OBTAINING PREPARATIONS FOR THE DIFFERENTIAL STAINING OF CHROMOSOMES

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UDC 576.312.32.086.15

A study of correlation between preservation of the three-dimensional structure of chromosomes and their ability to undergo differential staining showed that chromosomes shaped like a convex half-cylinder give a regular pattern of longitudinal differentiation, but those shaped like concave half-cylinders do not. It was found that if the chromosomes are exposed for a brief period to the action of water during the staining procedure their outlines are changed and they become shaped like concave half-cylinders. Another method of obtaining chromosome preparations – ruling out possible exposure to water and increasing both the percentage of metaphase plates with differential staining of the chromosomes and also the quality of the staining – is thus suggested.
KEY WORDS: chromosomes – differentiation; staining.

In recent years several new methods of longitudinal differential staining of metaphase chromosomes have appeared in cytogenetics [2-4, 6-8], and these are now subdivided into the Q-, G-, R-, and C-stains. On the basis of the picture of linear differentiation, which is strictly specific for each pair of homologues, it is now possible to identify the whole chromosome set of man and many species of mammals.

However, when these methods and, in particular, the G-stain are used, a number of difficulties may arise. First, differential staining of the metaphase chromosomes may be obtained in not all preparations. Second, the percentage of metaphase cells with clear differentiation of all chromosomes is small. Third, not all chromosomes can be differentiated within the same plate and stain uniformly, so that it is difficult to photograph them, and this reduces still further the number of cells suitable for analysis. Preparations obtained by the method of burning out the fixative on the slide cannot be stained differentially or the results are poor. In addition, as many authors have shown [2, 5, 6], chromosome preparations made more than 2 months before staining cannot be stained differentially by the G-technique.

This accounts for the fact that the number of new modifications of the differential staining technique is increasing all the time and now amounts to more than 20. It must be emphasized that all these methods are concerned in some way or other with the process of differential staining of preparations already made.

During a long period of work aimed at improving the G-technique the writer concluded that one possible reason for difficulty in regularly reproducing differential staining of high quality is concerned with the actual process of making the chromosomal preparation. The writer showed previously that, depending on the method used to obtain the preparations and the nature of the experimental procedures used, the 3-dimensioned structure (relief) of the chromosome may undergo specific changes [1]. In particular, after exposure to water the relief is modified and, instead of the usual shape of a convex half-cylinder, the chromosome assumes the shape of a concave half-cylinder (Fig. 1a, b). If the relief of the individual chromosomes is subsequently compared in preparations made by the usual method with the quality and degree of differentiation after G-staining, those chromosomes with a relief most closely resembling the convex half-cylinder are found to differentiate best of all (Fig. 1e, f, g, h).

Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 8, pp. 120-122, August, 1974. Original article submitted December 11, 1973.

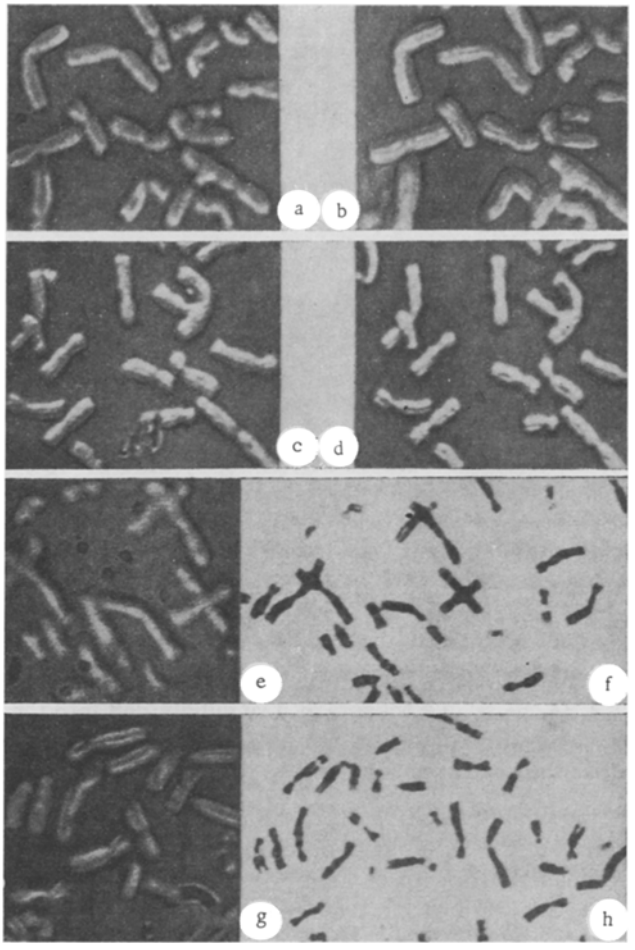

Fig. 1. Changes in relief and quality of differential staining of metaphase chromosomes after brief exposure to water (explanation in text).

In preparations made in the usual way, i.e., on slides wetted with water, all the types of chromosome relief mentioned above can be seen. If the relief of the chromosome shaped like concave half-cylinders is restored by exposing the preparations in fresh methanol–acetic acid fixative for 20-30 min (Fig. 1c, d), after treatment in this way the preparations can be differentially stained with Giemsa stain.

The above observations suggested that the ability of chromosomes to undergo differential staining is affected by the action of water on them. It was therefore decided to abandon the traditional method of applying the cell suspension to slides wetted with water. Instead, clean, defatted, dry slides were placed in fresh methanol–acetic acid fixative (3 : 1) and left for a few hours in the freezing compartment of a refrigerator. The cell suspension was applied to cold slides wetted with the fixative and quickly dried above the flame of a burner. Since the cells do not spread out in a layer immediately if the cell suspension is dropped on a slide wetted with the fixative, the slide must be tilted several times until the first sign of "graining" appears on the surface, indicating that the cells are starting to adhere to the surface of the slide. To obtain differential staining, the preparations are first exposed for 8-10 min to a solution containing 50 ml phosphate buffer (pH 6.8) and 0.05 ml of 0.25% trypsin solution. They are then transfered into a buffered solution of ordinary Romanovsky–Giemsa stain, diluted with phosphate buffer in the ratio of 1 : 100, and stained for 30-40 min at room temperature.

Definite correlation thus exists between the ability of metaphase chromosomes to undergo differential staining and the nature of their three-dimensional structure. Chromosomes with a relief of the convex half-cylinder type regularly give linear differential staining. Of all the factors influencing the three-dimensional morphology of the chromosomes during the production of chromosome preparations the most important was the effect of water. By preventing exposure of the fixed chromosomes to water their normal relief can be preserved and in that way the conditions for regular and uniform differential staining can be ensured.

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