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In vivo Sister Chromatid Exchange Frequencies in Fetal & Adult Cells of Mouse

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Sister chromatid exchange (SCE) frequencies *in vivo* in bone marrow and spleen cells of the adult and in the liver cells of fetal mice have been determined by bromodeoxyuridine (BrdU)—Giemsa staining technique. Analysis of SCEs in second cycle metaphases revealed that the mean SCE frequencies in adult bone marrow and spleen cells are 4.24 SCE/cell and 5.48 SCE/cell, respectively. In comparison, the fetal liver cells from the same animals show only 1.53 SCE/cell. It appears that the differences in the SCE frequencies in adult and fetal cells *in vivo* are due to different dosages of BrdU available to adult and fetal cells on account of placental barrier so that while in fetal cells the basal level of spontaneous SCEs is seen, in the adult tissues some SCEs are induced by BrdU itself.

IN view of the high potential of sister chromatid exchange (SCE) frequencies in monitoring genetic damage induced by various agents[1], the 5-bromodeoxyuridine (BrdU)—Harlequin technique has been widely applied *in vitro*; and recently several studies have been made to apply this technique to detect spontaneous and induced SCEs in mammalian cells *in vivo*[2-6]. These and other studies have established that a certain basal level of SCEs occur spontaneously *in vivo* as well as *in vitro*. However, different studies have provided a varying estimate of spontaneous *in vivo* SCE frequency in same or different cell types of different mammals[2-6]. The results of the present study show that the *in vivo* spontaneous SCE frequencies in fetal and adult cell types of the same animal may differ considerably and such differences are presumably related to different dosages of BrdU available to different cell types *in vivo*.

Two female house mice in advanced stages of pregnancy were given eight hourly intraperitonial injections of BrdU (Sigma) dissolved in water (3 mg/ml)[2]. The volume of every injection was adjusted to give 0.06 mg BrdU/g body wt of the animal every hour. Fourteen hours after the last BrdU injection, colchicine (5 μg/20g body wt) was injected and 2.5 hr later the animals were sacrificed by cervical dislocation. Bone marrow and spleen from the adult and liver tissue from the fetuses of each animal were taken out. Chromosome preparations from these adult and fetal tissues were made in the usual manner. Hoechst 33258 stained and sunlight exposed[7] slides were stained with Giemsa to obtain differential staining of unifilarly- and non-BrdU-substituted sister chromatids[2,3]. The frequencies of SCEs in second cycle metaphases in adult bone marrow and spleen and in fetal liver preparations were scored.

Among the 136 second cycle metaphases examined from fetal liver preparations of the two animals, the mean SCE frequency was found to be 1.53/cell. In 48 of these fetal metaphases, no SCE was detected (Fig. 1a) while in 81 cells the SCEs varied between 1 and 4/cell and in the remaining few cells there were 5 to 8 SCEs. In contrast, the adult bone marrow (60 cells) and spleen (134 cells) show a higher incidence of SCEs with mean values being 4.24 SCE/cell and 5.48 SCE/cell, respectively. Majority of the adult metaphase cells had more than 3 SCEs/cell (Fig. 1b).

The low SCE frequency in fetal cells in comparison with the adult cells of the same individuals observed in the present study may be either because of basic physiological differences in the adult and fetal cells with respect to occurrence of SCEs or these differences may be related to different dosages of BrdU received by the different cell types. The later possibility, which seems likely, would imply that the injected levels of BrdU may by themselves induce some SCEs in the adult cells but not in the fetal tissue. This seems likely since in bone marrow cells of adult rats, Tice *et al.*[4] have reported a low *in vivo* SCE frequency (1.5/cell), which is comparable to that observed in the fetal cells here. These authors have further shown that increasing the level of administered BrdU causes an increase in *in vivo* SCE frequency. Therefore, it would appear that the relatively high fequencies of SCEs observed in the adult cells in the present study may include BrdU-induced SCEs. In this context it may be noted that Mazrimes and Stetka[8] have provided evidence for the incorporated BrdU to directly induce SCEs and have further shown that the BrdU induced SCEs are proportional to the degree of BrdU substitution. The placental barrier may transport a lesser level of BrdU to fetal cells and therefore the SCEs observed in this tissue are of the same order as detected by Tice *et al.*[4] in adult bone marrow cells with the lowest BrdU dosages. It may be noted here that Basler[6] has reported equally high SCE frequencies (3.3 to 4.1/cell) in adult bone marrow and in fetal liver cells of Chinese hamster. However in that study, a 50 mg tablet of BrdU was implanted subcutaneously in the animal. This amount is much higher than the cumulative dose of BrdU injected in the mice in present experiments and therefore, a higher dosages of BrdU may have been available to fetal cells in Basler's study, which in turn would cause a higher SCE frequency. It may also be noted that the *in vivo* SCE frequency observed in spermatogonial cells of mouse is also lower (1.3 to 1.8/cell) than the SCE frequencies noted either in adult bone marrow[2] or in tumor cells[5]. Again it seems that the low SCE frequency in gonial cells may be related to a poor transport of BrdU across the testes sheath.

Finally it may be said that the factors like differential transport of BrdU or the test chemicals should be considered when evaluating the mutagenic potential by *in vivo* BrdU-Harlequin SCE technique.

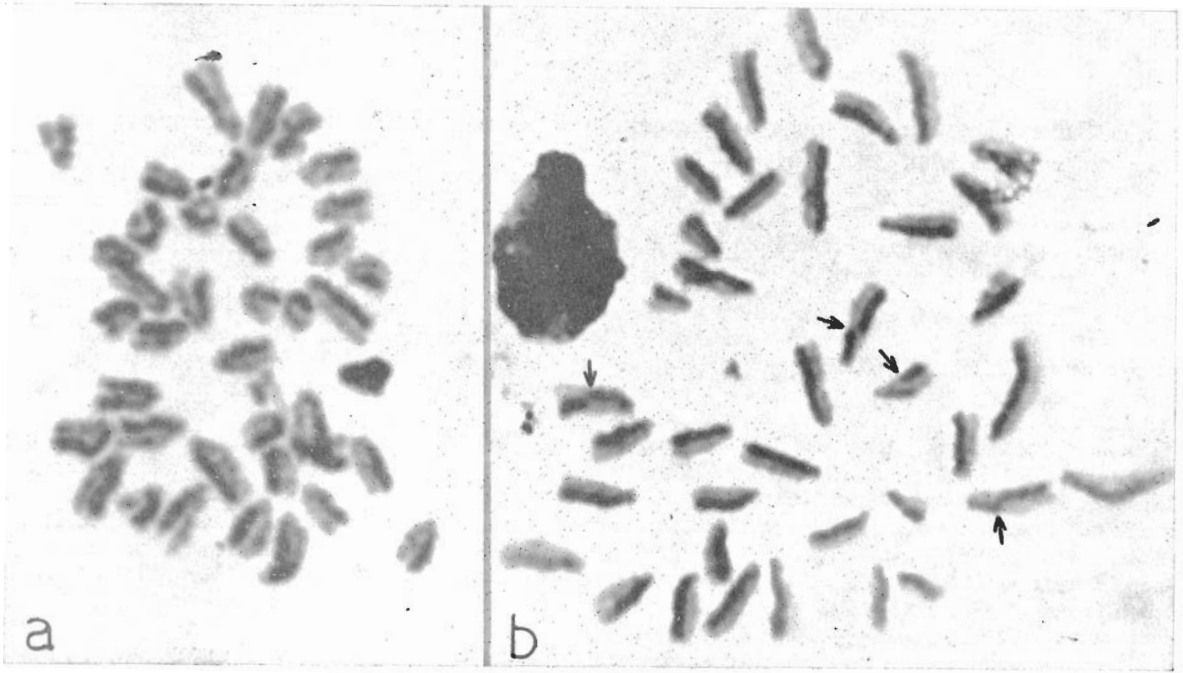

Fig. 1 — BrdU-Giemsa stained metaphase plates from fetal liver (a) and from adult spleen (b). Arrows indicate the points of sister chromatid exchange, × 2400

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