Steroid hormones enhanced sister-chromatid exchange in cultured CHO cells [1]

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Summary. The influence of steroid hormones on the induction of sister-chromatid exchange (SCE) in cultured CHO cells was studied. It was observed that estradiol-17 β, estriol, estrone and ethynyl estradiol treatments enhanced SCE rates compared to the controls. Overall, these compounds produced a dose response effect. The importance of a detailed study on the long-term genetic effects of steroids on mammalian cells is emphasized.
Key words. Chromosomal aberrations; steroid hormones; oral contraceptive; lymphocytes; 5-bromo-2'-deoxyuridine.

Enhancement of sister-chromatid exchange (SCE) by various environmental chemicals has been established to be a sensitive test to assess DNA damage in mammalian cells [2–4]. Cytogenetic studies conducted on the possible chromosome-damaging effect of steroid hormones so far are inconclusive and controversial [5]. Since the introduction of oral contraceptives, which are mainly the steroids, a large number of women of childbearing age have been taking these for relatively long periods of time. The experimental data obtained on these compounds have failed to reveal their mechanism of action and biological significance. For this reason it is immensely important that the long-term genetical effects of steroid hormones be thoroughly investigated.

For the past few years we have been conducting investigations in our laboratory to determine the effect of steroid hormones on mammalian chromosomes using cultured Chinese hamster ovary (CHO) cells. This investigator [6] recently reported that estrogens like estradiol-17 β, estrone, estriol and ethynyl estradiol were effective in causing various types of chromosomal aberrations. In the current report we describe the influence of similar compounds on the changes produced in the frequency of SCE in cultured CHO cells. As compared to other studies [7, 8] that had utilized cultured lymphocytes, we find SCE assay on cultured CHO cells to be a rapid, sensitive and simpler approach for detecting changes produced in the chromosomes.

Materials and methods. Exponentially growing cultures of CHO cells were used in the present study. The cells were routinely grown in 75-cm^2 Corning plastic flasks in 10 ml of McCoy's 5a medium containing 10% fetal bovine serum and antibiotics consisting of penicillin-G, (100 U/ml) and streptomycin (100 µg/ml). The cultures were kept in an atmosphere of 5% CO_2 in air. In order to test the influence of steroid hormones on SCE, the cultures were set up one day prior to the exposure of test chemical by seeding approximately 8×10^5 cells per flask. The CHO cells were obtained from Environmental Health Research and Testing, Lexington, KY. These cells were not used at a passage level of more than 15 after cloning, and were thawed routinely from liquid nitrogen storage and maintained by transferring twice a week. The steroid hormones used in this study were estradiol-17 β (hereafter referred to as estradiol), estriol, estrone, and ethynyl estradiol. The compounds were purchased from Sigma Chemical Co., St. Louis, MO.

The treatments were given by adding the test compounds in concentrations of 10^{-5}, 2.5×10^{-5}, 5×10^{-5} and 7.5×10^{-5} M, dissolved in dimethyl sulphoxide (DMSO) and suspended in the medium for 24 h. The final concentration of DMSO did not exceed 0.5% of the culture medium. Simultaneously 5-bromo-2'-deoxyuridine (BrdU) at concentration of 10 µM was added to each flask, covered with foil and reincubated for 24 h. Control cultures containing 0.5% DMSO were kept under identical conditions. Four hours prior to harvest, colcemid (0.1 µg/ml) was added to each flask.

The cells were harvested by decanting the medium into labeled centrifuge tubes and retained; each flask was trypsinized and the contents resuspended in the medium in the corresponding centrifuge tube. The tubes were spun for 10 min at 550 rpm and the supernatant discarded. The cells (pellet) were suspended in 10 ml of 0.075 M KCl (37 °C) and incubated for 4 min. The tubes were then centrifuged for 4 min at 550 rpm and supernatant discarded. The pellet was resuspended in 1 ml of cold fixative (3 : 1 methanol : glacial acetic acid), centrifuged for 10 min at 550 rpm and supernatant discarded once again. This procedure was repeated twice. Steps until the second fixation were carried out under OC Kodak safe light to avoid photoactivation of BrdU. Concentrated cell suspension was dropped on clean, wet and chilled slides at an angle of 45°. The slides were air dried overnight.

The slides were then stained with Hoechst dye essentially according to the procedure of Perry and Wolff [9] except for the black light modification introduced by Goto et al. [10]. SCE was scored by counting 25 metaphases per concentration and the experiments were replicated. Only chromosome spreads of 21 ± 2 chromosomes were scored. To calculate SCE/cell, the number of total SCE was divided by 25.

Results. The effect of steroid hormones on the induction of SCE frequencies in CHO cells is described in the table. The data indicate that all steroid treatments significantly enhanced SCE frequency. However, estradiol seemed to have the most pronounced effect as each concentration of this compound produced an increase in SCE rates. The highest level of estradiol (7.5×10^{-5} M) produced close to 3-fold increase in SCE compared to the BrdU controls. A similar dose of estriol and estrone produced more than 2-fold increase in SCE frequency over the controls. Ethynyl estradiol

Induction of sister-chromatid exchange (SCE) by steroid hormones in cultured Chinese hamster ovary (CHO) cells.

Treatment	Concentration (M)	SCE/cell ± SE[a]	Range
Control		4.08 ± 0.27	2–7
Estradiol-17 β	10^{-5}	5.36 ± 0.43[b]	3–12
	2.5×10^{-5}	7.12 ± 0.46[d]	3–11
	5×10^{-5}	10.04 ± 0.51[d]	7–16
	7.5×10^{-5}	11.80 ± 0.46[d]	8–17
Estriol	10^{-5}	5.32 ± 0.34[c]	2–9
	2.5×10^{-5}	7.68 ± 0.41[d]	4–11
	5×10^{-5}	7.60 ± 0.37[d]	3–11
	7.5×10^{-5}	9.12 ± 0.36[d]	6–13
Estrone	10^{-5}	6.08 ± 0.26[d]	3–9
	2.5×10^{-5}	6.80 ± 0.30[d]	4–10
	5×10^{-5}	7.20 ± 0.31[d]	5–11
	7.5×10^{-5}	8.48 ± 0.33[d]	7–12
Ethynyl estradiol	10^{-5}	6.16 ± 0.33[d]	4–10
	2.5×10^{-5}	6.88 ± 0.30[d]	5–11
	5×10^{-5}	8.00 ± 0.27[d]	6–10
	7.5×10^{-5}	–	–

[a] Standard error of the mean; [b] Significant at $p < 0.05$ by Student's t-test; [c] Significant at $p < 0.01$; [d] Significant at $p < 0.0001$.

was also effective in enhancing SCE levels; however, the highest concentration of this compound was very toxic to the cells and hence no recognizable SCE was noticed. As seen in the table all compounds, overall, produced a dose response influence on the rates of SCE. Attempts were made to determine the effect of higher levels (than employed here) of these compounds on same parameter, but the concentrations proved to be too toxic to reveal clear cut SCE.

Discussion. For a long period of time the scientific community has been unsure of the possible role of steroid hormones or hormonal contraceptives in causing chromosome changes in mammalian cells. However, since the discovery of new, high resolution techniques for detecting subtle changes in chromosomes, there have been a few studies to resolve this controversy. Murthy and Prema [7, 8] showed that women using estrogen-progestogen combination contraceptive exhibited an increased frequency of SCE in cultured peripheral bloody lymphocytes compared to subjects consuming no oral contraceptives and pregnant women. These investigators suggested that alteration in SCE in the contraceptive-using women may be due to mutagenic activity of the estrogens or their metabolites. However, another group of workers [11] found no evidence at all of the elevated SCE in women using a similar combination of contraceptives.

The current study, utilizing the SCE technique, does indicate that the steroid hormones are capable of producing elevated SCE in cultured CHO cells. This is in complete agreement with our earlier study [6] which showed that estrogens, namely estradiol, estrone, estriol and ethynyl estradiol, were very effective in producing various types of chromosomal alterations in CHO cells. Also, an earlier study conducted on cultured HeLa cells by Rao and Engleberg [12] showed that similar estrogens showed a high degree of structural specificity in the induction of mitotic chromatid non-disjunction that resulted in undivided chromosomes in two daughter cells.

A study performed by Littlefield and coworkers [13] on the lymphocyte cultures of normal women, pregnant women and women taking oral contraceptives showed profound variations in the frequency of chromosome breakage among the consecutive cultures from the same person and among cultures from different individuals. However, a similar study [14] failed to show any significant increase in numerical or structural chromosome aberrations in women who had used the pill.

Despite existing controversies found in the literature, our studies do indicate that steroid hormones or the pill can contribute to chromosome changes in mammalian cells. These changes suggest an increased mutagenic environment in these cells, which may be due to steroids themselves or their metabolites. The mechanism by which these compounds bring about these changes is not clear, nor is the biological significance. Therefore, further studies are definitely needed to clarify the situation, and until such time it will be wise to view the current information with caution.

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2 Abe, S., and Sasaki, M. J., Natl Cancer Inst. *58* (1977) 1635.
3 Takehisa, S., and Wolff, S., Mutat. Res. *58* (1978) 103.
4 Wolff, S., Research in Photobiology, p. 721. Ed. A. Castellani. Plenum, New York 1977.
5 Bishun, N., Smith, N., and Mills, J., Mutat. Res. *39* (1976) 97.
6 Kochhar, T. S., Toxic. Lett. *29* (1985) 201.
7 Murthy, P. B. K., and Prema, K., Mutat. Res. *68* (1979) 149.
8 Murthy, P. B. K., and Prema, K., Mutat. Res. *119* (1983) 351.
9 Perry, P., and Wolff, S., Nature, Lond. *251* (1974) 156.
10 Goto, K., Maeda, S., and Sugiyama, T., Chromosoma *66* (1978) 351.
11 Husum, B., Wulf, H. C., and Niebuhr, E., Mutat. Res. *103* (1982) 161.
12 Rao, P. N., and Engleberg, J., Expl Cell Res. *48* (1967) 71.
13 Littlefield, L. G., Lever, W. E., Miller, F. L., and Goh, K. O., Am. J. Obstet. Gynec. *121* (1975) 976.
14 Matton van Leuven, M. T., Thiery, M., and de Bie, S., Contra *10* (1975) 25.

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Heterochromatin associated with active versus inactive centromeres of mouse replicates at different times

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Summary. A subline of mouse L-cells carries a dicentric chromosome in which one centromere always separates prematurely. This centromere is not involved in the dynamics of chromosome migration and is considered inactive. By use of anti-BRdU antibody binding to BRdU-treated chromosomes it is shown that the pericentric constitutive heterochromatin associated with the prematurely separating centromere replicates earlier than its counterpart associated with the active centromere and even before several euchromatic regions in the genome. These results point to a possible mechanism by which dicentric chromosomes segregate equationally.

Key words. Heterochromatin; dicentrics; DNA replication; L-cells; mouse L-cells; anti-BRdU antibody.

Certain mouse, rat and human cell lines have several dicentric and multicentric chromosomes. These chromosomes undergo orderly segregation in spite of the existence of more than one centromere along their length. It has been demonstrated that all but one of the centromeres in the dicentrics or multicentrics separate prematurely during prophase/early metaphase. This premature separation is related to the inactivity of the 'accessory' centromere [1–3]. By use of antikinetochore antibody found in the serum of scleroderma patients we demonstrated that the permanent dicentric and multicentric chromosomes in mouse carry only one kinetochore located at the site of the centromere which separates during late metaphase [4, 5]. It was therefore postulated that premature separation of a centromere precludes its binding to microtubules. However, this does not explain why premature centromere separation occurs. This report shows that premature separation is related to early or premature replication of the pericentric heterochromatin.

All genomes so far analyzed show a definite sequence of centromere separation. This sequence is dependent upon the