MODIFICATION OF GIEMSA'S METHOD OF STAINING HUMAN CHROMOSOMES TO DETECT THEIR LINEAR DIFFERENTIATION

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If the preparations of human chromosomes are stained in buffered (pH 6.8) solution of Giemsa's stain their linear differentiation is revealed. The differentiation pattern is specific. Differential staining of the chromosome disappears after hydrolysis of the preparations with hydrochloric acid. The nature of the differential staining is discussed. A role of chromosome proteins in the exhibition of differential staining is postulated.

Interest in Giemsa's method for staining chromosomes has recently increased as the result of work, [6] which showed that after treatment before hybridization of mouse satellite DNA in situ in cytological preparations of mouse chromosomes Giemsa's dye selectively stains the heterochromatic zones of the chromosomes around the centromere. Workers [2] who have used this method to stain human chromosomes also observed selective staining of certain chromosomes with this dye in zones around the centromere. Dutrillaux and Lejeune [3], who treated chromosome preparations by heating them to 87°C and staining them in buffered (pH 6.5) solution of Giemsa's stain, obtained differential staining along individual chromosomes. These workers propose the use of this method to identify individual chromosomes in the human karyotype. Sumner et al. [7], to obtain the pattern of differential staining of human chromosomes, simply heat the material to 60°C and stain it in buffered (pH 6.8) Giemsa's solution. Arrighi and Hsu also regard this as the pH optimum for the solution of the dye when demonstrating the staining differences between heterochromatic and euchromatic zones of chromosomes. In all the papers cited, preliminary alkaline or thermal treatment of the chromosomes is regarded as an essential step in the obtaining of differential staining, and on this basis it is suggested that the phenomenon is based on "denaturation" followed by "renaturation" of the chromosomal DNA.

When choosing optimal conditions for staining human chromosomes differentiated along their length after treatment with β-mercaptoethanol the writer found that chromosomes in control cultures, not treated with mercaptoethanol, can also be differentially stained in buffered Giemsa's solution, and that no preliminary heating of the preparations is necessary.

EXPERIMENTAL METHOD

A series of 72-h cultures of human peripheral blood obtained by a modified Hungerford's method [5] was used. To accumulate metaphases in the cultures, demecolcine (0.06 g/ml) was added to them 2 h before fixation. Hypotonic treatment was carried out at room temperature with 0.75 M KCl solution (pH 5.7-5.8), previously warmed to 37°C. The duration of the hypotonic treatment was 10-12 min. Fixation was carried out with a mixture of methanol and glacial acetic acid (3:1) at room temperature (3 changes of fixative at intervals of 10-15 min). Cytological specimens of chromosomes were made by the drying method. Slides with the cells were stained by immersion for 15 min in a solution consisting of 50 ml

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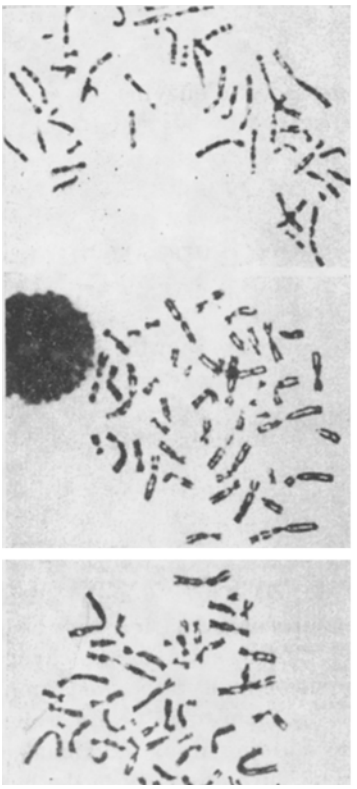

Fig. 1

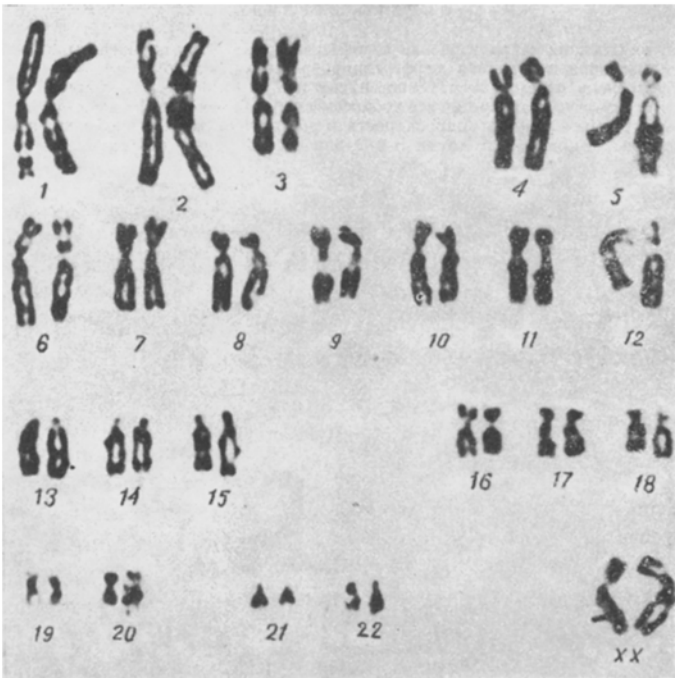

Fig. 2

Fig. 1. Examples illustrating linear differentiation of staining of chromosomes. Three metaphase plates with different degrees of helical conformation are shown. Giemsa, 630 ×.

Fig. 2. Karyotyped metaphase plate. Difference between linear differentiation of individual chromosomes (pairs) and similarity between homologous chromosomes (by comparing the chromosomes of each pair) will be apparent.

phosphate (0.7 M) buffer (pH 6.8) and 1 ml of a commercial solution of azure-eosin. Staining must be carried out under microscopic control. If necessary the slides must be differentiated quickly in 50% ethanol solution and washed in distilled water.

EXPERIMENTAL RESULTS

Microscopic study of metaphase plates stained by this method revealed longitudinal differentiation of the chromosomes. This differentiation was due to alternation of darkly and lightly stained zones of the chromatids (Fig. 1). Virtually all the chromosomes of the set were found to have a differential structure. The pattern of differentiation was sufficiently specific if judged with respect to chromosome A1 and chromosomes of the B and D groups, the fine structure of which has previously been studied by other methods [2, 8]. Similarity of the segmental structure of the homologous chromosomes could be seen in the karyotyped metaphase plate (Fig. 2). Similarity of differentiation of the chromosomes when stained by this method with their differentiation revealed by staining after preliminary heating by Sumner's method will be apparent. Attention must be drawn to the appreciable, at least in the large chromosomes of group A, "reversal" of the pattern of stained and weakly stained zones of chromosomes when the present method and certain other methods were used [3, 8].

In their discussion of the nature of differential staining of chromosomes by Giemsa's method Sumner et al. emphasize the lack of correlation between the content of high-repeating human DNAs (17%) and the ratio between the darkly and lightly stained zones of the chromosomes (on the average 1:1) and they point out that this staining phenomenon cannot be explained purely by the presence of zones of chromosomes

containing high-repeating DNAs (Giemsa-positive) and "low-repeating" zones (Giemsa-negative). The present writer has emphasized that after hydrolysis of the preparations with hydrochloric acid differential staining of the chromosomes disappears and becomes uniform. Dutrillaux et al. observed the phenomenon of differential Giemsa staining of human chromosomes treated with the proteolytic enzyme pronase [4]. Consequently, it can be postulated that differential staining by Giemsa's method is determined, at least in part, by the nonhomogeneity of the protein component of the chromosome. This nonhomogeneity may be the result of asynchronous formation of the nucleoprotein complex during mitotic helix-formation between early and late replicating segments of the chromosome [8]. Proteins of the nucleoprotein complex of the chromosome can be imagined to undergo stepwise denaturation in different segments. It is known [1] that denatured proteins have a high affinity for dyes. Finally, if differential staining is explained from the standpoint of staining of chromosomal proteins, which are amphoteric compounds, it will be clear why the hydrogen ion concentration in solutions of the dye is important in the manifestation of this phenomenon.

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