consists of chromosomes of groups D and G, ten chromosomes in all. On hybridization of 28S and 18S RNA with DNA from patients with Down's syndrome an increase in the number of ribosomal cistrons by 10% was found [2].

The writers previously found a sharp decrease in the incorporation of ^{3}H-uridine into total RNA of PHA-stimulated lymphocytes of patients with Down's syndrome after incubation of the cells with the mitogen for 24 and 48 h. Synthesis of RNA in these experiments was assessed from the rate of incorporation of radioactive precursor into $1 \cdot 10^6$ cells [1].

This decrease can be presumed to be based upon a disturbance of the function of the nucleolar organizer. This hypothesis is also supported by the data of Rigas [7], who found a decrease in DNA synthesis in PHA-stimulated patients' lymphocytes. For the cell to enter the phase of DNA replication activation of rRNA synthesis in the G-phase is known to be necessary. However, in the investigation described above no difference was found in the efficiency of rRNA synthesis in lymphocytes trisomic for chromosome 21 compared with the control values. In this connection the decrease in incorporation of ^{3}H-uridine into total RNA found previously was probably due to a decrease in the percentage of cells responding to the mitogen.

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DEPENDENCE OF CYTOGENETIC ACTION OF TEPA ON ITS CONCENTRATION IN HUMAN LYMPHOCYTE CULTURES

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The cytogenetic action of TEPA [tris(2-methyl-1-aziridinyl)-phosphine oxide; mol. wt. 173.154] on cultures of human lymphocytes was investigated. With an increase in concentration of the mutagen from 0.125 to 16.0 μg/ml the cytogenetic effect increased: the proportion of aberrant metaphases rose from 6.0 to 61.0% and the total number of breaks from 7.96 to 116.3. A method of finding the smallest effective concentration of a test substance compared with a control is suggested, and for TEPA its value is 0.120 μg/ml. The fraction of chromatid breaks remained constant at 51.72% when different TEPA concentrations were used. The distribution of chromosome breaks among the cells is satisfactorily described by a geometric distribution.

KEY WORDS: cultures of human lymphocytes; chromosomal aberrations.

An important factor in the testing of chemical compounds for mutagenic activity is the determination of their smallest effective concentration compared with a control. In the recommended techniques [1] the need

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TABLE 1. Action of Various Concentrations of TEPA on Chromosomes of Human Lymphocyte Cultures

Concentration of TEPA, in μg/ml	Number of cell	Fraction of aberrant metaphases,	Number of breaks per 100 cells	Fraction of single breaks, %	Number of breaks in exchanges per 100 cells	Parameters of equations	
						for fraction of aberrant metaphases	for number of breaks per cell
—	200	1,5	1,5	100,0	0	K = 0,16096	K=0,14037
0,125	200	6,0	7,96	61,5	0	α =0,54430	α =0,51226
0,25	200	11,5	13,9	44,0	1,5	F_r = 224,37	F_r =394,64
0,5	200	15,0	18,4	58,8	1,5	F_{in} =0,20550	F_{in} =0,2668
1	200	23,5	27,4	47,0	2,0		
2	300	33,6	44,5	45,3	3,0		
4	300	40,1	64,8	43,1	11,0		
6	100	49,0	84,0	58,2	12,0		
8	226	54,4	98,2	52,7	10 ,6		
10	100	60,0	101,9	48,9	7,0		
12	100	58,0	116,8	61,6	14,0		
14	100	63,0	140,7	51,4	19,0		
16	100	61,0	116,3	51,1	23,0		

Legend. K, α) coefficients; F_r) regression ratio; F_{in}) inadequacy ratio.

for determining the effect of concentration when assessing the mutagenic activity of chemical agents is stated. In this way the action of a substance with which man may come into contact can be determined in the presence of concentrations of that substance actually found in the human body.

Using TEPA as the example, the character of the relationship between its cytogenetic action and its concentration in cultures of human lymphocytes was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on human peripheral blood lymphocytes. The conditions of cultivation, preparation, and staining of the preparations and also the principles of the cytogenetic analysis were standard.

We used TEPA [tris(2-methyl-1-aziridinyl)-phosphine oxide; mol. wt. 173.154] in concentrations of between 0.125 and 16.0 μg/ml. The mutagen was added 48 h after the beginning of cultivation and was left until fixation, 72 h after the beginning of cultivation. Colchicine was added 2 h before fixation. Experiments were carried out in two or three repetitions for several concentrations. At least 100 metaphases were analyzed for each concentration.

Statistical analysis of the results was carried out in accordance with the recommendations of Draper and Smith [3], and the χ^2 criterion also was used. The 95% confidence interval for the number of aberrant metaphases was calculated by the equation in [5]:

$$\left.\begin{matrix}\arcsin\sqrt{P_2}\\ \arcsin\sqrt{P_1}\end{matrix}\right\} = \arcsin\sqrt{\frac{n\tilde{p}\pm\frac{1}{2}}{n}} \pm \frac{E_0}{2\sqrt{n}},$$

where P_2 is the upper limit and P_1 the lower limit of the 95% confidence interval; $\tilde{p}$ the fraction of aberrant metaphases in the experimental series; n the number of metaphases analyzed.

EXPERIMENTAL RESULTS

The results (Table 1) showed that TEPA in a concentration of 0.125 μg/ml induced chromosomal aberrations in 6.0% of cells, significantly more than the spontaneous level of chromosomal aberrations (1.5%). With an increase in concentration of the mutagen from 0.125 to 16 μg/ml both the number of cells with chromosomal aberrations (from 6.0 to 61.0%) and the number of breaks per cell (from 7.96 to 116.3) increased, indicating considerable mutagenic activity of TEPA. Single breaks were the main type of chromosomal aberration.

Fraction of Aberrant Metaphases. It was found by the method of least squares that the results can be best described by the equation:

$$\rho = 1 - e^{-(KluC-\alpha)^2}, \quad (1)$$

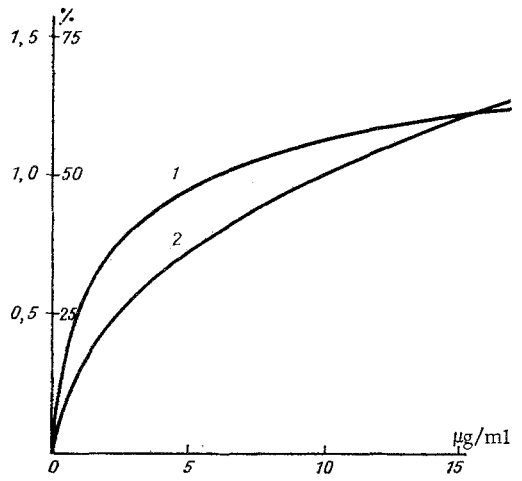

Fig. 1. Theoretical curves showing fraction of aberrant metaphases (1) and number of chromosome breaks per cell (2) as functions of TEPA concentration. Ordinate, left: number of chromosome breaks per cell, right: fraction of aberrant metaphases (in %); abscissa, TEPA concentration (in μg/ml).

where ρ is the function of aberrant metaphases; C the concentration of the mutagen, and K and α coefficients whose values are given in Table 1. Regression for this equation is highly significant ($P < 0.01$) but inadequacy of the experimental data is not significant ($P > 0.05$).

Number of Chromosomal Breaks. The total number of chromosomal breaks is satisfactorily described by the equation:

$$X = e^{(KluC+\alpha)^2-1}, \quad (2)$$

where X is the number of chromosome breaks per cell and the other symbols have the same meanings as in Eq. (1). Equation (2) is obtained by combining Eq. (1) and the equation of the geometrical distribution of chromosome breaks among cells in [2]. For Eq. (2) regression also was shown to be significant and the inadequacy of the experimental data not significant just as for Eq. (1). The values of the coefficients for Eq. (2) are given in Table 1. The expected values of the percentage of aberrant metaphases and the number of chromosome breaks in 100 cells, calculated by Eqs. (1) and (2) are given in Fig. 1.

Fraction of Chromatid Breaks. Investigation of the fraction of chromatid breaks as a proportion of the total number of breaks and its dependence on changes in the concentration of the mutagen by linear regression analysis showed that linear regression was not significant ($P > 0.05$) and that the experimental data for it were adequate ($P > 0.05$). This shows that with all TEPA concentrations used the fraction of chromatid breaks remained constant, with a mean value of 51.72%.

The number of breaks participating in exchanges (Table 1) increased with an increase in the TEPA concentration, but it was difficult to determine how this growth depended on function. The reasons were, first, the relatively small number of chromatid exchanges (there were no exchanges of chromosomal type), and second and more important, the absence of a satisfactory model of exchange formation suitable for quantitative description.

Character of Distribution of Chromosome Breaks among Cells. This was analyzed for TEPA in concentrations of 4 μg/ml and higher. It was shown that for all points except one (10 μg/ml) this distribution differed from the Poisson type ($P < 0.05$) but was satisfactorily described by a geometrical distribution. However, it will be noted that the distribution of breaks among the cells was described best of all by a Polya distribution [4] with a coefficient β of between 0.3 and 0.6 depending on concentration. For the Poisson distribution $\beta = 0$ and for the geometrical distribution $\beta = 1$.

The following approach was suggested in this investigation in order to determine the smallest effective concentration of mutagen compared with the control that would approximate to the concentration actually found in the human body. Initially, an adequate regression equation was chosen for the experimental data for a series

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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