Effect of Alkali Chlorides on Human Chromosomes Studied by the Method of Poly-L-lysine Binding

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Alkali Chlorides, Human Chromosomes, Poly-L-lysine Binding

Human metaphase chromosomes A_1 and A_3 did bind more tritiated poly-L-lysine ([^{3}H]PL) when they were isolated from lymphocyte cultures treated with LiCl rather than with NaCl or KCl. A_2 chromosome did not show this differential behavior and in all three cases did bind more [^{3}H]PL than the control A_2 chromosome. The effect was dependent on the time of exposure of cells to the salts in tissue culture and on the degree of chromosomal contraction. The observed differences in [^{3}H]PL binding are probably due to differences in surface morphology of the three chromosomes caused by treatment with alkali metal salts.

In the previous communications [1, 2] we have described the effect of two psychotropic drugs, chlorpromazine and imipramine, on the morphology of human metaphase chromosomes. In this report we wish to present evidence indicating that salts of alkali metals when added to tissue cultures of human lymphocytes also exert specific effects dependent on the nature of alkali metal ions, their concentration and the time of exposure to the salts.

It has been reported [3] that lithium salts when administered to patients suffering from manic-depressive psychosis had an adverse effect on chromosomes. A significantly higher frequency of breaks was found in chromosomes isolated from lymphocyte cultures of these patients as compared with that of the control chromosomes. On the other hand, no higher incidence of structural abnormalities was found in chromosomes isolated from lymphocyte cultures treated *in vitro* with the non-toxic doses of lithium salts.

In our experiments we employed human peripheral lymphocyte cultures to study the effect of LiCl, NaCl, KCl, RbCl and CsCl on the structure of metaphase chromosomes. Using non-toxic concentrations of these salts (1 to 3 meq/l) we were able to prepare a sufficient number of metaphase spreads under standard conditions [4] to perform structural analyses of chromosomes treated with all salts except RbCl. We have used tritiated poly-L-lysine ([^{3}H]-PL) binding to metaphase chromosomes as a method for studying chromosomal structure at the molecular level [5].

Representative karyotypes of LiCl-treated, NaCl-treated and of control chromosomes are shown in Fig. 1 *. The arrangement of chromosomes into groups was aided by the application of a Giemsa banding procedure after preliminary treatment of chromosomes with trypsin [6, 7]. In all cases, between 89 to 98% of total silver grains was located over the chromosomes and the remainder over the background area. A large number of karyotypes was analyzed for grain density and grain distribution on the chromosomes (9 control, 81 alkali salt-treated) to see if the labeling 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 between 20 – 30% of the mean value.

The [^{3}H]PL binding results obtained for the chromosomes of A group are shown in the Table. The data are given for each salt treatment in terms of $G_e - G_c$ values, *i. e.*, the differences in the average grain density between the treated and the control chromosomes, and in terms of $\Sigma\, \Delta G$ values that show differences in binding due to chromosomal contraction. LiCl-treated A_1 and A_3 chromosomes bind more [^{3}H]PL than NaCl- or KCl-treated chromosomes. A_2 chromosome appears to bind more [^{3}H]PL after exposure to LiCl, NaCl or KCl than the control A_2 chromosome (positive $G_e - G_c$ values). The effect is dependent on the length of exposure of cells to the salts. It is larger when the salts were added at 48 hours than at 4 hours before termination of cultures. There seems to be little, if any, dependence on the dose of NaCl or KCl (1 or 2 meq/l) when they were added at 48 hours. The magnitude of [^{3}H]PL binding also depends on the degree of chromosomal contraction. Longer chromosomes isolated from the LiCl-treated cultures bind more [^{3}H]PL than shorter chromosomes. The opposite effect is observed for the KCl-treated chromosomes. NaCl-treated chromosomes exhibit variable effect. Generally, the effect appears to be larger in those cases when the cells were exposed to the salts for longer periods of time.

Alkali chlorides-treated chromosomes appeared similar to the control chromosomes under a light microscope in agreement with the previous results [3], but the observed differences in [^{3}H]PL binding indicate that their surface morphology is different from that of the controls. Moreover, there are also differences in morphology between the three sets of

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* Figs 1 a–c see Tables on pages 304 a to 304 c.

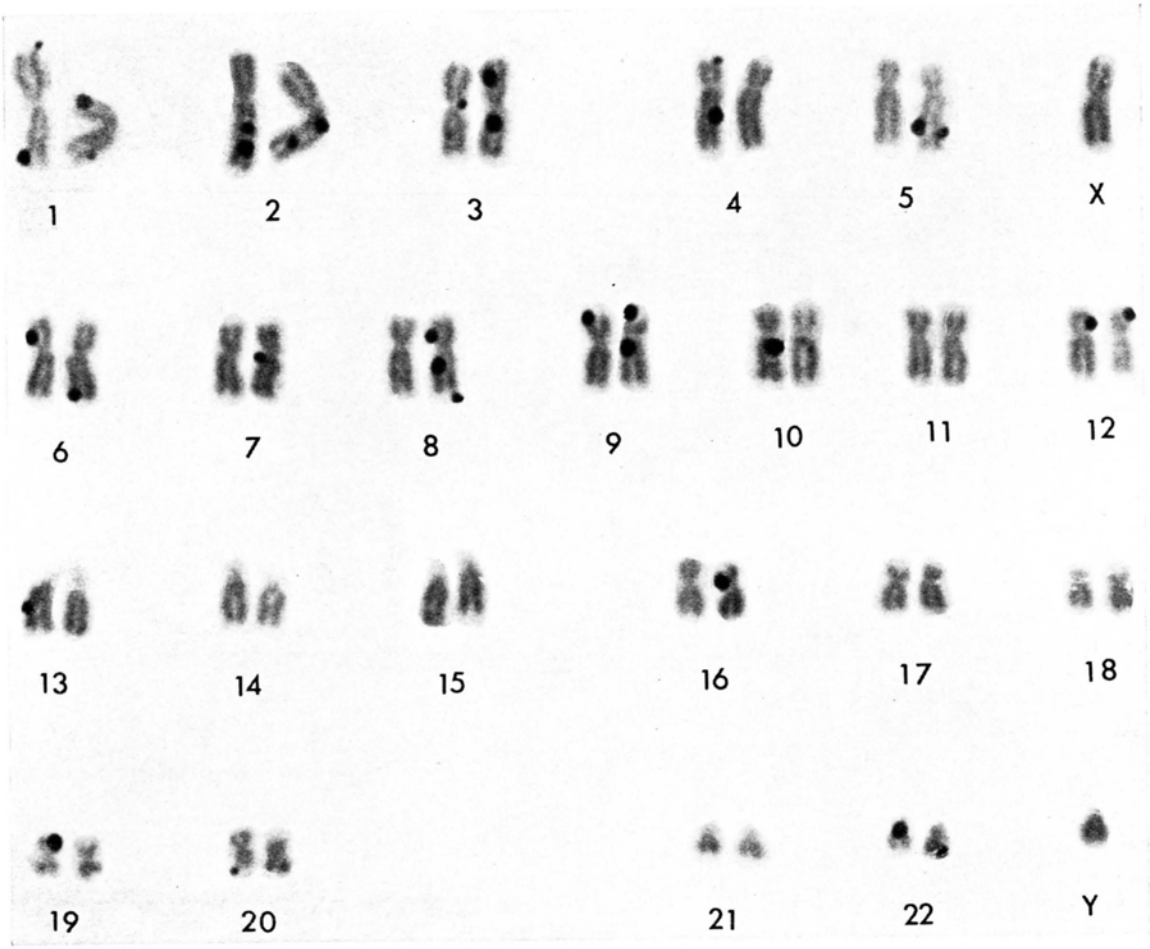

Fig. 1 a.

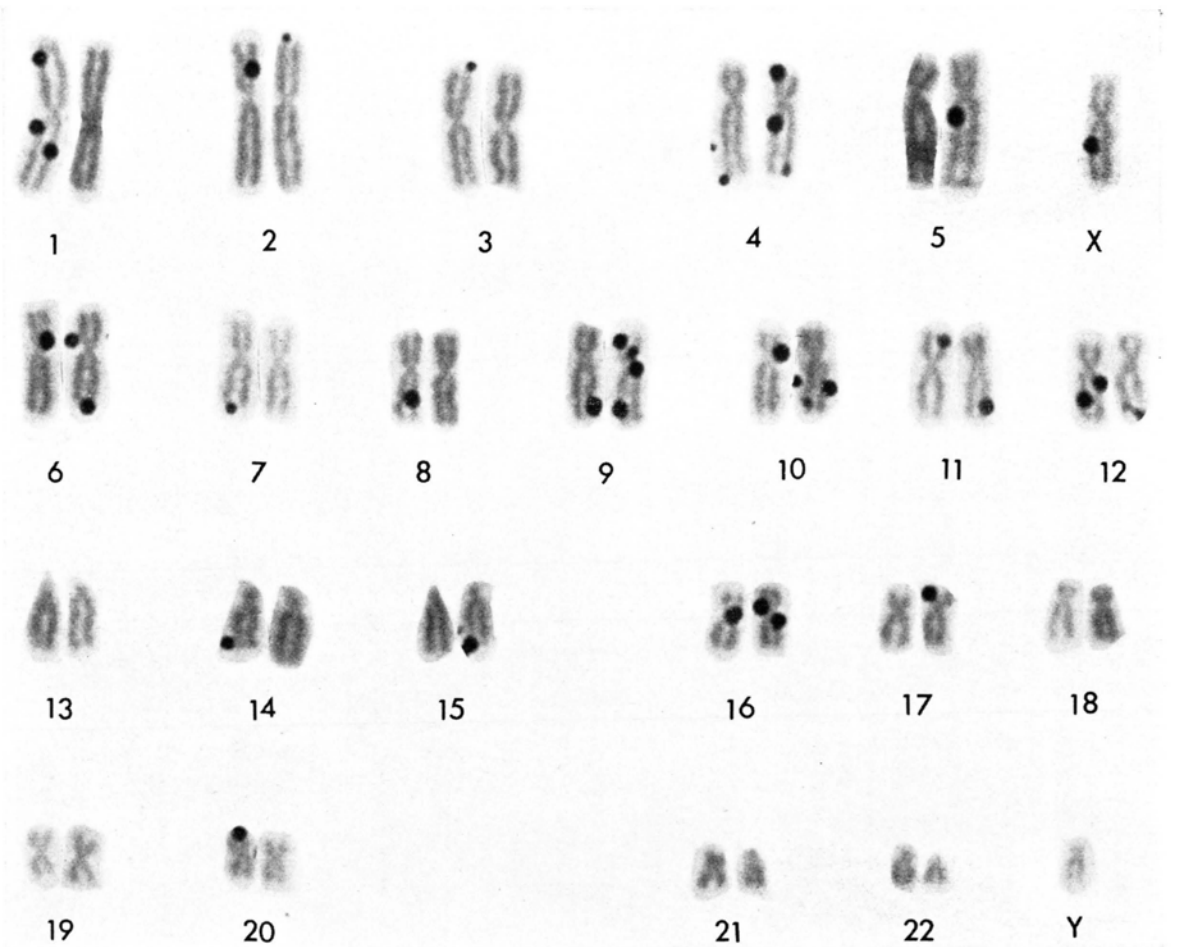

Fig. 1 b.

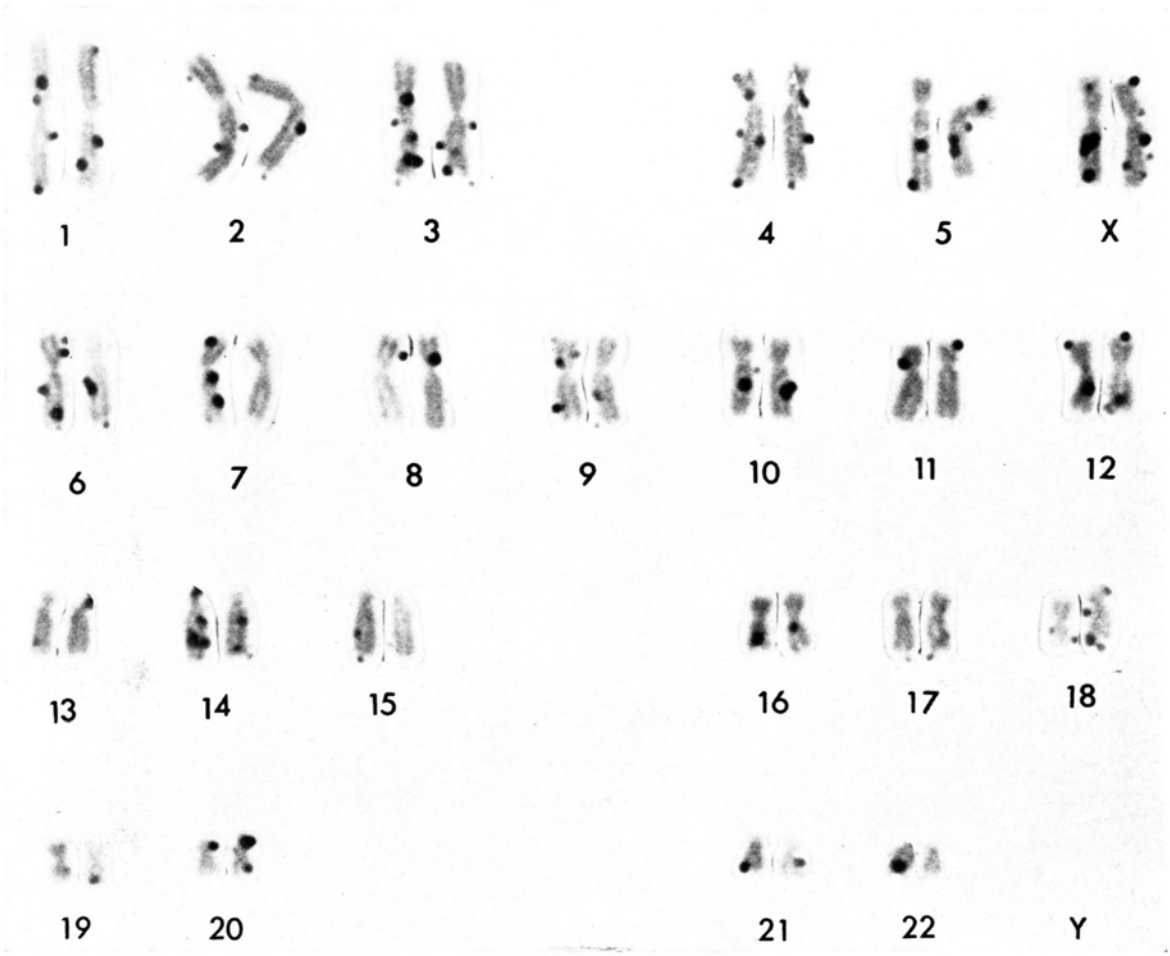

Fig. 1 c.

Fig. 1. Typical karyotypes of human metaphase chromosomes with bound tritiated poly-L-lysine. a, chromosomes isolated from LiCl-treated culture (2 meq/l, added at 4 hours before termination of culture). b, chromosomes isolated from NaCl-treated culture (1 meq/l, added at 4 hours before termination of culture). c, control chromosomes. Slides containing metaphase spreads prepared by a standard method (ref. 4) were treated with [^{3}H]PL (molecular weight 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 slip was removed, the slide washed with water followed by acetone and air dried. The [^{3}H]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).

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 alkali salt-treated chromosomes (G_e) was determined on 81 karyotypes (LiCl, 19; NaCl, 35; KCl, 18; CsCl, 9) separately for each class of chromosomes (short, medium and long). Average number of grains per unit weight of chromatid of control chromosomes (G_c), obtained on 9 karyotypes prepared from the same cultures, was subtracted from G_e to show more clearly the effect of salt 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.

$G_e - G_c$ per chromatid (top) and $\Sigma \Delta G$ per chromatid X 10 (bottom)

Chromosome	LiCl		NaCl				KCl		CsCl	
	4 hours	48 hours	4 hours	24 hours	48 hours		48 hours		48 hours	Control
	2 meq/l	3 meq/l	1 meq/l	1 meq/l	1 meq/l	2 meq/l	1 meq/l	2 meq/l	3 meq/l	
A_1	2.21	3.23	−1.63	2.03	−0.79	−0.07	−1.80	−0.15	0	—
	0.68	0.20	0.46	2.16	−2.34	2.72	−0.72	−3.44	−0.18	−2.06
A_2	2.70	8.58	1.40	6.10	2.72	2.54	2.66	2.64	−0.36	—
	0.28	2.20	0.20	4.78	3.72	5.60	−9.38	−6.26	1.00	1.58
A_3	−4.43	0.50	0.13	−0.20	−2.30	−3.03	−4.17	−3.00	0.43	—
	−0.82	4.20	−7.14	1.94	−3.16	0.84	−2.20	0.64	−5.40	1.32

treated chromosomes. Although the physico-chemical basis of the observed differential effect of the tested ions on chromosomal structure is not known, it is likely that this effect could arise from the differences in their membrane permeability properties and/or from their differential interaction with the macromolecular components of chromosomes [8].

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