution also can be replaced by isotonic sodium chloride solution. However, better contrast between staining of the sister chromatids and better quality of staining were obtained by the method suggested above. Instead of acridine orange it is possible to use an aqueous solution of Hoechst 33258 (1 μg/ml) with the same effect. If barium hydroxide treatment was prolonged or if the temperature of the solution rose to 37°C, both chromatids were palely stained and the phenomenon of differential staining did not occur.

If 5-bromodeoxyuridine was added to a culture of Chinese hamster cells under the conditions described above an effect of differential staining of the sister chromatids was observed in 95-98% of cells, and if added to a culture of human lymphocytes it was observed in 75-90% of cells depending on the individual. These variations can evidently be attributed to differences in the duration of the cell cycle in different individuals and also with the more rapid asynchronization of the human lymphocyte culture than the culture of Chinese hamster cells.

The level of sister chromatid exchanges in Chinese hamster cells was determined in the course of the experiments. On analysis of 500 cells the mean number of sister exchanges per cell was 6.004 with a dispersion of 7.376 and a 95% confidence interval of the mean from 4.768 to 7.557 exchanges per cell. The number of exchanges per cell varied from 0 to 15. The level of sister chromatid exchanges in human lymphocytes was determined after different methods of fixation and with the use of cultures from the same donor. In each case 50 metaphases were analyzed. With fixation at 72 h the mean number of exchanges per cell was 7.00, and after 96 h it was 6.92, or virtually the same. Later fixation can thus be used in order to obtain a larger number of mitoses in cultures of human lymphocytes, for the time of fixation does not affect the number of sister chromatid exchanges.

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METHOD OF COMBINED HISTOLOGICAL STAINING OF THE LIVER WITH ALCIAN BLUE AND CARBOL FUCHSIN

G. G. Avtandilov, N. N. Litvinov, and V. A. Popov

UDC 616.36-076.1+611.36-086.15

A modification of Novelli's combined histological staining method whereby the functional state of hepatocytes can be determined is suggested.

KEY WORDS: liver; hepatocytes; alcian blue.

The differentiation of functional states of cells during the study of native and fixed tissues continues to attract the attention of experimental workers and pathomorphologists. There are indications in the literature of differences in the functional state of cells and the lability of cellular structures in the organs under normal and pathological conditions [1, 5, 6].

Novelli [7] suggested a method of double staining – with alcian blue and carbol fuchsin – of different organs of experimental animals, including the liver. Novelli concluded that this method can be used to differentiate cells on the basis of their functional state. Parallel experiments on isolated nuclei and with staining by the

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Central Pathological Anatomical Laboratory, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 243-245, February, 1978. Original article submitted April 13, 1977.

0007-4888/78/8502-0254$07.50