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TECHNICAL FACTORS IN VARIABILITY OF SILVER STAINING OF THE NUCLEOLUS ORGANIZER OF HUMAN CHROMOSOMES

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In recent years after development of the technique of selective staining of the nucleolar organizers (NO) of chromosomes in the metaphase of mitosis by means of silver nitrate interest in the study of these regions, containing ribosomal genes, has increased considerably. It has been shown that the number of NO stained with silver and the degree of staining are stable characteristics of the karyotype of a given individual. Nevertheless, many workers have noted variability of silver staining of some usually palely stained NO within the same tissue [1, 2]. The reasons for this phenomenon, which is of considerable theoretical and practical interest, have been studied, and attention has been concentrated on factors of a technical character.

The aim of the present investigation was to study the effect of the time of silver staining and of some other conditions on intercellular variability of NO in human chromosomes, to determine the optimal conditions for silver staining, and to select for analysis metaphase plates (MP) with maximally stained NO.

EXPERIMENTAL METHOD

Peripheral blood lymphocyte cultures from four persons conventionally identified by the numbers 1, 2, 3, and 4, were used. Preparations of metaphase chromosomes were obtained by the standard method and kept for 1 week in an incubator at 37°C. Silver staining was carried out by two methods. The first method was the Ag-1 method suggested by Bloom and Goodpasture [3]: two drops of a 50% aqueous solution of $AgNO_3$ were dropped on the preparation, a coverslip was applied, and it was introduced into a wet chamber and incubated at 37°C for 12, 24, 48, or 72 h. The second method was the writer's own modification of the Ag-1 method: preparations placed in $AgNO_3$ solution in the wet chamber were kept under a DRT-230 UV lamp for 20, 40, 60, or 80 min. After removal of the coverslip and washing of the preparations in water they were incubated in 0.2 N NaCl solution for 6-7 min at 65°C, taken through a series of alcohols, and stained with a 2% phosphate buffer solution of Giesma stain to obtain longitudinal differential staining of the chromosomes. With the first method of staining 50 MP were analyzed in each case, with the second method from 10 to 50 MP. The state of NO was estimated from the intensity of staining, expressed in points: 0) no staining, 1 point) pale staining, 2 points) average, 3 points) intense staining. The mean ability of MP to take up the dye in a given sample was determined as the sum of the points divided by the number of MP.

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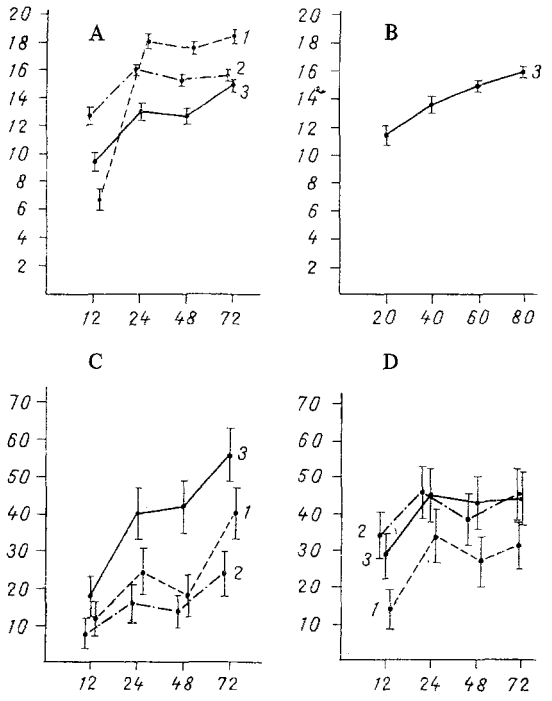

Fig. 1. Changes in intensity of silver staining of NO of chromosomes as a function of staining time. A) Mean intensity of staining of MP when first method used; B) mean intensity of staining of MP when second method used; C) demonstrability of varying silver-containing chromosome when first method was used; D) number of chromosomes intensely stained with silver when first method was used. Abscissa, time (in h); ordinate: A, B) points; C, D) percentages. 1, 2, 3, 4) Conventional identification number of individual.

EXPERIMENTAL RESULTS

Analysis of preparations stained for 12 h by the first method and for 20 min by the second method showed that most MP contained no grains of silver, and that stained plates were located chiefly at the periphery of the coverslip. A low average rating of MP staining (Fig. 1A) indicates that silver staining of the NO was incomplete. Regions of the short arms (satellites and satellite threads) typing NO of acrocentric chromosomes differed considerably at these times of silver staining from those of intact chromosomes. The difference was the appearance of a homogeneous, palely stained spot in these regions instead of distinctly outlined satellites and satellite threads. The proximal part of the short arm, like the long arm of the chromosomes, remained unchanged. The dynamics of the change in morphology of the short arms during silver staining was further observed in a number of MP by photographing them in stages (Fig. 2). When long satellite threads were present and the quantity of silver-containing material was small, the changes described above affected only the region of the satellite threads, and the satellites themselves were stained by the Giesma method. In some MP a few grains of silver also were observed against the background of a homogeneously palely stained spot in NO. With lengthening of the staining time these regions always stained with silver (Fig. 2). This observation, compared with data in the literature on the mechanism of selective staining of RMP in the NO region [4-6, 8, 9], suggests that the region of the short arm of human acrocentric chromosomes contains material which is achromatic under the ordinary conditions of staining with azure-eozin. However, under the influence of facts of the silver staining procedure, changes taking place in this material increase its susceptibility to Giesma stain and create conditions for precipitation of silver grains. The phenomenon we have discovered is important when assessing the completeness of silver staining: if it is observed in any particular acrocentric, the staining must be regarded as incomplete.

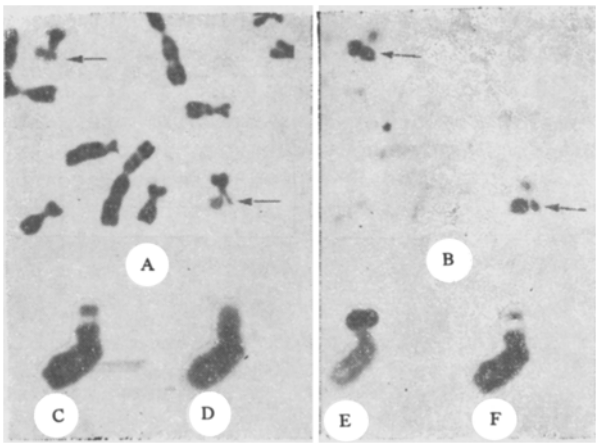

Fig. 2. Dynamics of changes in character of staining of chromosomal NO during silver impregnation. A) NO after staining with silver before appearance of grains; B) NO with optimal silver saturation; C) intact chromosome stained with azure—eosin; D) initial stage of silver staining before appearance of grains; E) optimal silver saturation of NO; F) staining with azure—eosin after washing out silver.

After incubation of the preparations with $AgNO_3$ for 24 and 48 h the mean intensity of silver impregnation of the chromosomes was considerably increased, the number of stained MP also increased, but nevertheless significant differences were observed in the intensity of staining along the edges of the coverslip and in the middle of the preparation, where the NO remained unstained. After staining for 72 h, the mean intensity of silver impregnation of MP was similar to that after staining for 24-48 h, except in individual 3, when it was a little higher (Fig. 1a). All MP were characterized by the presence of grains of silver in NO, but nonspecific precipitation of silver also was observed along and around MP, and took place after staining for 24-48 h at the periphery of the coverslip. Analysis of the results of staining of NO by the second method showed that saturation of NO with silver occurred within the time interval from 40 to 60 min. In preparations stained for 60 min all MP contained silver, the mean intensity of silver impregnation of MP was 13.9 (individual 2) and 14.9 (individual 3), and it varied from metaphase to metaphase less than on staining by the first method, but nonspecific precipitation of silver was found less frequently. Keeping the preparations under the UV lamp longer than 2 h has a result that the chromosomes did not stain with Giemsa dye, whereas against the background of palely yellow chromosomes, the silver-containing NO stood out clearly. In some plates, regions near the centromere also stained with silver.

Data on quantitative changes in a chromosome with varying silver staining depending on the staining time by the first method are given in Fig. 1C. The maximal number of silver-containing chromosomes was observed during long incubation of the preparations in $AgNO_3$ solution. On comparison of these data with the results shown in Fig. 1D it will be clear that the strongly stained NO reach optimal silver saturation in the earlier stages of staining. Since the contribution of highly stained NO to the average intensity of silver staining of MP is much greater than that of the varying NO, the mean intensity of staining of MP also remained stable between 24 and 72 h. The number of varying chromosomes discovered by staining by the second method was 30% (individual 2) and 68% (individual 3).

It can be concluded from the results of this investigation that the time of silver staining has a considerable influence on intercellular variability of silver-containing NO, as is seen particularly clearly with respect to ability to detect palely stained NO. In this connection selection of fully stained MP for analysis assumes great importance, as already stated above. MP in which, besides silver-containing NO there are also acrocentrics with an unchanged, "routine" structure of the short arm region, satellite threads, and satellites must also be selected for analysis.

Our modification of the Ag-1 method, consisting of the use of UV irradiation of the preparations, enables NO of acrocentric chromosomes to be stained in much shorter times, and with a high yield of fully stained MP. The long arm of acrocentric chromosomes still remained capable of longitudinal differential staining. If preparations of metaphase chromosomes obtained from a culture of lymphocytes grown in the presence of BDU throughout the period of culture and at the end of the S period were stained by the second method, silver staining could be obtained in chromosomes with different degrees of staining of the sister chromatids, but with shorter introduction of BDU-Ag + R-like staining (individual 4). In the first case it is possible to identify the order of mitosis after introduction of BDU, and in the second case to identify individual acrocentrics.

In MP with optimal silver saturation the characteristic morphology of deposition of silver grains can be observed. For strongly and weakly stained NO it was so specific that often it enabled individual acrocentrics to be identified. The whole diversity of localizations of silver grains in NO is such that they can be divided into three clearly distinguished types: I) along the lateral sides of the satellite threads; II) along both sides of each satellite thread; II) between satellites. Different combinations of these types also are possible. The morphology of deposition of grains of silver, determined by the size and mutual arrangement of the silver-containing material, satellite threads, and satellites, is a stable and characteristic feature of individual acrocentrics with a narrow range of variation from cell to cell. Other workers have made similar observations [7].

The new information obtained on changes in morphology of NO during and after the end of silver impregnation, and also the new modification of the Ag-1 method of staining nucleolar organizers will facilitate the further study of functioning of the ribosomal genes in man under normal and pathological conditions.

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