8

CHROMOSOME ABERRATIONS

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INTRODUCTION

Chromosomal aberrations have been implicated in carcinogenesis since 1914, when Boveri proposed the somatic mutation theory of cancer. Boveri held that a "wrongly combined chromosomal complex" in a somatic cell was the heritable cause of abnormal cell proliferation.

We now know that structural changes in specific chromosomes may indeed be involved in the etiology of some malignant diseases. In most instances, however, the pathogenetic significance of chromosomal aberrations—if any—is yet unknown. Whatever their significance for an individual's ultimate risk of disease, aberrations in chromosome number or structure are increasingly being used in monitoring populations exposed to environmental hazards (Office of Technology Assessment, 1983, 1986). This chapter reviews the methodology of human chromosome analyses and explores their application in epidemiologic studies.

Although it is beyond the scope of this chapter, the history of cancer cytogenetics is itself a fascinating subject. Readers may want to consult any of several recent reviews (e.g., Hsu, 1979, 1987; Therman, 1986).

METHODOLOGY OF THE LYMPHOCYTE ASSAY

To analyze chromosomal aberrations, one needs to use cells that are capable of rapid division in culture. Because they are easy to obtain, white blood cells are the samples epidemiologists encounter most frequently. (Erythrocytes have no nucleus and, therefore, no chromosomes.)

In the preparation of a lymphocyte culture, a sample of peripheral blood is obtained and mixed with heparin to prevent clotting. The sample is centrifuged so that the white blood cells form a distinct layer that can be collected and placed in a tissue culture

medium. The addition of a mitogen (mitosis-producing agent), usually phytohemagglutinin (PHA), will stimulate the cells to divide. The PHA, an extract of the kidney bean, is capable of stimulating cell division by an as yet unknown mechanism. The culture is then incubated for 48 to 72 hours. Although the optimal incubation time is a point of contention (see "Technical factors in aberration type and yield"), in general, most investigators prefer 48-hour incubation.

The steps just described are known collectively as *culturing*. A process called *harvesting* allows one to obtain cells for analyses from cultured cells. The first step in harvesting is the addition of a very dilute solution of colchicine, which binds specifically to the tubulin of spindle microtubules. Because colchicine arrests mitosis at metaphase, cells in metaphase accumulate in the culture. A hypotonic solution is then added to cause the cells to swell from the influx of water. This allows the chromosomes to disperse freely within the cell membrane, separating the paired chromatids but leaving them still attached at the centromere. The cells are then fixed, to harden the chromatin and enhance the morphology. The fixed cells are spread on slides and may be stained (discussed later). The goal of slide preparation is to disperse the chromosomes well, leaving little overlap between them.

Microscopic examination of the slides involves counting the number of chromosomes per cell in a specified number of cells. After observing the morphology of each chromosome, one may want to take photomicrographs for a permanent record. Chromosomes can be cut from photographs of individual cells to form a karyotype. The normal human karyotype contains 46 chromosomes, arranged and numbered by size and centromere position from largest (1) to smallest (22). There are 44 autosomes (22 pairs) and two sex chromosomes.

Similar procedures are used for specimens of bone marrow cells. Because a portion of the cells in the bone marrow are already in the cell cycle, however, PHA is not necessary to stimulate mitosis. Modifications of this methodology for use with bone marrow cells are described by Dale (1980).

This is a general outline of the methodology. For complete technical details of the peripheral blood lymphocyte technique consult Evans and O'Riordan (1975).

Shipping and Storage of Samples

It is often desirable to take blood samples in a field setting. Blood specimens taken far from the cytogenetics laboratory should be maintained at about 4°C during shipping and storage. Cultures prepared from freshly drawn blood samples have more favorable growth characteristics than cultures prepared from stored blood. In general, storage times longer than 5 to 7 days will probably result in poor growth.

Technical Factors in Aberration Yield and Type

The length of time cells are maintained in culture is the most important in vitro factor that can influence the chromosome aberration result. After 48 hours in culture at 37°C, most of the lymphocytes are undergoing their first mitosis. Thus, cultures incubated for longer periods, that is, 72 hours, will contain increasing numbers of cells in their second or even third divisions. An important issue is the differential survival and

multiplication of healthy versus genetically damaged lymphocytes. For example, if lymphocytes with chromosome-type damage fail to undergo repeated mitoses in vitro because of genetic damage or mechanical difficulties, an examination of cells at 72 hours will underestimate the true aberration rate, as some aberrant cells could not enter their second division. Moreover, a proportion of the observed chromosome-type aberrations seen later may actually be "derived" aberrations, resulting from the duplication of aberrations that were initially of the chromatid-type (see "Types of chromosome damage"). For this reason, many investigators (e.g., Evans and O'Riordan, 1975) strongly recommend the 48-hour harvest time, and one consensus report of cytogeneticists deems fixation after 54 hours "unacceptable" (Bloom, 1981).

Efficiency, however, favors the 72-hour harvest time because it yields a considerably greater harvest of metaphase chromosomes. Several studies (reviewed in Gebhart, 1982) report that the observed chromosome aberration rates obtained in 48- and 72-hour samples show no significant difference. In some cases higher chromosome aberration rates were seen in the 48-hour samples, but in others, the reverse was observed. Thus, it would seem that the choice of sampling time per se, 48 versus 72 hours, may not be critical. The 72-hour period, however, is clearly advantageous in terms of cell yield (Gebhart, 1982).

The culture medium used to incubate the cells is also a relevant factor, particularly combined with sampling time. In 72-hour samples, the proportion of cells in their first, second, and third cycles varies with the culture medium (Gebhart, 1982). For example, when Obe et al. (1975) compared two media, TC medium 199 and Ham's F-10, they found that DNA synthesis and mitoses occurred much earlier and to a greater extent with Ham's F-10. These investigators reported that sufficient quantities of exclusively first mitoses were obtained in 48 hours using Ham's F-10. Because accurate determinations of aberration frequencies should include only cells in their first mitoses, culture techniques that provide adequate numbers of dividing cells using the 48-hour harvest time may be the procedure of choice.

A possible "compromise" method, which insures that only first division cells are analyzed, involves adding bromodeoxyuridine (BrdUrd) to the culture medium and then performing a staining procedure that detects first, second, and subsequent divisions. This technique has been widely used in radiation studies. The principal drawback of this method is the possibility that BrdUrd itself may alter the aberration frequency, either alone, or by interacting with DNA damage induced by a chemical agent. Advantages and disadvantages of the BrdUrd technique are briefly discussed by Preston (1984).

AN OVERVIEW OF CHROMOSOME STRUCTURE

Brief *user-friendly* overviews of chromosome nomenclature are available in Yunis (1981) and Larson (1983). Readers who want more detailed accounts of human chromosome structure can consult the recent volumes by Therman (1986) and by the International System for Cytogenetic Nomenclature (ISCN) (1985). For the present, however, here is a brief review. A metaphase chromosome is composed of two identical (sister) chromatids, attached at a constricted region, the centromere. The

centromere divides the chromosome into two short arms, designated "p" (for petite), and two long arms, designated "q." The position of the centromere in human chromosomes can be in the middle of a chromosome (metacentric), near the middle (submetacentric), or very near the end (acrocentric).

At anaphase, each chromosome divides longitudinally at the centromere, and each sister chromatid is incorporated into one of the two daughter cells.

It should be noted that the term *chromosome* is potentially ambiguous, as it refers to different structures at different stages of the cell cycle. To preclude confusion, a brief outline of the cell cycle may be helpful.

The Mitotic Cycle

Mitotic division comprises only a small portion of a cell's life cycle (Fig. 8-1). After mitosis, the new daughter cells enter a postmitotic phase during which no DNA synthesis occurs. This is G_1 (Gap 1). *Resting cells,* those not preparing for cell division, are considered to be in a subphase of G_1 called G_0. The next stage is S, the period of DNA synthesis. During S, the DNA content of the cell doubles, as each DNA molecule acts as a template to make a complementary copy of itself. The S phase is followed by a second nonsynthetic period, G_2 (Gap 2). G_2 ends with the onset of mitosis (M). The complete cell cycle in cultured human cells may last 12 to 24 hours, only 1 hour of which involves mitosis (Thompson and Thompson, 1986).

The potential ambiguity of the term chromosome stems from the fact that the chromosomes of G_1 cells are made up of one chromatid, whereas those of G_2 cells are made up of two chromatids. Cells in S contain chromosomes made up of both one and two chromatids.

As we will see later, the stage of the cell cycle that cells are in when they are exposed to a clastogenic (chromosome-breaking) agent is a major determinant of the type of chromosome aberration observed.

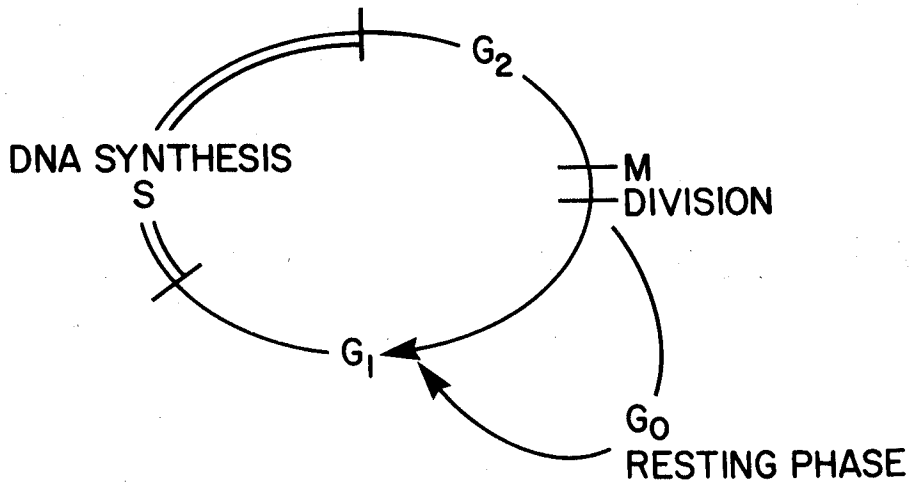

Figure 8-1. The cell cycle. (Modified after Thompson and Thompson, 1986.)

Banding Techniques

Chromosomes are identified by their morphology and staining characteristics. Cytogenetic staining methods are known as *banding techniques*. Banding refers to the appearance of horizontal alternating light and dark areas along the length of a chromosome. The advent of banding techniques in the early 1970s revolutionized cytogenetics, because it permitted the identification of individual chromosomes, a feat previously possible for only a few chromosomes (Hsu, 1987).

Three types of banding are in general use: G-banding, Q-banding, and R-banding. Most laboratories generally use routinely one or another of these techniques in identifying chromosome abnormalities.

G-banding

G-banding is the most popular banding technique. The G stands for Giemsa, the dye generally used to visualize the bands. G-bands are produced by pretreating slides with trypsin, which denatures chromosomal protein, and then staining the slides with Giemsa. A simple method for G-banding is described by Sanchez et al. (1973).

The numbering of G-bands follows an internationally standardized system that permits precise definition of numerical and structural aberrations (ISCN, 1978). This convention is as follows. The centromere divides the chromosome into short and long arms. Each arm is divided into several regions by specific bands that serve as landmarks. Numbering begins at the centromere and proceeds outwards in each arm. To designate a specific band one lists the chromosome number, the arm symbol, the region number, and the band number in order, without spacing. Thus, band 1q42 indicates chromosome 1, long arm, region 4, band 2. Subbands may be described by placing a decimal point after the band designation, followed by the number assigned to the subband (ISCN, 1981).

Q-banding

Q-bands, the first bands described, are the reference bands for the standard classification. The Q stands for the dye quinacrine, which causes the bands to fluoresce under ultraviolet light. With a few minor exceptions, Q-bands are comparable to G-bands. Unlike G-bands, however, Q-bands are not permanent: after several photomicrographic exposures the fluorescence fades too much to be usable. For this reason, G-bands may be more suitable for routine work.

R-banding

The R in R-banding stands for reverse. Reverse-banding involves pretreatment with hot alkali and subsequent staining with Giemsa or with flurochromes. R-bands are the reverse of Giemsa bands, dark where G is light and light where G is dark. R-banding is not common in the United States, but is used extensively in European laboratories. Figure 8-2 illustrates G, Q, and R bands.

Several other banding techniques (e.g., high-resolution banding) may be used to study specific chromosomes or specific areas of certain chromosomes. These are rarely employed in epidemiologic studies unless some hypothesis predicts specific chromo-

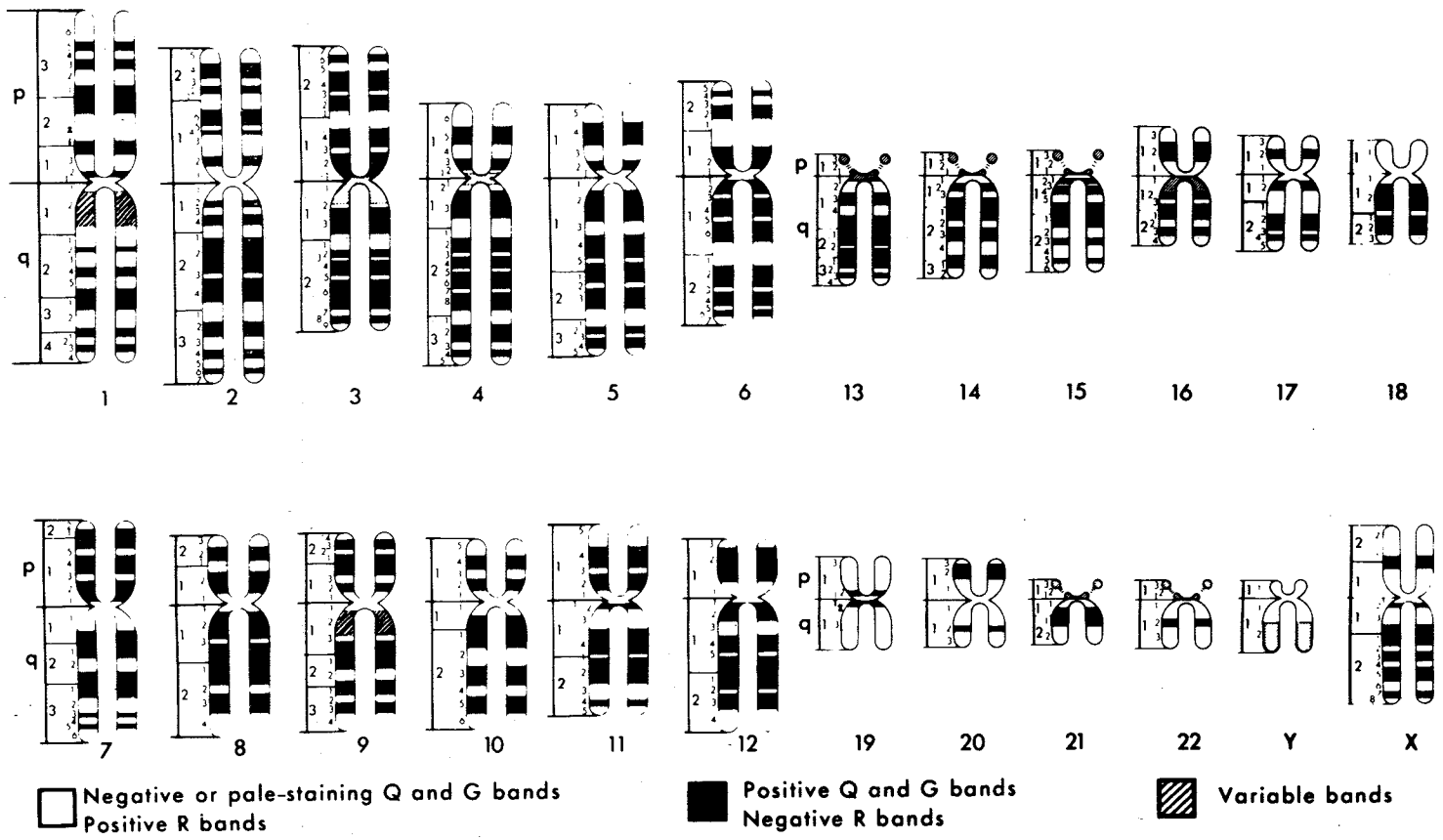

Figure 8-2. Diagrammatic representation of G, Q, and R banded chromosomes. Reprinted from Paris Conference (1971). Standardization in human cytogenetics. *Birth Defects:*1972;8(7).

some breaks. For technical details of these techniques, see Dale and Jurgens (1980), Yunis (1981), and Therman (1986).

From the standpoint of identifying chromosome aberrations, banded preparations are optional: most aberrations can be detected without banding (Preston, 1984). Banded preparations, however, increase the sensitivity of the lymphocyte assay for several common classes of structural aberrations: terminal deletions, inversions, and reciprocal translocations, as discussed later.

TYPES OF CHROMOSOME DAMAGE

Numerical Abnormalities

Chromosomal abnormalities may be either *numerical* or *structural*. Numerical aberrations denote any deviation from the normal human complement of 46 chromosomes. Numerical aberrations usually result from an error in division at metaphase, nondisjunction. When nondisjunction occurs during mitotic division, the sister chromatids fail to separate. As a result, one daughter cell receives an extra chromosome, and the other daughter cell receives none. An extra or whole missing chromosome is indicated by a "+" or "-" placed before the chromosome number. Probably the best known numerical anomaly is trisomy 21, which results in the phenotype of Down syndrome (Lejeune et al., 1959).

Structural Abnormalities

Two types of induced structural chromosome damage can be distinguished at metaphase: *chromosome-type* and *chromatid-type*. In chromosome-type damage, the unit of breakage and reunion is the whole chromosome (i.e., both chromatids at the same locus). Chromosome-type damage occurs when exposure to ionizing radiation or to other mutagens occurs during the G_0 or G_1 phase of mitosis. This may seem paradoxical, as chromosomes in the G_1 phase consist of only one chromatid. However, if a chromosome break occurs during G_1, the break will be duplicated during the S phase and, therefore, will affect both chromatids when they are observed in the following metaphase.

Chromatid-type damage takes place if exposure to these mutagens occurs during S or G_2. In this case, usually only a single chromatid is involved. Some agents, such as some alkylating agents, are exceptions in that they produce only chromatid-type aberrations even though the cells are exposed in G_0 or G_1 (Preston, 1984).

Chromosome-Type Aberrations

Numerous classification systems for structural chromosome aberrations abound (e.g., Evans and O'Riordan [1975], Savage [1975], and ISCN [1985]). Because they are essentially similar, any comprehensive classification system that permits clear and accurate identification of aberrations is acceptable. This chapter follows the classification of Evans and O'Riordan (1975).

This classification system distinguishes seven classes of chromosome-type aberrations. Types 1–5 involve only a single chromosome and are, therefore, called *intra-*

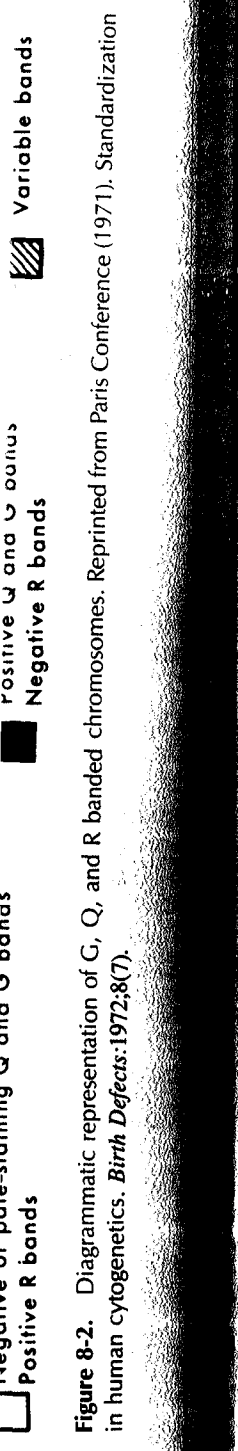

Figure 8-2. Diagrammatic representation of G, Q, and R banded chromosomes. Reprinted from Paris Conference (1971). Standardization in human cytogenetics. *Birth Defects*:1972;8(7).

changes; types 6 and 7 exchange material between chromosomes and thus are *interchanges.*

1. *Terminal deletions* are paired acentric fragments that result from a simple break across the chromosome.

2. *Minutes* are pairs of acentric fragments that are smaller in size than terminal deletions. Minutes appear as small spheres of chromatin (hence their name) and probably represent intercalary, rather than terminal deletions. Synonyms are *interstitial, isodiametric* or *dot deletions.*

3. *Acentric rings* are paired segments of chromatid without a centromere, joined physically to form a ring.

4. *Centric rings* are ring structures containing a centromere. Centric rings are generally accompanied by an acentric fragment.

5. *Inversions* may be of two types: *paracentric,* in which both points of breakage and reunion lie on the same arm of the chromosome, and *pericentric,* in which the points of breakage and inversion lie on opposite sides of the centromere. That is, pericentric inversions are intrachromosome exchanges between the p and q arms.

The relative position of the centromere may not be altered in inversions and will appear to change only if the broken arms are of dissimilar size. As a result, inversions are generally detectable only in banded preparations.

6. *Reciprocal translocations* involve the breakage of two chromosomes and the reciprocal exchange of their broken segments. If reciprocal exchanges occur between segments of equal size, the translocations will not be detectable unless banded preparations are used (see inversions). Interchanges may occur in the centromere regions and thus result in whole arm exchanges. When these translocations involve acrocentric chromosomes, they are referred to as *Robertsonian translocations* or *centric fusions.*

7. *Dicentric or polycentric aberrations* involve exchanges between two or more chromosomes and result in a dicentric or polycentric chromosome, plus an associated acentric fragment.

When aberration types 1–3 (terminal deletions, minutes, and acentric rings) are not associated with a chromosome rearrangement, they are often lumped together as *acentric fragments.* Fragments associated with chromosomal exchange (e.g., a minute associated with a dicentric) are scored as part of that exchange, and not as aberrations in their own right. The seven classes of chromosome-type aberrations are illustrated in Figure 8-3.

Chromatid-Type Aberrations

Chromatid-type aberrations are induced by ionizing radiation and other mutagens during the S or G_2 stage of the cell cycle. Viruses and many chemical agents, however, will cause only chromatid-type aberrations even though cells are exposed in G_1 and are examined in their first mitosis. These chromatid-type aberrations result from errors in DNA replication that occur during the S phase. Thus, these chromatid-type aberrations are not primary genetic lesions (lesions arising from direct mutagenesis), but are secondary lesions resulting from defective DNA repair.

The nomenclature of chromatid-type aberrations parallels closely that of chromosome-type aberrations, as follows.

1. A *chromatid and isochromatid gap* (= *achromatic lesion*) appears as a nonstaining, constricted region in the chromatid arm. The apparently broken segments of the

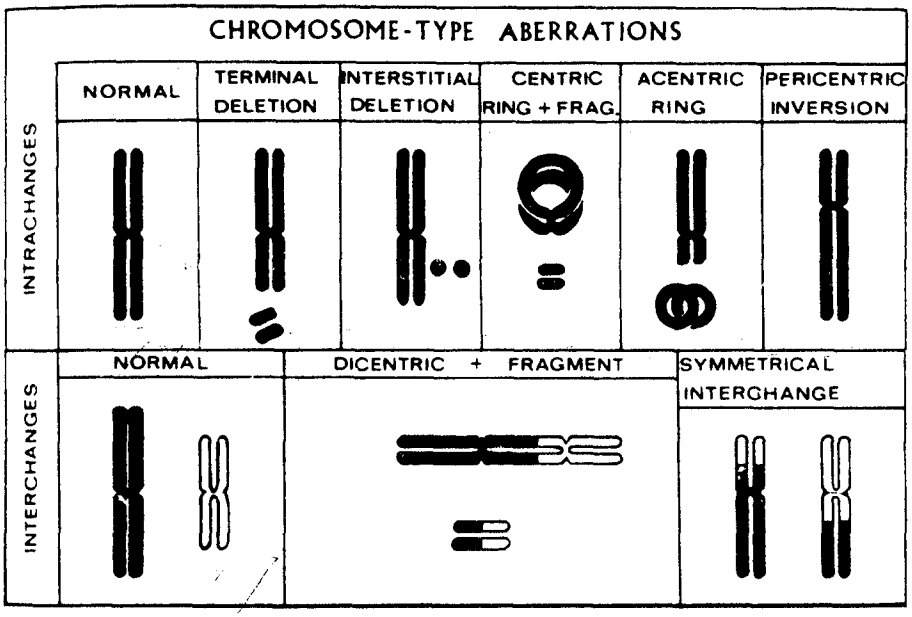

Figure 8-3. Seven classes of chromosome-type aberrations distinguishable at mitotic metaphase. (From Evans, 1984.)

chromatid are aligned. Gaps involving both chromatid arms at the same position are termed *isolocus* or *isochromatid gaps*.

2. A *chromatid break* is a discontinuity and displacement in the chromatid arm such that the broken chromatid ends are not aligned. *Simple* chromatid breaks result in a terminal deletion.

3. *Chromatid minutes* are unpaired intercalary fragments.

4. *Chromatid acentric* rings are intercalary fragments joined to produce ring structure.

5. *Centric rings* are intrachanges that result in rings that include a centromere.

6. *Inversions* can be *paracentric* or *pericentric*, as in *chromosome-type* inversions. Paracentric inversions are generally not scorable.

7. *Isochromatid aberrations* involve exchanges between paired chromatids (= *sister chromatid exchanges*). These can be confused with *chromosome-type* terminal deletions, but can be distinguished because isochromatid breaks usually involve a reunion of the broken chromatids.

8. *Symmetrical interchanges* involve an exchange(s) between two (or more) chromosomes. Exchanges between one chromatid in each of two chromosomes results in a four-arm structure termed a *quadri-radial.*

9. *Asymmetrical interchanges* are exchanges between two or more chromosomes resulting in one or more dicentric chromatids.

Unlike chromosome-type aberrations, chromatid breaks, and especially, chromatid gaps, are unreliable indicators of exposure-induced genetic damage. In part, this is because the scoring of gaps and breaks can be extremely subjective. Moreover, many gaps are technical artefacts—the results of poor culture conditions and drastic pro-

cedures during slide preparation (Evans and O'Riordan, 1975). For this reason, many investigators (e.g., Bloom, 1981; Therman, 1986) recommend that chromatid gaps not be counted in the total aberration score. If gaps are scored, it is important that control cell cultures be prepared at the same time as the exposed cultures to estimate the background rate of gap formation (Preston, 1984).

Other Chromosome Aberrations

1. *Shattering.* In shattering, chromosomes seem to have fractured into many small pieces. Shattering may affect all, or only a small number of the chromosomes in a cell. Shattered cells should be recorded separately and should not be included in the total number of cells analyzed (see Scoring of Aberrations).

2. *Pulverization.* Pulverization is similar to shattering, except that usually the entire cell is affected. As in shattering, pulverized cells should be recorded separately and should be excluded from the total cells analyzed.

3. *Polyploidy.* Cells with more than two full sets of chromosomes (polyploidy) may be observed. Aberrations observed in polyploid cells should be recorded separately (as above).

SCORING OF ABERRATIONS

It is important that each of the aberration types described be recorded separately. Results should be reported as frequency of aberration types per cell and frequency of aberrant cells per aberration type. The score "total breaks per cell" obscures valuable information and is no longer acceptable as state of the art (Bloom, 1981; Preston, 1984). For statistical analysis, however, it is often appropriate to combine frequencies of aberration types, such as chromatid-type exchanges and chromosome-type exchanges.

Much of the inconsistency observed in the results of chromosome aberration studies may reflect the fact that currently there is widespread variation in the way abnormalities are scored. The adoption of consistent rules might help avoid some of this cytogenetic information bias. Therman (1986) has suggested the following five rules for chromosome-breakage studies:

1. If the analysis is done on cultured cells, the control cells should be from the same individual and cultured at the same time. If this is not possible (for instance, in the study of whole-body irradiation), the control person should be of the same age and sex. In animal studies, the control should belong to the same inbred strain.
2. Diagrams or drawings should illustrate scored abnormalities, and particularly show what is scored as a gap or a break.
3. The chromosome slides of treated and control cells, made and stained at the same time, ought to be randomly coded to avoid investigator bias.
4. The investigator should establish clear rules about which metaphases are included in the study. Furthermore, published results should state whether the whole chromosome complement has been analyzed in all cells or only in those with obvious abnormalities.

5. The analysis should be done by a competent cytogeneticist, not by part-time, inexperienced laboratory helpers (Therman, 1986, p. 85).

Specific guidelines for scoring aberrations as well as a sample score sheet are available in Bloom (1981).

As Therman notes, it is essential that the cytotechnologist analyzing the slides be blind to the group (exposed or unexposed) to which they belong. Commonly several individuals and laboratories share the analysis. Thus, precautions must be taken so that possible bias caused by interindividual or interlaboratory variation will not influence the result. For example, control slides should not be scored exclusively by Laboratory A and exposed slides by Laboratory B. If possible, researchers (or laboratories) should keep photographic records of all aberrant and suspect cells. It is also desirable to produce and keep a permanent banded karyotype for each individual in the study, whether or not banded preparations are employed in the analysis of chromosomal aberrations themselves. (Banded preparations may be judged too time consuming to warrant the small added gain in sensitivity [see Preston, 1984, p. 132].) The banded karyotype ensures that any observed chromosomal abnormalities cannot be attributed, at some later date, to a congenital chromosome abnormality.

It is not possible to determine exactly the number of cells to be analyzed from any sample, as this number will depend on the expected aberration frequency, and hence, on the specific exposure under study. In general, however, 200 cells should be analyzed from each sample (Preston, 1984; see Sample Size and Power Considerations).

STATISTICAL ANALYSIS OF CHROMOSOME ABERRATIONS

The goal of analysis is to determine whether there is evidence of prior exposure to chemical mutagens, ionizing radiation, or both. Bloom (1981) provides an excellent discussion of the logic of data analysis on structural aberrations. Highlights of that discussion follow.

Analysis Strategies

The data of any individual studied consist of the number of aberrant cells identified from the total number of cells examined. In other words, the data are proportions. Thus, the number of cells with aberrations should conform to a binomial distribution with parameters n (sample size) and p (the true population of aberrant cells in the individual sampled). The basic statistical task is to compare the sets of proportions observed among the exposed and unexposed groups and to test the hypothesis that, on average, the proportions are greater in the exposed group.

The analysis that yields the greatest statistical power involves pooling data across individuals within exposure groups. The total number of cells examined in each group is used as the denominator for the estimate of the overall aberration rate. Because large aberration scores in a few individuals can influence the pooled analysis (e.g., 10 aberrations in a single individual is "equivalent" to one aberration in each of 10 individuals), it is important to ensure that the observed interindividual variability among the proportions does not exceed the variability expected from repeated sampling

from a *single* binomial distribution. Fisher's variance test is recommended for this purpose (Cochran, 1954).

If one can assume a single aberration frequency within each group, one can analyze with statistical techniques for comparing two binomial frequencies. The data can be cast in the familiar 2 x 2 table, as follows:

	Exposed	Not Exposed	Total
Aberrant cells	a	b	a + b
Nonaberrant cells	c	d	c + d
Total cells	a + c	b/(b + d)	N
Proportion aberrant	a/(a + c)	b/(b + d)	

The question central to the analysis is whether the observed proportion in the exposed group, a/(a + c), is greater than the observed proportion in the unexposed group, b/(b + d), and whether this difference is sufficiently large to rule out chance as a competing explanation.

If there is an apparent difference between the exposed and control groups, either in terms of frequencies of cells with aberrations or frequencies of aberrations per cell, then further analysis is necessary to ascertain whether there is a dose–response relationship and whether interactions exist with potential confounding variables (Bloom, 1981).

Sample Size and Power Considerations

The number of cells examined can greatly influence a study's conclusions and is thus of particular importance in interpretating apparently negative findings. A valuable discussion of sample size and power as they pertain to human cytogenetic studies appears in papers by Whorton (1985) and Whorton et al. (1979).

To illustrate the importance of sample size, let us consider the following: If an aberration frequency of 1 percent is expected in the control group (as might occur if all aberrations were tallied), then it will be necessary to score 325 cells in each group to detect a fourfold increase in aberration frequency in an exposed group at the 5 percent significance level, with 80 percent power. Detecting a threefold increase would require some 600 cells, and detecting a relative risk of two would require nearly 2,000 cells per group. (These estimates assume equal numbers of exposed and unexposed in each group.)

If an exposed group as a whole shows an elevated frequency of aberrations, then the specificity with which an individual's aberration frequency can be ascertained becomes of interest. In general, it is necessary to have large quantities of data to estimate small frequencies accurately. For example, if no aberrations are detected in 100 cells from a given individual, that individual's aberration frequency can be determined only as less than 4 percent. To conclude that the true aberration rate is less than 1 percent at the 95 percent confidence level would require 375 normal cells. Of course, such precise determinations generally may not be cost effective. One feature of studies using chromosome aberrations, however, is that, given an adequate supply of stored blood, it is relatively easy to increase sample sizes.

Finally, it should be noted that there is considerable variation in frequency of chromosome breakage among cultures from different individuals and among consecutive cultures from the same individual (Littlefield et al., 1975; Galloway et al., 1986). This suggests that the frequency of chromosome aberrations in peripheral lymphocytes is influenced by many factors that are yet unidentified.

COST OF THE LYMPHOCYTE ASSAY

Analyzing metaphase chromosomes is a costly and labor-intensive process. Apart from the expense of obtaining blood samples, the processes of culture handling, slide preparation, and analysis of cells requires approximately 4.5 person-hours per culture of 100 cells (Evans and O'Riordan, 1975). Because most of this time—4 hours—is required for scoring aberrations, the lymphocyte assay requires a considerable expenditure of a trained cytogeneticist's time.

Attempts have been made to automate chromosome analysis (e.g., Philips and Lundsteen, 1985). But, although there are semiautomated techniques that can expedite the analysis of a relatively small number of cells (those needed for fetal chromosome diagnosis, for example), techniques for automated analysis of the large numbers of cells required for epidemiologic purposes are not currently practical.

ACCURACY OF THE LYMPHOCYTE ASSAY

To illustrate the limitations of the lymphocyte assay as a method of detecting aberrations and predicting possible exposure to chromosome damaging agents, we need to consider several aspects of the assay and of particular clastogens themselves. Discussions of the sensitivity of the lymphocyte assay appear in papers by Preston (1984), Buckton and Evans (1982), and Schinzel and Schmid (1976).

The accuracy of the lymphocyte assay varies according to the type of clastogenic exposure. In general, the assay is a sensitive tool for detecting exposure to radiation; it is much less sensitive for detecting exposure to chemicals.

Radiation Exposures

Radiation and a small number of chemical agents (the truly *radiomimetic* drugs, e.g., cytosine arabinoside, bleomycin, streptonigrin, and 8-methoxy caffeine) cause aberrations in all stages of the cell cycle: chromosome-type aberrations in G_1 and chromatid-type aberrations in S and G_2. In contrast, most chemical agents induce aberrations only when the cell is in the S phase or when it passes through the S phase between exposure and observation at mitosis. These aberrations occur either at the time of DNA replication or as a postreplication event, and are exclusively of the chromatid-type. This difference in the mode of aberration induction has important consequences for the ability of the assay to detect aberrations after radiation or chemical exposures.

Peripheral lymphocytes are in a noncycling stage of G_1 (generally referred to as G_0) until they are stimulated in vitro to reenter a cycling phase. If these noncycling cells are exposed to radiation, then chromosome-type aberrations will be induced directly. The

frequency of aberrations observed at the first mitotic division in vitro will be the induced frequency, hence the importance of analyzing cells in their first in vitro division.

There is ample evidence, from studies that made physical estimates of exposure, that the frequency of observed chromosome aberrations is proportional to the radiation exposure (Awa, 1975). In individuals exposed to radiation, it is thus possible to use the chromosome aberration frequency to estimate exposure with a reasonable degree of accuracy (Preston, 1984). We must note, however, that this can be done reliably only in instances in which blood samples are taken relatively soon after exposure—within 6 weeks.

For samples taken at much longer intervals after acute radiation exposure (e.g., in the atom bomb survivors in Hiroshima and Nagasaki), attempts to infer exposure levels become more complex because of the importance of cell turnover in the lymphocyte population. Although no selective disadvantage has been ascribed to aberrant cells in G_0, repopulating cells tend to be those that do not contain cumbersome, cell-lethal aberrations such as rings, dicentrics, and acentric fragments. The selective death of aberrant cells means that the aberration frequency in samples taken at increasing intervals after exposure will contain fewer and fewer aberrant cells. The numbers of newly arising cells will dilute the numbers of aberrant cells. Because a portion of lymphocytes are very long-lived, however, a small proportion of aberrant lymphocytes can be detected in the circulating population 30 years or more after exposure.

Unlike the majority of observed chromosome-type aberrations (dicentrics, rings, and acentric fragments), reciprocal translocations and inversions usually are not cell-lethal. Because these can be transmitted with a high probability, the numbers of cells containing these aberrations will decline less after exposure. Although one might want to analyze these types of aberrations, the sensitivity of the assay for these aberrations is only about 50 percent, even with banded preparations (Preston, 1984).

In the case of long-term chronic exposure to radiation (e.g., in nuclear dockyard workers), some aberrations in the G_0 cells will accumulate in the population of the long-lived lymphocytes and can indicate that an exposure has occurred. Unlike samples taken after acute exposure, the observed aberration frequency will not be a direct measure of the actual amount of exposure.

In summary, the fact that chromosome aberrations induced by radiation occur at the same stage of the cell cycle as that in which exposure occurs means that, under appropriate circumstances, these aberrations can be reliable dosimeters of exposure. The lymphocyte assay is less sensitive in measuring chronic exposures, or if it is performed long after acute radiation exposure has occurred. Such exposures are detectable, however, in a long-lived subpopulation of lymphocytes.

Exposure Specificity and Marker Persistence

The lymphocyte assay is not a one-size-fits-all detector of exposure to all clastogenic agents, simply because the nature and longevity of the aberrations vary from one agent to another. For example, occupational exposures to some chemicals, such as arsenic and benzene, are associated with very long-lived chromosomal aberrations that probably reflect cumulative exposure (for a review of these exposures, see Office of Technology Assessment, 1983). Conversely, aberrations seen with exposure to vinyl chloride

monomers are very short-lived, often disappearing within weeks or even days. In this instance, chromosomal aberrations could be used to document recent, but not chronic exposure. What mechanism underlies this difference is not known.

Such differences in the persistence of induced aberrations indicate the need to determine the appropriateness of chromosomal endpoints in occupational studies on a case by case basis. Ideally, meaningful use of the assay requires some prior information about the type and persistence of the aberrations expected. For an illustration of how to use the lymphocyte assay, see the case of ethylene oxide (under "Occupational Monitoring").

Chemical Exposures

The interpretation of the lymphocyte assay is less certain and its potential sensitivity is less well understood in cases of exposure to chemical agents. As described previously, most chemicals induce aberrations only when the cell passes through an S phase between exposure and observation. Because the peripheral lymphocyte is in G_0, the first S phase takes place in vitro after mitogenic stimulation. DNA damage induced in G_0 will be manifest in the S phase as chromatid-type damage, due in part to aberration-induced misrepair. That is, the frequency of chromatid-type aberrations will depend on the amount of chromosome damage, whose preservation and/or misrepair results in chromatid aberrations that remain at replication. Several factors influence these aberrations including the amount of initial damage and the amount and accuracy of repair that takes place in G_0 and G_1 after mitogenic stimulation.

The time between exposure to a chemical agent and sampling is a far more important consideration in the case of chemical agents than for radiation. This is because several factors operate to reduce the amount of DNA damage present at the time of replication and thus to reduce the aberration frequency. A striking illustration of the importance of time in diluting the frequency of observed chromatid-type aberrations can be seen in patients who have received cytostatic drug chemotherapy. Such people have very high aberration frequencies in lymphocyte samples taken during therapy, but virtually no induced aberrations in samples taken 2 months after therapy ends (Schinzel and Schmid, 1976).

Furthermore, because chromosomes have complex mechanisms of repair, the frequency of aberrations observed in a cell population exposed to a chemical agent is not likely to be directly related to exposure. However, one might find a proportional relationship. Unlike exposure to radiation, in which an observed aberration frequency that is not significantly elevated above background can be used to rule out exposure of a given magnitude, it is impossible to estimate a maximum possible exposure to chemical agents. Again, study of patients exposed to cytostatic drugs shows that, even after exposure to massive doses of clastogens, a normal frequency of aberrations can be found after enough time has passed (Schinzel and Schmid, 1976).

As with acute chemical exposures, the lymphocyte assay is also an unreliable tool to indicate chronic chemical exposures because of the repair that constantly occurs in the G_0 lymphocyte. This constant repair is reflected in less damage observable in the S phase. As a result, the chromosome aberration frequency observed will be very low, and the probability of detecting a frequency significantly above background will be small (Preston, 1984).

Table 8-1 Radiation Versus Chemically Induced Chromosome Aberrations in Peripheral Lymphocytes

	Radiation	Chemicals
Aberration type	Chromosome	Chromatid
Reliability	Good	Uncertain/poor
Dose-response	Yes	Variable
Persistence of marker	Long duration	Short/variable duration

In conclusion, because aberrations caused by nonradiomimetic chemicals are not induced at the time of exposure to the clastogenic agent, and as several time-dependent repair processes reduce the induced aberration frequency, the lymphocyte assay is much less sensitive as a measure of exposure to chemicals than of exposure to radiation. Table 8-1 presents a comparison of different properties of the lymphocyte assay for chemical and for radiation exposures.

CONTROL GROUPS AND THE ISSUE OF "BACKGROUND" FREQUENCY

To gauge whether the frequency of chromosome aberrations is elevated in a human population exposed to some factor, it is necessary to compare this frequency with the frequency of aberrations in an unexposed, or control, group. How one selects an appropriate reference group will depend on the type of exposure: whether to radiation or to chemicals.

In the case of suspected exposure to radiation, determining background frequencies of chromosome aberrations is fairly straightforward. Radiation-induced aberrations are of the chromosome-type. Because they can be scored easily and reliably, dicentric chromosomes are generally used to estimate exposure. Because the frequency of dicentrics in unexposed populations is low (about 1/1000 cells), it is easy to detect the effect of even small radiation doses. Moreover, because most other environmental agents that influence the background rate of aberrations are expected to induce chromatid-type aberrations, these factors should not be important confounders in comparing radiation-exposed and nonexposed groups. In general, the factors that induce chromosome-type aberrations are very well studied (Awa, 1975), and Lloyd et al. (1980) have assembled a compendium of data on the frequencies of chromosome-type aberrations in numerous groups occupationally exposed to radiation.

As discussed, most chemical agents induce chromatid-type aberrations. Thus, factors that influence the frequency of these aberrations in exposed and nonexposed populations are important. The Office of Technology Assessment (1983) has compiled background frequencies for chromosomal aberrations in unexposed populations from several reported studies. Although diverse laboratory methodologies make accurate comparisons among these studies difficult, the range of reported frequencies of individual aberrations per cell was:

Chromosome breaks	0.11–6.72%
Chromatid breaks	0.1–3.0%
Exchange aberrations	0–0.34%
Cells containing any aberrations	0.2–8.5%
Sister chromatid exchanges	5.8–16.2 per cell

The wide variations in the range of normal values for chromosomal endpoints emphasize the potential importance of individual differences in laboratory methodology and illustrate the difficulty in making quantitative comparisons of aberration frequencies across studies. It is especially noteworthy that the 40-fold differences reported for normal values of chromosome aberrations are far in excess of those reported in individual studies between occupationally exposed and unexposed groups (Office of Technology Assessment, 1983).

"Matched" Controls

Most investigators recommend comparing the aberration frequency observed in an exposed group with that of a matched control group. The matching variables, such as gender, age, socioeconomic status, geographic location, and others, are attempts to ensure that the aberration frequency observed in a control population is, save for the exposure, similar to the background frequency of the exposed population. Because it is not clear what factors actually influence the background frequency of chromatid-type aberrations, attempts to provide a matched control group should be viewed as attempts to control for unmeasured confounders.

In occupational exposures, the ideal controls clearly would be the individuals themselves. That is, blood samples could be taken from individuals before they began work in a potentially hazardous environment. Although this has been done rarely, it certainly should be considered in planning studies. Further discussions on selecting control populations appear in Preston (1984).

Potential Confounders

Many factors have been reported to influence the background rate of chromosome aberrations, including recent viral infections, age, gender, smoking habits, oral contraceptive use, and season of the year (see Office of Technology Assessment, 1983, for complete citations). Of these, only viral infection is an undisputed and strong independent risk factor. Age and smoking status probably are risk factors, albeit moderate ones.

Acute viral infections, particularly with measles virus, are potent clastogens. Chromosome damage caused by viral infection varies from single chromatid breaks to pulverization of the entire chromosome complement (Therman, 1986). The mechanism(s) of virus-induced chromosome breaks are poorly understood. For a review of the role of viruses in clastogenesis, see Nichols (1983).

There are many conflicting reports on the role of age. Some studies report an increase in background aberration frequency with age, and others report no effect. One study with a large sample size ($> 4{,}000$ cells) supports the hypothesis that aberration frequency does increase with age. Kuhn and Therman (1979) report that an analysis of

2,324 cells from subjects under 40 years of age yielded an average of 0.8 percent of cells with chromosome aberrations (excluding gaps). A sample of about the same size from individuals with a mean age of 55.8 years gave an average of 2.4 percent abnormal cells. Galloway et al. (1986) also found an increase in chromosome aberrations with age. Similarly, Bochov and Kuleshov (1972) reported that the frequency of chromosome aberrations induced in vitro by alkylating agents increased sequentially in lymphocytes from newborns, young adults (mean age 23), and elderly persons (mean age 70). In this much smaller study, however, these authors found no increase with age in the background aberration rate.

There has been considerable controversy about the clastogenic effects of cigarette smoke. Many positive and negative studies have been reported (see Littlefield and Joiner, 1986, for citations). Few have used comparable methodologies. In a recent carefully conducted study, Littlefield and Joiner (1986) compared the incidence of chromosome aberrations in 500 first-division metaphases from 48-hour lymphocyte cultures from six nonsmokers and from six people who had smoked at least one pack of cigarettes per day for at least 20 years. The subjects were similar in age and in their consumption of beverages containing caffeine, a potent co-clastogen. Analysis of coded slides revealed a total of three aberrations in the 3,000 slides from the nonsmokers (an aberration rate of 0.1%) versus 22 aberrations in the 3,000 slides from the smokers (an aberration rate of 0.7%). Thus, these data support the hypothesis that cigarette smoke is clastogenic. Therefore, in epidemiologic studies, it is important to ensure that smokers are not distributed disproportionately between the unexposed and the exposed groups under study. It is also possible that smoking may modify the clastogenic effects of other exposures.

In conclusion, established potential confounders of an exposure-chromosome aberration association include current viral infections, age, and heavy smoking. Thus, it is necessary to obtain smoking histories on all participants in a planned study. Potential subjects with exposures to ionizing radiation, chemotherapy, and recent vaccinations should be excluded.

BIOLOGICAL SIGNIFICANCE OF CHROMOSOME ABERRATIONS

An empirical association between chromosome damage and carcinogenesis has been established in animal studies. We must emphasize, however, that the lymphocyte assay is a marker of exposure or biological response to exposure. Except in rare cases, such as the Philadelphia chromosome described later, it is not a general marker of disease or of an individual's susceptibility to disease.

An important issue that needs to be clarified is the difference between the epidemiologic significance of an increased aberration frequency in populations, and the clinical significance of an elevated aberration frequency in a given individual.

Ongoing epidemiologic studies of the Japanese survivors of the atomic bomb have established that these populations have an increased risk for many types of cancer, particularly leukemias, cancers of the thyroid, female breast, and lung (Awa, 1975). All of these cancers have shown a dose-dependence for radiation, although the shape of the dose–response curve differs for the different cancers. Extensive cytogenetic investigations of the survivors have also revealed a dose-dependent increase in chromosomal

aberrations. Evidence of chromosomal aberrations, malignancy, and other clinical findings studied among individuals in the Hiroshima population, however, seemed not to show correlations among these factors (King et al., 1975). Thus, although chromosome aberrations can serve as dosimeters of exposure to known carcinogens, they appear to be predictive of an increased disease frequency at the ecological level only.

A notable exception to the generalization that chromosomal aberrations are not predictive of disease is the case of the Philadelphia chromosome—a translocation of the long arm of chromosome 22, usually to the long arm terminus of chromosome 9. This translocation correlates highly with chronic myelogenous leukemia: about 90 percent of patients having this marker chromosome later develop the disease (see Sandberg et al., 1986, for a review of the Philadelphia chromosome). Specific aberrations, such as the Philadelphia chromosome, which carry a probable prognosis of serious disease raise ethical questions for investigators who discover them during cytogenetic analysis. Has the investigator a responsibility to inform an individual that that he or she is at risk? As yet, the epidemiologic literature has rarely addressed such questions.

CHROMSOME ABERRATIONS VERSUS SISTER CHROMATID EXCHANGES

Sister chromatid exchange (SCE) was first described by Taylor in 1958. In the 1970s, SCE analysis was shown to be applicable to both in vivo and in vitro mutagenicity testing (for a review, see Latt et al. (1980)). Chapter 6 presents a detailed discussion of the SCE assay. In the context of the present review, however, several points bear repetition.

In the late 1970s, cytogenetics witnessed a period of "SCE-euphoria" during which many investigators substituted the comparatively simple and less costly SCE test for classic cytogenetic analyses (Gebhart, 1981). Two major developments have since tempered this euphoria: the realization that the biological consequences of SCEs are unknown and the recognition that the results of SCE and chromosome aberration tests often do not agree.

Gebhart (1981) has summarized the world literature on the concordance between chromosomal aberrations and SCEs. He found a 30 percent qualitative disagreement between chromosome aberrations and SCEs in both in vivo and in vitro tests of the same chemical. Ionizing radiation offers a clear illustration of the disparity between aberrations and SCEs. Radiation doses of about 400 R, which can cause a 100-fold increase in the rate of chromosome breaks, raise the baseline rate of SCEs only twofold. This suggests that the mechanism(s) through which particular agents interact with DNA to produce SCEs may differ from the mechanism(s) that produces chromosomal aberrations.

Compared with SCEs, chromosome breakage in untreated cells is very rare. In one laboratory (Gebhart, 1981), the average baseline frequency of SCEs is 5.6 per metaphase. In contrast, the average breakage rate is 0.02 per metaphase, yielding a SCE-to-break ratio of 280:1. This large disparity between the baseline frequencies of SCEs and chromosome breaks should be remembered when one compares results from both systems.

OCCUPATIONAL MONITORING: ETHYLENE OXIDE

Having set out the basic methodology for using the lymphocyte assay in epidemiologic studies, we can now compare several recent investigations that have examined occupational exposure to the same chemical agent, ethylene oxide.

Ethylene oxide, an alkylating agent, has been produced commercially since 1921 and is used worldwide as a chemical intermediate in the production of manufactured goods and in sterilizing medical supplies.

Ethylene oxide has been shown to induce mutations in bacterial, plant, and animal test systems (see Glazer, 1979, for a review). Several investigators (e.g., Embree et al., 1977) estimate that the genetic risk from exposure to ethylene oxide may be as high as that from exposure to ionizing radiation. Because of the compound's high volatility, the opportunities for human exposure are great, especially among industrial workers, medical staff, and patients.

The current Occupational Safety and Health Administration (OSHA) standard for ethylene oxide is 50 ppm, time-weighted average. There are two reports of increased leukemia among people industrially exposed to this chemical (Högstedt et al., 1979a, 1979b).

In 1967, Kalling reported an increased chromosome aberration rate in exposed workers' lymphocytes 18 months after an accident involving ethylene oxide. He also reported that 2 hours of acute exposure to the chemical produced a significant increase in the number of cells with chromosome aberrations. The report, however, gave no details about the actual study methods used.

The first completely reported work of chromosome aberrations in workers exposed to ethylene oxide was a study by Thiess et al. (1981). They studied 43 men (average age 47.1 years) who were exposed to the chemical in a German plant. The men were divided into four groups: group 1, long-term exposure of 20 years or more; group 2, exposure less than 20 years; group 3, long-term plus accidental exposure, and group 4, accidental acute high exposure only. Thiess and colleagues were not able to make accurate measurements of past exposure levels to ethylene oxide, but they estimated that the accidental exposure caused acute exposure levels as high as 1,900 ppm.

The control group of 25 men (average age 38.6 years) was composed mostly of office workers from the West German Occupational Medicine and Health Protection Department (the authors' institution).

The investigators carried out chromosome analyses using a 70- to 72-hour culture period, but did not use BrdUrd. They coded and examined 100 metaphases from each exposed man and each control.

The results indicated that the mean frequency of aberrant metaphases 6.4 percent (excluding gaps) in the long-term exposure group (group 1) was significantly higher that the mean frequency of aberrations in the control group, 4 percent. Aberration frequencies for groups 2 through 4 had slightly, but not significantly increased aberration frequencies (6.0, 4.7, and 5.2%, respectively). On resampling group 1 and the control group 1 year later, there was still a significantly elevated aberration frequency. The authors concluded that the increased chromosome aberration rate in group 1 reflected a mutagenic exposure. But, as the workers had been exposed to many other

agents (e.g., benzene), the authors noted that the results did not support the assumption that the risk is specific to ethylene oxide.

This study can be criticized on several grounds. First, the culturing period, 70 to 72 hours, was too long. No attempts were made to ensure the analysis of only first division cells. Other criticisms apply to the selection of the controls, who were roughly 10 years younger than the exposed groups (thus creating a potential bias away from the null), and largely drawn from a population, health professionals, probably unlike the factory population. It is, thus, possible that other exposures, such as smoking, could be partly responsible for the observed effect.

Sarto and colleagues (1984) investigated cytogenetic damage (SCEs and chromosome aberrations) in sanitary workers exposed to ethylene oxide in eight hospitals in Venice. They studied two exposure groups: one was exposed to 10.7 ± 4.9 ppm ethylene oxide (19 individuals), and the other to 0.35 ± 0.12 ppm (22 individuals). Each exposed worker was matched to a control of similar age and smoking habits. The controls also were sanitary engineers from the same hospitals who were not occupationally exposed to ethylene oxide, cytostatic drugs, anesthetic gases, or ionizing radiation.

The authors cultured lymphocytes for 48 to 52 hours, analyzed 100 metaphases for each person, and scored them blind to exposure status.

The results revealed a significant elevation of SCEs for both the low- and high-exposure groups (i.e., a dose-response). Similarly, in the higher exposure group, the number of both chromatid- and chromosome-type aberrations was significantly increased. In the lower exposure group, the frequency of chromosome-type aberrations only was significantly higher than in controls. A reexamination of 10 individuals 18 months after exposure was ended or lowered indicated that the elevated frequencies of both SCEs and chromosome-type and chromatid-type aberrations had changed little. Of the cytogenetic changes, the chromosome aberrations were more stable. The frequency of cells with chromosome aberrations was found to be weakly but significantly correlated with ethylene oxide exposure, but not correlated with smoking, age, or SCE frequency.

The work by Sarto and colleagues represents a large methodologic advance over the earlier work by Thiess et al. (1981). The Sarto study took particular care to ensure the comparability of exposed and unexposed persons (matching by job title and hospital) and to minimize confounding (matching on age and smoking status). As in the previous study, Sarto and colleagues noted chromosome aberrations of apparently long duration. Because the aberrations in the Sarto study were seen in individuals not occupationally exposed to other known clastogens (e.g., benzene), this result suggests that ethylene oxide may indeed be the cause of the long-lived aberrations.

Van Sittert and colleagues (1985) examined the occurrence of chromosome abnormalities and other hematologic parameters in workers in an ethylene oxide manufacturing plant. They studied 36 individuals (mean age 32 years) who had been employed up to 14 years in ethylene oxide manufacture. They studied two exposure groups: one with exposure of more than 5 years duration (17 workers), the other with exposure of less than 5 years (19 workers). The exposed workers were matched to individuals who had no exposure to ethylene oxide manufacturing or to other known or suspected clastogens. They obtained information on smoking habits and previous occupational history for all groups.

For the cytogenetic analysis, they incubated cells either 48 hours or 72 hours using Ham's F-10 medium. The percent of cultures left for 72 hours was unspecified.

The results indicated no statistically significant differences between the plant workers and the control group with respect to chromosome aberrations or any of the other hematologic parameters investigated. The duration of employment in the ethylene oxide industry, however, was correlated positively with the frequency of chromosome breaks. This effect could not be attributed to the older age of the workers with longer work histories.

Although the Van Sittert study did not find an increased aberration frequency in ethylene oxide-exposed workers compared with nonexposed workers, there are several reasons why the study might not have detected such exposure effects. Most salient among these is the type of manufacturing plant. Unlike the workers in previous studies, these workers were employed in a modern, open-air manufacturing plant. The work site had the lowest exposures of any in the previous studies. Furthermore, the exposure durations (years worked) were lower than in the Thiess et al. study. The fact that the duration of work exposure correlated significantly with the frequency of chromosome breaks is consistent with previous findings and suggests that the otherwise negative findings may be due to the low level and relatively short duration of exposure.

The most recent study of chromosome aberrations in workers exposed to ethylene oxide was reported by Galloway et al. (1986), who studied the aberration frequencies of 61 employees potentially exposed to ethylene oxide. They studied three different worksites. For each potentially exposed worker, a randomly selected control matched for sex and age was identified in the same plant. They also selected a second control group of age-matched and sex-matched individuals who lived in the same community and were not exposed to ethylene oxide. They obtained a smoking history from all study participants.

The authors processed blood samples from potentially exposed and unexposed individuals concurrently. They processed lymphocytes from community controls on separate occasions from the other groups. Cells were incubated for 48 to 51 hours.

The results indicated an elevated chromosome aberration rate in exposed workers in worksite III, the site with the greatest historical exposure to ethylene oxide. Worksite III was subdivided a priori into high and low exposure categories. The frequency of cells with aberrations was 5.6 percent in the two workers in the high exposure category and 2.6 percent in the 23 individuals in the low exposure category. The overall frequency of aberrations in the 304 matched control individuals was 1.4 percent.

Within the 304 control individuals, the authors found significant increases in aberrations with smoking and with increasing age. The authors also compared the frequency of chromosome aberrations with data they had previously reported on SCEs (Stolley et al., 1984). There was only a weak overall association between SCEs and chromosome aberrations. The correlation was found in potentially exposed but not in control groups. For a given individual, they found that they could not use data on SCE frequency to predict the frequency of chromosome aberrations.

The study by Galloway and colleagues represents the best in state of the art epidemiology using cytologic endpoints. The study is distinguished by its large sample sizes, use of multiple worksites, and of community and worksite controls, and complete reporting of aberration subtypes and frequencies.

SUMMARY: ADVANTAGES AND DISADVANTAGES OF THE PERIPHERAL LYMPHOCYTE ASSAY

Advantages

1. Peripheral blood lymphoctyes are readily available. A few milliliters of peripheral blood can be easily and repeatably obtained from an individual. Each milliliter of blood contains $1–3 \times 10^6$ lymphoctyes.

2. The lymphocytes circulate through all tissues in the body. A portion of them are long-lived.

3. Blood lymphocytes are a synchronized population; virtually all the cells are in the same G_0 or G_1 stage of mitotic interphase.

4. A proportion of the lymphocytes can be stimulated to undergo mitosis in culture and provide a supply of dividing cells for the study of chromosome abnormalities.

5. Lymphocytes from unexposed people have a low spontaneous aberration frequency.

6. Exposures to ionizing radiation produce persistent aberrations that permit estimations of received dose.

Disadvantages

1. Although lymphocytes are a synchronized population, there are different subpopulations of cells within the same individual. These may have varying responses to mitogenic and clastogenic agents.

2. It is probable that less heavily damaged cells will grow preferentially in culture; in Thilly's (1985) words, "dead cells don't form mutant colonies." This will underestimate the true clastogenic effect of any agent under study.

3. In humans, only the T or thymus-derived lymphocytes can be stimulated to undergo mitosis in culture. Because T lymphocytes are involved in immune responses, any previous immunologic stimulus may positively or negatively alter the number of cells with chromosome aberrations, depending on when the cells are drawn from the individual.

4. Although the spontaneous aberration frequency is low, the assay's interlaboratory and even interculture variation is large. This can confound real associations of interest.

5. In general, the lymphocyte assay is an uncertain indicator of exposure to chemicals. The need to consider exposures on a case-to-case basis complicates matters when individuals are exposed to more than one chemical.

6. The assay is expensive, time intensive, and requires trained personnel.

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