3. Cytogenetic Studies on the Indian Spiny Mouse, *Mus platythrix*. I

Frequency of Sister Chromatid Exchanges in Lung Primary Cultures^{*)}

By Toshihide H. YOSIDA and Toru INOUE^{**)}

National Institute of Genetics, Misima

(Communicated by Sajiro MAKINO, M. J. A., Jan. 12, 1981)

It has been shown that the karyotype of the Indian spiny mouse (*Mus platythrix*) is characterized by 12 acrocentric autosome pairs and XY sex chromosomes, and further that several pairs of the chromosome complement of this animal would be produced as a result of tandem fusion of certain acrocentric chromosomes in the house mouse (*M. musculus*) (Yosida 1979, 1980a). Further, it was revealed that the total length of chromosomes of the Indian spiny mouse showed about 12.8% less in comparison with that of the house mouse due probably to the deletion of centromeres and telomeres. The number of sister chromatid exchanges (SCEs) of the house mouse in the presence of 0.5 $\mu\text{g/ml}$ BrdU is reported to be $8.5 \pm 0.4/\text{cell}$ in the second cell cycle (Kato 1977a). Since there is no information on the SCE value for the Indian spiny mouse, the present authors undertook a study along this line, particularly on the number of SCEs of the Indian spiny mouse under the treatment with several concentrations of BrdU, in comparison with that of the house mouse.

Material and methods. The study was carried out with lung primary cell cultures of the Indian spiny mice which were maintained in our laboratory. Exponentially growing cells were treated with BrdU at several concentrations (0.1 to 25.0 $\mu\text{g/ml}$) in the dark for 24 to 36 hrs. Colcemid was added one hour prior to fixation. Chromosome preparations were proceeded according to the conventional air-drying method and stained with Giemsa solution following the PFG method (Perry and Wolff 1974) modified by Kato and Sandberg (1977).

Results. 1) *Examination of inoculation time with BrdU.* To know the most effective inoculation times for labeling of BrdU at the second cell cycle, the lung cells growing *in vitro* were treated with

^{*)} Dedicated this paper to the author's colleague, late Dr. H. Kato, in the third anniversary of a death. Contribution no.1350 from the National Institute of Genetics, Japan. Supported by a grant-in-aid for the scientific research from the Ministry of Education, Science and Culture (nos. 339025, 501001).

^{**)} Present address: The College of Dairying, Nopporo, Hokkaido.

5 $\mu\text{g/ml}$ BrdU for 24, 30 and 36 hrs. The results were presented in Table I. As seen in the table the highest frequency of labeled cells with BrdU at the stage of the second cell cycle was found to occur at 30-hrs inoculation (72.2%) (Fig. 1). Then, the following experiment was carried out on 30-hrs inoculation samples with BrdU.

Table I. Frequencies of successive mitoses labeled with BrdU in cells of lung cultures in the Indian spiny mouse

Fixation time (hrs after 5 $\mu\text{g/ml}$ BrdU inoculation)	Frequencies of successive mitoses			Total no. of cells observed
	First	Second	Above third	
24	33(43.4%)	36(57.6)	—	69(100.0)
30	4(5.1)	57(72.2)	18(22.7)	79(100.0)
36	1(1.5)	12(18.2)	53(80.3)	66(100.0)

Fig. 1. Sister chromatid exchanges by BrdU treatment and Giemsa staining in a lung cultured cell of the Indian spiny mouse.

Table II. Number of SCEs in cells of lung cultures in the Indian spiny mouse treated with different doses of BrdU (30-hrs inoculation)

Doses of BrdU ($\mu\text{g/ml}$)	No. of cells counted	No. of SCEs/cell			
		Mean	Variance	S. D.	S. E.
0.1	25	7.6	14.1	3.76	0.75
0.25	40	10.2	30.3	5.50	0.87
0.5	50	11.1	32.0	5.66	0.80
1.0	50	11.3	18.6	4.31	0.61
2.5	50	13.3	23.5	4.85	0.68
5.0	50	13.0	27.5	5.24	0.74
10.0	50	13.7	19.5	4.42	0.63
25.0	50	18.1	43.6	6.60	0.93

2) *Dose effect of BrdU for SCEs.* To find out the dose effect for SCEs in the cultured cells of the Indian spiny mouse, BrdU was used at dose levels from 0.1 to 25.0 $\mu\text{g}/\text{ml}$. As seen in the Table II and Fig. 2 the number of SCEs increased gradually from 7.6 ± 0.75 to 18.1 ± 0.93 , but the curve became flat in the samples under the dose of 0.25 to 1.0 $\mu\text{g}/\text{ml}$. The occurrence of SCEs in the dose of 0.5 μg BrdU was considered as of spontaneous frequency in the house mouse (Kato 1977). In the Indian spiny mouse the spontaneous occurrence of SCEs seems to be accepted as 11.1/cell at the dose of 0.5 μg BrdU.

Fig. 2. Frequency of sister chromatid exchanges in lung cultured cells of the Indian spiny mouse treated with various doses of BrdU (30-hrs inoculations).

Remarks. By comparison of the G-band karyotypes between the Indian spiny and the house mice the present author (Yosida 1979, 1980a) suggested that the karyotype of the former species was derived due to the tandem fusion of some chromosome pairs of the latter species. According to the author, the loss of the total length of the Indian spiny mouse was about 12.8% in comparison with that of the house mouse, probably due to the delation of centromere and telomeres of the latter species by the tandem fusion.

The spontaneous occurrences of SCEs of the lung cultured cells have suggested that the distribution of SCE within chromosomes in the house mouse (*Mus musculus musculus*) under the treatment with 0.5 $\mu\text{g}/\text{ml}$ BrdU was 8.58/cell (Kato 1977), while in the Indian spiny mouse it was estimated as 11.1/cell at the same dose of BrdU following the same procedure (Table II). The total chromosome length of the

house mouse is longer than that of the Indian spiny mouse, whereas the spontaneous occurrence of SCEs in the former species is lower than that of the latter. The spontaneous occurrence of SCEs was suggested to be random and it was proportional to the length of metaphase chromosomes (Dutrillaux *et al.* 1974; Geard 1974; Ikushima and Wolff 1974; Latt 1974; Galloway and Evans 1975; Kihlman and Kronborg 1975). In contrast, there were many investigators who emphasized nonrandomness, and further that there are some discrepancies on the occurrence of SCEs by investigators. The region of constitutive heterochromatin has less SCE rate than that of the euchromatin (Carrano and Wolff 1975; Hsu and Pathak 1976; Hoo and Parslow 1979; Schneider *et al.* 1980). According to some authors, the heterochromatin has higher SCE rate than the euchromatin (Natarajan and Schmid 1971; Natarajan and Klášterská 1975). On the other hand, the preferential SCEs were found to occur at the heterochromatin and euchromatin junctions (Carrano and Wolff 1975; Bostock and Christie 1976; Kato 1979).

The distinct heterochromatic C-bands have been shown in the centromeric regions in almost all chromosomes of the house mouse (Chen and Ruddle 1971; Hsu *et al.* 1971; Takagi 1971; Forejt 1973; Dev. *et al.* 1975; Miller *et al.* 1976), whereas the C-bands were very small and unclear in all chromosome pairs in the Indian spiny mouse (Yosida 1970, 1980a). The higher occurrence of the spontaneous SCE in the Indian spiny mouse can be explained by the less constitutive heterochromatin in this species than in the house mouse. Yosida (1980b), one of the present authors, is of opinion that the differentiation of inner-structure of chromosomes involving the different distribution of the constitutive heterochromatin is significantly associated with karyotype evolution and species differentiation. The occurrence of quantitative difference of the spontaneous SCE between the Indian spiny mouse and the house mouse seems to be a good example for the above consideration.

Summary. Sister chromatid exchanges (SCEs) in the lung cultured cells of the Indian spiny mouse were examined at several concentrations of BrdU. The spontaneous occurrence of SCEs in this animal was determined to be 11.1/cell at the dose of 0.5 μ g BrdU. The frequency value was slightly higher than that of the house mouse. This may be due to less C-band heterochromatin in the Indian spiny mouse than in the house mouse.

Acknowledgment. The authors are grateful to Emeritus Professor Sajiyo Makino, M. J. A., for revision of the manuscript with keen interest.

References

- Bostock, C. J., and Christie, S.: *Chromosoma (Berl.)*, **56**, 275-287 (1976).
Carrano, A. V., and Wolff, S.: *ibid.*, **53**, 361-369 (1975).
Chen, T. R., and Ruddle, F. H.: *ibid.*, **34**, 51-72 (1971).
Dev, V. G., Miller, D. A., Tantravahi, R., Schreck, R. R., Roderick, T. H., Erlanger, B. F., and Miller, O. J.: *ibid.*, **53**, 335-344 (1975).
Dutrillaux, B., Fosse, A. M., Prieur, M., and Lejuene, J.: *ibid.*, **48**, 327-340 (1974).
Forejt, J.: *ibid.*, **43**, 187-201 (1973).
Galloway, S. M., and Evans, H. J.: *Cytogenet. Cell Genet.*, **15**, 1-16 (1975).
Geard, C. R.: *Mutat. Res.*, **23**, 67-78 (1974).
Hoo, J. J., and Parslow, M. I.: *Chromosoma (Berl.)*, **73**, 67-74 (1979).
Hsu, T. C., Cooper, J. E. K., Mace, M. L., and Brinkley, B. R.: *ibid.*, **34**, 73-87 (1971).
Hsu, T. C., and Pathak, S.: *ibid.*, **58**, 269-273 (1976).
Ikushima, T., and Wolff, S.: *Exp. Cell Res.*, **87**, 15-19 (1974).
Kato, H.: *Intern. Rev. Cytology*, **49**, 55-97 (1977).
——: *Chromosoma (Berl.)*, **74**, 307-316 (1979).
Kato, H., and Sandberg, A. A.: *Exp. Cell Res.*, **109**, 423-427 (1977).
Kihlman, B. A., and Kronborg, D.: *Chromosoma (Berl.)*, **51**, 1-10 (1975).
Latt, S. A.: *Science*, **185**, 74-76 (1974).
Miller, D. A., Tantravahi, R., Dev, V. G., and Miller, O. J.: *Genetics*, **84**, 67-75 (1976).
Natarajan, A. T., and Klášterská, I.: *Hereditas*, **79**, 150-154 (1975).
Natarajan, A. T., and Schmid, W.: *Chromosoma (Berl.)*, **33**, 48-62 (1971).
Perry, P., and Wolff, S.: *Nature (Lond.)*, **251**, 156-158 (1974).
Schneider, N. R., Chaganti, R. S. K., and German, J.: *Chromosoma (Berl.)*, **77**, 379-389 (1980).
Takagi, N.: *Japan. J. Genet.*, **46**, 361-363 (1971).
Yosida, T. H.: *Proc. Japan Acad.*, **55B**, 270-274 (1979).
——: *Cytologia (in press)* (1980a).
——: *Cytogenetics of the Black Rat—Karyotype Evolution and Species Differentiation*. Univ. Tokyo Press, Tokyo, and Univ. Park Press, Baltimore, 256 pp. (1980b).