By using this experimental technique it is evidently possible to estimate quantitatively the powers of association of NOR of chromosomes, which cannot be done in mononuclear cells with respect to each chromosome. In the present case chromosomes 1 and 2 in mononuclear cells form associations with one another extremely rarely, and this makes it difficult to determine the associative activity of these chromosomes.

Polykaryocytes consisting of micronuclei can therefore be used to assess the powers of association of chromosomes carrying NOR and taking part in associations in mononuclear cells.

LITERATURE CITED

1. A. F. Zakharov and N. A. Egolina, Tissue Culture in Oncology [in Russian], Moscow (1968), p. 247.
2. S. E. Bloom and C. Goodpasture, Hum. Genet., 34, 199 (1976).
3. V. I. Stobetskii (V. I. Stobetsky), Biol. Zbl., 97, 587 (1978).
4. V. I. Stobetskii (V. I. Stobetsky), Biol. Zbl., 97, 595 (1978).

COMPARISON OF SOME METHODS OF MEASURING C SEGMENTS OF HUMAN CHROMOSOMES

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KEY WORDS: chromosomes; structural heterochromatin; C segments; morphometry

The effect of the quantity of structural heterochromatin in the genome on formation of the human phenotype still remains an unsolved problem. To endow the results of investigations with an objective character, it has been suggested that the size of regions of structural heterochromatin (C segments of metaphase chromosomes 1, 9, 16, and Y) be estimated quantitatively. Many different methods have been suggested to measure the size of C segments [2-7]. The absence of a unified method of characterizing their size may be the reason why investigators using different methods of quantitative assessment of the size of C segments, studying identical clinical groups, have obtained different results.

The aim of this investigation was to study the comparability and reproducibility of the results of determination of the absolute dimensions of C segments, obtained by the use of known methods of quantitative measurement of their size.

EXPERIMENTAL METHOD

Preparations of metaphase chromosomes of blood lymphocytes, obtained in the course of a double investigation of three healthy women, stained by the C method [11], were used. Altogether 120 metaphase plates (20 in each culture) were photographed on Mikrat-300 film. Negatives were projected on the screen of a "Mikrofot" instrument and the boundaries of the C segments and euchromatin regions of chromosomes 1, 2, 9, and 16 were outlined in accordance with the recommendations in [3]. The results of the measurements were estimated in microns, allowing for total magnification from the original size of the chromosome by 3000 times. Homologous chromosomes were studied separately. The absolute dimensions of the C segments of chromosomes 1, 9, and 16 were determined in 5 metaphase plates with a length of chromosome 2 of between 5 and 10 μ, by the method in [4] and by regression correction [2] in the modification described in [5, 7]. Method [3] was used on 5 metaphase plates with a length of chromosome 2 of 7-9 μ. Absolute dimensions of C segments obtained by the above methods, and in paired cultures, were compared by the t test collectively for chromosomes 1, 9, and 16 of each individual studied.

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TABLE 1. Absolute Dimensions of C Segments of Chromosomes 1, 9, and 16 in Paired Cultures of Individual 1

Chromosome	1		2		3		4	
	A	B	A	B	A	B	A	B
1′	2,01±0,17	2,00±0,08	1,72±0,13	1,75±0,04	2,00±0,07	1,72±0,03*	1,83±0,07	1,77±0,03
1″	1,79±0,12	1,86±0,06	1,48±0,09	1,63±0,03	1,79±0,08	1,61±0,03	1,67±0,08	1,64±0,03
9′	1,76±0,05	1,92±0,14	1,57±0,10	1,68±0,10	1,71±0,09	1,65±0,09	1,68±0,09	1,70±0,09
9″	1,59±0,04	1,70±0,06	1,35±0,08	1,50±0,05	1,49±0,04	1,50±0,05	1,46±0,04	1,49±0,05
16′	0,91±0,07	1,19±0,05*	0,86±0,06	1,05±0,03*	0,99±0,03	1,10±0,02	0,90±0,03	1,04±0,02*
16″	0,87±0,07	1,01±0,05	0,79±0,06	0,89±0,04	0,92±0,04	0,90±0,04	0,86±0,04	0,89±0,04*

Legend. Here and in Tables 2 and 3: 1) Method in [4]; 2) Method in [3]; 3) Regression correction by method in [5]; 4) Regression correction by method in [7]. A) 1st culture; B) 2nd culture, dimensions of C segments given in microns. Apostrophes indicate homologous chromosomes. When results obtained by methods 1, 2, 3, and 4 were compared, in all cases $p > 0.05$; when results in cultures A and B were compared, cases for which $p < 0.05$ are indicated by an asterisk.

TABLE 2. Absolute Dimensions of C Segments of Chromosomes 1, 9, and 16 in Paired Cultures of Individual 2

Chromosome	1		2		3		4	
	A	B	A	B	A	B	A	B
1′	1,76±0,06	1,72±0,11	1,66±0,07	1,79±0,14	1,77±0,99	1,65±0,11	1,71±0,07	1,54±0,11
1″	1,60±0,07	1,55±0,08	1,44±0,06	1,61±0,09	1,49±0,06	1,52±0,07	1,46±0,06	1,46±0,07
9′	1,37±0,05	1,40±0,08	1,24±0,06	1,45±0,11	1,35±0,08	1,53±0,09	1,32±0,07	1,58±0,09
9″	1,15±0,03	1,21±0,06	1,10±0,06	1,25±0,10	1,15±0,07	1,15±0,07	1,13±0,06	1,11±0,07
16′	0,98±0,05	1,10±0,05	0,88±0,04	1,13±0,03	0,92±0,04	1,13±0,02*	0,90±0,04	1,11±0,02*
16″	0,90±0,02	0,98±0,03*	0,87±0,03	1,02±0,05	0,91±0,04	1,02±0,04	0,89±0,03	1,05±0,04*

TABLE 3. Absolute Dimensions of C Segments of Chromosomes 1, 9, and 16 in Paired Cultures of Individual 3

Chromosome	1		2		3		4	
	A	B	A	B	A	B	A	B
1′	1,53±0,07	1,49±0,04	1,56±0,09	1,51±0,04	1,43±0,11	1,45±0,03	1,39±0,12	1,49±0,03
1″	1,30±0,07	1,41±0,05	1,37±0,06	1,42±0,03	1,36±0,06	1,33±0,03	1,35±0,06	1,39±0,03
9′	1,15±0,05	1,33±0,02*	1,16±0,05	1,30±0,03*	1,12±0,05	1,30±0,03*	1,11±0,06	1,30±0,03*
9″	0,83±0,05	1,06±0,04*	0,87±0,05	1,07±0,03*	0,79±0,07	1,07±0,03*	0,78±0,07	1,07±0,03*
16′	1,01±0,04	1,13±0,07	1,03±0,05	1,17±0,05	0,96±0,06	1,15±0,05*	0,92±0,08	1,16±0,05*
16″	0,89±0,03	1,04±0,05*	0,93±0,04	1,05±0,04	0,90±0,04	0,99±0,04	0,88±0,05	1,03±0,04

EXPERIMENTAL RESULTS

Analysis of the data in Tables 1-3 shows that there was no difference in size between the absolute dimensions of C segments of chromosomes 1, 9, and 16, obtained by the methods indicate above [3, 4, 5, 7] ($p > 0.05$). All the methods used demonstrated good reproducibility of the results in a repeated culture for chromosomes 1 and 9. For chromosome 16, statistically significant differences were found in all individual tested between the size of the C segment in paired cultures, and the reason for this may be variability of their size due to artifacts [8, 9].

The results are evidence that it is correct to use the methods examined above for quantiative measurement of C segments of chromosome 1, 9, and 16 within the intervals of mitotic condensation of chromosome 2 specified.

LITERATURE CITED

1. L. N. Bol'shev and N. V. Smirnov, Tables of Mathematical Statistics [in Russian], Moscow (1983).
2. G. P. Lelikova, V. A. Benyush, and T. S. Tsvetkova, Genetika, 13, 2195 (1977).
3. O. A. Podugol'nikova, Zh. D. Sycheva, and A. A. Prokof'eva-Bel'govskaya, Tsitologiya, 21, 667 (1979).
4. P. Balicek, J. Zizka, and H. Skalska, Hum. Genet., 38, 189 (1977).

5. T. Brown, F. W. Robertson, B. M. Dawson, et al., Hum. Genet., 55, 367 (1980).
6. B. Erdtmann, F. M. Salsano, M. S. Mattevi, and R. Z. Flores, Hum. Genet., 57, 58 (1981).
7. U. Friedrich and A. J. Therkelsen, Hum. Genet., 60, 139 (1982).
8. H. Schmiady and K. Sperling, Hum. Genet., 35, 107 (1976).
9. C. Staessen and C. Susanne, Clin. Genet., 19, 537 (1981).
10. C. Staessen, M. Kirsch-Volders, and C. Susanne, Hum. Genet., 61, 68 (1982).
11. A. T. Summer, Exp. Cell Res., 75, 304 (1972).

QUANTITATIVE COMPARISON OF THE CYTOGENETIC EFFECT OF THIOPHOSPHAMIDE ON MONKEY LYMPHOCYTES IN VIVO AND IN VITRO

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KEY WORDS: mutagenesis; thiophosphamide; monkey lymphocytes; in vivo; in vitro

Before regulations governing the use of chemicals with mutagenic activity can be drawn up, their mutagenic effect must be quantified. One approach to the solution of this problem is to establish the principles of extrapolation of data obtained during testing of chemicals on mutagenicity in vitro to the intact organism. It was shown previously that in the case of the action of thiophosphamide on rabbit lymphocytes [1, 3] and of cyclophosphamide on human lymphocytes in vitro and in vivo similar dose–effect relationships are observed during analysis of sister chromatid exchanges (SCE) and of chromosomal aberrations (CA) provided that certain principles of the conduct of chemical dosimetry and evaluation of the effects are observed.

The aim of this investigation was to make a quantitative comparison of induction of SCE and CA during the action of thiophosphamide on monkey lymphocytes in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on three sexually mature male rhesus monkeys. Before the animals were treated with cyclophosphamide samples of 8 ml of blood were taken for the control tests and for the experiments in vitro. Thiophosphamide, diluted in 4 ml of distilled water, was injected intravenously in a dose of 3 mg/kg body weight. During the 4 h after injection of the compound blood was taken several times from a vein in a volume of 3-4 ml, and part of it was used to determine the mutagenic effect in vivo, the rest to determine the thiophosphamide concentration in the nitrobenzylpyridine test by the method described previously [2].

In experiments in vitro 8.5 ml of Hanks' solution with thiophosphamide in a final concentration of 2 to 15 μg/ml, in different versions of the experiments, were added to 1.5 ml of blood. The mixture was incubated for 1 h at 37°C.

In the experiments in vivo and in vitro the lymphocytes were washed 3 times with 10 volumes of Hanks' solution to remove the mutagen. The cells were cultured in the usual way for 73 h with 5-bromodeoxyuridine, which was added at the beginning of culture in a final concentration of 10 μg/ml. Concanavalin A (Sigma, USA) in a final concentration of 15 μg/ml was used as the mitogen.

CA were counted in the preparation in the first mitoses, and SCE in the second mitoses, during anlaysis of 2500 and 600 metaphases, respectively.

To quantify the mutagenic effect the dose of mutagenic action (D) was determined by the method described previously [1] as the integral of the function of the change in thiophos-

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