of concentrations by the method of least squares. In this case it was Eq. (1). Next on the basis of the fraction of aberrant metaphases in the control, the upper 95% confidence limit was calculated by the equation given in the section "Experimental Method." In the present case, three aberrant metaphases were found in the control series of 200 cells (1.5%). The upper confidence limit was 4.025%. Let this figure be substituted in Eq. (1) and let the smallest effective concentration be calculated: In this case it is 0.120 μg/ml. This concentration does not serve as the threshold dose, as might be supposed, but it is in reality the threshold of accuracy, depending on the choice and level of chromosomal aberrations in the control.

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ANALYSIS OF THE MORPHOLOGY OF SHORT ARMS OF HUMAN ACROCENTRIC CHROMOSOMES BY SEQUENTIAL STAINING FOR G AND C BANDING

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Sequential staining of acrocentric chromosomes from eight individuals for G and C banding showed that a large heterochromatin region is present more often in homologs of chromosome 15 than of 13 and 14, and in the G group it is found more often in pair 22 than in pair 21. As a rule the size of the heterocheomatin region does not correspond to the size of the short arm of the acrocentrics when stained for G banding. The frequency of occurrence of satellites in all eight individuals was approximately the same for all five pairs of acrocentrics. Staining for constitutive heterochromatin revealed heteromorphism for the presence of satellites frequently in the homologs.

KEY WORDS: G and C banding of chromosomes; acrocentric chromosomes; heterochromatin.

The participation of human acrocentric chromosomes in the formation of the nucleolus and, consequently, in ribosome synthesis is generally accepted. This fact determines the approach to the study of function of the acrocentric chromosomes. Ribosomal cistrons are located in secondary constriction bands (satellite threads) of acrocentric chromosomes. A noteworthy feature is the extreme variation of the short arms of acrocentric chromosomes – the presence or absence of a secondary constriction, an increase or decrease in its size, the presence of absence of satellites of different shapes: enlarged, double, and so on. It is interesting to study the limits of variability of human acrocentric chromosomes, whether this reflects variability in the phenotype, and

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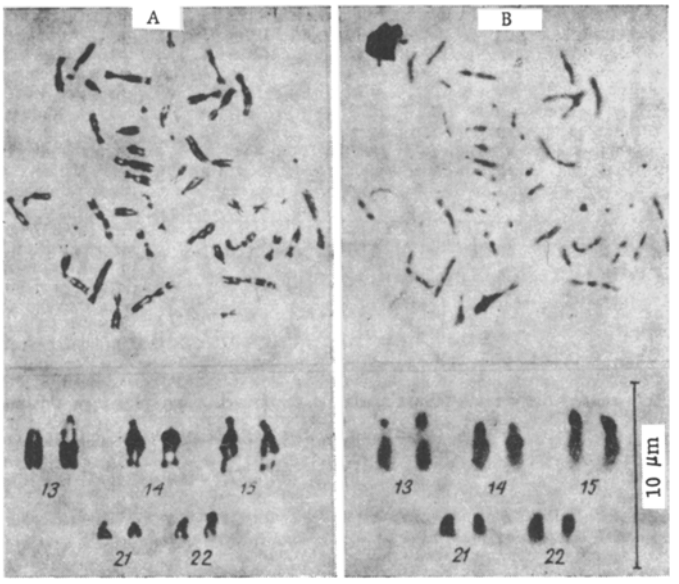

Fig. 1. Metaphase plate and acrocentric chromosomes after differential Giemsa staining (G banding; A) and after staining for constitutive heterochromatin (C banding; B).

whether it is pathological in nature. The study of the structure of the short arms of each homolog of individual pairs of acrocentric chromosomes will do much to facilitate the approach to the solution of these problems.

Whereas until recently all human acrocentrics were divided into two groups, D and G, and identification of chromosomes within the groups was impossible, with the appearance of methods of differential staining of chromosomes this task has become quite practicable. Each pair of acrocentric chromosomes can now be easily identified both by fluorescence staining of the chromosomes and by differential Giemsa staining [3, 14, 15]. Staining for constitutive heterochromatin (C banding of chromosomes) is of great interest [7-9, 12]. Staining for heterochromatin has revealed polymorphism of the human karyotype [4, 5, 10]. Considerable variation has been found on staining human acrocentric chromosomes for heterochromatin. For instance, some acrocentrics have a large heterochromatin region, whereas in others it is only a single point; satellites are sometimes stained and sometimes not [5, 10]. However, by staining for heterochromatin it is impossible to identify chromosome pairs. Only a combination of staining for G and C banding makes it possible to determine to what extent variation in size of the heterochromatin of acrocentric chromosomes is connected with any particular pair. An appropriate investigation was accordingly undertaken.

EXPERIMENTAL METHOD

Chromosomes were stained in preparations of human peripheral blood lymphocyte cultures obtained by the standard method. Fixation was carried out after cultivation for 72 h, the hypotonic solution consisted of 0.075 M KCl, fixative was methanol : acetic acid (3 : 1), and drying was carried out without ignition. The quality of staining depends essentially on the time the preparation has been kept. Best results were obtained with 6-10-day preparations kept at room temperature.

Acrocentrics were identified by differential Giemsa staining (G band). The preparation was treated with 0.2 M CsCl solution at 60-65°C for 40 min. The hot sections were placed in dye made up from standard Giemsa stain in phosphate buffer (0.067 M KH_2PO_4 + 0.067 M Na_2HPO_4), pH 6.8, in the ratio of 1 : 40 and staining continued for 10-15 min. The sections were analyzed, photographed, and then decolorized in acidified alcohol (concentrated HCl : H_2O : C_2H_5OH absolute ethanol in the ratio of 1 : 12 : 37). The sections were then rinsed in ethanol of increasing concentrations (70, 96, and 100%), dried, and stained for constitutive heterochromatin (C band). To do this, the sections were treated with 0.2 N HCl solution for 10 min, washed with water, and dried. The sections were then placed in 0.07 N NaOH solution for 40 sec and then taken through ethyl alcohols of increasing concentration. The dried preparation was kept in 0.2 M CsCl solution for 10-15 min at 60-65°C. It was stained for 45-50 min in Giemsa stain, made up as described above.

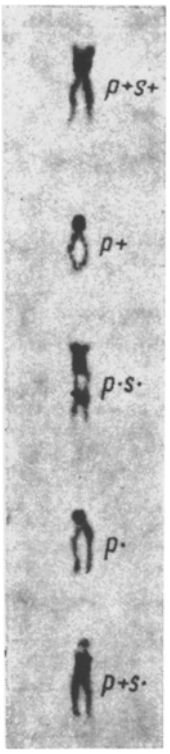

Fig. 2. Some variants of short arms of human acrocentric chromosomes after staining for constitutive heterochromatin: p) heterochromatin region equal in size to that in other autosomes excluding 1, 9, and 16; s) presence of palely stained satellite; p^+) heterochromatin approximately doubled in size; s^+) darkly stained satellites.

TABLE 1. Morphology of Short Arms of Human Acrocentric Chromosomes Stained for Constitutive Heterochromatin

Observations	Group D			Group G	
	13	14	15	21	22
1	p·s·/p·	p·s·/p·	p+s·/p·	p·s·/p·s·	p·s·/p·
2	p·s·/p·	p+s·/p	p+s·/p+	p·/p·s·	p+s·/p+
3	p·s·/p·	p·s·/p+	p+/p·	p·s·/p·	p+s·/p+
4	p·s·/p·	p·/p·	p+s·/p+	p·s·/p·	p·s·/p+
5	p·s·/p·	p·/p·	p+s·/p+	p·/p·	p+/p·
6	p·/p·	p·s·/p+	p+s·/p·	p·/p·	p+/p·
7	p·s+/p·	p·s·/p·	p+s+/p+s·	p·s·/p·	p+s·/p·s·
8	p·s·/p·	p·s·/p·	p·s·/p+	p·s·/p·	p+s·/p·

Legend. Symbols as in Fig. 2.

Acrocentrics were identified in accordance with the Paris nomenclature [11] (Fig. 1). The size of the heterochromatin region was estimated in accordance with the scheme in Fig. 2.

The technique of sequential staining for G and C banding of chromosomes is readily reproducible provided that the times of treatment of the preparations are strictly adhered to. Constitutive heterochromatin also was investigated in all cells analyzed by the G method. The stain is best made up a few minutes before use, and the "film" which sometimes forms on its surface must be removed with filter paper.

EXPERIMENTAL RESULTS

Lymphocytes of eight individuals were analyzed by sequential staining for G and C banding of the chromosomes. A minimum of 50 cells was analyzed for each individual. The results of the analysis are given in Table 1. Clearly in homologs of chromosome 15 a large heterochromatin region was found more often than in chromosomes 14 and 13. In all individuals studied at least one homolog of chromosome 15 had a large heterochromatin region, and two were present in four individuals. Four individuals had a large heterochromatin region in chromosome 14, but no large heterochromatin regions were observed at all in chromosome 13. In group G chromosomes 21 and 22 also differed in their heterochromatin content. A large heterochromatin region was observed only in pair 22. The size of the heterochromatin region usually did not correspond to the

size of the short arm observed on staining for G banding. The frequency of discovery of satellites was about the same for all five pairs of acrocentrics in the individuals studied; homologs were often heteromorphic for the presence of satellites. It was also found that in some cases of differential staining for G banding that the satellites were clearly defined in both homologs, whereas staining for C banding showed absence of satellites in these homologs. This could probably be indirect evidence that the stained substances determining the G and C banding differed in nature and even that the satellites of acrocentric chromosomes are heterogeneous in nature, for some of them stained for heterochromatin whereas others did not.

The tendency for chromosomes 15 and 22 to have a large heterochromatin region more often than chromosomes 13, 14, and 21 is in agreement with observations of other workers [1, 13]. According to some writers, for example, the size of the heterochromatin region corresponds to the degree of repetition of the corresponding DNA fractions [6, 9], and this determines the nonspecific conjugation of the acrocentric chromosomes. Nonspecific conjugation, on the other hand, determines the degree of participation of a given acrocentric chromosome in the formation of satellite associations [2]. These ideas naturally suggest that chromosomes 15 and 22 take part in association more often than the rest, as appropriate investigations have confirmed [1, 13]. It will be noted that the tendency thus observed is by no means absolute. Each of the individuals studied had his own characteristic distribution of heterochromatin among the acrocentrics. Consequently, the frequency of participation of each of the homologs of the different individuals in association differed somewhat [13]. The frequency with which acrocentrics participate in associations is probably largely determined by the quantity of heterochromatin in their short arm. It is not yet clear, however, to what degree the frequency of associations depends on the functional activity of the cell, and to what it is determined by the hereditary constitution of the organism (the size of the heterochromatin region is an inherited character). The technique of sequential staining of chromosomes for G and C banding may help to solve these problems.

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