Sister Chromatid Exchanges (S.C.E.) in Normal and Abnormal Bovine (*Bos taurus* L.) Karyotypes¹

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The addition of BUDR (5-bromodeoxyuridine), a thymine analogue, to cell cultures causes various chromosomic changes, depending on the length of treatment and when the BUDR is incorporated into the DNA molecule. A short BUDR treatment of cells in the second half of the S-phase causes symmetrical chromosome segmentation (Zacharov *et al.* 1971), while prolonged contact over two division cycles induces sister chromatid asymmetry (Zacharov and Egolina 1968). In the first case, chromosomes may be individually recognized due to segmentation specificity (Dutrillaux 1973), while asymmetry accompanied by differential staining permits observation of sister chromatid exchanges (Latt 1973).

The replication method (Villard and Latt 1976, Nadan *et al.* 1976) and DNA polarity (Lin and Davidson 1974) can be equally studied by BUDR-induced asymmetry and exchange frequency serves as an indicator of the mitogenic activity of some chemical products (Allen and Latt 1976).

The pattern and frequency of chromatid exchange have been studied up to now in normal or pathological cases in man and in some laboratory species as the mouse (Lin *et al.* 1974, Hsu and Markvong 1975). In this report, we present the distribution and frequency of chromatid exchange in karyotypes of normal *Bos taurus* L., and in a carrier of a Robertsonian-type translocation.

Material and methods

Eighteen animals were used in this study, 17 of which had a normal karyotype ($2n=60$, XY) and one presented centric fusion between the largest and probably the smallest chromosome (59, XY, t^+), relatively frequent in several cattle breeds (Popescu 1975).

Chromosomic preparations were made as usual according to the method of Grouchy *et al.* (1964) from whole blood. They were cultured 70 hours and BUDR was added to the medium at a final concentration of 200 $\mu\text{g/ml}$ for the whole culture time.

To show exchange, we used Wolf and Perry's method (1974). The slides stained 15 min in a 5% solution of Hoechst Fluorochrome 33258 were mounted in water and exposed to daylight. Twenty-four hours later they were unmounted, incubated 2 hours at 65°C in a salt-citrate solution ($2\times\text{SSC}$) and then stained with

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Fig. 1. Metaphase (a) and karyotype (b) of a normal cell with sister chromatid exchange.

Giemsa. Exchanges were studied on the photos taken with a Leitz Ortholux microscope in normal daylight.

Results

After two cell cycles in presence of BUDR, the chromosomes present a very dark chromatid, intensely stained with Giemsa, and another slightly longer and weakly stained (Figs. 1a and b). The first, appearing under fluorescence after intense acridine green staining (Dutrillaux *et al.* 1974), incorporated the BUDR in a single DNA strand (T-B). The other chromatid, weakly stained with Giemsa and pale green under fluorescence, fixed BUDR on the two DNA strands (B-B).

Table 1. Chromatid exchange frequency in normal cattle karyotypes and in the carrier of 1/29 centric fusion

Karyotype	No. of cells studied	No. of chromosomes studied	Total no. of exchanges	Exchange mean per cell	Exchange mean per chromosome	Exchanges between homologous chromosomes	Quadriradial formation
Normal	52	2974	602	10.37	0.202	7	1
Aberrant	8	452	130	16.26	0.287	—	—
Total	60	3426	732	12.2	0.213	7	1

From all the cells studied we recorded a total of 732 sister chromatid exchanges, or a mean of 12.2 exchanges per cell (Table 1). The mean number of exchanges per chromosome, depending on the number of exchanges per cell, is 0.21.

An analysis of variance done on 11 normal animals to compare their mean number of exchanges per cell shows a significant difference between individuals at the 0.5% threshold ($F=3.41$ for 10 and 35 DF).

Bos taurus autosomes are all acrocentric and thus difficult to identify. However, there are more exchanges on large-sized chromosomes.

The localization of chromosome exchange is very variable because it occurs all along the chromatid. The exchanges observed are found in various sites along the chromosome; slightly more are seen in the telomeric regions.

Exchanges between homologous chromosomes are rarer; we only saw 7 on all the chromosomes studied (Figs. 2b, c and d.). A quadriradial formation (Fig. 2a) was identified in one animal presenting more than four exchanges between homologous chromosomes on eight cells studied.

The animal carrying 1/29 centric fusion had a mean number of 16 (26 exchanges per cell), comparable to those of some normal animals. On the fused chromosome, we observed from 0 to 4 exchanges on various cells, generally located on the long arm corresponding to the largest chromosome of the complement (Figs. 3a-d).

Discussion

The differential staining of chromosomes after two cell cycles in presence of BUDR has not yet been satisfactorily explained. Latt (1973) thinks the fluorescence

Fig. 2. a, quadriradial formation (shown by arrow) in a normal cell. b, c and d, homologous chromosome exchange from 3 different cells.

of Hoechst fluorochrome 33258 decreases 20% when bound to DNA in which BUDR is substituted for thymine. However, this cannot explain the reduced affinity of this same B-B chromatid for Giesma. Thus, Goto *et al.* (1975) and Sugiyama *et al.* (1976) believe it is due to rapid DNA photolysis in the B-B chromatid; its mechanism, however, is unknown.

Sister chromatid exchange was seen for the first time by Taylor *et al.* (1957) using autoradiography after tritiated thymidine incorporation. Based on sub-

Fig. 3. a, metaphase carrying 1/29 centric fusion. Abnormal chromosome is shown by arrow. b, c and d, abnormal chromosome in 3 different cells.

stituting BUDR for thymine, the method proposed by Latt (1973) and improved by Wolff and Perry (1974) seems better than autoradiography; it is simpler and permits more detailed analysis.

The mean exchange frequency per cell in man varies rather widely from one individual to another. While several authors found a mean of 14 exchanges per cell (Latt 1973, Kim 1974, Chaganti *et al.* 1974, Galloway and Evans 1975), others find 4 or 5 (Beek and Obe 1975, Sperling *et al.* 1976), 8 (Sperling *et al.* 1975), 12 (Latt 1974) or 27 (Dutrillaux *et al.* 1974). The mean frequency of 12.2 (1.5–31) in cattle is similar to that often found in man. However, the *F* value shows rather wide variation between individuals. Homologous chromosome exchanges are rare in normal subjects, but their frequency increases in individuals presenting the Bloom syndrome who also show increased sister chromatid exchange frequency (German *et al.* 1974, Chaganti *et al.* 1974, Sperling 1976). Among homologous chromosome exchanges observed in this syndrome, some have a very characteristic quadriradial configuration (Chaganti *et al.* 1974). In the animals studied we found a very low homologous chromosome exchange frequency with the exception of the animal which had 5 and thus a quadriradial configuration. The frequency of sister chromatid exchange in that animal was 15.2 per cell.

The biological role of homologous chromosome exchange, which can be interpreted as mitotic crossing-over, is unknown but it may furnish interesting data on nucleus organization at interphase.

Homologous chromosome exchange leads us to think that some chromosomes are drawn together, or even paired off, at some time during the interphase (Vogel and Schroder 1974). A better knowledge of interphasic order, which can be studied by this technique, would be useful for understanding the formation of some abnormalities as Robertsonian translocations.

Exchange frequency does not seem altered on the chromosome of the 1/29 translocation. In fact, this chromosome, resulting from fusion of the largest autosome with the smallest, seems to have a number of exchanges in proportion to its size and comparable to its free homologues.

Summary

Sister chromatid exchanges are studied in 17 normal animals and one carrier of 1/29 centric fusion. A mean of 12.2 exchanges per cell is found on the total animals studied. An analysis of variance done on 11 normal subjects to compare their mean number of exchanges per cell shows a significant individual difference at the 0.5% threshold. Homologous chromosome exchanges are very rare with the exception of one animal which had 4 on 8 cells studied and a quadriradial formation. The fused chromosome presents a variable number of exchanges comparable to those of free homologues.

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