# Effect of Chlorpromazine on Human Chromosomes Studied by a New Method

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It has been reported [1] that metaphase chromosomes from cultured lymphocytes of patients receiving chlorpromazine (CPZ) had more structural abnormalities (gaps, breaks, hypodiploid cells) than did control chromosomes. However, when we cultured lymphocytes of non-treated individuals in the presence of CPZ (5, 10 or 20 $\mu$g/ml) during periods of 4, 24 and 48 hours before termination of culture, the morphology of chromosomes examined under a light microscope was in all cases essentially similar to that of controls. If CPZ induced alterations in chromosomal structure at the molecular level, they would not be seen under the microscope but might be revealed by treatment with tritiated poly-L-lysine [2] (PL). PL would be expected to bind preferentially to those regions of metaphase chromosomes which contain exposed (not extensively covered with proteins) segments of nucleic acids whose negatively charged phosphodiester linkages can form electrostatic bonds with positively charged $\varepsilon$-amino groups of the PL.

A typical karyotype of the CPZ-treated and of control chromosomes is shown Fig. 1 *. Identification of individual chromosomes was aided by the application of a Giemsa banding procedure after treatment of chromosomes with trypsin [3, 4]. More than 95% of total grains was located over the chromosomes and the remainder over the background. The grain density and the relative location of grains over chromatids was different for the CPZ-treated and for control chromosomes. The results obtained for A group chromosomes are summarized in the Table. As an example, $A_1$ chromosome from the CPZ-treated culture binds less PL than $A_1$ control. Furthermore, there appears to be a dependence of this differential binding $(\overline{G}_e - \overline{G}_c)$ on CPZ concentration and on the time of CPZ exposure.

It was also observed that the extent of PL binding depends on chromosomal contraction (Table). Therefore, the $(\overline{G}_e - \overline{G}_c)$ values were computed separately for karyotypes containing short, medium or long chromosomes. For example, a long $A_1$ chromosome was found to bind more PL than did a short $A_1$ chromosome even though both of them were isolated from the same CPZ-treated culture. The opposite result was obtained for the control $A_1$ chromosome. This difference in binding due to chromosomal contraction ($\Sigma \Delta G$ per chromatid) was also dependent on CPZ concentration and on CPZ exposure time.

A large number of karyotypes was examined (at least 3 for each experimental condition, over 100 in total) to make sure that the results were reproducible. The probable error computed from the data of several groups of karyotypes obtained under the same experimental conditions ranged from about 20 to 30% of the mean value. It appears that CPZ

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 CPZ-treated chromosomes ($\overline{G}_e$) was determined on at least 3 karyotypes of each treatment and separately for each class of chromosome (short, medium and long, approximately 90 in total). Average number of grains per unit weight of chromatid of control chromosomes ($\overline{G}_c$), also obtained on at least 3 karyotypes from the same experiment, was subtracted from $\overline{G}_e$ to show more clearly the effect of drug treatment. For each treatment, the differences in grain density for each chromatid between the three classes of chromosomes were also calculated ($\overline{G}_L - \overline{G}_M$, $\overline{G}_L - \overline{G}_S$ and $\overline{G}_M - \overline{G}_S$). A sum of these differences ($\Sigma \Delta G$) is given in the Table.

| | $\overline{G}_e - \overline{G}_c$ per chromatid (top) or $\Sigma \Delta G$ per chromatid $\times 10$ ( bottom) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chromosome | 4 hours | | | 24 hours | | | 48 hours | | |
| | 5 $\mu$g/ml | 10 $\mu$g/ml | 20 $\mu$g/ml | 5 $\mu$g/ml | 10 $\mu$g/ml | 20 $\mu$g/ml | 5 $\mu$g/ml | 10 $\mu$g/ml | Control |
| $A_1$ | −2.67 | −6.10 | −8.47 | −6.13 | −5.20 | −0.13 | −5.20 | −3.53 | — |
| | −3.82 | 6.18 | 0.36 | 5.06 | 0.60 | 1.68 | 4.00 | 3.34 | −.3.36 |
| $A_2$ | 1.44 | −6.40 | −5.66 | −8.00 | −1.76 | 3.58 | 2.76 | 1.62 | — |
| | 16.02 | 9.79 | 2.62 | −3.02 | −8.34 | 10.81 | −5.74 | 4.1 | −6.20 |
| $A_3$ | −1.63 | −3.20 | −1.40 | 3.13 | 4.03 | 27.83 | 8.37 | 0.50 | — |
| | 4.05 | 2.24 | 5.74 | 7.78 | −2.64 | 49.52 | 9.78 | 11.40 | −5.2 |

* Fig. 1 see Table on page 306 a.

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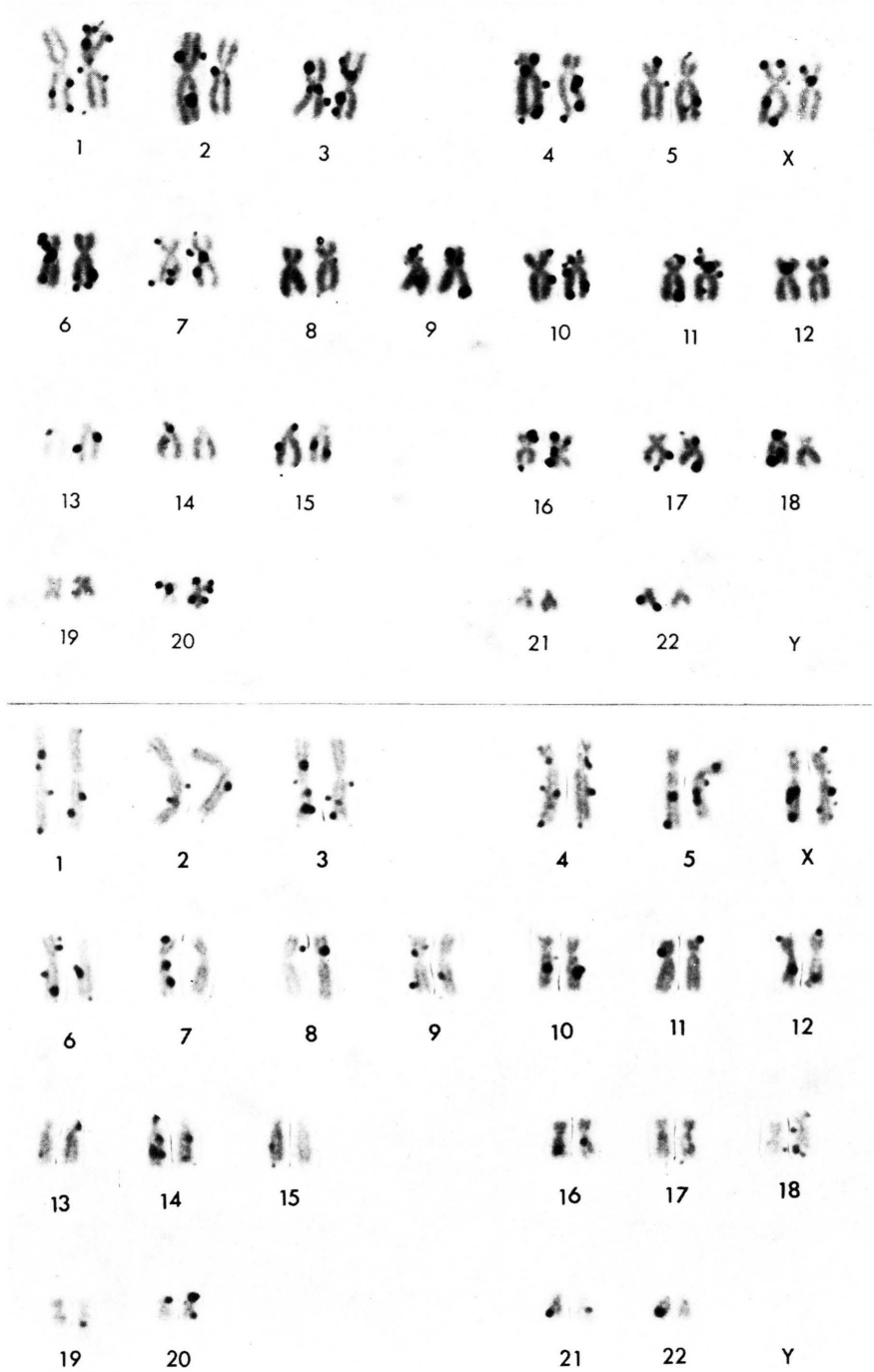


Fig. 1. Two typical karyotypes of human metaphase chromosomes with bound tritiated poly-L-lysine. Top, chromosomes isolated from CPZ-treated culture (20 $\mu$g/ml added at 4 hours before termination of culture; bottom, control chromosomes. Slides containing metaphase spreads prepared by a standard method (ref. 5) were treated with PL (mol.wt 50,000—100,000, specific activity $3.32 \times 10^3$ cpm/mg) al 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 washed with water and 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 $\mu$ (short chromosomes), 10.0 $\mu$ (medium chromosomes) and 12.5 $\mu$ (long chromosomes).

added to tissue culture medium had an effect on chromosomal structure as revealed by the above method of examination. It is likely that the organization of nucleic acids and proteins in chromatids was different in the CPZ-treated chromosomes than it was in the controls. Under our conditions of *in vitro* CPZ treatment the observed differences in structure were not manifested as gross-morphological changes which were observed under the conditions of *in vivo* treatment [1]. Such parameters as CPZ concentration, length of exposure and the presence of CPZ metabolites might be responsible for the different results obtained under the two sets of conditions. It could be, however, that the observed changes at the molecular level constitute an initial stage in the process of alteration of chromosomal morphology.

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