LEUCOCYTE MORPHOLOGY AND CHROMOSOME MORPHOLOGY

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The banding techniques currently employed in human cytogenetics for the identification of the individual chromosomes have been used to stain PHA lymphocytes and circulating leucocytes. The capacity of these techniques to localize singular chromosomes or chromosomal regions has been investigated.
It has been observed that among the four major categories of bands (Q, G, E and R) only the quinacrine staining is informative in interphase nuclei, because of its peculiarity to stain the long arm of the Y chromosome and few other heterochromatic regions. Interphase nuclei treated according to the C-bands show the presence of several heterochromatic masses, corresponding to the centromeric areas of individual chromosomes, but as such they cannot be recognized accurately. More specific and selective techniques, like G-11 and G-Y protocols, appear to be suitable to localize the centromeric regions of chromosome no. 9 and the long arm of Y chromosome. Variation of the incubation time in the alkali-saline solutions and of pH values have proven to be appropriate for the demonstration of other heterochromatic regions in interphase nuclei and in circulating leucocytes.
The "nuclear" approach to the study of specific heterochromatic regions of human chromosomes may be of practical interest into the investigation of several biological problems and into the detection of individuals carrying chromosome variants.

INTRODUCTION

The leucocyte morphology in patients carrying syndromes due to chromosome aberrations displays a wide spectrum of well established abnormalities (review in Dallapiccola 1969, 1973). In *autosomal trisomies* these abnormalities are of no diagnostic value, but belong to the peculiar combination of signs, which characterize these syndromes. The best established leucocyte patterns in autosomal syndromes include:

— decreased nuclear segmentation in Down's syndrome (Turpin and Bernyer 1947);

— presence of nuclear projections in the neutrophils in Patau's syndrome (Huhens et al. 1964);

— presence of drumstick-like appendages in a proportion of monocytes in Edwards' syndrome (Dallapiccola 1969).

On the contrary, the leucocyte pattern in syndromes due to *sex chromosome aberrations* is quite peculiar and its study appears to be extremely useful for diagnostic purposes. The so-called "leucocyte test" for the X chromatin is based on the identification of specific nuclear formations (drumsticks) which were first described and recognized as important by Davidson and Smith (1954).

Although the correlation between numerical and structural anomalies of sex chromosomes

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and the female chromatin pattern is less obvious and at times difficult to detect in peripheral blood, as compared to the chromatin pattern in interphase nuclei of epithelial cells, it is beyond doubt that both Barr bodies and drumsticks are the late replicating X chromosomes in different cell types (Mittwoch 1965).

The remarkable advances in human cytogenetics following the introduction of staining techniques which employ fluorescent agents have made possible the positive nuclear sexing of the male cells and the demonstration of additional characteristics in human leucocytes (Dallapiccola 1971, Polani and Mutton 1971, Thuline 1971). In fact, the distal part of the long arm of the Y chromosome fluoresces very strongly after quinacrine staining and retains its fluorescence during interphase, when can be perceived as a small luminous spot (Zech 1969, Caspersson et al. 1969, George 1970, Pearson et al. 1970). In most cases, no other chromosome exhibits a fluorescent spot of similar intensity and size, when stained with quinacrine or similar dyes. Therefore, the presence of such a small fluorescing body in the cell nucleus can be regarded as a fairly certain sign of the presence of the Y chromosome in that particular cell. Variations in size and in number of the Y chromosomes are reflected in size and in number of the Y-chromatin bodies.

Several practical and theoretic problems related to the study of the Y chromatin in all types of white blood cells have been investigated up to date:

(1) Taking into account the information obtained from the study of the female and male sex chromatin on quinacrine-stained polymorphs, a method for the indentification of the individual sex complement has been devised (Dallapiccola 1971, Polani and Mutton 1971, Thuline 1971).

(2) It has been demonstrated that the Y chromosome in interphase nuclei (especially lymphocytes) is spatially associated with the nucleolus (Bobrow et al. 1971, Gagné et al. 1972, Dallapiccola and Malacarne 1973), and may contain a specialized form of genetic material related to nucleolar function.

(3) In granulocytes, the Y chromosome is often situated in a small protrusion of the nucleus (so-called " small club ", Dallapiccola and Franceschini 1973). This peculiarity is of practical value in diagnosing individuals carrying very long Y chromosomes (Dallapiccola 1971, Ricci et al. 1971).

(4) In a particular case, the Y-chromatin test on W.B.C. has been the indicator of illegitimate paternity, suggesting a blood groups study, which eventually led to paternity exclusion (Dallapiccola and Castoldi 1972, Dallapiccola 1973).

(5) The presence of Y-chromatin positive leucocyte in pregnant women carrying a male fetus has been occasionally reported as a valuable method for prenatal sexing (Schröder and De la Chapelle 1972).

A significant progress to the study of relationship between cell morphology and chromosome pattern has been obtained in recent years by the demontration of specific cytological staining techniques. At least ten different types of banding patterns have been identified in human chromosomes (Table). Some of these techniques (Caspersson et al. 1969, Gagné et al. 1971, Bobrow et al. 1972, Gropp et al. 1972) and molecular hybridization techniques (Jones and Corneo 1971) have demonstrated that individual heterochromatic areas of metaphase chromosomes are specifically enriched with repetitive DNA sequences. The heterochromatic regions of human chromosomes number 1, 3, 9, 13, 16 and Y differ in respect to various staining dyes and staining conditions (Caspersson et al. 1970, Arrighi and Hsu 1971, Sumner et al. 1971). At least two different kinds of heterochromatic regions have been observed

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by combined staining with fluorochrome and Giemsa (Gagné et al. 1971), and in molecular studies (Corneo et al. 1970, 1971, 1972) at least four satellite DNA fractions have been isolated from the main DNA band.

The applications of these new banding techniques, particularly those staining heterochromatic regions, to PHA stimulated lymphocytes or to peripheral blood leucocytes gives a practical possibility for studying heterochromatic polymorphism in human interphase nuclei and to obtain further information about leucocyte morphology.

MATERIAL AND METHODS

Preparation and Staining of Metaphase Chromosomes and Interphase Nuclei

Metaphase chromosomes were prepared by conventional air-drying technique (Moorhead et al 1960) from 10 healthy individuals (5 males and 5 females), one subject with "uncoiler" polymorphism of no. 1 (1qh), one patient with elongated secondary constriction on chromosome no. 9 (9qh), and two individuals with long Y chromosomes (Y = D).

Interphase nuclei were obtained from all these subjects by cultivating peripheral blood lymphocytes in the presence of PHA. After treatment with colchicine and hypotonic shock with citrate 1% at 37° C, for 20 minutes, the cells were fixed in ethanol-acetic acid (3:1) and air-dried on glasses.

Slides were treated according to the following banding techniques:

Q-bands: the fluorescence technique described by Caspersson et al. (1969) was slightly modified by using quinacrine hydrocloride (Sigma, St. Louis, Missouri).

G-bands: slides were incubated for 15 min. in 0.9% NaCl at 65° C, washed in tap water and stained for 7 min. in a Giemsa-saline solution (3 ml Giemsa, 100 ml 0.9% NaCl), prepared immediately before use.

E-bands: the Seabright technique (1971), slightly modified by us, was employed.

R and T bands: were obtained according to the "destaining" technique described by Dallapiccola and Ricci (1975).

N-bands: the original technique described by Matsui and Sasaki (1973) was used.

C-bands: slides were incubated for 5 min. at 90° C in an alkali-saline solution, washed in tap water and stained for 30 min. in a Giemsa-saline solution.

G-11: the technique described by Bobrow et al. (1972) was followed.

G-Y: slides were processed according to the technique of Dallapiccola and Ricci (1975).

A-bands: were obtained by the treatment of "aged" slides according to the protocol of Crossen (1974).

Staining of Circulating Leucocytes

A series of peripheral blood films was obtained from the same individuals in which studies on metaphase and interphase cultured lymphocytes were performed. Since the circulating leucocytes, especially polymorphs, are not resistent to some of the conventional treatments by which the metaphase chromosomes are processed to obtain banding patterns, the following stains were used:

(1) The *fluorescence* technique described by Dallapiccola (1971) for the *Y* chromosome.

(2) The original technique of Matsui and Sasaki (1973) for *N-bands*.

(3) In order to demonstrate with Giemsa fluorochrome-binding and nonfluorochrome-binding heterochromatic regions (e.g., Yq, 1qh, 9qh), several procedures have been developed:

(*a*) slides are fixed for 1 min. in methanol, washed in running tap water and air-dried;

(*b*) the slides are immersed in solutions consisting of 0.9 NaCl, adjusted with NaOH to different pH values, ranging between 10 and 11. The duration of treatment is between 15 and 30 sec.;

(*c*) blood films are washed in tap water and stained in a freshly prepared Giemsa solution, consisting of 5 ml Giemsa in 95 ml 0.9 NaCl solution.

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In our experimental conditions, shorter incubation times in alkali-saline solution are adequate for the demonstration of the heterochromatic spot corresponding to the long arm of the Y chromosome (10 to 15 sec.); slightly longer incubations (15 to 25 sec.) are needed for the demonstration of the dark blocks corresponding to the paracentromeric regions of chromosome no. 1. Values of pH ranging between 10 and 10.5 are appropriate for the detection of these heterochromatic areas, while higher values, ranging between 10.5 and 11 are needed for the detection of secondary constriction of chromosome no. 9.

RESULTS

Metaphase and Interphase Nuclei

Metaphase plates stained for Q, G, E, R and T banding techniques allow the identication of each individual metaphase or prometaphase chromosome, according to a distinct pattern of bands [1]. The T bands (Dutrillaux 1973), which are part of the R system, specifically stain the telomeric (terminal) regions, but no T bands are recognizable in chromosomes 3, 6, 18, X and Y. However, for pratical purposes, we consider Q, G, E, R and T bands together, because of their peculiarity to stain the whole chromosome set, or most of all the chromosomes, and to be uniformative in interphase nuclei.

Q-Bands

The only exception is quinacrine staining. After staining with quinacrine or similar dyes, in most cases, no other chromosome exhibits a fluorescent spot of similar intensity and size like the Y chromosome. Therefore, the presence of such a small fluorescing spot in interphase nucleus may be considered to be a fairly certain sign of the presence of a Y chromosome in that cell. Variation in size of the Y are reflected in the size of the Y-chromatin. When more than one Y chromosome is present, the number of the Y-chromatin bodies in interphase nuclei corresponds to the number of the Y chromosomes.

On the other hand, in subjects displaying an unusual strong quinacrine fluorescence of other chromosome regions (as, e.g., the centromeric region of chromosome no. 3 or the satellites of acrocentric chromosomes), the corresponding fluorescing small spots may confuse the accurate diagnosis of interphase nuclei and require further examinations.

G, R, E, and T Bands

The intranuclear heterochromatic blocks which are visible after staining according to G, R, E, and T banding techniques, hardly can be classified. Neither they can be identified when, following fluorescence technique, the nuclei are restained by one of these methods. An improvement into the identification of singular interphase heterochromatic blocks can be obtained, by staining the cells with fluorochrome and restaining with standard Giemsa. According to these procedures a rather comparable pattern is obtained, and the distal part of the Y and some faintly fluorescing parts of chromosomes (e.g., the centromeric regions of 1, 9 and other regions) can be recognized as dark intranuclear masses.

[1] A chromosome band is defined as a part of the chromosome that is clearly distinguishable from its adiacent segments, by appearing darker or lighter in either of the technique (Paris Conference, 1971).

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TABLE

MAIN BANDING PROCEDURES AND STAINING CURRENTLY EMPLOYED IN HUMAN CYTOGENETICS

Q	Quinacrine	Caspersson et al. 1971
G	Giemsa	Sumner et al. 1971
R	Reverse	Dutrillaux and Lejeune 1971
E	Enzyme	Dutrillaux et al. 1971
T	Telomere	Dutrillaux 1973
C	Constitutive Heterochromatin	Arrighi and Hsu 1971
G-11	Giemsa, pH 11	Bobrow et al. 1972 Gagné and Laberge 1972
G-Y	Giemsa Y	Dallapiccola and Ricci, 1975
N	Nucleolar Organizers	Matsui and Sasaki, 1973
A	Aged Slides	Crossen 1974

C, G-11, and G-Y Bands

The C-bands technique localizes the heterochromatic regions in human chromosomes, mainly at the centromeric areas. Interphase nuclei, treated according to these procedures reveal the presence of several heterochromatic masses (Fig. 1). When interphase cultured lymphocytes, stained for this type of bands are compared to the corresponding C-band patterns of methaphase chromosomes, tentatively many of these blocks can be identified. As a general rule, larger spots correspond to chromosomes Y, 1, 9 and 16, which often display an " ectopic pairing "; but also the heterochromatic blocks corresponding to chromosomes 6, 7, 11, 13, 19 and X may be distinctly visible.

A better approach to the selective study of few of these heterochromatic masses is possible by means of G-11 and G-Y banding techniques, which specifically stain the paracentric secondary costriction at the proximal long arm of chromosome no. 9 (Bobrow et al. 1972, Gagné and Laberge 1972), and the long arm of Y chromosome (Dallapiccola and Ricci 1975). Working at high pH values with alkali-saline solutions these regions stain metachromatically in a very dramatic and rather selective fashion (Figs. 2 and 3).

N-Bands

The N-bands technique differentially stains the satellite bodies of human acrocentrics with Giemsa, after extraction of both nucleic acids and histones. In human metaphase plates these bands appear as distinctive purplish red spots restricted to satellite regions of most of the acrocentrics. The same tiny spots are clustering within the nucleoli in human interphase cultured lymphocytes and their peculiar distribution in the cell nucleus is apparent until late prophase.

A-Bands

The unusual banding that occurs after treatment with NaOH on slides that are aged for periods of 3 months or more (so-called A-bands) are artefacts, resulting from prolonged storage. These bands are intensely stained, mainly located near the centromere, and their

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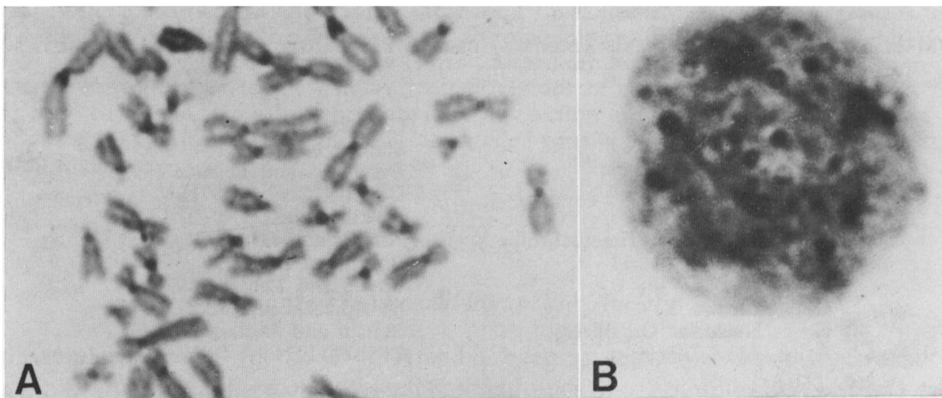

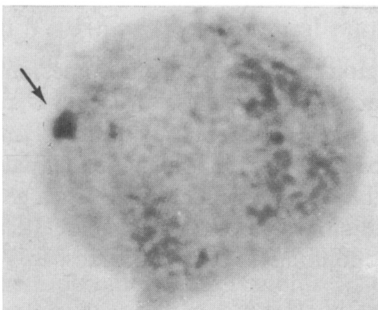

Fig. 1. A: Metaphase plate from a patient carrying a long Y chromosome, treated according to the technique for the C-bands.
B: PHA lymphocyte from the same patient treated for the C-bands technique: the intranuclear heterochromatic blocks cannot be classified.

Fig. 2. PHA lymphocyte from a female subject carrying a 9qh chromosome variant, treated according to the G-11 banding technique.

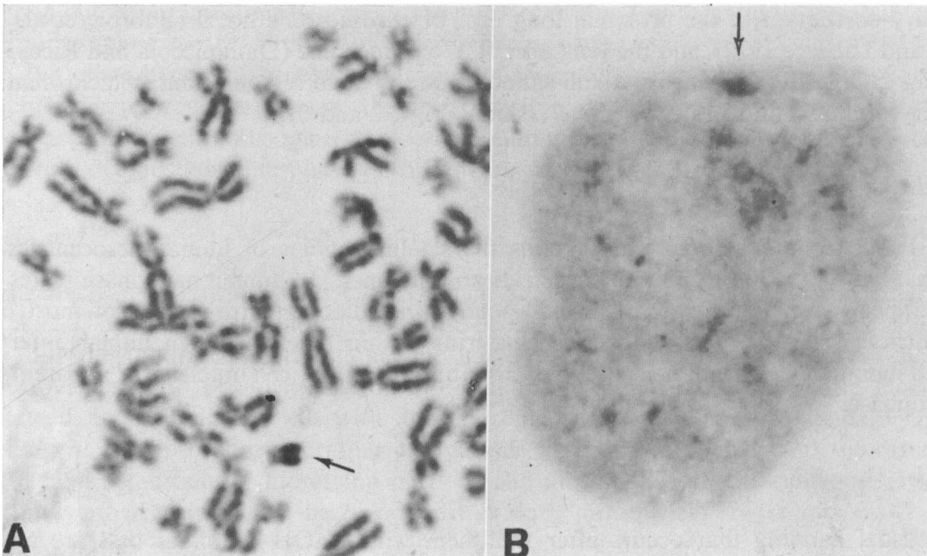

Fig. 3. Metaphase plate (A) and PHA lymphocyte (B) from a normal male, treated according to the G-Y technique.

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distribution on the chromosomes, although non specific, is not random. Therefore, interphase nuclei, treated according to the A-bands technique are not suitable to identify any precise chromosomal structure, but, in our experimental procedures, they have been capable to reveal at least one of the possible mechanisms involved in their formation. In fact, the nuclei appear well delimitated at their boundary or crossed by intensely stained lines, which correspond to folds of the nuclear membrane. In particular conditions the same folds can be recognized on metaphase plates within the " cloth " of proteins which covers the chromosomes. In correspondence of the points in which the folds cross the chromosomes, folding results in dark bands, which are in fact the A-bands.

Circulating Leucocytes

Quinacrine Stains

Peripheral blood films of normal males stained with quinacrine display the presence of Y-chromatin in all types of W.B.C. In lymphocytes, it is usually somewhat easier to detect than in granulocytes; in polymorphs, the Y-chromatin is often situated in a small protrusion of the nucleus (so-called " small club "). A fluorescent body of the size of a small Y-chromatin can be observed in about 1 out of 200 W.B.C. in females. These fluorescing spots are usually situated within the nucleus and in granulocytes they do not form extra protrusions like the small clubs. Similar criteria are often adequate to exclude the presence of a Y-chromosome, and probably reveal quinacrine-positive autosomal spots.

Alkali-Saline Stain

By means of alkali-saline treatment and Giemsa-saline staining, we could demonstrate also in circulating leucocytes the presence of heterochromatic blocks, corresponding to the long arm of the Y chromosome and to the centromeric regions of chromosomes 1 and 9. However, until now, we were unable to devise techniques which give differential staining of the heterochromatic regions of singular chromosomes according to a selective and reproducible method. As a general rule, a shorter incubation time in alkali-saline appears to be adequate for the demonstration of the Y chromosome (Fig. 4) as compared to the heterochromatic paracentromeric region of chromosome no. 1 (Fig. 5). Prolonged incubation in alkali-saline results in the appearance of several heterochromatic blocks, corresponding to chromosomes Y and 1 (Fig. 6). Higher pH values (around 10.5 - 11) are appropriate for the detection of the long arm secondary constriction of chromosome no. 9.

In the absence of techniques capable to give a direct evidence of the nature of these dark spots, indirect evidence was obtained for the Y chromatin, by previous, staining of the nuclei with quinacrine or through the study of leucocytes from individuals with long Y chromosomes (Fig. 4). In the cells in which the Y-chromatin body was localized by means of fluorescence technique the fluorescing spot after the alkali-saline incubation appeared darkly stained with the Giemsa-saline solution. In subjects carrying long Y chromosomes, the dark heterochromatic spot was found in the drumstick-like appendage of polymorphs (Fig. 4A). There is now circumstantiated evidence for the conclusion that these extra protrusions are formed by the Y chromosomes (Ricci et al. 1971, Dallapiccola 1971, Lamborot-Manzour

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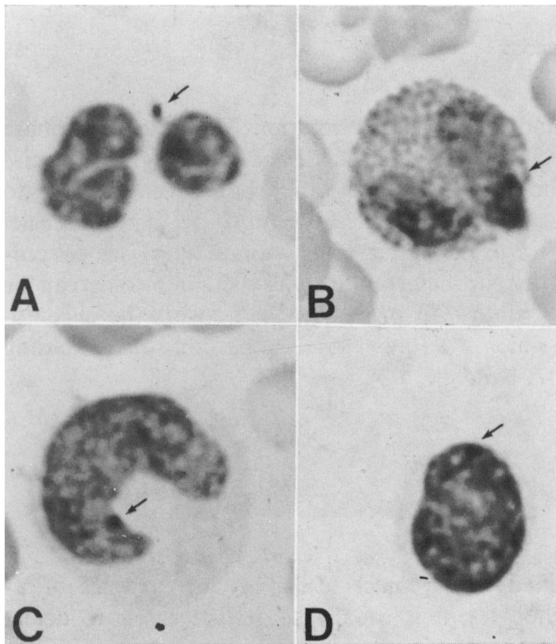

Fig. 4. Circulating neutrophil (A), eosinophil (B), monocyte (C) and lymphocyte (D) from a male carryng a long Y chromosome, treated for the demonstration of the Y chromatin.

Fig. 5. Metaphase plate (A) and PHA lymphocyte (B) from an individual carrying a 1qh chromosome variant. The arrows point to the heterochromatic regions of chromosomes no. 1.

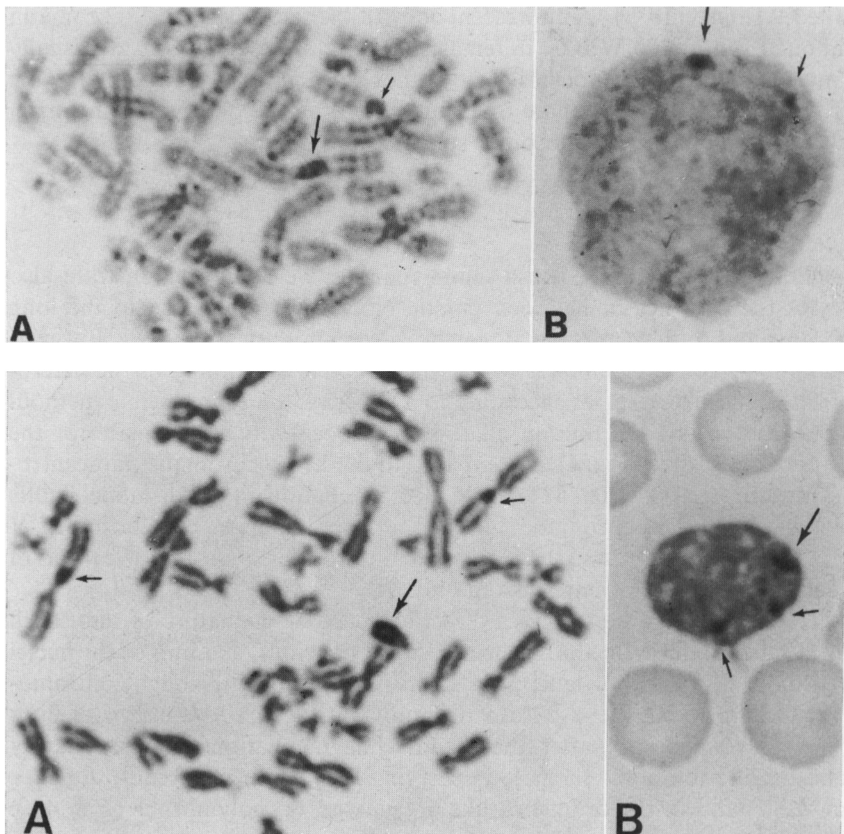

Fig. 6. A: Metaphase plate from a male patient with a long Y chromosome, treated according to a modified G-Y technique; the arrows point to the long Y chromosome and the heterochromatic regions of chromosome no.1.
B: A circulating lymphocyte, treated according to a similar technique, reveal the presence of three spots, which are tentatively assigned to the Y (long arrow) and the heterochromatic regions of chromosomes no.1 (short arrows).

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et al. 1971). Indirect evidence for the possibility to demonstrate in circulating leucocytes also the heterochromatic blocks corresponding to the paracentromeric regions of other chromosomes was obtained by studying subjects with ectopic pairing of chromosomes n. 1 and no. 9. In all the leucocyte series a single large and dark intranuclear mass was observed. The chromosomes of these individuals, stained according to the C-bands technique, did not reveal additional heterochromatic blocks, comparable for size and intensity of staining to the " uncoiler " region of chromosome 1 or to the paracentric secondary constriction of the long arm of chromosome 9 respectively: therefore the heterochromatic spots have been assigned to these structures.

DISCUSSION

Until now most of the information that could be obtained from the study of interphase nuclei in relation with their chromosomal set concerned the female and male sex chromatin (Barr bodies and drumsticks; Y-chromatin). In this respect, prior to the introdution of the modern techniques of study in human cytogenetics, investigations of interphase nuclei were unable to give any indirect evidence about autosome morphology. Furthermore, three out of four main categories of banding techniques, that stain the whole chromosome (G, R and E bands) provide important methods for the identification of each individual chromosome in metaphase plates, but result minimally informative when used to study interphase nuclei.

It is now well established that specific information on some autosomal structures can be obtained through the study of constitutive heterochromatin, and this evidence has been widely demonstrated both with cytological and molecular studies, in human metaphase chromosomes and in interphase nuclei (Kim 1974). Heterochromatin is considered as genetically inert region, stained in interphase (Heinz 1928), when it appears in form of a compact mass, late replicating by autoradiographic labelling (Lima de Faria and Jaworska 1968), and transcriptionally inactive (Yunis and Yasmineh 1972). Heterochromatic regions are localized by various stainings and fluorochromes, for istance also by Q and R bands, but specific staining techniques are more suitable to identify and classify these blocks in metaphase chromosomes (C-bands) or to visualize some of these regions both in chromosomes and in interphase nuclei (G-11, G-Y, N-bands).

The staining technique currently referred to as C-band methods, which have developed from the original description of Arrighi and Hsu (1971), localize constitutive heterochromatin, i.e., repetitive DNA, mainly in the centromeric areas of human chromosomes. Interphase nuclei, treated according to these procedures reveal the presence of several heterochromatic blocks, which correspond to the distal part of the Y and some other chromosomal parts, showing some faintly fluorescence, when stained with fluorochromes (e.g., the centromeric regions of 1, 9 and some other unidentified regions). Peripheral blood films cannot be studied according to these techniques, because leucocytes are not resistant to treatments, which require the denaturation of chromosomal DNA (alkali) and reassociation in a saline-citrate solution (SSC) at relatively high temperatures (60-65° C).

Characteristic patterns of constitutive heterochromatin in human metaphase chromosomes and in interphase nuclei are shown by treatment with enzymes, variation of incubation time in standard SSC, and staining with fluorochromes and Giemsa. According to Kim (1974) DNAse-resistant regions of metaphase chromosomes are present in intensely fluorescent parts of Y, 3 and group D and G; C-bands develop rapidly on chromosomes, 19, 1, 16, and

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later on chromosomes 2, 8, 9 and Y. Therefore, some heterochromatic regions in interphase can be differently identified by variations of paramethers involved in their formation, for instance by variations of the incubation time. It has been said that the centromeric region of chromosome no. 19 shows the first Giemsa staining reaction in comparison with all other chromosomes (Kim 1974), while after removing the basic proteins with HCl (Gall and Pardue 1971) all these heterochromatic regions appear nearly at the same time. At lower pH values, both centromeric regions and interarm bands are deeply stained. At higher pH values the centromeric region of C_9 and the long arm of the Y and some parts of chromosomes 1, 5, D and G are visible (Bobrow et al. 1972, Gagné and Laberge 1972, Dallapiccola and Ricci 1975). G-11, G-Y and N-bands methods prove that different DNA fractions can be cytologically shown in a selective way, by means of specific techniques. However, while demonstrating that a peculiar kind of DNA is mainly located on some chromosomal parts, these techniques cannot exclude its location also on other chromosomes. For example, the G-11 protocol not only stains the heterochromatic region of chromosome no. 9, mainly consisting of DNA III, but also stains about one third of the heterochromatic block of chromosome no. 1. A similar pattern is observed when metaphase plates are treated according to the G-Y technique: in addition to the long arm of the Y, this method stains in a lighter way the paracentromeric region of the chromosome no. 1, both possessing substantial amounts of single-sequence DNA (Jones and Corneo 1971, Marx et al. 1973). These considerations must be taken into account, when considering the results obtained in interphase nuclei or in WBC processed for these bands. Only a proportion of nuclei displays a singular spot corresponding to the heterochromatic part of a given chromosome, even when they are treated for a rather specific technique like G-11 and G-Y; therefore, not in every case a precise assignment of heterochromatic blocks to a definite chromosomal region can be made. As a general rule, comparisons between the results obtained with banded metaphase chromosomes and the corresponding interphase nuclei are necessary for a correct interpretation of intranuclear spots.

Among the various techniques devised so far, the N-bands method seems to be the more specific and selective. It stains a peculiar kind of heterochromatin which is situated adiacent to the nucleolar organizer regions (NOR) in most instances, and probably reflects nonhistone proteins (Faust and Vogel 1974). This technique may serve not only as an excellent genetic marker to the study of the variation in size and in number of this particular heterochromatin in different acrocentrics and in different individuals, but also gives useful information in interphase nuclei about the behaviour of genetic material associated to the nucleolar function.

Further technical progresses in this field possibly will give more satisfactory and reproducible results concerning the identification of singular part of autosomes in interphase, similar to those already obtained for metaphase plates. The application of these staining techniques to interphase nuclei appears to be informative for the approach to several biological problems. For example, they may be employed to establish topographic relationships of certain chromosomes in interphase (Dallapiccola and Franceschini 1973, Hoehn and Martin 1973), which are necessary for the evaluation of such concepts such as genome segregation and ectopic pairing (Martin and Sprague 1969, Hoehn and Martin 1973), and to test the hypothesis that the propensity to ectopic pairing is determined by molecular factors (Barr and Hellison 1972). However, at the moment, they are not suitable to be employed in routine analyses or in screening programs to detect autosomal abnormalities or variants.

There is an increasing evidence that chromosomal variants represent not only an aspect of human polymorphism, but also that such a variability increases the risk for more severe

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chromosomal disorders in the carriers' offsprings. Recently, Nielsen et al. (1974*a*, *b*) have found on a large sample of parents of children with major chromosome abnormalities 3.5% individuals with 9qh, as compared with 0.1% in population studies. These data indicate that such heterochromatin duplication may affect pairing and segregation and thus gives an increased risk of nondisjunction, deletion, translocation and duplication in the progeny. Therefore, the " nuclear " approach to the study of these chromosome variants appears to be recommended.

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RIASSUNTO

Morfologia Leucocitaria e Morfologia Cromosomica

Dieci diverse tecniche di bandeggiamento cromosomico sono state impiegate per analizzare i nuclei linfocitari coltivati in presenza di PHA e strisci di sangue periferico, ottenuti da soggetti normali e portatori di varianti cromosomiche. È stata esaminata la possibilità di localizzare, attraverso queste tecniche, singoli cromosomi o aree cromosomiche sui nuclei in interfase.
È stato osservato che, fra le principali tecniche di bandeggiamento (bande Q, G, E ed R), solo la prima, che impiega derivati fluorescenti di acridina, è informativa sui nuclei in interfase, per la sua peculiarità di colorare, e perciò di identificare il braccio lungo dell'Y ed occasionalmente altre aree cromosomiche.
I nuclei colorati secondo la tecnica per le bande C rivelano l'esistenza di numerosi blocchi eterocromatici, che non permettono comunque di riconoscere le regioni cromosomiche dalle quali derivano. Altre tecniche più specifiche, quali la G-11 e la G-Y consentono invece di localizzare le regioni paracentromeriche del 9 ed il braccio lungo dell'Y, rispettivamente. Variazioni nel tempo di incubazione dei vetrini nelle soluzioni saline-alcaline e variazioni del pH sono in grado di dimostrare anche altre regioni eterocromatiche (ad esempio la

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porzione paracentromerica del cromosoma 1). Con appropriate tecniche, le stesse strutture sono state evidenziale sui leucociti del sangue periferico.
I risultati ottenuti indicano l'utilità di queste analisi, nello studio di svariati problemi biologici e nell'individuazione di soggetti portatori di varianti cromosomiche.

RÉSUMÉ

Morphologie Leucocytaire et Morphologie Chromosomique

Dix différentes techniques de bandage chromosomique ont été employées afin d'analyser les noyaux lymphocytaire cultivés en présence de PHA et des lamelles de sang périphérique obtenus de sujets normaux et de sujets porteurs d'anomalies chromosomiques. La possibilité a été étudiée de localiser, moyennant ces techniques, les chromosomes individuels, ou des zones chromosomiques, sur les noyaux en interphase.
Il a été observé que, parmi les différentes techniques de bandage (bandes Q, G, E et R), la première seulement, qui utilise des dérivés fluorescents de acridine, donne des informations sur les noyaux en interphase, étant donnée sa caractéristiques de colorer, et donc d'identifier, le bras long de l'Y et, occasionnellement, d'autres secteurs chromosomiques.
Les noyaux colorés d'après la technique pour les bandes C révèlent l'existence de nombreux blocages hétérochromatiques qui ne permettent pas de reconnaître leurs régions chromosomiques de provenance. D'autres techniques plus spécifiques, telles que la G-11 et la G-Y, permettent par contre de localiser, respectivement, les régions paracentromériques du 9 et du bras long de l'Y. Des variations du temps d'incubation des lamelles dans les solutions salines alcalines et des variations de pH peuvent aussi démontrer d'autres régions hétérochromatiques (par example la portion paracentromérique du chromosome 1). Par des techniques appropriées, les mêmes structures ont été individuées sur les leucocytes de sang périphérique.
Les résultats obtenus indiquent l'utilité de ces analyses dans l'étude de différents problèmes biologiques et dans l'individuation de sujets porteurs d'anomalies chromosomiques.

ZUSAMMENFASSUNG

Die Morphologie von Leukozyten und Chromosomen

Um die zusammen mit PHA und mit Abstrichen aus peripherem Blut gezüchteten Lymphozytenkerne normaler Versuchspersonen mit verschiedenen Chromosomenvarianten zu analysieren, wurden zehn verschiedene Chromosomenbandtechniken angewandt. Es wurde versucht, mit Hilfe dieser Techniken auf den Zellkernen in Interphase einzelne Chromosomen oder Chromosomenzonen zu lokalisieren.
Man beobachtete, dass von den verschiedenen Bandentechniken (Q, G, E und R) nur die erste, die fluoreszierende Acridinderivate verwendet, durch ihre besonderen Färbungsmöglichkeiten Information über die Zellkerne in Interphase erteilt, wodurch es möglich ist, den langen Schenkel des Y-Chromosoms sowie gelegentlich auch andere Chromosomenzonen zu identifizieren.
Die nach der Technik für C-Bande gefärbten Nuklei weisen zahlreiche heterochromatische Blöcke auf, doch lässt sich daraus nicht erkennen, aus welchen Chromosomenzonen sie stammen. Andere spezifischere Techniken, wie z.B. die G-11 und die G-Y, gestatten hingegen, auf den Nuklei in Interphase die parazentromeren Zonen des 9. bzw. den langen Schenkel des Y-Chromosoms zu lokalisieren. Durch Aenderung der Inkubationszeit der Präparate in den Salz-Alkalilösungen und durch Variation des pH lassen sich auf denselben Zellkernen auch andere heterochromatische Chromosomenzonen (z.B. die parazentromere Portion des Chromosoms 1) nachweisen. Mit geeigneten Techniken wurden dieselben Strukturen bei Leukozytem aus peripherem Blut aufgezeigt.
Die Ergebnisse zeigen die Nützlichkeit dieser Analysen bei Untersuchung der verschiedensten biologischen Probleme und zur Individuierung der Träger von Chromosomenvarianten.

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