ON THE NATURE OF SISTER-CHROMATID EXCHANGES IN 5-BROMODEOXYURIDINE-SUBSTITUTED CHROMOSOMES

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ABSTRACT

Sister-chromatid exchanges (SCE) were studied in Allium cepa L. meristematic cells at the second and third divisions after BrdUrd-substitution during just the first or during the second and third cycles, respectively. The observed SCE nonreciprocal/reciprocal ratios detected at the third division in both experiments, as well as comparison of the lowest SCE frequency observed per cycle and expressed per picogram of DNA with data from different species expressed accordingly, strongly suggest that most of the exchanges detected in BrdUrd-substituted chromosomes are BrdUrd-dependent events. Hypotheses suggesting some different mechanisms are discussed to explain the formation of these BrdUrd-dependent SCEs.

Although SCEs were inferred by McClintock (1938) while studying the behavior of ring chromosomes in maize, the first unequivocal visual evidence of their occurrence was provided by Taylor, Woods and Hughes (1957) in radioautographs of well-spread chromosomes at the second division after H3-Thd incorporation. Twenty years have elapsed and the question as to whether SCEs occur spontaneously, or are induced by the treatments required to differentiate between sister chromatids, still remains open (Kato 1977a; Wolff 1977). Both H3-Thd and BrdUrd are known to increase the yield of SCEs under certain circumstances (Brewen and Peacock 1969; Gibson and Prescott 1972; Kato 1974; Latt 1974a; Wolff and Perry 1974; Lambert et al. 1976; Miller, Aronson and Nichols 1976; Schwartzman and Cortés 1977; Mazrimas and Stetka 1978), and the ring chromosome behavior leading to the inference of SCEs may well be interpreted, at least partially, as a consequence of switches in polarity of chromosomal DNA (Wolff, Lindsley and Peacock 1976). In spite of the extensive research of SCE phenomena undertaken during the last few years, a general estimation of the “spontaneous levels” of SCEs, if they occur, remains a difficult problem (Wolff 1977).

Since the analysis of SCEs in the chromosomes of cells at the third division after the beginning of DNA labelling permits a more accurate calculation of

1 This paper is dedicated to the memory of Prof. Hatao Kato, who largely contributed to the study of sister-chromatid exchanges.

2 Abbreviations used in text: SCE = sister-chromatid exchange; BrdUrd = 5-bromodeoxyuridine; H3-Thd = tritiated thymidine; Thd = thymidine; FPG = fluorescence plus Giemsa; FdUrd = 5-fluorodeoxyuridine; Urd = uridine.

First interphase with BrdU

First mitosis

Second interphase with Thd

Second mitosis

Third interphase with Thd

Third mitosis

First interphase with BrdU

First mitosis

Second interphase with BrdU

Second mitosis

Third interphase with BrdU

Third mitosis
the yield of SCEs per cycle (Gead 1974; Tice, Chaillet and Schneider 1975; Miller, Aronson and Nichols 1976), we decided to estimate the frequency of SCEs in Allium cepa L. meristematic cells that had completed two and three consecutive cycles in the presence of BrdUrd, as well as in cells that had completed only the first treated cycle with BrdUrd and were then exposed to unlabelled Thd for the next one or two cycles. As the number of BrdUrd-substituted polynucleotide chains per chromosome should vary in each cycle in both experiments, this could be a useful method of determining whether the SCEs detected in BrdUrd-substituted chromosomes occur spontaneously (Kato 1974; Tice, Chaillet and Schneider 1975) or are induced, the yield in each cycle depending upon the number of equally BrdUrd-substituted polynucleotide chains.

**Rationale**

That DNA segregation in sister chromatids occurs following a semi-conservative pattern has been extensively demonstrated in different organisms (see reviews by Kato 1977a; Wolff 1977). Additionally, Kato (1977b) and Schwartzman and Cortes (1977) demonstrated that in the same material a differential staining of sister chromatids can be achieved between bifilarly BrdUrd-substituted and unifilarly BrdUrd-substituted chromatids, as well as between unifilarly BrdUrd-substituted and unsubstituted ones. By means of the FPG staining technique, the lesser BrdUrd-substituted chromatid stains more deeply than the more BrdUrd-substituted one (Wolff and Perry 1974), so that if cells complete only the first replication period with BrdUrd, third division chromosomes would show three out of four chromatids deeply stained, (Figure 1A) while they would only show one out of four chromatids preferentially stained if they underwent the three cycles in the presence of BrdUrd (Figure 2A).

SCEs result from the breakage and reunion of the four polynucleotide chains, and their reunion occurs only between those showing the same polarity (Taylor, Woods and Hughes 1957). Taking these facts into account and assuming different hypotheses on the nature of SCEs, predictions can be made regarding the SCE frequencies that would be expected in the second and third-division

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**Figures 1 and 2.**—Diagrams of expected BrdUrd-labeling patterns and chromosome differential staining of sister chromatids throughout three consecutive division cycles, where only the first replication round occurs in the presence of BrdUrd (Figure 1), and where all three replication periods occur in its presence (Figure 2). Both diagrams are based on the uninemic model of chromosome structure and the semi-conservative pattern of DNA replication and segregation. Bars represent polynucleotide chains immediately after DNA replication (continuous = unsubstituted; discontinuous = BrdUrd substituted). Arrows indicate the occurrence of SCEs. (A) when no exchange occurred. (B) shows that approximately only half the exchanges that occur at the third cycle could be detected in every case. This would not apply when cells underwent only the first cycle with BrdUrd, if exchanges are assumed to be BrdUrd-dependent events, because in such a case every exchange should be detected. (C) and (D) show different SCE frequencies and the nonreciprocal/reciprocal ratios expected assuming that exchanges are spontaneous events (C), as well as if SCEs are assumed to be BrdUrd-dependent events (D). At the third division, white triangles point to reciprocal SCEs, while black triangles indicate nonreciprocal exchanges.
chromosomes in the case that only the first replication round occurred in the presence of BrdUrd (Figure 1C and D), as well as if cells undergo the three replication periods with this Thd analogue (Figure 2C and D). Third division chromosomes would show two different types of SCEs, those detected as "reciprocal" would be the ones formed during the last cycle, while those appearing as "nonreciprocal" would have been produced during either the first or second cycles (Figures 1 and 2).

If SCEs are spontaneous events, their frequency should be independent of whether cells developed one or three replication rounds in the presence of BrdUrd. On the other hand, if SCEs are BrdUrd-dependent events, their frequency would be expected to vary according to the number of replication periods that cells developed in the presence of BrdUrd. These two hypotheses may be experimentally tested by comparing the SCE frequencies observed at the second and third divisions in each set of experiments, as well as by comparing the SCE nonreciprocal/reciprocal ratios detected in the third-division chromosomes in each case.

If it is assumed that exchanges are spontaneous events, the SCE frequencies detected at the second and third divisions would be expected to be the same in both sets of experiments (Figure 1C and Figure 2C). Also, since the yield of SCEs would be the same in each cell cycle and only half the exchanges that would have occurred during the last cycle would have been detected (Figure 1B and Figure 2B), the SCE nonreciprocal/reciprocal ratio observed in the third division chromosomes would be 4.0:1 in both sets of experiments (Figure 1C and Figure 2C).

On the other hand, if exchanges are assumed to be BrdUrd-dependent events, the SCE frequencies detected at the second and third divisions should be significantly lower in those cases in which cells completed only one cycle with BrdUrd. In these cases, the third-division chromosomes would show all the exchanges that had taken place throughout the three cycles, since no exchange would have occurred in unsubstituted chromosome segments (Figure 1B). In this case, the SCE nonreciprocal/reciprocal ratio would be 6.0:1 (Figure 1D). But in the third-division chromosomes of cells that completed the three cycles in the presence of BrdUrd, again approximately only half the exchanges that had occurred during the third cycle would be detected (Figure 2B); the SCE nonreciprocal/reciprocal ratio would then be about 3.3:1 (Figure 2D).

MATERIALS AND METHODS

Root meristems of *Allium cepa* were employed. The bulbs, 15 to 30 g in weight, were grown in the dark at a constant temperature of 25 ± 0.5° in cylindrical receptacles of about 80 ml capacity in tap water renewed every 24 hr and continuously aerated by bubbling air at a rate of 10 to 20 ml per min. The bulbs were so placed that only their bases remained submerged, and all treatments began when roots reached a length of 15 to 20 mm. The cell cycle duration under these growing conditions was estimated according to GONZALEZ-FERNANDEZ, LOPEZ-SAEZ and GIMENEZ-MARTIN (1966). BrdUrd substitution was performed by exposing the growing roots to a treatment solution containing $10^{-4} \text{M} \text{BrdUrd}$, $5 \times 10^{-8} \text{M} \text{FdUrd}$ and $10^{-6} \text{M} \text{Urd}$ throughout one or more cycle times. This solution, when necessary, was renewed every 24 hr.
In those cases in which Thd incorporation was pursued after the roots had been exposed to the treatment solution throughout one cycle time, the roots were exposed to $10^{-4}$ M Thd and $10^{-6}$ M Ur for the following one or two cycle times. Before fixing in ethanol-acetic acid (3:1) overnight, most roots were treated with 0.05% colchicine for three hr in order to accumulate cells in metaphase, while the others were exposed to $2 \times 10^{-3}$ M 8-hydroxyquinoline for four hr without aeration. The culture receptacles and the treated bulbs always remained protected from light by wrapping them in aluminum foil. Root tips were squashed after one hr treatment with 0.5% pectinase (Sigma, from Aspergillus niger) dissolved in citrate buffer adjusted to pH 4.2, at $37^\circ$. Coverslides were removed by the dry ice method, and preparations were hydrated by passing them through ethanol (100%, 96%, 70%, 50%, 30%) and distilled water. Then, slides were incubated with RNase (Sigma, Ribonuclease-A from bovine pancreas) at 25° for two hr.

The FPG staining technique was performed as described by Schvartzman and Cortes (1977) with slight modifications according to Goto et al. (1978). Slides were washed with a 50% SSC (sodium saline citrate) and treated with 33258 Hoechst for 0.5 hr at room temperature. The 33258 Hoechst solution was prepared by dissolving one mg of the fluorochrome in one ml of ethanol, 0.2 ml of this solution was added to 100 ml of 50% SSC. Then, slides were washed with McIlvaine buffer adjusted to pH 8.0, coverslides were applied and sealed with nail varnish, and the preparations were exposed to a fluorescent sun lamp radiating in the 280-380 nm band (Westinghouse FS20) at 10 cm distance on a hot plate at 30° for one hr. Afterwards, coverslides were removed and preparations were treated at 55° with the same McIlvaine buffer for one more hr. Finally, after several washes with phosphate buffer adjusted to pH 6.8, slides were stained with 3% Giemsa in the same phosphate buffer for nine min, washed again, air dried and mounted with Euparal.

RESULTS

Since all 16 chromosomes making up the karyotype of Allium cepa are similar in length and shape, and only the satellized pair can easily be recognized (Figure 3), SCE frequencies were estimated per chromosome. From this figure, the number of exchanges per genome or per picogram of DNA were calculated, assuming an equal DNA content for every chromosome of the complement.
Differential staining of sister chromatids was not detected in either set of experiments involving the chromosomes of cells at the first division after DNA labelling. The chromosome morphological patterns, obtained in cells at the second division in both sets of experiments, (Figure 4Aa) clearly show that DNA segregation occurs in *Allium cepa* chromosomes according to the semi-conservative pattern. This fact is confirmed by examining chromosomes of cells at the

**Figure 4.**—Harlequin-like chromosomes of *Allium cepa* stained by means of the FPG technique. (Aa) at the second division after DNA-substitution with BrdUrd throughout the two cycles. (Ab) cut-out chromosome showing a reciprocal SCE. (Ba) at the third division after DNA-substitution with BrdUrd only during the first cycle. (Bb) cut-out chromosome showing reciprocal as well as nonreciprocal SCEs. (Ca) at the third division after DNA-substitution with BrdUrd throughout the three cycles. (Cb) cut-out chromosome showing nonreciprocal SCEs.
third division in cases where only the first replication round occurred in the presence of BrdUrd (Figure 4Ba), as well as in cases where the three replication rounds occurred with BrdUrd (Figure 4Ca). Reciprocal and nonreciprocal SCEs can be clearly detected in the chromosomes of cells at the third division in both sets of experiments (Figure 4Bb and Figure 4Cb), while every exchange detected at the second division appears as reciprocal (Figure 4Ab).

In all the experiments, the frequency distribution of SCEs per chromosome occurs according to Poisson expectations. A comparison of the frequency distributions of SCEs per chromosome, obtained at the second division in each set of experiments, is presented in Figure 5. The mean value of SCEs per chromosome, obtained when cells underwent only the first replication round with BrdUrd, ($\bar{X} = 2.8$) differs from the one obtained when cells completed the two cycles in the presence of BrdUrd ($\bar{X} = 3.9$). Figure 6 shows the results obtained in the analysis of reciprocal and nonreciprocal SCEs observed in the third-division chromosomes in each set of experiments, respectively. The mean values of reciprocal, as well as nonreciprocal, SCEs observed in cells that completed only the first replication round in the presence of BrdUrd ($\bar{X}_{rec} = 0.4$; $\bar{X}_{nonrec} = 2.7$) again differ from those observed when cells completed the three replication peri-

![Figure 5](image-url)

**Figure 5.**—Frequency distributions of chromosomes at the second division showing different numbers of SCEs. The dotted bars represent the mean values and the horizontal marks the expected percentages derived from theoretical Poisson distributions. (A) after only the first replication period with BrdUrd. (B) after two replication rounds in the presence of BrdUrd. Data were calculated after studying 300 chromosomes in each case.
ods in the presence of BrdUrd ($\bar{X}_{rec} = 1.2; \bar{X}_{nonrec} = 3.6$). The SCE nonreciprocal/reciprocal ratio obtained in the first case is 6.7:1, while in the chromosomes of cells that developed the three replication rounds with BrdUrd, this ratio is 3.0:1. Table 1 indicates how the observed numbers compare to the expected ones, derived from both hypotheses under test. It is noted in both cases that the observed ratios agree with those calculated on the assumption that SCEs are BrdUrd-dependent events.

**DISCUSSION**

**Spontaneous or BrdUrd-dependent SCEs?** As soon at LATT (1973) and PERRY and WOLFF (1974) developed the techniques permitting an accurate visualization of SCEs after DNA-substitution with BrdUrd, it became clear that the concentration of BrdUrd in the culture medium was one of the most important factors to be considered when estimating the yield of SCEs (KATO 1974; LATT 1974a; WOLFF and PERRY 1974; LAMBERT et al. 1976). Of all these authors, only KATO (1974) maintain that BrdUrd-concentrations below some level (1.0 $\mu$g/ml) do not modify the yield of SCEs in Chinese hamster cultures. All the other
TABLE 1

Fitness of observed and expected nonreciprocal and reciprocal SCE numbers of third-division chromosomes in both sets of experiments according to the hypotheses under test

<table>
<thead>
<tr>
<th></th>
<th>Expected</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(according to the 4:1 ratio)</td>
<td></td>
<td>(according to the 6:1 ratio)</td>
</tr>
<tr>
<td>Nonreciprocal SCEs</td>
<td>744</td>
<td>810</td>
<td>797</td>
</tr>
<tr>
<td>Reciprocal SCEs</td>
<td>186</td>
<td>120</td>
<td>133</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 29.27 \quad \chi^2 = 1.48 \]
\[ d.f. = 1 \quad d.f. = 1 \]
\[ 0.01 > P \quad 0.30 > P > 0.20 \]

B. When the three replication periods occur in the presence of BrdUrd

<table>
<thead>
<tr>
<th></th>
<th>Expected</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(according to the 4:1 ratio)</td>
<td></td>
<td>(according to the 3.3:1 ratio)</td>
</tr>
<tr>
<td>Nonreciprocal SCEs</td>
<td>1080</td>
<td>1152</td>
<td>1105</td>
</tr>
<tr>
<td>Reciprocal SCEs</td>
<td>360</td>
<td>282</td>
<td>335</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 22.50 \quad \chi^2 = 2.43 \]
\[ d.f. = 1 \quad d.f. = 1 \]
\[ 0.01 > P \quad 0.20 > P > 0.10 \]

authors found, however, that the yield of SCEs is dependent upon the concentration of BrdUrd in the culture medium. In addition to the BrdUrd concentration in the culture medium, the cells concentration is also critical. Actually, it is the BrdUrd to base-pair ratio that is critical (STETKA and CARRANO 1977; MAZRIMAS and STETKA 1978).

The mechanism governing BrdUrd-dependent SCEs in cells that have not been exposed to light remains to be elucidated. If we assume, however, that only DNA-incorporated BrdUrd can act as a SCE-inducer, then, as the amount of DNA-incorporated BrdUrd per chromosome varies in each cycle (Figure 1 and Figure 2), the yield of these BrdUrd-dependent SCEs should also vary, according to the treatment to which the cells were exposed. However, TICE, CHAILLET and SCHNEIDER (1975) pointed out that, in human lymphocyte cultures, the yield of SCEs per cycle remained constant throughout three consecutive cycles in the presence of BrdUrd. WOLFF and PERRY (1975) reported that, when CHO endoreduplicated cells underwent two consecutive cycles in the presence of BrdUrd, the ratio of single to twin SCEs did not deviate from the 2:1 ratio theoretically expected on the assumption that SCEs occur with a similar frequency in each cycle. Our results clearly disagree with these observations. We found that the yield of exchanges per cell cycle varies according to the number of replication rounds that cells completed in the presence of BrdUrd. The mean numbers of observed SCEs are always lower in those cases in which cells completed only one cycle with BrdUrd (Figure 5 and Figure 6). In addition, the SCE nonreciprocal/reciprocal ratios, detected at the third division, favor, in both cases, the expected ratios calculated on the assumption that exchanges are BrdUrd-dependent events (Table 1).
One possible explanation for the discrepancy between our results, and those reported by Tice, Chaillet and Schneider (1975) and Wolff and Perry (1975), may be that, under the experimental conditions they used, BrdUrd concentration in the culture medium may have fallen with time, causing a variation in the amount of DNA-incorporated-BrdUrd in each cycle—always being lower during the last replication rounds (Kuebbing and Werner 1975; Miller, Aronson and Nichols 1976). We eliminated such a possibility by renewing the treatment solution every 24 hr.

SCE frequencies in different organisms: In order to compare SCE frequencies, reported in the chromosomes of different species, Kihlman and Kronborg (1975) suggested that the yield of exchanges should be proportional to the DNA content per cell, since the number of SCEs seems to be proportional to chromosome length (Dutrillaux et al. 1974; Ikushima and Wolff 1974; Latt 1974b). After DNA replication, cells are temporarily tetraploids; it is logical,

### TABLE 2

<table>
<thead>
<tr>
<th>Species and systems</th>
<th>DNA content per 4C metaphase (in picograms)</th>
<th>SCEs per genome after two cycles BrdUrd during one cycle</th>
<th>SCEs expressed per picogram of DNA BrdUrd during two cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human lymphocytes (in vitro)</td>
<td>46</td>
<td>12.0</td>
<td>4.1 (2)‡</td>
</tr>
<tr>
<td>Pig lymphocytes (in vitro)</td>
<td>40</td>
<td>10.0</td>
<td>7.2 (3)</td>
</tr>
<tr>
<td>Rabbit lymphocytes (in vitro)</td>
<td>44</td>
<td>12.6</td>
<td>4.4 (3)</td>
</tr>
<tr>
<td>Mouse (in vitro)</td>
<td>40</td>
<td>13.0</td>
<td>23.1 (4)</td>
</tr>
<tr>
<td>Mouse (in vivo)</td>
<td>40</td>
<td>13.0</td>
<td>3.4 (4)</td>
</tr>
<tr>
<td>Chinese Hamster (in vitro)</td>
<td>22</td>
<td>15.2</td>
<td>2.3 (5)</td>
</tr>
<tr>
<td>Chinese Hamster (in vivo)</td>
<td>22</td>
<td>15.2</td>
<td>3.3 (6)</td>
</tr>
<tr>
<td>Rat (in vivo)</td>
<td>42</td>
<td>12.2</td>
<td>1.5 (7)</td>
</tr>
<tr>
<td>Chicken embryo (in vivo)</td>
<td>78</td>
<td>4.6</td>
<td>0.7 (8)</td>
</tr>
<tr>
<td>Vicia faba (in vivo)</td>
<td>12</td>
<td>88.0</td>
<td>20.6 (9)</td>
</tr>
<tr>
<td>Allium cepa (in vivo)</td>
<td>16</td>
<td>108.0</td>
<td>44.8 (10)</td>
</tr>
</tbody>
</table>

†References: (1) Sparrow et al. 1972; (2) Beek and Obe 1975; (3) Bianchi et al. 1977; (4) Allen and Latt 1976; (5) Kato 1974; (6) Rajer 1978; Tice et al. 1976; (8) Bloom and Hsu 1975; Kihlman and Kronborg 1975; (10) This paper.

‡ Note that the lowest number reported corresponds to that of rats treated with BrdUrd in vivo for two cycles (for explanation, see text).
therefore, to consider DNA content. Table 2 compares the exchange frequencies expressed per genome and per picogram of DNA in different species and systems. Data were selected from the literature; when several authors referred to different values for the same material, we selected those reporting the smallest yield of SCEs for comparing. The lowest number of exchanges resulting per picogram of DNA is 0.12, deduced from data reported by Tice, Chaillet and Schneider (1976), in rats treated for two cycles with BrdUrd in vivo. Assuming that exchanges could have happened with a similar frequency in each cycle, 0.06 SCEs per picogram of DNA and per cell cycle would have occurred in this case. By comparing our own results, obtained at the third cycle in cells that completed only the first replication round in the presence of BrdUrd (Figure 1) with the DNA content, 0.05 SCEs would have occurred per picogram of DNA during that cycle. As we have already said, even such a low yield of SCEs seems to have been, at least partially, induced.

These observations strongly suggest that spontaneous occurrences of SCEs are significantly less frequent than generally reported, especially when estimated after DNA-substitution in vitro.

On the mechanism of BrdUrd-dependent SCE formation: After examining

![Diagram](image-url)

**Figure 7.**—Diagrams illustrating BrdUrd-labelling patterns as the DNA fork progresses in each of the situations studied, and according to some different hypotheses proposed to explain the mechanism of SCE-formation. At the upper part, arrows indicate lesions occurring only in BrdUrd-substituted polynucleotide chains. (A) expected exchanges assuming that BrdUrd-dependent SCEs occur at the replicating points. (B) expected SCEs assuming that these exchanges occur according to the Whitehouse (1963) model of genetic recombination. (C) expected exchanges assuming that BrdUrd-dependent SCEs occur according to the Meselson and Radding (1975) model of genetic recombination.
the yield of SCEs induced by fluorescent light irradiation on differentially BrdUrd-substituted chromosomes of the Chinese hamster, KATO (1977a) proposed that exchanges could arise via at least two different mechanisms: one operating at the replicating points (S-dependent) and the other acting only on the post-replicating DNA portion (S-independent). If we assume that most of the exchanges we detected are BrdUrd-dependent events, as seems to be the case, comparing the results from each set of experiments should be helpful in ascertaining whether or not these BrdUrd-dependent SCEs occur at the replicating points, and whether single-strand breaks are capable of initiating the process leading to the formation of SCEs. Figure 7 illustrates the different situations expected as the replication fork progresses. If BrdUrd-dependent exchanges are formed at the replicating points (Figure 7A), the yield of SCEs detected at the second division, in both sets of experiments, would be expected to be the same. Because we found a different frequency of SCEs in each case, it is logical to conclude that these BrdUrd-dependent SCEs were formed in the post-replicated DNA portion.

Two hypotheses have been proposed to explain the molecular mechanism leading to the formation of S-independent SCEs (Kato 1977a). The first one, based on the model proposed by Whitehouse (1963), to explain the molecular mechanism of genetic recombination, requires two breaks in complementary DNA strands to initiate the process leading to the formation of each SCE (Figure 7B). The second one, based on the model of Meselson and Radding (1975), assumes that single-strand breaks are enough to initiate the molecular process leading to the formation of each exchange (Figure 7C). Our results clearly favor this last hypothesis, since BrdUrd-dependent SCEs occurred when only one out of four polynucleotide chains appeared to be BrdUrd-substituted.

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LITERATURE CITED


Bajer, U., 1978 The in vivo induction of sister chromatid exchanges in the bone marrow of Chinese Hamster. II. N-nitrosodiethylamine (DEN) and N-isopropyl-........(2-methyl-hydrazino)-p-toluamide (NATULAN), two carcinogenic compounds with specific mutagenicity problems. Mutation Res. 56: 305-309.

Beek, B. and G. Ose, 1975 The human leukocyte test system. VI. The use of sister chromatid exchanges as possible indicators for mutagenic activities. Humangenetik 29: 127-134.


SISTER CHROMATID EXCHANGES


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