SISTER CHROMATID EXCHANGE RATES IN HUMAN TWINS

by

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A Thesis Submitted to the Faculty of the

COMMITTEE ON GENETICS (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ACKNOWLEDGMENTS

This work would not have been possible without the assistance of many contributors. I am very grateful to the Tucson Mothers of Twins Organization for providing the brave youngsters who contributed their time, blood and tears for the benefit of this project. Dr. Douglas Heustis, Ms. Marcia Kuhl and associated personnel at the Arizona Health Sciences Center graciously donated their services in the drawing and typing of blood samples. I would especially like to thank my major professor, Dr. Oscar G. Ward, for his advice, ideas, constructive criticisms, encouragement and support in guiding me to the successful completion of this research project. I also thank Dr. Robert Harris and Dr. John Endrizzi for reviewing this manuscript and for providing helpful suggestions. Many have assisted me in this research, be it in large ways or small, but the mainstay of my inspiration and my continual source of encouragement is my fiancée, Jean-Marie Kinder, to whom I am forever thankful.

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ABSTRACT

Sister chromatid exchanges (SCE's) represent reciprocal exchanges of chromatids within a chromosome. Variation in SCE numbers within a species may be conditioned by hereditary and/or environmental influences. In this study human twins were used to assess genetic and environmental components of SCE variation. SCE's were visualized for light microscopy by in vitro treatment of cells for two division cycles with the thymidine analogue, 5-bromodeoxyuridine (BrdU), followed by treatment with hot phosphate buffer and Giemsa staining. Treatment of three day lymphocyte cultures with 40 µg/ml BrdU produced, on the average, 8.4 SCE's per cell. No significant variation in the number of SCE's per cell was found within or between monozygous and dizygous human twins. Cells treated with 100 µg/ml BrdU exhibited increased numbers of SCE's per cell which were inconsistent within and between twin sets. SCE's at both BrdU concentrations were distributed among the chromosomes in proportion to chromosomal length. This study suggests that significant variation of SCE's above the baseline level of 8.4/cell does not reflect genetic differences between healthy individuals. Such variation may be indicative of disease states,

exposure to environmental hazards, or protocol variation in laboratories applying SCE tests.

INTRODUCTION

The phenomenon of sister chromatid exchange (SCE), first suggested to have taken place in maize (McClintock 1938, Schwartz 1955), was experimentally elicited by Taylor, Woods, and Hughes (1957) in their work with Vicia faba. They demonstrated that it was possible for segments of chromatids to exchange at coincident locations within the same chromosome. Cells grown in the presence of tritiated-thymidine for two replications were shown to have chromosomes with one chromatid unifilarly substituted, and the other chromatid bifilarly substituted, with the radioactive label. Sister chromatid exchanges were also detected.

Marin and Prescott (1964) demonstrated that the tritiated-thymidine was not solely responsible for SCE. Because no statistically significant difference in the exchange rate was detected over a 100-fold range of variation in the amount of incorporated label, they concluded that SCE's were spontaneous events.

In place of tritiated-thymidine, 5-bromodeoxyuridine (BrdU) may be incorporated into deoxyribonucleic acid (DNA). A technique based on the fluorescence sensitivity of the bis-benzimidazole dye, Hoechst 33258, to BrdU (Latt 1974) avoids many problems associated with autoradiography. After

two replication cycles in the presence of BrdU, the sister chromatid that contains DNA with one chain substituted with BrdU fluoresces more brightly than the sister chromatid which contains DNA with both chains substituted with BrdU. Fluorescence intensity thus can be used to identify SCE's in chromosomes.

Although the Latt technique is simple and provides for better resolution in the detection of SCE's, the Hoechst 33258 dye is prone to a degree of photoinstability. Photoinstability and the necessity for fluorescence microscopy can be circumvented by treating cells with BrdU, staining with both Hoechst 33258 and Giemsa stain (Perry and Wolff 1974), and examining them for SCE's with the ordinary compound light microscope. The Giemsa preparation is permanent and not subject to fading.

It has now become possible to eliminate the use of Hoechst 33258 altogether. Treatment of chromosome preparations with hot buffer followed by Giemsa staining (Korenberg and Freedlender 1974) provides for detection of SCE's (Figure 1) quickly and easily. This method was employed for the present study.

The numbers of sister chromatid exchanges have been investigated in a variety of plant and animal species, including Vicia faba (Scheid 1976), Mus musculus (Allen and Latt 1976), Cricetulus griseus (Wolff and Perry 1975),

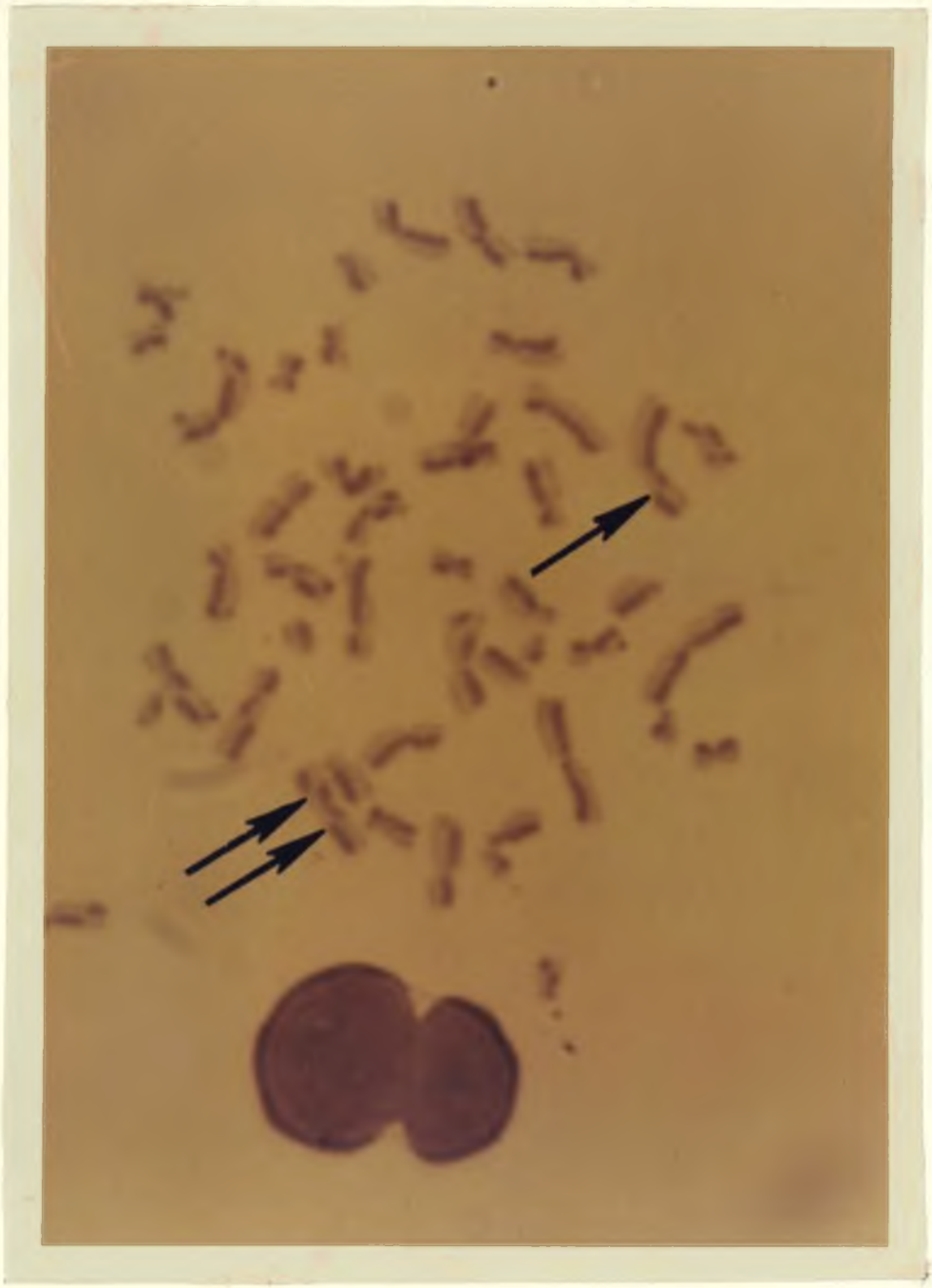

Figure 1. Sister Chromatid Exchanges in Chromosomes Derived from a Human Lymphocyte Culture.

Lymphocyte culture from individual 1a (Table 1, p. 11); each arrow indicates one SCE.

Dipodomys ordii (Bostock and Christie 1976), Muntiacus muntjac (Carrano and Wolff 1975), and Microtus agrestis (Natarajan and Klasterska 1975). Results have indicated that SCE is a natural occurrence in all species examined.

Researchers soon realized that SCE implied DNA breakage and subsequent repair. Since the assay is a very sensitive one, it seemed plausible that an increased rate of SCE might be observed in humans with inherited disorders involving DNA repair systems. The rate of SCE in cells from normal individuals varies from 4.10 to 27.33 per cell (Chaganti, Schonberg and German 1974; Dutrillaux et al. 1974; Kim 1974; Latt 1974; Beeck and Obe 1975; Galloway and Evans 1975; Sperling et al. 1975; Daoud, Shaw and Craig-Holmes 1976; Morgan and Crossen 1977; and P. Crossen, M. E. Drets, F. E. Arrighi, and D. A. Johnston in Morgan and Crossen 1977). A review of these reports suggests that the variation is dependent upon the number of subjects, the number of cells, and the final concentration of BrdU. An increase in the SCE rate has been observed in cells from patients with Bloom's syndrome (Chaganti et al. 1974; Shiraishi, Freeman and Sandberg 1976; Bartram, Koske-Westphal and Passarge 1976), whereas the rate of SCE is in the normal range in Fanconi's anemia (Sperling et al. 1975, Hayashi and Schmid 1975), ataxia telangiectasia (Chaganti et al. 1974, Galloway and Evans 1975, Bartram et al. 1976), xeroderma pigmentosum

(Wolff et al. 1975, Bartram et al. 1976) and Werner's syndrome (Bartram et al. 1976).

Increased rates of SCE have also been observed in cells treated with a variety of environmental mutagens (Carter, Wolff and Schnedl 1976; Stoll, Borgaonkar and Levy 1976; Wolff, Rodin and Cleaver 1977). BrdU induces breaks in chromatids (Hsu and Somers 1961) and increases radiosensitivity and aberration frequencies (Dewey and Humphrey 1965). Furthermore, viruses have been shown to increase the rate of SCE (Brown and Crossen 1976).

The foregoing suggest that SCE rates are affected by both hereditary and environmental influences. Consequently, this study was undertaken to 1) determine the extent to which variation in SCE rates is genetically conditioned, 2) assess the contribution of BrdU to an increased rate of SCE, and 3) establish a normal baseline number of SCE per cell and per chromosome for specimens treated according to the protocol used in this laboratory. Cells from healthy monozygous and dizygous human twins were grown for two replication cycles in the presence of BrdU, then scored for SCE after appropriate treatment. The variability in the rate of SCE in cells of presumed genetically identical individuals (monozygous twins) was compared to the variability observed in genetically nonidentical individuals (dizygous twins) whose fetal environments were closely related to those of their counterparts.

MATERIALS AND METHODS

Blood Samples and Culture Techniques

The individuals selected for the study ranged in age from six to ten years. From each individual of four twin pairs, approximately 10 ml of venous blood was drawn by personnel at the Arizona Health Sciences Center using standard blood-drawing techniques. A portion of each sample was used for ABO and Rh blood typing to verify the zygosity of each twin pair as stated by one parent in response to a questionnaire provided by the investigator. The questionnaire consisted of the following three inquiries:

1. Do you know if the birth membranes were examined following the birth of your twins?
2. Was this the basis for your physician's opinion as to whether your twins are identical?
3. What other reasons do you have for believing that your twins are identical?

All of the above procedures were carried out under the guidelines and with the approval of the Committee on Human Subjects Research at The University of Arizona.

The remainder of each blood sample was used to establish short-term lymphocyte cultures. Whole peripheral blood (0.6 ml) was added to 10 ml of culture medium consisting of

RPMI-1640 (Gibco) supplemented with 20% (v/v) fetal calf serum (Gibco), 1,000 units of penicillin, 1,000 µg of streptomycin, and 0.166 ml of reconstituted phytohemagglutinin (Wellcome, HA 15). Three parallel cultures of cells from each individual were established and incubated at 37°C in a CO_2 incubator in culture tubes. Approximately 24 hr after culture initiation and 48 hr prior to termination, 5-bromodeoxyuridine (BrdU) prepared in RPMI-1640 was added to one culture tube at a final concentration of 40 µg/ml. A second culture tube received BrdU at a final concentration of 100 µg/ml. The final culture tube received no BrdU. All culture tubes were wrapped in aluminum foil to insure that the cultures would be grown in complete darkness.

Slide Preparation

At the 69th hr of culture and 3 hr prior to termination, each culture received Colcemid (Calbiochem) at a final concentration of 0.06 µg/ml. This agent was used to arrest cells, producing a C-metaphase. Cells were harvested using a modified Moorhead et al. (1960) technique at 72 hr after the initiation of the culture. The cells were placed in 5 ml of hypotonic solution of 0.075 M KCl for 10 min at 37°C. They were then fixed with a 3:1 solution of absolute methanol:glacial acetic acid. The cells were washed three times in fresh fixative and stored overnight at 4°C. They were then resuspended in fresh fixative, dropped onto clean,

dry slides, air-dried, and the slides were coded. For best results, the slides were treated for observation the following day.

Slide Treatment and Staining

A modified technique of Korenberg and Freedlender (1974) was carried out as follows. Air-dried slides were pre-treated in 50% (v/v) acetic acid for 2 min in a 37°C water bath, then rinsed three times with distilled water. This was followed by treatment in a phosphate buffer (1 M Na_2HPO_4 adjusted to pH 8.1 with 1 M NaH_2PO_4) at 90°C for 10 min and a thorough distilled water rinse. The cells were stained for 2 min at room temperature in 5% (v/v) Giemsa (Harleco) prepared in distilled water, rinsed, and mounted with Permount (Fisher).

Scoring Procedure

The number of SCE's present in the chromosomes treated with each BrdU concentration was ascertained. A minimum of 10 cells and a maximum of 50 cells were scored for each culture. For each cell, both the number of chromosomes and SCE's were counted. Chromosomes were categorized according to chromosome groups A through G (Denver Conference 1960). The X chromosome was classified with the C group while the Y chromosome was placed with the G group. Terminal and interstitial SCE's that were clearly defined were scored separately from exchanges at the centromere.

Only those cells containing a complete complement of chromosomes were included in the tabulations.

RESULTS

The Rate of SCE per Cell

On the basis of blood-typing results and from the responses of the parents to the questionnaires, the zygosity of each twin pair was determined (Table 1). Three of the four twin pairs are monozygous.

Clearly defined terminal and interstitial SCE's were scored separately from apparent centromeric SCE's because the latter may have been due to twisting of the chromosome rather than actual exchange of chromatid segments.

Two twin pairs were examined for non-centromeric SCE's per cell. The frequency of combined terminal and interstitial SCE per cell was similar for all four subjects at the lower concentration of BrdU (Table 2). A t-test (Schefler 1969) demonstrated no significant difference between members of each twin pair when the rate of SCE per cell was examined. F-test (Schefler 1969) results indicated no significant difference between the dizygous twin pair 1 and the monozygous twin pair 2.

Results from the higher BrdU concentration (Table 3) were inconsistent with those from the lower BrdU concentration. T-tests for all four twin pairs confirmed that there was no significant difference between individuals of twin

Table 1. ABO Blood Types and Rh Factors Used to Assist in Zygosity Determination.

Twin Pair #	Individual	Age (Years)	Sex	ABO Blood Type	Rh Factor	Zygosity
1	a	6	M	A	+	Dizygous
	b	6	M	AB	+	
2	a	6	F	O	+	Monozygous
	b	6	F	O	+	
3	a	7	F	O	+	Monozygous
	b	7	F	O	+	
4	a	10	M	O	+	Monozygous
	b	10	M	O	+	

Table 2. Number and Range of Non-centromeric SCE's per Cell at Low BrdU Concentration‡.

Twin Pair #	Individual	Total Cells	Range per Cell	SCE's per Cell	Standard Deviation	t-value	F-value
1	a	50	2-18	8.3200	±3.6983	0.6902	0.5023
	b	50	2-19	8.8400	±3.7596		
2	a	50	1-16	8.2000	±2.9428	0.2170	
	b	50	3-16	8.0800	±2.6895		

‡ Final concentration 40 µg/ml.

Table 3. Number and Range of Non-centromeric SCE's per Cell at High BrdU Concentration‡.

Twin Pair #	Individual	Total Cells	Range per Cell	SCE's per Cell	Standard Deviation	t-value	F-value
1	a	50	3-25	10.8800	±3.8711	1.0560	
	b	50	4-21	10.0000	±4.3635		
2	a	13	3-12	8.1538	±2.9573	3.6452*	
	b	50	4-21	11.3200	±3.0818		3.4416*
3	a	19	9-28	13.5263	±3.9185	3.1133*	
	b	10	5-15	8.9000	±3.1445		
4	a	40	4-19	10.3500	±3.4609	0.4068	
	b	25	5-15	10.0000	±3.0854		

‡ Final concentration 100 µg/ml.
* Significant at p=0.05.

pairs 1 and 4, whereas there was a significant difference between members of twin pairs 2 and 3. F-test results indicated a significant difference between all twin pairs. This interpretation is questionable because of the smaller number of cells scored for twin pairs 2 and 3.

When centromeric SCE's were included in the tabulations (Tables 4 and 5), the results paralleled those described for Tables 2 and 3. There was no significant difference between and among individuals of twin pairs 1 and 2 at the lower BrdU concentration (Table 4). At the higher BrdU concentration, there was no significant difference between individuals of twin pairs 1 and 4, but there was a significant difference between the individuals of the monozygous twin pairs 2 and 3 (Table 5). Again, F-tests results indicated a significant difference between all twin pairs, but this was also questionable due to the small sample sizes. Therefore the inclusion or exclusion of centromeric SCE's does not change the interpretation of the data.

The trend in the data was that the rate of SCE per cell increased with BrdU concentration.

The Rate of SCE per Chromosome

The rate of SCE was estimated on a per-chromosome basis as follows. The sum of SCE's in one particular chromosome group (A through G, Denver Conference 1960) was divided by the number of chromosomes within that group. This

Table 4. Number and Range of Total SCE's per Cell at Low BrdU Concentration‡.

Twin Pair #	Individual	Total Cells	Range per Cell	SCE's per Cell	Standard Deviation	t-value	F-value
1	a	50	2-18	9.0600	±3.7358	1.2484	2.0877
	b	50	4-23	10.0400	±4.0297		
2	a	50	1-17	8.5400	±3.2200	0.0970	
	b	50	3-16	8.4800	±2.8930		

‡ Final concentration 40 µg/ml.

Table 5. Number and Range of Total SCE's per Cell at High BrdU Concentration‡.

Twin Pair #	Individual	Total Cells	Range per Cell	SCE's per Cell	Standard Deviation	t-value	F-value
1	a	50	5-26	11.3200	±3.7172	0.8227	
	b	50	4-21	10.6400	±4.4329		
2	a	13	3-12	8.3846	±2.8158	3.8898*	
	b	50	5-21	12.1200	±3.0896		3.7496*
3	a	19	9-30	13.9475	±4.8718	2.7104*	
	b	10	5-15	9.1000	±3.3897		
4	a	40	4-19	10.6250	±3.4618	0.2559	
	b	25	5-17	10.4000	±3.2863		

‡ Final concentration 100 µg/ml.
* Significant at p=0.05.

provided an estimate for the amount of SCE per chromosome. Thus, chromosome groups were used as a source of comparison and no distinction was made for chromosomes within the group. Data were compiled for SCE's per chromosome both with and without centromeric SCE's as before.

The results obtained for the lower concentration of BrdU, centromeric SCE's omitted, are shown in Table 6. For both individuals of each twin pair, the number of SCE's per chromosome within each group was similar. Twin pair 1 had a rate of SCE similar to that of twin pair 2 for all chromosome groups. Statistical tests were not performed to confirm this trend.

Centromeric SCE's were included in the data of Table 7. Whereas in most cases the rate of SCE was slightly increased over those rates shown in Table 6, the larger increases were generally observed to have taken place in the longer chromosomes, those of groups A, B and C.

After treatment with the higher BrdU concentration, the rate of SCE per chromosome, obtained as above, was increased over that of cells treated with the lower BrdU concentration (Tables 8 and 9). In some chromosome groups, the rate of SCE per chromosome did not change in response to the higher concentration of BrdU, and occasionally, was even lower than the rate of SCE obtained with the lower concentration of BrdU. However, the trend is that the number of sister chromatid exchanges in the chromosomes of

Table 6. Average Number of Non-centromeric SCE's per Chromosome in Denver Conference Groups at Low BrdU Concentration‡.

Twin Pair #	Individual	Chromosome Group A	B	C	D	E	F	G
1	a	0.3533	0.2900	0.2480	0.1467	0.0500	0.0200	0.0120
	b	0.3000	0.3550	0.2800	0.1667	0.0533	0.0100	0.0120
2	a	0.2733	0.2750	0.2663	0.1267	0.0467	0.0050	0.0350
	b	0.3000	0.2850	0.2300	0.1567	0.0700	0.0150	0.0100

‡ Final concentration 40 µg/ml.

Table 7. Average Number of Total SCE's per Chromosome in Denver Conference Groups at Low BrdU Concentration‡.

Twin Pair #	Individual	Chromosome Group A	B	C	D	E	F	G
1	a	0.3800	0.3050	0.2693	0.1533	0.0633	0.0400	0.0120
	b	0.3467	0.3850	0.3227	0.1767	0.0533	0.0100	0.0120
2	a	0.2767	0.2800	0.2850	0.1267	0.0467	0.0050	0.0350
	b	0.3200	0.2900	0.2450	0.1567	0.0733	0.0150	0.0100

‡ Final concentration 100 μg/ml.

Table 8. Average Number of Non-centromeric SCE's per Chromosome in Denver Conference Groups at High BrdU Concentration‡.

Twin Pair #	Individual	Chromosome Group A	B	C	D	E	F	G
1	a	0.4000	0.2800	0.3813	0.1633	0.0833	0.0250	0.0240
	b	0.3933	0.3550	0.3120	0.1700	0.1000	0.0100	0.0160
2	a	0.3846	0.2500	0.2692	0.0641	0.0256	0.0000	0.0000
	b	0.4233	0.3700	0.3363	0.2400	0.0800	0.0150	0.0650
3	a	0.5789	0.4868	0.3651	0.2895	0.0789	0.0000	0.0132
	b	0.3167	0.3250	0.2750	0.1667	0.0333	0.0000	0.0250
4	a	0.4417	0.2813	0.3367	0.1625	0.0667	0.0250	0.0100
	b	0.4000	0.4000	0.3013	0.2000	0.0267	0.0100	0.0160

‡ Final concentration 40 µg/ml.

Table 9. Average Number of Total SCE's per Chromosome in Denver Conference Groups at High BrdU Cencentration‡.

Twin Pair #	Individual	Chromosome Group A	B	C	D	E	F	G
1	a	0.4133	0.2900	0.3960	0.1633	0.0933	0.0350	0.0240
	b	0.4200	0.3750	0.3173	0.1733	0.0133	0.0200	0.0160
2	a	0.3846	0.2500	0.2788	0.0641	0.0385	0.0000	0.0000
	b	0.4500	0.3800	0.3613	0.2433	0.0933	0.0400	0.0700
3	a	0.5877	0.5000	0.3882	0.2895	0.0877	0.0132	0.0132
	b	0.3167	0.3250	0.2750	0.1667	0.0333	0.0500	0.0250
4	a	0.4417	0.2813	0.3467	0.1625	0.0750	0.0438	0.0100
	b	0.4200	0.4000	0.3173	0.2000	0.0267	0.0200	0.0160

‡ Final concentration 100 μg/ml.

a group is directly proportional to the length of the chromosome within that group, irrespective of twin pair zygosity.

DISCUSSION

That BrdU may itself increase the frequency of sister chromatid exchange has been previously demonstrated (Hsu and Somers 1961). However, Kato (1974) presented a dose-response curve of increasing concentrations of BrdU in a pseudodiploid Chinese hamster cell line wherein SCE frequencies remained stable at low (0.1 to 2.0 μg/ml) concentrations of BrdU but showed a linear increase with BrdU concentrations above an initial plateau. Wolff and Perry (1975) observed no plateau at BrdU concentrations of 0.25 to 1.0 μM (0.07 to 0.31 μg/ml) but found an increase in SCE frequency at these low BrdU concentrations and a saturation at higher BrdU concentrations. However, in vivo studies with Wistar rats by Tice, Chaillet and Schneider (1976) showed no significant variation in SCE frequency at low BrdU concentrations (1.9 to 7.5 μg/g wt/h). Concentrations of BrdU above a plateau increased SCE frequencies over the range of BrdU infusions utilized, and saturation occurred at concentrations above 50 μg/g wt/h. Galloway and Evans (1975) reported that, for in vitro systems, the influence of BrdU concentration is minimal within the range of 1.0 to 160 μM BrdU.

Two concentrations of BrdU were employed in this study. The first, 40 µg/ml, corresponds to approximately 129 µM and falls within the minimal range of Galloway and Evans (1975). The second, 100 µg/ml, corresponds to approximately 323 µM, thus is beyond the minimal range. Whether the higher BrdU concentration falls into the linear phase of a dose-response curve or is at the saturation point was not determined in this investigation.

In the present study, the number of SCE's found in the cells of the two members of any twin pair was essentially the same (Tables 2 and 4). The cells of the monozygous twin pairs responded in like fashion to those of the dizygous twin pair at the lower concentration of BrdU. A discrepancy in the SCE's between and within twin pairs was realized at the higher BrdU concentration (Tables 3 and 5). The rate of SCE appeared to be variable when the final concentration of BrdU was 100 µg/ml. The twin pairs exhibiting the inconsistency were both monozygous female twin pairs. Although the heterochromatic X chromosomes of females has been shown to exhibit an increased frequency of SCE (Schnedl et al. 1976), it seems unlikely that such a response would produce differences that would be detectable by the methods of this study. Since there was a significant difference between members of the same twin pair, it appears more likely that the difference was due to either sampling error or unequal response to BrdU at the higher concentration.

Therefore, the lower concentration of 40 μg/ml was used in order to establish a baseline rate of 8.4 SCE's per cell. Centromeric SCE's were not included in this figure due to their ambiguous interpretation. They should not be totally neglected, however, for the presence of SCE's at centromeres indicates exchange occurring within or near heterochromatic regions and such information may lend insight into the mechanism of SCE.

The rates of SCE per chromosome, based on group analysis, were also examined (Tables 6 through 9). Although the precise locations of SCE's on the chromosomes were not examined, the presence of SCE's appeared to be random within a given chromosome. The total number of SCE's for all chromosomes was not random, for the variation of SCE's per cell was distributed among the chromosomes on a per length basis: the longer chromosomes demonstrated a frequency of SCE higher than that exhibited by the shorter chromosomes. Therefore the number of SCE's within a chromosome is directly proportional to the amount of chromatin contained by that chromosome. Smallest chromosomes were probably under-represented due to the greater difficulty in scoring.

In conclusion, it has been demonstrated that 1) the variation in the number of SCE's per cell is relatively independent of genetic differences in normal individuals and 2) variation in SCE's per cell is distributed among the chromosomes in proportion to their length, at least over

concentrations of BrdU from 40 to 100 µg/ml. This investigation also supports the interpretation of Pathak, Ward and Hsu (1977) that variation in SCE is dependent upon amount rather than kind (species) of DNA present. This indicates that SCE is characteristic of the DNA of all species of eukaryotes and that their frequency per cell is dependent solely on the amount of DNA per genome. The present SCE estimations are in essential agreement with those of others who report SCE's per cell which range from 4.10 to 27.33 in unrelated human individuals. Therefore, the observations of the present study suggest that this apparent variation in SCE's in unrelated individuals reflects differences in laboratory techniques rather than differences among the subjects involved. This means that significant variation of SCE's above a defined baseline level may be reliably taken as an indication of a disease state or an environmental hazard rather than as an indication of genetic differences among the individuals participating in the study. The baseline level referred to above, however, may have to be defined by individual laboratories on the basis of their unique set of experimental conditions.

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