DIRECT QUANTITATIVE ESTIMATION OF LENGTH OF THE C SEGMENTS OF HUMAN CHROMOSOMES

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Several methods have now been suggested for quantitative assessment of the dimensions of the C segments of human chromosomes [2-5]. All are based on the preliminary obtaining of a photographic negative and subsequent measurement of heterochromatin blocks and the arms of individual chromosomes from photographic enlargements. However, the process of taking repeated photographs of chromosomes is laborious. In addition, it is by no means always possible in practice to standardize the exposure or parameters of development and fixation of the photographic films, with the result that the optical density of the negatives may vary, and this may give rise to artefacts when the test structures are measured.

The aim of this investigation was to compare the traditional method of analysis of the C segments of human chromosomes from photographic negatives and direct investigation of metaphases on the display screen.

EXPERIMENTAL METHOD

Chromosomes of peripheral blood lymphocytes of nine individuals were stained by the C method [6] and from 5 to 20 metaphases were analyzed in each depending on the aim of the investigation. Photomicrographs of the chromosomes were obtained on "Mikrat-300" film and the negatives were projected on a screen with a total magnification of 3200 times. The boundaries of the C segments of chromosomes 1, 9, 16, and Y, and also the dimensions of chromosome 2 were noted and investigated by the method described in [3].

For direct quantitative analysis of the dimensions of the chromosomes and their segments, metaphase plates were projected on the display screen with a total magnification of 5550 times by means of a microtelevision camera, mounted on a microscope. To measure chromosomes on the display screen, a slide caliper with scale division of 0.1 nm was used, each measurement of the segments of the chromosomes being repeated 5 times. Homologous chromosomes were investigated separately, with conventional distinction between chromosomes with a larger and smaller heterochromatin block. The absolute dimensions of the C segments of chromosomes 1, 9, 16, and Y were determined in metaphases with chromosome 2 having a length of between 6.5 and 8.5 μ [1].

EXPERIMENTAL RESULTS

Table 1 gives the results of comparative analysis of the absolute dimensions of C segments when two different methods were used to measure the chromosomes: from photographic negatives and from the display screen. To assess the error of each method of measurement, each chromosome in a cell chosen for study was measured 5 times. As the results show, those obtained by the different methods of measurement are satisfactorily reproducible. The standard errors of determination of the dimensions of the C segments varied in each case within identical limits (from 0.01 to 0.05 μ), with a mean value of 0.03 μ; the coefficient of variation varied from 2.1 to 11.5%, with a mean value of 6.7% for the first and 6.4% for the second measurement.

To study the error of reproducibility of the method of measuring C segments from the display screen, intercellular variation of their size was studied in 20 cells from the same

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TABLE 1. Comparative Analysis of Absolute Dimensions of C Segments of Chromosomes 1, 9, 16, and Y Using Different Methods of Measurement (M±m).

Method of measurement	Dimensions of C segments of chromosomes, μ						
	1′	1″	1′	9″	16′	16″	Y
From negative	1,17±0,03	0,95±0,05	0,96±0,02	0,92±0,03	0,77±0,04	0,71±0,02	1,35±0,01
From display screen	1,18±0,05	0,94±0,02	0,95±0,03	0,89±0,03	0,79±0,03	0,65±0,02	1,39±0,02

Legend. Here and in Tables 2 and 3, ′ and ″ denote homologous chromosomes.

TABLE 2. Intercellular Variation in Size of C Segments when Chromosomes Measured from Display Screen

Statistical parameter	Dimensions of C seg. of chromosomes, μ						
	1′	1″	9	9″	16′	16″	Y
M	1,23	1,08	1,16	1,02	0,96	0,72	1,01
$\pm m$	0,03	0,04	1,04	0,05	0,04	0,02	0,05
Coef. of variation, %	4,46	8,07	7,77	10,61	7,65	5,10	10,83

TABLE 3. Interindividual Variation of Dimensions of C Segments when Chromosomes were Measured from Display Screen

Statistical parameter	Dimensions of C seg. of chromosomes, μ						
	1′	1″	9′	9″	16′	16″	Y
M	1,60	1,35	1,39	1,20	1,13	0,93	1,23
$\pm m$	0,07	0,06	0,10	0,09	0,05	0,05	0,05
Coef. of variation, %	13,96	13,36	22,14	23,12	13,99	16,63	12,59

individual, divided into four samples, each of five cells (Table 2). Clearly when this method of measurement was used the standard error of determination of the size of the C segments varied from 0.02 to 0.5 μ, and the coefficient of variation lay between 4 and 11%. These values do not exceed the corresponding values obtained by analysis of chromosomes by the photographic method [3].

Interindividual variation of size of the C segments (Table 3), investigated from the display screen on chromosomes of nine individuals, exceeded intercellular variability by more than two times. In this case the standard error varied from 0.05 to 0.10 μ and the coefficient of variation from 13 to 23%, which also corresponds to data in the literature obtained by analysis of chromosomes from negatives [3].

The method of measurement of chromosomes from the display screen thus has undoubted advantages over the traditional method of quantitative analysis of C segments from negatives because it is much less laborious and gives better reproducibility of the results of the measurements, sufficient for population studies.

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