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HUMAN CHROMOSOME MAPPING WITH AN AMMONIACAL SILVER STAINING PROCEDURE

Preliminary Report

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SUMMARY

The application of an ammoniacal silver procedure to the staining of human metaphase chromosomes is described. A map based on the differential staining characteristics of chromosome segments is presented.

Ammoniacal silver as a nuclear stain was first utilized by Black and Speer (1958). Subsequently Black and Ansley (1964) applied this procedure to the giant chromosomes of the salivary glands of various diptera. In the past, one of us (Bartalos et al. 1966) attempted to apply this technique to human metaphase chromosomes. Recently, we have been able to standardize this procedure and obtain consistent staining. Our technique is as follows.

TECHNIQUE

Human peripheral blood cultures are processed in the usual manner (Bartalos and Baramki 1967). Carnoy's fixative (3 parts ethyl alcohol and 1 part glacial acetic acid) is used without flaming the slides. Air-dried slides are placed in 10% formalin neutralized with sodium acetate for 5 minutes. After brief rinsing in distilled water, the slides are placed in 5% ammoniacal silver solution for 10 minutes. (Ammoniacal silver solution is prepared by dropwise addition of concentrated ammonium hydroxide to 10% aqueous silver nitrate solution until the initially formed precipitate almost completely disappears. Thereafter, equal volume of distilled water is added, the solution is stored overnight, and is filtered before use.) After the ammoniacal silver treatment, the slides are again briefly rinsed in distilled water and are inserted into 3% formalin for 15 minutes. After repeat washing in deionized water, the slides can be dehydrated and mounted in the usual manner or the chromosomes can be counterstained by any one of the DNA stains. For this purpose, we presently prefer neutral red for 10 minutes. (The neutral red solution is made up by mixing 10 cc of 1%

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aqueous solution of neutral red with 0.2 cc of 1% aqueous acetic acid solution to which 100 cc of distilled water is added. The solution is filtered before use.) After neutral red staining, the slides are rinsed in distilled water, dehydrated through an alcohol series and mounted with permount. If the Giemsa stain is used for counterstaining, care must be exercised not to overstain the chromosomes. Should this happen, however, immersion of the slides into 3% formalin for 3 to 5 minutes will reduce the intensity of the Giemsa staining. For observation and photography we employed a Zeiss photomicroscope.

DISCUSSION

Chromosomes stained by this procedure reveal alternating areas of heavily and lightly stained segments (Fig. 1). The number of the segments and their position was found to be similar on repeat samples of chromosomes obtained from blood samples taken at five different times from the same individual. Identical patterns were subsequently seen in cells cultured from six females and four males.

Close examination of numerous good-quality photographs revealed that sister-chromatids in general do not have a completely identical pattern. Frequently, the staining pattern suggest a complementarity, insofar as the heavily stained areas of

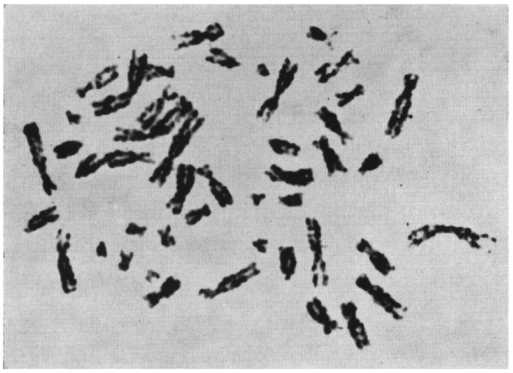

FIG. 1. *Metaphase spread of mitotic chromosomes of a human male obtained by short-term culture of peripheral leukocytes stained by an ammoniacal silver procedure and counterstained lightly with neutral red.*

one chromatid lie adjacent to corresponding lightly stained areas on the other chromatid. Thus, these metaphase chromosomes, in general, exhibit two sets of morphological characteristics, one for each sister-chromatid. Observation of the chromosomes revealed that homologous chromosomes have identical patterns, insofar as each sister-chromatid has a corresponding sister-chromatid in its homologous chromosome. Thus, identification of members of a homologous pair involve the comparison of the staining pattern of *both* sister-chromatids.

Ten individuals were studied for the purpose of establishing the constancy of the staining patterns. These included six females and four males. One male was from India while the rest had European ancestry.

A schematic representation of the staining characteristics of the different chromo-

somes is given in Fig. 2. Arrows indicate constant and readily observable sites of lightly staining areas. Although all members of the chromosome complement appear to posses a unique pattern, it is not known how this pattern corresponds to that obtained with the fluorescence procedures and the modified Giemsa procedures. There-

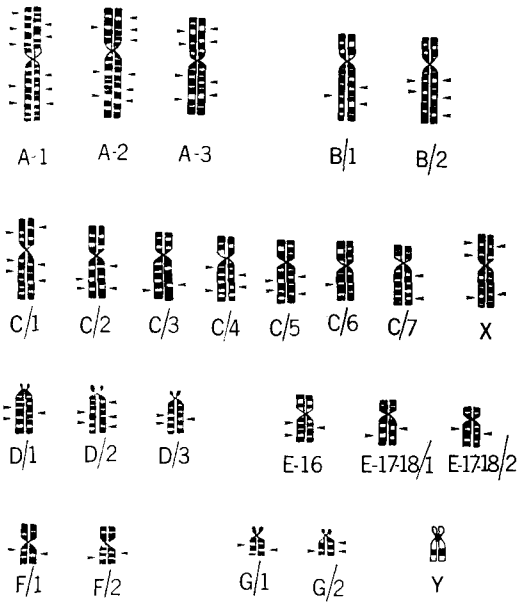

FIG. 2. *Schematic representation of a morphological map of human metaphase chromosomes obtained by short-term culture of peripheral leukocytes. The pattern represents the staining characteristics of individual chromosomes as revealed by the ammoniacal silver technique.*

fore, to avoid future confusion, members of the B, C, D, E (17-18), F, and G groups are not designated by specific numbers; rather, the numbering indicates only that each chromosome is a unique and identifiable member of a given group.

It is of interest that in females no appreciable difference was observed between the two X-chromosomes and that the X-chromosome in males is indistinguishable from that of the females. Photographic reproduction is more difficult in the case of the smaller chromosomes. However, the available evidence indicates that members of the G group and the Y-chromosome do have different patterns.

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Riassunto

Viene descritta l'applicazione di argento ammoniacale alla colorazione dei cromosomi metafasici nell'uomo. Viene presentata una mappa basata sulle caratteristiche di colorazione differenziale dei segmenti cromosomici.

Résumé

L'application d'argent ammoniacal à la coloration des chromosomes métaphasiques chez l'homme est décrite et une carte est présentée, basée sur les caractéristiques de coloration différentielle des segments chromosomiques.

Zusammenfassung

Es wird die Anwendung von Ammoniaksilber zur Färbung der Metaphasenchromosomen beim Menschen beschrieben und eine Karte gezeigt, aus der die besonderen Kolorationsdifferenzen der einzelnen Chromosomensegmente hervorgeht.

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