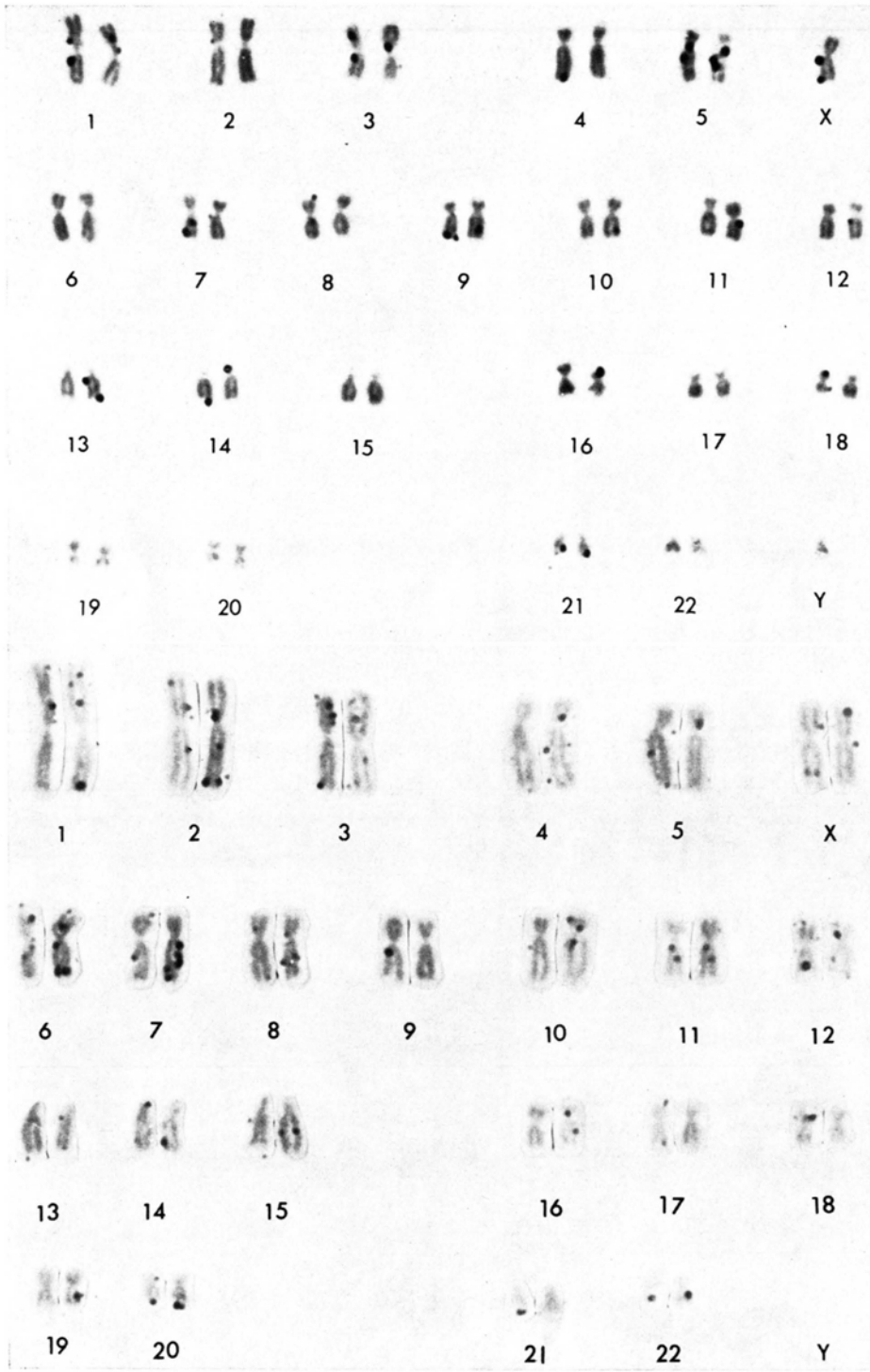

Figure. Two typical karyotypes of human metaphase chromosomes with bound tritiated poly-L-lysine. Top, chromosomes isolated from imipramine-treated culture (1 μg/ml added at 24 hours before termination of culture; bottom, control chromosomes. Slides containing metaphase spreads prepared by a standard method (ref. 2) were treated with tritiated PL (mol. wt. 50,000—100,000, specific activity 3.32×10^3 cpm/mg) as follows. 0.1 ml of 0.1% ^{3}H-PL in 0.1 N acetic acid was applied over the chromosomes and the slide was covered with a cover slip. After 20 min at room temperature the cover slips were removed, the slides were washed with water followed by acetone and air dried. The PL-treated slides were exposed to NTB-2 emulsion for 7 days. The grouping of metaphase spreads according to the degree of contraction was based on the measurement of A_1 chromosome. The average length of this chromosome in the three states of contraction was 8.3 μm (short chromosomes), 10.0 μm (medium chromosomes) and 12.5 μm (long chromosomes).

Effect of Imipramine on Human Chromosomes Studied by the Method of Poly-L-Lysine Binding

S. P. Marfey and M. G. Li

Department of Biological Sciences, State University of New York at Albany

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Imipramine, Human Chromosomes, Poly-L-lysine Binding

Metaphase chromosomes of group A isolated from the imipramine-treated lymphocyte cultures did bind more tritiated poly-L-lysine (^{3}H-PL) than the control chromosomes. The increase in binding was dependent on the length of treatment and on contraction of chromosomes. The effect of contraction was more pronounced for the treated chromosomes than for controls and was larger when the treatment was done 4 hours before termination of culture. The observed differences in the ^{3}H-PL binding probably reflect differences in the chromosomal surface morphology.

In the previous communication [1] we have described the effect of chlorpromazine (CPZ) on the structure of human metaphase chromosomes. Now we wish to report on the effect of imipramine, 5-(3-dimethylaminopropyl)-10,11-dihydro-5H-dibenz[b,f]azepine, which is widely used as an antidepressant drug and whose molecular geometry is similar to that of CPZ.

Imipramine was added to tissue cultures of human peripheral lymphocytes at concentrations of 1, 5 or 10 μg/ml at times 4, 24 and 48 hours before termination of cultures. The cultures were set up according to a standard procedure [2] for a total of 72 hours. Colcemid (0.04 μg/ml) was added 3 hours before harvesting the cells. Metaphase spreads prepared on slides [2] were treated with tritiated poly-L-lysine (PL) and then exposed to NTB-2 emulsion to visualize the sites on chromosomes where PL binding did occur. The binding of PL is expected to take place predominantly in those regions of chromosomes which have segments of nucleic acids exposed to the external environment [1]. It occurs by means of electrostatic interactions between the positively charged ε-amino groups of PL and the negatively charged phosphodiester bonds of nucleic acids. At least 10–20 such ionic bonds are required per molecule of PL to form a stable complex because the energy of interaction per mole of ion pairs is of the order of 1 Kcal.

A representative karyotype of an imipramine-treated and of an untreated control is shown in the Figure *. The identification of individual chromosomes was aided by the use of a Giemsa binding method applied to chromosomes after preliminary treatment with trypsin [3, 4]. Between 93 and 99% of total grains was located over the chromosomes and the remainder over the background. A large number of karyotypes was examined for grain density and for grain location over the chromosomes (12 control and 80 imipramine-treated karyotypes) to make sure that the results were reproducible. An estimate of the probable error was made on several groups of karyotypes obtained under the same conditions of treatment, and it was found to be approximately 30% of the mean value.

The results of PL binding expressed in terms of $G_e - G_c$, *i. e.*, the difference in average grain

Table. Binding of tritiated poly-L-lysine to human metaphase chromosomes. Average number of grains per unit weight ($g \times 10^{-15}$) of chromatid of imipramine-treated chromosomes (G_e) was determined on 6–12 karyotypes for each treatment (80 in total) and separately for each class of chromosome (short, medium and long). Average number of grains per unit weight of chromatid of control chromosomes (G_c), also determined on 4 karyotypes for each class of chromosomes (12 in total) and obtained from the same experiment, was subtracted from G_e to show more clearly the effect of drug treatment. For each treatment, the differences in grain density per chromatid between the three classes of chromosomes were also calculated ($G_L - G_M$, $G_L - G_S$ and $G_M - G_S$). A sum of these differences ($\Sigma \Delta G$) is given in the Table.

Chromosome	$G_e - G_c$ per chromatid (top) or $\Sigma \Delta G$ per chromatid × 10 (bottom)									Control
	4 hours			24 hours			48 hours			
	[1 μg/ml]	[5 μg/ml]	[10 μg/ml]	[1 μg/ml]	[5 μg/ml]	[10 μg/ml]	[1 μg/ml]	[5 μg/ml]	[10 μg/ml]	
A_1	2.43	3.00	2.53	1.90	0.20	4.23	1.23	3.13	6.43	–
	0.28	0.08	0.32	−0.30	0.30	0.02	−0.08	−0.30	−0.04	−0.12
A_2	−0.14	3.34	7.88	6.46	0.96	1.94	7.54	14.40	10.40	–
	0.70	−0.64	0.12	−0.24	−0.06	0.36	0.96	2.46	0	0.16
A_3	8.57	4.00	2.67	5.43	−0.77	−2.43	5.00	4.33	4.00	–
	1.76	−0.06	−0.46	−0.50	0.36	0.10	−0.36	−0.24	0.64	**0.32**

Requests for reprints should be sent to Prof. Dr. S. P. Marfey, Department of Biological Sciences, State University of New York at Albany, *Albany, New York*, 12222, U.S.A.

* Figure see Table on page 650 a.

density between the imipramine-treated and the control chromosomes, and in terms of $\Sigma \Delta G$, *i. e.*, the difference in the average grain density between the long and short chromosomes, are summarized in the Table for A group chromosomes. It can be seen that all chromosomes after treatment with imipramine did bind more PL than did the corresponding controls (positive $G_e - G_c$ values). The largest difference was observed for A_2 chromosome. Furthermore, treatment with the drug at 48 hours before harvest produced larger effect than treatment at 24 hours, *i. e.*, the effect was dependent on time of drug exposure. The effect of drug concentration varied depending on the conditions of drug treatment.

The effect of chromosomal contraction on the PL binding was more pronounced for imipramine-treated chromosomes than for controls. All treated chromosomes in most instances did bind more PL when in an elongated than in a contracted state (positive $\Sigma \Delta G$ values). The largest effect was observed for the A_2 chromosome. The PL binding of control chromosomes was less dependent on the degree of contraction. In all cases a larger effect was observed for the chromosomes treated at 4 hours before harvest than at 24 hours. No uniform effect of drug concentration was observed. It varied depending on the condition of drug treatment.

Tritiated PL binding appears to be a sensitive method for studying chromosomal structure at the molecular level. Although imipramine-treated chromosomes appear to be similar to the control chromosomes under a light microscope, their surface organization is different as judged by the ^{3}H-PL binding. It appears that the imipramine-treated chromosomes contain more regions with exposed nucleic acids and, consequently, they bind more PL than do the control chromosomes.

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[1] S. P. Marfey and M. G. Li, Z. Naturforsch. **29 c**, 306 [1974].
[2] P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford, Exp. Cell Res. **20**, 613 [1960].
[3] T. C. Hsu, Mammalian Chromosome Newsletter **13**, 43 [1972].
[4] M. Seabright, Lancet **II**, 971 [1971].