6

SISTER CHROMATID EXCHANGES

TIMOTHY C. WILCOSKY AND SUSAN M. RYNARD

INTRODUCTION

Sister chromatid exchanges (SCEs) occur during cell replication when a chromosome duplicates its genetic material, forming a pair of chromosomes (sister chromatids) attached at the centromere. Through mechanisms that involve DNA breakage and rejoining, sister chromatids can exchange seemingly identical segments of DNA without known alteration of cell viability or function (Allen et al., 1983).

SCE analysis has been used widely as an in vitro method for assessing the mutagenic potential of chemicals (Allen et al., 1983), but interest is growing in SCEs as markers of DNA lesions in humans exposed to certain types of chemical carcinogens (NTP, 1984). Although SCEs themselves do not necessarily lead to adverse health outcomes, elevated levels of SCEs apparently indicate that cells have been exposed to a mutagen. This chapter explains the principles of the assay, considers some of the issues governing its use in epidemiologic research, and briefly discusses its application in human studies to date.

CHARACTERISTICS OF SISTER CHROMATID EXCHANGES

Important characteristics of SCEs include the mechanisms that lead to their occurrence, the agents that produce them, the time necessary for their appearance, and their persistence in the body.

Mechanism of Occurrence

Two major theoretical models of SCE formation have been proposed. The recombination model is based on chromatid exchange as part of a postreplication repair process (Bender et al., 1974; Kato, 1977), whereas the replication model involves recombina-

tion during DNA replication (Painter, 1980; Ishii and Bender, 1980). The articles cited present the details of these complex models. Although the mechanisms responsible for the appearance of SCEs are still under investigation, SCEs apparently reflect processes distinct from those that produce chromosome aberrations (Wolff et al., 1977).

Sister Chromatid Exchange-Producing Agents In Vitro

Although a variety of physical and biological agents, including viruses, ionizing radiation, clastogens, and mutagens, can produce SCEs (Gebhart, 1981), this chapter focuses on the chemical induction of SCEs. A wide range of chemicals can induce SCEs in mammalian cell lines (Latt et al., 1981). Those most likely to produce SCEs in vitro include alkylating agents and other DNA-binding agents, certain DNA-base analogs, and chemicals that interfere with DNA repair or cause single-strand breaks in DNA. In contrast, exposures that cause double-strand DNA breaks, such as bleomycin and ionizing radiation, are efficient producers of chromosome breakage but not of SCEs (NTP, 1984; Carrano, 1986).

For most of the SCE-inducing chemicals tested, induction increases linearly with dose (NTP, 1984). Studies in vitro also indicate that some chemicals, but not others, show a linear association between SCE induction and single-gene mutations. A possibility also exists that some DNA lesions can produce either a mutation or an SCE (Carrano, 1986). In general, SCEs compared with chromosome breakage are a more sensitive indicator of DNA damage; the assay responds to a wider variety of agents and responds at doses that are 10- to 100-fold lower (NTP, 1984). The marker is nonspecific in that many different chemical exposures lead to the same response.

Temporal Aspects of Exposure, Sister Chromatid Exchange Appearance, and Sampling

SCEs occur when certain DNA lesions are present during cell division. When SCEs are used as markers of DNA lesions in humans, the lesions occur in vivo, but the resulting SCEs are measured in sampled cells that have been stimulated to divide in culture. The DNA lesions should occur as soon as the exposure agent reaches the cell. If DNA repair takes place before replication, however, SCEs may not occur.

Persistence of Sister Chromatid Exchange

The persistence of detectable SCEs depends both on the rate of DNA repair and on the normal half-life of the affected cells. For different lymphocyte subpopulations, for example, the life spans range from a few months to 20 years (Carrano, 1986). When stimulated to divide in culture, a sample of long-lived cells with unrepaired DNA lesions could give a good estimate of dose integrated over time. Cells with rapid turnover or cells with transient lesions could provide estimates of more recent exposures, if such cells can be separated from the total lymphocyte population. An exposure itself may increase the rate of cell turnover and alter the proportion of long- and short-lived lymphocytes. The persistence of SCE-inducing chemicals within the body after the ambient exposure ends also affects the persistence of detectable SCEs.

The persistence of elevated SCE levels may increase with increasing dose (NTP,

1984), but few data exist to support or reject the possibility. A study of only one cigarette smoker suggests that a transient elevation in SCEs returns to normal after 18 hours of nonsmoking (Lambert et al., 1982). Other studies found that, for unknown reasons, SCE levels increased for several months after long-term smokers quit (Wulf et al., 1985; Tucker et al., 1988). As summarized by Lambert et al. (1982), cancer chemotherapy may increase SCE frequencies for weeks or months. Workers with acute high exposure to ethylene oxide showed a 40 percent elevation in SCEs 5 days after exposure, but their SCE levels were normal 2 years later (Laurent, 1988). Chronic ethylene oxide exposure, on the other hand, apparently increases SCE levels for at least 1 to 2 years after exposure is reduced or ends (Stolley et al., 1984; Sarto et al., 1984).

Accessibility of Tissues with Sister Chromatid Exchanges

Although SCEs can apparently occur in any type of dividing cell, most studies of SCEs in humans focus on circulating lymphocytes because they are so easily available. For cancer etiology, cells in bone marrow or other tissues are of greater interest, as cancer can arise from such cells but apparently not from mature circulating lymphocytes. Mutagenic exposures that increase SCE levels in lymphocytes, however, presumably also affect other cells that can form tumors; therefore, lymphocyte SCEs should be a good biological response marker. Because the sampling procedure for lymphocytes is less invasive than that for most other cells of interest, population studies of SCEs have typically used lymphocytes (Bloom, 1981). For this reason, the rest of this discussion focuses on SCEs in lymphocytes.

THE SISTER CHROMATID EXCHANGE ASSAY

The SCE assay is a process in which one member of each chromatid pair in replicating cells is stained differently from its sister chromatid. This differential staining enables investigators to detect genetic material exchanged between sister chromatids. The assay is based on the incorporation of 5-bromodeoxyuridine (BrdUrd), a thymidine analog, into replicating chromosomes, so that BrdUrd-sensitive staining procedures allow visualization of newly formed SCEs.

Specimen Collection and Cell Culturing

In the human lymphocyte SCE procedure, summarized in Table 6-1, 10 ml of whole blood are centrifuged to yield leukocytes (white blood cells). Roughly 20–30 percent of the white cells are lymphocytes, the cell type used in the assay (Langley, 1971). The white cells are placed in a culture medium containing nutrients, BrdUrd, and phytohemagglutinin (PHA); PHA is a mitogen that stimulates lymphocytes (especially T-cells) to divide. Because cells are scored during their second division metaphases in culture, the culture duration should be selected to yield a high percentage of cells in their second division (Bloom, 1981). A culture length of about 72 hours is recommended (Lambert et al., 1982). When comparing exposed and nonexposed groups, some investigators report the proportion of cells that have undergone one, two, and

Table 6-1 SCE Assay Procedure

A. Collect 10 ml venous blood; add PHA and separate white cells and plasma by centrifugation.
B. Establish cell culture in medium containing antibiotics, autologous human serum, and BrdUrd.
C. Incubate for at least 72 hours at 37°C; protect from light.
D. When ready to harvest cells, add colchicine to inhibit further cell division.
E. Harvest cells by centrifugation.
F. Fix cells in 3 : 1 methanol : acetic acid; drop cells on slides and air dry.
G. Stain prepared slides in fluorescent 33258 Hoechst.
H. Irradiate stained slides under UV light.
I. For permanent preparation, counterstain with Giemsa.
J. Number-code slides to assure blind analysis.
K. Analyze for SCEs.

three divisions during a specific culture length. These proportions can be interpreted as indicators of cytotoxicity or mitotic delay.

BrdUrd Uptake in DNA Synthesis

As summarized in Chapter 8, dividing cells pass through different phases. Cells preparing to replicate enter a period of DNA synthesis called the S phase. During this phase, the DNA of chromosomes is duplicated.

In the S phase under assay conditions, BrdUrd partially replaces thymidine, a DNA base that is one of the chromosome's normal components. During the first metaphase in culture, each sister chromatid has one newly formed BrdUrd-substituted DNA strand and one parent strand of normal DNA without BrdUrd substitution, as Figure 6-1 shows. After mitosis, the double-stranded DNA of chromosomes in the two daughter cells also contains one BrdUrd-substituted strand and one parent strand. During DNA synthesis in the second cell division in culture, BrdUrd again partially replaces thymidine in the newly synthesized strands of DNA in each chromatid. Then, when the daughter cells enter metaphase, one of the sister chromatids includes a parent strand of DNA and a BrdUrd-substituted strand of DNA, and the other contains two strands of BrdUrd-substituted DNA. The asymmetrical distribution of BrdUrd-substituted DNA in the second metaphase allows visualization of SCEs: the chromatid containing the parent DNA strand stains with a different intensity compared with the chromatid with BrdUrd in both strands. If an SCE has occurred, it appears as a discontinuity in the stain intensity along the chromatid (Figs. 6-1 and 6-2). Note that the SCE assay cannot detect a DNA lesion formed in vivo unless it persists until the first DNA synthesis in vitro when BrdUrd is present.

Sister Chromatid Exchange Scoring

Investigators use various approaches to score SCEs. Some researchers perform replicate cell cultures from each individual in the study and prepare multiple slides from each culture (Anderson et al., 1986). They then select cells for scoring from the multiple slides prepared for each person. Because random fluctuations in SCE numbers among slides and cultures would tend to cancel each other, this procedure helps minimize the effect of variation among slides and cultures when estimating the mean number of SCEs per cell for an individual.

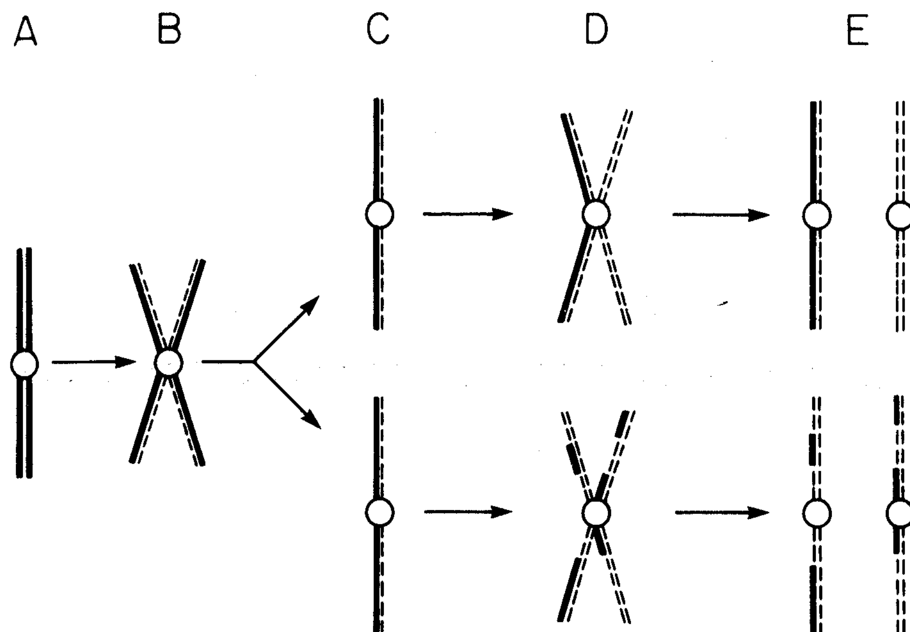

Figure 6-1. BrdUrd uptake and the visualization of SCEs. A schematic representation of a chromosome is shown for two cell cycles. **A.** During G_0, the chromosome has two arms, each having two DNA strands. **B.** When the cell enters its first metaphase in culture, each chromosome replicates in the presence of BrdUrd forming a pair of sister chromatids. Newly formed DNA strands, indicated by *dashed lines,* incorporate BrdUrd; the parent DNA strands are shown as *heavy dark lines.* Note that each sister chromatid in metaphase of the first cell cycle contains one BrdUrd-substituted DNA strand and one parent strand. **C.** After mitosis, each of two daughter cells, therefore, has one parent strand and one BrdUrd-substituted strand of DNA. **D.** When the daughter cells enter metaphase in the second cell division, one of the sister chromatids includes a parent strand of DNA and a BrdUrd-substituted strand of DNA; the other sister chromatid contains two strands of BrdUrd-substituted DNA. The asymmetrical distribution of BrdUrd-substitution in the second metaphase allows visualization of SCEs: the chromatid containing the parent DNA strand stains with a different intensity compared with the chromatid with BrdUrd in both strands. SCEs appear under a microscope as a discontinuity in stain intensity along the chromatids, as depicted in the lower chromosome, which shows three SCEs. **E.** After the second mitosis, the daughter cells have BrdUrd substitution as shown.

Although normal human cells contain 46 chromosomes, cells stained for SCE scoring may have fewer than 46 detectable chromosomes. Some researchers require that all scored cells have 46 clearly visible chromosomes (Lambert et al., 1982), whereas others score cells with 45 (Anderson et al., 1986) or even 40 chromosomes (Soper et al., 1984). Although the inclusion of cells with fewer than 46 chromosomes leads to a smaller average number of SCEs per cell for an individual, Soper et al. (1984) found this bias to be negligible in their analysis. Most investigators, however, who score fewer than 46 chromosomes normalize the SCE count to the frequency expected for 46 chromosomes.

The number of cells scored per individual varies among studies. Bloom (1981)

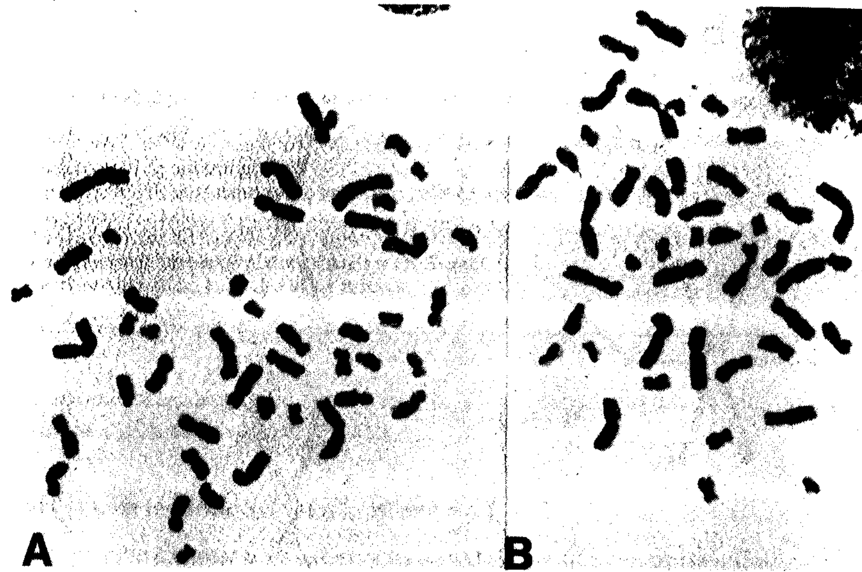

Figure 6-2. SCEs in human lymphocytes. Each panel shows differentially stained chromosomes from a human lymphocyte in metaphase. **A.** Cell from a control (unexposed) culture shows eight SCEs. **B.** Cell from a culture exposed to 0.1 mg/ml of vinyl carbamate shows approximately 40 SCEs. (Photograph from Allen et al. [1984] reprinted with permission from publisher and author.)

mentioned study hypotheses, heterogeneity of the study population, and the desired precision of results as factors that influence the number of cells scored. Many investigators score 50 cells (25 from each of two replicate cultures), as Anderson et al. (1986) and others suggest.

Hirsch et al. (1984) performed a computer simulation study that indicated that scoring 50 cells per person is appropriate. Scoring additional cells gives only modest increases in statistical power to detect differences between groups, unless variation in SCEs among cells within individuals is unusually high. Hirsch et al. point out that increasing the number of individuals in the study, scoring additional cells per person, or both can improve the ability to detect differences in SCE levels between groups. They provide tables that can be used to help estimate the number of persons and cells per person required to detect SCE percentage differences of varying magnitudes between groups.

Effects of Protocol Variations in the Assay

A number of protocol variations can influence the outcome of the assay. Important factors include the duration of sample storage before culture, the BrdUrd concentration, the time at which the mitogen is added, whether the investigator uses the macroculture or microculture technique, and other variables. The upcoming sections review these factors.

Sample Storage Before Culture

If possible, researchers should begin lymphocyte culture immediately after collecting the blood. Although heparinized blood can be stored for several days at 4–37°C, storage can lead to lymphocyte death, reduction of mitogenic response, and selective loss of cells with lesions that can result in SCE formation (Bloom, 1981). In a limited study of blood storage conditions and SCE formation, Lambert et al. (1982) found that storing blood for 8 to 24 hours caused a modest reduction in the mean number of SCEs per cell and in the number of cells with high SCE frequencies. Carrano et al. (1980) found small reductions in SCE frequencies after a week of storage in a study of four persons. More extensive studies with longer storage durations under various conditions would be helpful to design protocols for field studies of SCEs.

BrdUrd Concentration

Because BrdUrd itself causes SCEs in a dose-dependent manner, the BrdUrd concentration in the culture medium is a factor of great importance in determining the measured background level of SCEs (Lambert et al., 1976; Crossen, 1982; Lambert et al., 1982). BrdUrd induction of SCEs apparently depends on the ratio of BrdUrd concentration to the number of dividing cells per culture; within any given study, an investigator will need to standardize this ratio across cultures as much as possible (Stetka and Carrano, 1977; Bloom, 1981; Crossen, 1982). Otherwise, different numbers of cells across cultures will be competing for a given amount of BrdUrd. Because BrdUrd causes SCEs, current techniques cannot give a direct measure of the true background rate of SCEs. The artifactual background level of SCEs does not necessarily cause validity problems in comparisons of different groups, because an identical assay should produce the same baseline levels in all groups. One should remember, however, that the assays in no way measure the frequency of SCEs that actually occur among humans in vivo, because the assays are conducted on cultured cells, and the measurement procedure itself causes SCEs. Nevertheless, the marker allows investigators to make inferences about relative frequencies of in vivo alterations among individuals.

Timing of PHA Addition

To stimulate mitosis, an investigator adds a mitogen, usually PHA, to the culture. The timing of this addition, that is, whether PHA is added to whole blood before separation of cells or added directly to the culture, can affect the SCE frequency in the culture (Lambert et al., 1982). According to these researchers, differential selection of lymphocyte subpopulations may account for this observation.

Lymphocyte cultures contain T and B lymphocytes and other lymphocyte subpopulations, all of which vary in their proliferative response to specific mitogens. Because lymphocyte subpopulations vary in their half-lives, the detectable prevalence of exposure-related DNA lesions in a cell culture may depend on the types of lymphocytes that are stimulated to divide. Most human studies are based on T lymphocytes because PHA primarily stimulates this subpopulation. Although possible differential selection of lymphocytes is a nuisance in some respects, comparison of subpopulations could theoretically allow investigators to use markers in cells with different half-lives to estimate the timing of past exposures (Perera, 1987).

Macroculture Versus Microculture Technique

An alternative to the so-called macroculture technique described previously uses 0.5 ml rather than 10 ml of whole blood. In a study of 19 persons, Lambert et al. (1982) found that the microculture technique yielded a 40 percent lower mean SCE frequency compared with the macroculture. They attributed the difference to the fact that the microculture technique uses fetal calf serum as a nutrient instead of the autologous human serum typically used in macrocultures. Lambert et al. found higher SCE frequencies in cultures that used autologous serum compared with cultures that used fetal calf serum. They speculated that variation in SCE frequencies among individuals could result from different factors in their sera that influence SCEs. In contrast, Crossen (1982) found no differences in SCE frequencies between cultures that used autologous human serum and cultures that used fetal calf serum, and other researchers found lower frequencies when they used autologous serum (Gosh and Nand, 1979). Crossen (1982) suggested that different batches of fetal calf serum vary in their content of SCE-inducing factors and recommended pretesting to eliminate batches that increase SCEs. If human serum does vary with regard to SCE-inducing factors, the use of fetal calf serum in the culture medium may reduce the variance in measured SCE frequencies among humans.

Additional Variables in the Assay

In addition to BrdUrd concentration, PHA timing, and culture technique, other variations in SCE assays can also affect the measured frequency. For example, exposing the culture to light (other than red or yellow) dramatically increases SCE frequencies, and different culture media also influence frequencies (Crossen, 1982). In view of the numerous laboratory parameters that are determinants of observed SCE frequencies, the large variation in SCE scores observed across studies (Crossen, 1982) is not surprising.

In Vivo Sister Chromatid Exchange Assays

In addition to in vitro assays, extensive in vivo testing has been performed in both mammalian and nonmammalian systems. The protocols involve exposing test animals both to agents of interest and to BrdUrd (Latt et al., 1981; NTP, 1984). The major advantage of in vivo SCE assays is the incorporation of host mediation, that is, metabolism of the agent of interest. Furthermore, in vivo testing of animals also allows investigators to examine tissues that are not readily available from humans, such as germ cells or internal organ cells. In vivo assays thus permit comparisons of the sensitivity of various tissues to the mutagenic effects of agents of interest. Similarly, comparisons of in vivo animal assays with human in vitro assay data may provide insight into differences between the exposure-specific responses of humans and of animal models. Although this chapter focuses on in vitro laboratory methods and studies of human populations, in vivo testing in animal systems should be considered a valuable adjunct for evaluating SCEs.

EPIDEMIOLOGIC CONSIDERATIONS IN SISTER CHROMATID EXCHANGE TESTING

Epidemiologists must consider more than just sources of assay variability in determining the usefulness of SCEs for their research. Such issues as the sensitivity and specificity of the assay, the distribution of SCEs in individuals and groups, potential confounders, and research opportunities are concerns that need to be addressed in an evaluation of the SCE assay. These topics are discussed in the following sections.

Exposure Sensitivity and Specificity

Epidemiologists define exposure sensitivity as the proportion of exposed individuals identified by the measurement technique. This is analogous to the epidemiologic use of the term *sensitivity* in disease screening, where it denotes the correctly classified proportion of diseased persons (Mausner and Bahn, 1974). As Chapter 3 points out, other scientific disciplines use definitions of sensitivity and specificity that differ from the epidemiologic definition. For the SCE assay, estimates of exposure sensitivity in the epidemiologic sense are essentially nonexistent.

If exposure sensitivity is the measure of interest, some standard for ascertaining true exposure status must be identified. With regard to SCEs and a given chemical, *true exposure* could be defined variously as contact with any ambient level of the chemical, any level of internal dose, or at least one DNA lesion resulting from the chemical exposure. Consequently, the sensitivity of SCEs as a marker of a particular exposure depends on the definition of the exposure, as all ambient exposures will not necessarily produce internal doses high enough to cause DNA lesions. Given their biological properties, SCEs are theoretically most useful as markers of DNA lesions, rather than as more general markers of internal dose. Exposure sensitivity in the epidemiologic sense, then, ideally should be calculated to be the proportion of persons with DNA lesions who show elevated levels of SCEs.

The above definition of exposure sensitivity raises many operational problems. One must decide how many DNA lesions constitute an exposed state, what level of SCEs is higher than background, how the SCE level in one biological sample compares with the levels in other tissues and cell populations, and what the "gold standard" for estimating the true underlying prevalence of DNA lesions will be. Because of these operational issues, an epidemiologic definition of exposure sensitivity with respect to SCEs may have limited utility.

An alternative formulation of exposure sensitivity might use an approach like that of Latt et al. (1981), who reported the exposure concentrations that doubled the background level of SCEs for different chemicals in a variety of animal species and tissues. For those exposures that did double the level, the required doses of active chemical were usually about 10^{-4} to 10^{-5} mole/kg body weight, although many exceptions occurred outside of this range. In human studies, one might estimate the dose required to produce a 50 percent or other detectable percent increase in SCEs over the background level in a nonexposed group. Precise exposure information will usually

be unavailable, however, so most statements about exposure sensitivity and SCEs in human populations must be qualitative rather than quantitative.

Exposure specificity in the epidemiologic sense is the proportion of nonexposed persons who are correctly classified as nonexposed by the measurement technique. Again, this definition is analogous to the epidemiologic definition of specificity used in disease screening, where it indicates the correctly classified proportion of nondiseased individuals (Mausner and Bahn, 1974). In general, the operational difficulties that apply to SCEs and sensitivity also apply to specificity. Nonmutagenic exposures in vitro usually do not cause an increase in SCEs (NTP, 1984), but, as discussed in greater detail later, confounders can cause false-positive results in human populations, because many exposures in addition to the exposure of interest can elevate the number of SCEs.

Sister Chromatid Exchange Distribution

With some exceptions, SCEs occur randomly among the chromosomes, and the number of SCEs per chromosome correlates positively with chromosome length (Block, 1982). Almost all lymphocytes from an individual contain at least one SCE as measured with current assays. A single cell may have 20 or more SCEs, however, and the distribution among cells is positively skewed (Crossen, 1982; Soper et al., 1984). That is, many cells in a sample have a small number of SCEs, but relatively few cells contain a large number; therefore, the frequency distribution of SCEs among cells within individuals has an asymmetrical shape.

Hirsch et al. (1984) and Anderson et al. (1986) examined a number of different theoretical frequency distributions to identify those that best describe the distribution of SCEs in human lymphocytes. Identification of appropriate distributions helps in selecting statistical tests for SCE differences between populations of individuals. For most people in their samples, these researchers found that the negative binomial and normal distributions adequately fit the distribution of SCEs among cells. The Poisson distribution, on the other hand, fit the data poorly, presumably because the scored lymphocytes include a heterogeneous mixture of subpopulations with differences in longevity, sensitivity to mutagens, or other biological differences (Carrano and Moore, 1982; Margolin and Shelby, 1985). The SCEs within each lymphocyte subpopulation may follow a Poisson distribution, and the negative binomial distribution can fit a mixture of Poisson distributions.

The individual means and the individual variances are significantly correlated; that is, persons with a large mean number of SCEs per cell also show a large variance in the number of SCEs per cell. As a result, Anderson et al. (1986) and Soper et al. (1984) recommended using a log transformation of each person's mean SCEs per cell as a dependent variable in analysis of variance (ANOVA) models. Hirsch et al. (1984) applied log or square root transformations to the SCE frequency for each cell.

Although the distribution of SCEs per cell is positively skewed within a given individual, the mean SCE frequency per cell among individuals follows a symmetrical normal distribution (Crossen, 1982). Researchers typically use the mean SCE frequency per cell to characterize an individual's SCE levels and base comparisons of groups on the overall mean of individuals' means. In addition, Carrano and Moore (1982) hypothesized the existence of cells with abnormally high SCE frequencies, which they termed high frequency cells. According to these investigators, the presence of high

frequency cells may be at least as important as mean frequencies in analyzing SCEs, because they may reflect persistent, cumulative effects or the existence of particularly sensitive lymphocyte subpopulations, or both. They also conclude that analyses of high frequency cells have greater power to detect individuals with elevated SCE frequencies, whereas comparisons of mean SCE levels have more power to detect differences in SCE levels between groups. Margolin and Shelby (1985) have proposed the H value, an index of heterogeneity, as an appropriate measure of high frequency cells. The H value is the variance to mean ratio estimated from the SCE distribution in each person's sample of cells.

Ideally, the variability in SCE frequencies between individuals should be greater than that within individuals, and different scorers should show close agreement. Anderson et al. (1986) found substantially more variation between individuals than between replicate cultures from the same people, although they did find significant variation between replicate cultures. When they resampled 32 individuals over about 7 weeks, they found similar SCE frequencies on both occasions. Crossen (1982) found nonsignificant variations in mean SCEs per cell in 17 of 20 people sampled repeatedly at 3-month or greater intervals; he attributed the significant differences in the other three people to possible exposure to SCE-inducing factors. Tucker et al. (1987) found larger variations in mean SCE levels between individuals than within individuals resampled at daily or twice-weekly intervals, but the temporal variation was statistically significant. Because the proportion of high frequency cells varied less over time than did mean SCE levels, Tucker et al. suggest that analyses of high frequency cells may be superior to comparisons of mean SCE levels. Soper et al. (1984) also found more variation between persons than for the same persons resampled at 6-month or yearly intervals. In general, most studies indicate that SCE frequencies remain fairly constant over time if exposures and assay conditions are also constant (Crossen, 1982), although differences in SCE frequencies between samples increase with increasing time between samples (Tucker et al., 1988).

Differences in scoring may influence the variability found in SCE distributions. Soper et al. (1984) found significant interreader variability that weakened observed associations between SCEs and various independent variables. Anderson et al. (1986), on the other hand, found no significant variation in SCE scores among different readers in a laboratory with very careful quality control. Although Tucker et al. (1987) found statistically significant differences between readers, they felt that the differences were small enough to ignore in most studies.

Statistical techniques for SCE analysis are still under development. Temporal elements, choice of controls, underlying response model, and departures from the response model may be relevant areas of statistical concern (Archer, 1984). Margolin and Shelby (1985) have urged that investigators publish individual SCE data, rather than simple group means, to serve as a catalyst for the development of SCE data analysis techniques.

Potential Confounders in Studies of Sister Chromatid Exchanges

Because many different chemical exposures can increase SCE frequencies, observed differences in SCEs between groups in an epidemiologic study could result from exposures other than the one of primary interest. Cigarette smoking provides an excel-

lent example. Smokers have about 20 percent more SCEs than do nonsmokers (Lambert et al., 1982; Anderson et al., 1986), and confounding can occur if smokers are differentially distributed among groups being compared with respect to some other exposure. Most studies have found no association between SCEs and age (e.g., Anderson et al., 1986), although some (e.g., Soper et al., 1984) found a weak positive association.

Recent studies indicate that women have roughly 10 percent more SCEs than do men (Anderson et al., 1986; Bender et al., 1988). Margolin and Shelby (1985) reviewed data from 12 major SCE studies and found a small but significant increase of 0.5 SCEs per cell for women as compared to men. Preliminary findings from Tucker et al. (1987) suggest that SCE frequencies show three different peaks during the menstrual cycle. These authors also summarize the conflicting studies of SCEs with regard to pregnancy and oral contraceptive use.

The Margolin and Shelby (1985) analysis of data from Butler's (1981) study showed differences in SCE levels by race using comparisons based on H values, but systematic scoring differences across races may have contributed to the race effect. Bender et al. (1988) found no SCE differences between whites and blacks in a larger study with careful scoring. They compared SCE means rather than H values, however, and SCE means were not sensitive to race differences in Butler's (1981) data.

Other potential confounders include vaccinations and drug therapy. Crossen (1982) summarized limited data that indicate that smallpox and measles vaccinations increase SCEs for days or weeks. Although naladixic acid therapy and treatment with melphalan resulted in elevated SCE levels, therapy with known mutagenic drugs such as metronidazole, actinomycin D, and PUVA (8-methoxypsoralen combined with ultraviolet A light) caused no significant increase in SCEs (Lambert et al., 1982). Lambert speculates that the negative findings may result from differences in metabolism between in vivo and in vitro test systems, or that clinical dosages were too low to produce detectable SCEs above background.

Opportunities for Retrospective Studies

Although some mutagenic exposures may produce only transiently elevated SCE levels, retrospective case-control studies of chronic diseases using SCEs as exposure markers are possible if cells are frozen at liquid nitrogen temperatures and properly thawed to maintain viability. Case-control studies might be nested within cohorts selected on some criterion other than exposure status, such as blood donation or clinic attendance. In such situations, although each cohort member initially provides blood samples, SCE assays are conducted only on a subset of controls and cases with a disease outcome of interest. This kind of study could answer the question of whether SCE levels in lymphocytes are themselves associated with subsequent cancer or other diseases, and the design would be more efficient than would a prospective cohort design requiring assays of all baseline blood samples. Alternatively, the nested case-control approach could make use of stored blood from a cohort with a particular exposure; the samples could be used to study the relationship between different exposure levels and the subsequent development of disease.

HUMAN SISTER CHROMATID EXCHANGE STUDIES

SCEs have been investigated in numerous human studies, including investigations of life-style factors, occupational exposures, disease states, and therapeutic exposures. Table 6-2 summarizes studies of diet, tobacco use, and medical therapy. Table 6-3 summarizes occupational SCE studies.

The investigations described here were chosen to represent a variety of exposures and populations. Compared with the literature on other markers (e.g., urine mutagenesis), these studies show such superior design elements as relatively large sample sizes, greater attention to choice of control groups, and increased control of confounders.

Diet, Trace Minerals, and Tobacco Use

Wulf et al. (1986) studied SCE frequency and diet among three geographically separate groups of Greenlandic Eskimos with a total sample of 147 persons. The different groups had different levels of seal consumption, which is a key element of traditional Eskimo diet and one with a high heavy metal content. The investigators scored 30 cells per person (some consider this a small number), and expressed the results as mean SCEs per cell. Multiple regression analyses considered sex, age, tobacco use, diet, area of residence, and blood levels of lead, mercury, selenium, and cadmium. For the regression model that included lead and mercury (but not selenium and cadmium), diet, residence, age, tobacco use, and mercury were all significantly related to the number of SCEs per cell with a linear dose–response. In both this model and one that also included selenium and cadmium, the traditional seal meat diet was the most important predictor of increased SCEs. A possible bias in Wulf's work lies in the relatively long time lag between blood sample collection and SCE testing; in many instances, 4–7 days elapsed before testing. If samples from the communities with the highest seal consumption (presumably the most remote) experienced the longest delays, and if the delays reduced SCEs, Wulf's results may understate the true association.

Adhvaryu et al. (1986) examined small groups of East Indian patients suffering from oral submucous fibrosis (SMF), a chronic fibrotic change of the oral tissues

Table 6-2 Studies of SCEs and Life-Style or Therapeutic Exposures

Reference	Exposure	Sample size	Cells scored per person	Reported results
Wulf et al. (1986)	Diet	147	30	+
Adhvaryu et al. (1986)	Tobacco/betel nut chewing	35	25	+
Kelsey et al. (1986)	Asbestos and cigarette smoking	32	50	+
Hou et al. (1985)	Acute leukemia	60	20–50	+
Kärki et al. (1986)	Multiple sclerosis	28	25	+
Aronson et al. (1982)	Drug and radiation therapy	54	50	+

Table 6-3 Studies of SCEs and Occupational Exposures

Reference	Exposure	Sample size	Cells scored per person	Reported results
Pohlová et al. (1986)	Cytotoxic drug manufacturing	57	30	+
Stiller et al. (1983)	Cytotoxic drug handling	19	25	–
Jordan et al. (1986)	Cytotoxic drug handling	36	30	–
Stolley et al. (1984)	Ethylene oxide	234	80	+
Yager et al. (1983)	Ethylene oxide	27	50	+
Mäki-Paakkanen et al. (1984)	Rubber industry	90	30	+
Nagaya (1986)	Chromium plating industry	48	25	–

thought to be a cancer precursor. Blood was drawn from 20 long-time tobacco/betel nut chewers, 10 of whom had SMF, and from 15 controls who had never used any form of tobacco or betel nut, and who had been screened for recent viral infections and current medication use. Mean SCE scores for each individual and group were calculated from 25 scored cells per person. Group means were significantly higher for chewers (both SMF patients and so-called normal chewers) than for controls, whereas SMF patients and normal chewers had essentially the same mean number of SCEs.

In a study of workers occupationally exposed to asbestos, Kelsey et al. (1986) examined 22 male asbestos-exposed workers (11 smokers and 11 nonsmokers) and 10 unexposed workers (four smokers and six nonsmokers) of the same age. Mean SCE scores were calculated from 50-cell scores, and the data were analyzed by *t*-tests and one-way ANOVA. Cigarette smoking significantly increased the baseline number of SCEs. Asbestos exposure was associated with a small nonsignificant increase in SCEs, and a positive interaction between asbestos and smoking approached nominal statistical significance.

Sister Chromatid Exchanges in Disease States

The SCEs have been investigated both as markers of disease states and as indicators of therapy. Several studies have shown extremely high SCE levels in patients with Bloom's syndrome. Other genetic DNA repair defects, however, apparently do not cause dramatically elevated SCE levels (Zakharov, 1982).

Hou et al. (1985) studied SCEs in 49 cases of acute leukemia and found increases among patients before therapy. Mean levels of SCEs were significantly higher for 36 acute nonlymphocytic leukemias and for 13 cases of acute lymphocytic leukemia compared with 11 healthy controls. In nine treated cases where complete remission occurred within 6 months of treatment, SCE levels were significantly lower than pretreatment levels, but significantly higher than among healthy controls.

Kärki et al. (1986) studied SCEs in patients with multiple sclerosis. Fourteen clinically established multiple sclerosis patients were matched by age and sex with healthy controls drawn from a population of laboratory workers and blood donors. Mean SCE levels were significantly higher among patients compared with controls

s scored person	Reported results
30	+
25	–
30	–
80	+
50	+
30	+
25	–

:-time tobacco/betel
never used any form
viral infections and
l group were calcu-
ificantly higher for
or controls, whereas
n number of SCEs.
Kelsey et al. (1986)
nonsmokers) and 10
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after a standard 72-hour incubation, but prolonged incubation (9 days) reduced the relatively high level of SCEs among multiple sclerosis patients.

A study of acute and long-term effects of drug and radiation therapies in childhood cancer (Aronson et al., 1982) included SCEs and chromosome aberrations. Pretreatment cancer patients, patients undergoing therapy, posttherapy patients, and adult noncancer controls provided a total of 53 individuals. Fifty cells were scored per person. Among pretreatment patients and posttherapy patients, SCE levels were comparable with those of controls. The SCEs increased, however, among patients undergoing chemotherapy compared both with healthy controls and to pretreatment patients. The authors speculated that the considerable variability among individuals may be due to the broad representation of tumor types included in the study. The use of adults, 30 percent of whom smoked cigarettes, as noncancer controls was probably inappropriate. Data on chromosome breakage followed the same general pattern as the SCE findings.

Occupational Exposures and Sister Chromatid Exchanges

Two areas of occupational exposure have been extensively investigated for an association with SCEs: health-care workers handling cytotoxic drugs and workers exposed to ethylene oxide. Pohlová et al. (1986) investigated SCEs, chromosomal aberrations, and urine mutagenicity among 38 chemists and plant workers who were exposed to alkylating agents and other drugs during research and pilot production of newly developed cytotoxic drugs. Nineteen controls were chosen from presumably unexposed plant librarians and clerks, and matched on age and sex. All participants completed questionnaires on health history, smoking, and x-ray exposures. For the SCE assays, 30 cells were scored per participant, and differences between group means were evaluated with a *t*-test. Exposed workers had significantly elevated levels of SCEs (8.94 per cell compared with 5.81). Frequency of chromosome aberrations (defined as breaks) and urine mutagenicity was also significantly higher among exposed workers.

Two other studies, however, found no significant elevation in SCEs among hospital workers handling cytotoxic drugs. Stiller et al. (1983) assayed SCEs and chromosomal aberrations in 9 exposed workers and 10 unexposed controls and found no differences between exposed and unexposed participants. Six controls, however, smoked cigarettes and only two exposed workers smoked, so smoking differences could have masked SCE elevations from the drug exposures. Jordan et al. (1986) found no difference in the mean number of SCEs between two groups of 18 nurses each. One group handled cytotoxic agents an average of 3 days per work week, whereas the other never came in contact with oncology patients. All members of both groups were women, and comparable in age, smoking history, medication history, and alcohol intake. Regression analyses showed no association between mean number of SCEs and number of drug-handling days. Although the data suggested a relatively high prevalence of high frequency cells (i.e., individual cells with a large number of SCEs) among the exposed group, the difference was not statistically significant. The investigators scored only 30 cells per person. Although the reasons are unclear for the differences in results between the studies of plant workers and hospital workers, possible differences in study design and analysis, exposure levels, and drug toxicity may contribute to the disparate findings.

In a study of workers exposed to ethylene oxide, Stolley et al. (1984) identified three industrial sites with low (site I), intermediate (site II), and high (site III) exposures to the agent. At each site, workers were further characterized by potential exposure levels based on job classification. At sites I and II, the researchers chose worksite controls; at site III, they used both worksite and community controls. Blood samples were collected from a total of 234 persons at the start of the study and at 6-, 12-, and 24-month intervals. In over 91 percent of the assays, the researchers scored at least 80 cells. Age, sex, smoking history, and variability among scorers were controlled using ANOVA. At site III (high exposure), they found large differences in SCEs among high- and low-potential exposure groups that persisted throughout the 24-month study period. At site II, the investigators found a higher frequency of SCEs in the high-potential exposed group than in other groups, but found no consistent differences at site I.

Yager et al. (1983) studied ethylene oxide exposures among 14 hospital workers and 13 unexposed controls. They characterized exposure as high or low based on 6-month cumulative estimates from self-reports that were supported by hospital records that documented the use of ethylene oxide sterilizers. Age, sex, medical histories, and caffeine, alcohol,and other drug use were similarly distributed between exposed workers and controls. Although an equal number of exposed workers and controls smoked, smokers among the exposed workers smoked an average of nine cigarettes per day compared to 21 for controls. Fifty cells were scored in each assay, and data were analyzed with the Mann-Whitney U test. The mean frequency of SCEs was significantly higher among exposed workers compared with controls. Furthermore, a significant increase appeared when high-exposure workers were compared with those with low exposure.

A small number of studies have focused on SCEs and chromosome aberrations among rubber workers and metal plating workers. Mäki-Paakkanen et al. (1984) studied chromosome aberrations and SCEs among rubber workers in two manufacturing plants. They compared 55 workers exposed to complex mixtures of rubber manufacturing compounds with 35 controls from office jobs in the same factory or from a research institute. Thirty cells per assay were scored for SCEs, and a single scorer rated all the assays. Among nonsmokers, there was a significant increase in SCEs among exposed compared with unexposed persons in both factories. For both smoking workers and smoking controls, mean SCE frequencies were higher than for nonsmokers.

Nagaya (1986) compared SCEs among 24 workers in the chromium plating industry with 24 unexposed office workers matched for sex and age. An attempt was made to match on smoking history, but smokers averaged from 5–40 cigarettes per day, and "nonsmokers" included anyone who had not smoked for the previous 2 years, regardless of lifetime tobacco consumption. Twenty-five cells per assay were scored for SCEs, and urine chromium was analyzed by atomic absorption. The SCE levels and urinary chromium were not correlated, and mean SCE frequencies did not differ significantly between the chromium platers and the unexposed controls. The SCEs among smokers of both exposed and control groups, however, were significantly higher than SCEs among nonsmokers.

Numerous other studies have compared SCE levels across groups defined by lifestyle, demographic, exposure, or other characteristics, and virtually all epidemiologic studies of SCEs use them as dependent variables rather than as exposure markers in

studies of exposure–disease associations. The link between SCEs and disease risk remains unclear. The fact that many mutagenic exposures cause SCEs, however, suggests that they may be associated with increased disease risk. Descriptive studies of SCEs are necessary, because the determinants of SCE levels in healthy individuals are still a subject of controversy. Only recently, for example, have most investigators agreed that SCEs vary by sex and smoking status, and studies of age and race effects are still inconclusive. The current limited state of knowledge about SCEs and their measurement suggests that their greatest contributions to epidemiology lie in the future.

SUMMARY

1. SCEs are a form of chromosomal alteration. Although a background level of SCEs exists in the absence of identifiable mutagenic exposure, an increased SCE level is generally interpreted as an exposure or response marker. Theoretically, SCEs are detectable through in vivo and in vitro assays using any cell type; most human studies, however, use circulating lymphocytes.

2. The DNA lesions thought to produce SCEs undergo repair processes that may prevent SCE formation. Therefore, the number of observed SCEs can depend, in part, on the time between exposure and the sampling of biological material.

3. The SCE assay is most useful in detecting exposures from alkylating chemicals, agents that induce single-strand DNA breakage, DNA-binding agents, and some DNA-base analogs.

4. Several laboratory issues can substantially affect the interpretation of assay data. Lag time between sample collection and culturing, standardization of culturing protocol, and scoring procedure are points that require attention. Because cell half-lives vary from days to several years, the specific cell type under study also affects assay interpretation.

5. Estimates of the sensitivity and specificity (in the epidemiologic sense) of the SCE assay for exposure assessment are not available, largely because of operational problems with the epidemiologic definition of sensitivity and specificity in the context of SCEs. One alternative definition of sensitivity is the exposure level that produces a specified percentage increase in SCEs compared with the background level.

6. Human studies of SCEs have found associations with such life-style factors as diet and smoking, occupational exposures, and therapeutic exposures. Different studies, however, often show conflicting results.

7. Given the importance of variations in laboratory methods, comparisons across studies should most likely be limited to qualitative assessments unless assay protocols are very similar or identical.

8. A clear connection between the presence of SCEs and morbidity has not yet been established. Furthermore, the assay is nonspecific in the sense that it cannot discriminate the effects of several different exposures. Thus, SCE data should be evaluated conservatively as indicators of damage to genetic material.

SCE analysis has been used primarily as an in vivo (in animals) and in vitro method of screening chemicals for genotoxic activity. It is now gaining importance as a useful marker of exposures to genotoxic agents in epidemiologic studies. Its utility is limited,