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Current status of cytogenetic procedures to detect and quantify previous exposures to radiation *

Michael A Bender [1,**], Akio A. Awa [2], Antone L. Brooks [3], H. John Evans [4], Peter G. Groer [5], L. Gayle Littlefield [5], Carlos Pereira [6], R. Julian Preston [7] and Bruce W. Wachholz [8]

[1] Brookhaven National Laboratory, Associated Universities Inc., Upton, NY 11973 (U.S.A.), [2] Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima City 732 (Japan), [3] Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185 (U.S.A.), [4] Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH5 2XU (Great Britain), [5] Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117 (U.S.A.), [6] Universidade de Sao Paulo, Sao Paulo (Brazil) and Operations Research Center, University of California, Berkeley, CA 94720 (U.S.A.), [7] Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 (U.S.A.), and [8] Radiation Effects Branch, National Cancer Institute, Landow Building, Bethesda, MD 20892 (U.S.A.)

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Correspondence: Dr. R. Julian Preston, Biology Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831 (U.S.A.).

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** Chairman, Cytogenetics Working Group.

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Summary

The estimation of the magnitude of a dose of ionizing radiation to which an individual has been exposed (or of the plausibility of an alleged exposure) from chromosomal aberration frequencies determined in peripheral blood lymphocyte cultures is a well-established methodology, having first been employed over 25 years ago. The cytogenetics working group has reviewed the accumulated data and the possible applicability of the technique to the determination of radiation doses to which American veterans

might have been exposed as participants in nuclear weapons tests in the continental U.S.A. or the Pacific Atolls during the late 1940s and 1950s or as members of the Occupation Forces entering Hiroshima or Nagasaki shortly after the nuclear detonations there.

The working group believes that with prompt peripheral blood sampling, external doses to individuals of the order of about 10 rad (or less if the exposure was to high-LET radiation) can accurately be detected and measured. It also believes that exposures of populations to doses of the order of maximum permissible occupational exposures can also be detected (but only in populations; not in an individual). Large exposures of populations can also be detected even several decades after their exposure, but only in the case of populations, and of large doses (of the order of 100 to several hundred rad). The working group does not believe that cytogenetic measurements can detect internal doses from fallout radionuclides in individuals unless these are very large.

The working group has approached the problem of detection of small doses ($\leqslant 10$ or so rad) sampled decades after the exposure of individuals by using a Bayesian statistical approach. Only a preliminary evaluation of this approach was possible, but it is clear that it could provide a formal statement of the likelihood that any given observation of a particular number of chromosomal aberrations in a sample of any particular number of lymphocytes actually indicates an exposure to any given dose of radiation. It is also clear that aberration frequencies (and consequently doses) would have to be quite high before much confidence could be given to either exposure or dose estimation by this method, given the approximately 3 decades of elapsed time between the exposures and any future blood sampling.

Additional research on the problem is clearly needed, but at the moment it appears unlikely that determination of chromosomal aberration frequencies in peripheral blood lymphocytes will prove a useful method of determining ionizing radiation doses to individual veterans (though it might prove useful in showing that doses to veterans as a population were not greatly in excess of those presently estimated).

2. Introduction

2.1. Mission statement

During a Senate Veterans Affairs Committee hearing in 1984 the question was raised as to whether or not it was possible to determine or estimate (1) radiation doses which might have been received in the early period following World War II by military personnel who were present in Hiroshima or Nagasaki as members of the occupation forces and (2) radiation doses that may have been received by military personnel as a result of exposure during atmospheric testing of nuclear weapons (24–42 years ago). Subsequent to this hearing Congress enacted legislation which was signed into law by the President on October 24, 1985, as Public Law 98-542. Section 7(a)(2) of this law instructs the Secretary of Health and Human Services, through the director of the National Institutes of Health, to "... (A) conduct a review of the reliability and accuracy of the scientific and technical devices and techniques (such as 'whole-body counters') which may be useful in determining previous radiation exposure; (B) submit to the Administrator of Veterans' Affairs and the Committees on Veterans' Affairs of the House of Representatives and the Senate, ... the results of such review, including information concerning the availability of such devices and techniques, the categories of exposed individuals as to whom use of such devices and techniques may be appropriate, and the reliability and accuracy of dose estimates which may be derived from such devices and techniques. ..." The National Institutes of Health (NIH) requested the National Cancer Institute (NCI) to accept responsibility for this review.

At the request and under the guidance of the NCI, the Medical and Health Sciences Division (MHSD) of Oak Ridge Associated Universities (ORAU) undertook a review of devices and techniques which may be useful in determining previous radiation exposure. To accomplish this task, 4 expert working groups were formed to review the applicability of biological indicators, bioassay, whole-body counting, and cytogenetic techniques

in radiation dose assessment and to author 4 separate reports on these subjects. These expert working groups reviewed the status of current technology and methods in these fields as well as new experimental techniques being investigated. The present report evaluates cytogenetic techniques.

2.2. Background

Cytogenetic detection and measurement of human exposures to ionizing radiation have become well-established methodologies during the past 2 or 3 decades.

As is now well known, the genetic material of humans (and of virtually all other organisms as well) is the double helical macromolecule, deoxyribonucleic acid (DNA), composed of a pair of intertwined polynucleotide chains. In most organisms, including humans, the DNA is organized into discrete "packages", called "chromosomes", the number and size of which is characteristic for each species. During the interphase of the cell cycle the chromosomes are extended and thus not visible with the ordinary light microscope. Instead, one can only see a fairly homogeneously distributed DNA-containing material, called "chromatin", contained within a usually spherical nucleus. It is only during cell division, mitosis or meiosis, that the chromosomes can be easily visualized and studied. The microscopic study of chromosomes is termed "cytogenetics"; and the study of chromosomal changes induced by ionizing radiation is often referred to as "radiation cytogenetics".

Exposure to ionizing radiation may result in the breakage and rearrangement of chromosome structure. Early work in radiation cytogenetics during the late 1930s and 1940s established not only the types and patterns of breakage and rearrangement (chromosomal aberrations) by ionizing radiation, but also quantitative relationships relating the frequency of aberrations in irradiated cells to the level of radiation exposure. It was obvious even then that chromosomal aberrations could be used as a kind of biological dosimeter. Instead of reading some kind of physical meter, aberration frequencies could be determined in cells from an exposed organism or tissue. Then, from already established calibration curves previously determined from relevant cells exposed to known physically measured exposures, the unknown dose could be calculated.

For technical reasons, however, early work was confined to plant systems, such as onion or broad bean root tips, or the microspores of flowers, such as Trillium or Tradescantia species, characterized by low numbers of very large chromosomes. Mammalian chromosomes, generally much smaller, were not suitable for such cytogenetic analysis. Indeed, even the chromosome number characteristic of the human species itself had been incorrectly determined to be 48, and it was not until the development of new methods for the preparation of mammalian chromosomes in the 1950s that it was found that the correct number was actually 46. However, these methods enabled rapid progress to be made thereafter. Particularly notable was the discovery that human peripheral blood lymphocytes, a cell type which does not normally undergo cell division, could be made to divide in short-term tissue culture if the cells were treated with certain plant lectins, the first of which to be applied was an aqueous extract of common beans called "phytohemagglutinin". This development gave the cytogeneticist, for the first time, easy access to samples of dividing cells from human subjects. As chromosomal abnormalities were found to be characteristic of, and often diagnostic for, many human diseases, cytogenetic analysis rapidly became a widely used clinical procedure.

Almost as soon as the methodology became available, it was demonstrated that ionizing radiation induced chromosomal aberrations in human cells, whether exposed in culture (in vitro) or in the body (in vivo), just as it did in the plant systems so extensively studied earlier. In vitro human lymphocyte calibration curves were quickly developed (though not without controversy), and during the early 1960s the cytogenetic dosimetry technique was tested in a number of cases of accidental human radiation exposure for which physical information allowed dose reconstructions good enough to provide at least crude tests of the cytogenetic dosimeter's utility.

By the later 1960s it could be said that the technique was well established. It continues to be applied routinely to all suspected cases of radiation exposure in several countries, most notably

by the National Radiological Protection Board in the United Kingdom. In fact, it appears that the technique was used to provide dose estimates for victims of the recent Chernobyl disaster in the U.S.S.R. (Gale, 1986). Large-scale studies have also been undertaken to determine chromosomal aberration frequencies in peripheral lymphocytes from large human populations exposed either to moderate-to-large doses many years in the past, as for example the ongoing studies of irradiated survivors at Hiroshima and Nagasaki, Japan, or to chronic exposures at occupational radiation levels.

Using the large body of data accumulated over the past almost 30 years, it is possible to assess both the sensitivity and the selectivity of the human lymphocyte chromosomal aberration frequency technique for detecting human radiation exposure. It is also now possible to evaluate its potential as a possible means of determining the previous radiation exposure of American veterans who participated in atmospheric nuclear tests or the occupation of Hiroshima and Nagasaki prior to July 1, 1946.

To understand the way in which this technique works, and perhaps more important, its inherent limitations in terms of sensitivity and selectivity, it is necessary to understand something of the underlying biology.

3. Chromosomal aberrations

3.1. Cell reproductive cycle

Various cells which make up the human body reproduce, or divide, from time to time, each giving rise to a pair of daughter cells, each having the physical structure and genetic information possessed by the original parent cell. The frequency with which cells in the various tissues of the body undergo such cell division is quite variable. In the adult, certain tissues, for example the red bone marrow, contain many cells engaged in fairly rapid reproduction. Other tissues, for example the brain, contain few if any reproducing cells. Tissues with the former characteristic are often cell-renewal systems, producing a steady supply of new cells which differentiate to replace those lost by attrition. Tissues of the latter sort are already highly differentiated, with little cell turnover and thus little need for cell replacement. Notable examples of cell-renewal systems (in addition to the red bone marrow) are the lymphoid organs, the skin, the lining of the gastrointestinal tract, and the spermatogonial cells of the male testis, which are responsible for maintaining a steady supply of spermatozoa.

Cellular reproduction is usually described in terms of a "cell cycle". Somatic cells actually divide by an elaborate mechanism termed "mitosis". (In germ-line cells, such as those leading to the production of spermatozoa, a more complicated form of cell division, termed "meiosis", occurs. However, because there is very little quantitative information on radiation-induced chromosome damage in meiotic cells in humans, all subsequent discussion will be confined to the somatic mitotic cell divisions.)

Cell division, easily observable in the microscope, ends each cell cycle, with each of the 2 resulting daughter cells free to begin a new cell cycle of its own. Cytogenetically, the only visible events associated with the cell cycle, other than the cells' growth in preparation for a new cell division, are those associated with the division itself. The remainder of the cell cycle (originally thought of as essentially featureless) is termed the "interphase". With the development of particular biological molecules labeled with suitable radioisotopes, it was found in the early 1950s that the interphase was not completely featureless after all. Specifically, it was discovered that the synthesis of the important biomolecule, DNA, did not occur continuously throughout the cell cycle but instead was limited to a particular segment of the interphase.

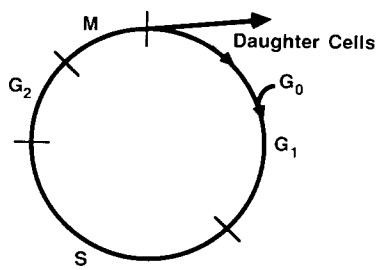

Fig. 3.1. Schematic of the cell replication cycle. S, DNA synthetic phase; M, mitosis; G_1 and G_2, first and second "gap" phases.

This phenomenon was subsequently used as a marker to differentiate 3 separate interphase subphases: prior to the onset of DNA synthesis the interphase cell is said to be in the G_1 stage of the cell cycle (resting, or noncycling, G_1 cells are termed G_0); this is followed by the period during which DNA synthesis takes place, termed the S phase; finally, prior to cell division there is a second "gap" phase, termed G_2. This is illustrated schematically in Fig. 3.1. These 3 stages of the interphase of the cell cycle are of singular cytogenetic significance.

3.2. Chromosomes

On the cytogenetic level the first sign of cell division, "M" in Fig. 3., is that the diffuse chromatin material within the cell nucleus begins to condense into discrete objects while the membrane separating the nucleus from the cell cytoplasm begins to break down. This is called "prophase" (Fig. 3.2). During the next division phase, the "metaphase", the individual chromosomes become shorter and more condensed, and seem to be double structures. Chromosomes, as viewed at metaphase, have a definite anatomy as shown in Fig. 3.3. Each is composed of 2 parallel, more or less rod-like, structures called "chromatids", attached to each other at a specific point along their length called the "centromere" (the 2 identical chromatids are in fact about to become the daughter chromosomes when they are separated at anaphase). The elaborate spindle mechanism responsible for the distribution of the daughter chromosomes to future daughter cells also develops, to which the fully condensed metaphase chromosomes become attached. The centromere (or more specifically the "kinetochore" contained therein) is actually the point of attachment for each of the chromosomes to the spindle apparatus.

During the third phase of the mitotic cell cycle, "anaphase", each of the chromosomes separates into 2 identical daughter chromosomes; the daughters then separate into 2 groups. During "telophase", the fourth phase of the division process, the 2 groups of chromosomes begin to decondense and become diffuse, and new nuclear membranes appear, surrounding and separating the 2 chromosome groups. In the final division process, called "cytokinesis", the now binucleated parent cell pinches in two, resulting in 2 mononucleate daughter cells, each just at the beginning of its own new cell cycle.

Each species, including humans, is characterized by a specific number, size, and form of chromosomes. Not only are the lengths of different chromosomes characteristic, but so are the centromere locations. When the centromere is located at or near the middle of the chromosome length, the chromosome is called "metacentric". When it is near one end, it is termed 'acrocentric", and when it is somewhere in between, the chromosome is termed "submetacentric".

In higher (diploid) organisms, such as humans, there are 2 sets of chromosomes in somatic cells, one complete set derived from each of the parents. Humans possess 23 such chromosome pairs. Based upon length and centromere position, as well as special staining techniques which will be described

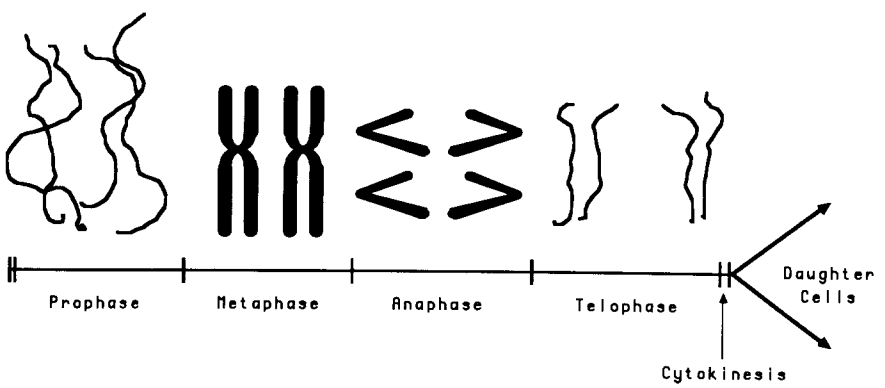

Fig. 3.2. Schematic diagram of the stages of mitosis, with chromosomal appearance shown above.

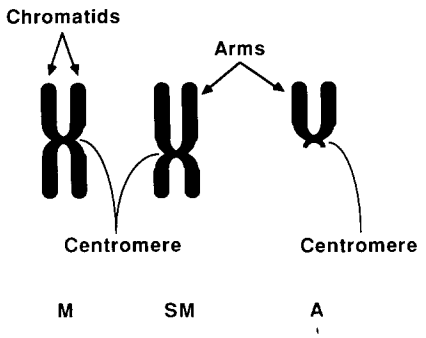

Fig. 3.3. Schematic diagram of metaphase chromosomes. M, metacentric; SM, submetacentric; A, acrocentric.

presently, each of the normal human chromosomes may be recognized.

Sex in humans and other mammals is determined by the inheritance of one chromosome "pair", the X and the Y, which are dissimilar in size and shape. Females have 2 X chromosomes and no Y; males have 1 X and 1 Y. Thus, in males the sex chromosome pair is not a morphological pair, though in females it is.

While the normal set of human chromosomes, or "karyotype", is observed in most somatic cells of most individuals, a number of human disorders, including a variety of congenital abnormalities, endocrine problems, and certain forms of cancer, are characterized by an abnormal chromosome set. In some cases, the abnormalities are numerical — an extra, or a missing, chromosome, for example. In some cases, however, there are structural aberrations resulting from chromosome breakage and (sometimes) the rejoining of broken ends in "illegitimate" ways to give rise to new structures of abnormal length, centromere position, and genetic constitution.

3.3. DNA damage and repair

Ionizing radiations cause a number of different kinds of damage to DNA in exposed cells. These include a variety of types of chemical changes involving aberrations of the 4 purine and pyrimidine bases attached to the sugar–phosphate backbone of the polynucleotide chains, as well as double and single breaks in the DNA double helix.

It is well established that the repair of DNA damaged by ionizing radiation or other agents is, in human and other cells, accomplished by elaborate enzymatic repair systems. Such repair mechanisms normally eliminate the bulk of the damage ionizing radiation causes in DNA, so that only a fraction actually result in aberrations. However, it is clear that DNA repair mechanisms are also intimately involved in the actual formation of chromosomal aberrations as well.

There are a number of human genetic clinical conditions which involve, or at least appear to involve, some defect or deficiency in the cells' ability to enzymatically repair certain classes of DNA damage. Cells from affected individuals display an abnormally high sensitivity to the induction of chromosomal aberrations by agents producing DNA lesions for which the individual's DNA repair system is defective. Only a few clear-cut examples of such a sensitivity to ionizing radiation are known, however. An example is the syndrome called "ataxia telangiectasia", a very rare condition with obvious clinical symptoms including extreme radiosensitivity. Because they are so rare and easily identified, individuals with this syndrome appear to present no obstacle to the use of cytogenetic dosimetry for determining the magnitude of most radiation exposures. It is possible that lesser degrees of sensitivity also occur among the general population, but none has as yet been discovered.

3.4. Chromosomal aberrations

A number of chemical and physical agents in addition to ionizing radiation induce chromosomal aberrations. It is important to recognize, as well, that aberrations also arise spontaneously, without any known exposure to chromosome-damaging agents (termed "clastogens"). In fact, clastogens, like ionizing radiation, do not induce any new or novel types of aberrations but simply increase the frequency of those which occur at low frequency without any exposure.

Aberrations may be classified in several important ways. Early cytogeneticists working with ionizing radiation noticed that if cells were irradiated at the beginning of the cell cycle, in what is now termed the G_1 stage, the chromosomes behaved as though composed of a single unit. Each break or aberration seen at the subsequent mitosis involved the entire chromosome (i.e., when the chromosome was viewed at metaphase both

chromatids were always affected in the same way at the same point along their length). However, when cells were irradiated late in the cell cycle, in what is now termed the G_2 phase, the chromosomes generally behaved as though they had become double structures, composed of 2 parallel units either of which could be affected independently of the other; thus breaks and aberrations usually affected only 1 of the 2 metaphase chromatids. This was easily understood in terms of the single appearance of the daughter chromosome distributed to the daughter cell during mitosis and the double nature of the metaphase chromosome, consisting of 2 parallel chromatids (or daughter chromosomes).

Each of the single daughter chromosomes had clearly replicated itself at some time during the interphase; that is, at a time corresponding to that at which the chromosome began to behave toward radiation as though double. It is now widely recognized that the G_1 chromosome contains a single DNA double helix running its length, and that aberration formation involves direct or indirect breakage of the double helix and interactions between the broken ends. When the DNA double helix containing deletions or other aberrations replicates, these are replicated as well, giving rise to the class of aberrations, known as "chromosome-type" aberrations, involving both chromatids identically. Once the DNA has replicated, each double helix behaves generally as an independent "target" giving rise to aberrations called "chromatid-type" aberrations, involving only one or the other chromatid at any given location.

It is interesting that this pattern of aberration production, which has been thoroughly studied for ionizing radiation over many decades, and which became the model for our thinking about "clastogenesis", is not actually characteristic of aberration production by most clastogenic agents. Both ultraviolet light, another physical clastogen, and the vast majority of the many known chemical clastogens display an entirely different pattern in which only chromatid aberrations are seen in the first mitosis after interphase exposure, a phenomenon known as "S-dependence". It is characteristics of S-dependent clastogenic agents that they induce few, if any, aberrations of the chromosome type, and treated cells must go through an S phase for any significant aberration production to result.

As will be described more fully in Ch. 4, the human peripheral lymphocyte is the cell most often used for biological radiation dosimetry. Because it is in a nondividing pre-DNA-synthesis stage (G_1 or G_0) in the cell cycle, radiation exposure induces exclusively chromosome-type aberrations; whereas, in contrast, exposure to most chemical agents induces only chromatid-type aberrations. This fortunate circumstance allows the cytogeneticist to distinguish effects caused by many possible environmental mutagens from those which might have been caused by a radiation exposure.

Chromosomal aberrations may also be categorized as to the number of breaks involved and the subsequent interactions between broken ends. The situation is essentially the same whether the aberrations are of the chromosome or the chromatid type; chromosome-type aberrations have chromatid-type analogs. The great majority of the lesions or breaks induced in interphase chromosomes are repaired, or "restituted", and no aberration is visible at metaphase. If unrepaired (or misrepaired), single breaks give rise to metaphase chromosomes from which a portion has been broken off, either at the chromosome or chromatid level. These are called "deletions". The deleted portion, no longer attached to the rest of the chromosome, is called an "acentric fragment". Of course, 2 or more unrepaired or misrepaired breaks can occur in the same cell. This could give rise to multiple deletions; however, multiple breaks can also interact, or rejoin, to give rise to new and sometimes bizarre chromosome forms called "rearrangements" or "exchanges". It is simplest to consider only the case of 2 breaks in the same cell, remembering that, although substantially rarer at low aberration frequencies, exchanges involving more than 2 breaks do occur. Fig. 3.4 illustrates some typical chromosome and chromatid aberration types.

Two breaks may either be in the same or in different chromosomes. Furthermore, any set of 4 broken chromosome or chromatid ends resulting from 2 breaks can rejoin with each other in 2 quite different ways (if the broken ends do not simply rejoin the way they were). Either the 2 broken ends on the chromosome portions still bearing

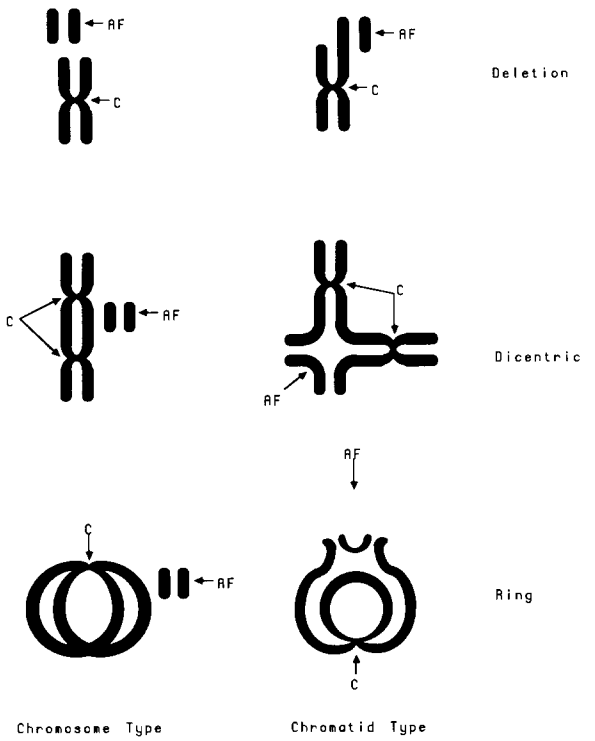

Fig. 3.4. Schematic diagram of typical chromosomal and chromatid aberration types. C, centromere; AF, acentric fragment.

centromeres can rejoin, leaving the 2 distal acentric fragments to rejoin with each other, or one centric end can rejoin with the acentric broken end from the other chromosome or chromosome arm, leaving the other 2 ends to rejoin similarly. The cytogeneticist terms the former case "asymmetrical" and the latter "symmetrical".

If the 2 breaks involve but a single chromosome, then asymmetrical rejoining gives rise to a ring chromosome or chromatid (i.e., an asymmetrical intrachange) while symmetrical rejoining gives rise to an inversion within a chromosome (i.e., a symmetrical intrachange). If the 2 breaks are in different chromosomes, asymmetrical rejoining yields a "dicentric" chromosome or chromatid plus an acentric fragment (i.e., an asymmetrical interchange), while symmetrical rejoining gives rise to a translocation (i.e., a symmetrical interchange). The four 2-break exchange types are illustrated in Fig. 3.5. The new topologies created by some of these aberrations may have important consequences during cell division.

3.5. Aberration fates

For purely mechanical reasons, chromosomal aberrations may be modified or lost during cell division. Thus the aberrations evident at the first metaphase following their induction may appear again in different form in subsequent metaphases, or alternatively, they may not be evident at all in subsequent metaphases (should the cell survive long enough to enter another division). Acentric fragments, lacking the centromeric attachment to the spindle apparatus, fail to move with the centric chromosomes during anaphase, and are often not included in the daughter cells' nuclei. Often a small nuclear membrane forms around such acentric fragments, and they appear in the cytoplasm of daughter cells as micronuclei. Even though the acentric fragments resulting from simple deletions thus tend to be rapidly lost as a function of cell division, the centric portion of the chromosome from which a fragment was deleted will often persist. However, deletions are usually recognized

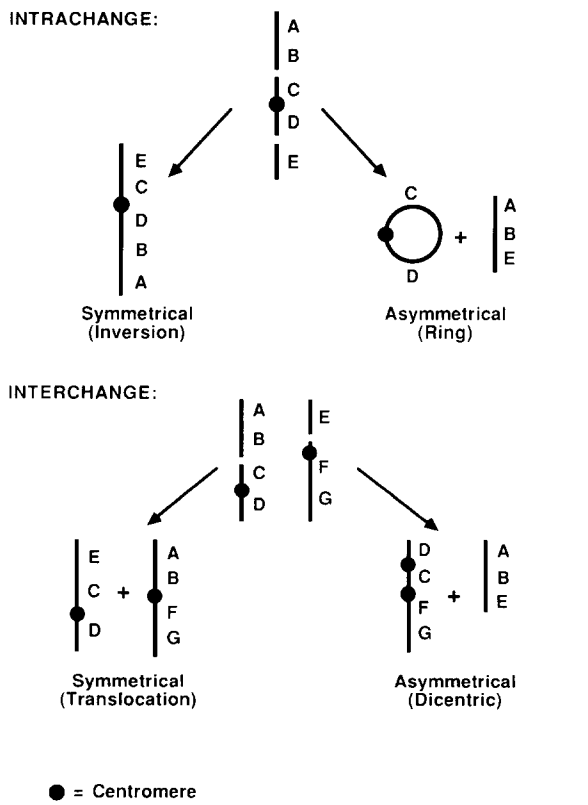

Fig. 3.5. Schematic diagram of symmetrical and asymmetrical exchange.

by the presence of the acentric fragment, so even though the deleted chromosomes may still be there in subsequent divisions, they are generally not detected, except in cells stained by special techniques.

Not only do asymmetrical exchanges, which always produce an acentric fragment, lose those fragments at cell division, but the rearrangement may also be lost for mechanical reasons. A dicentric chromosome or chromatid, having more than 1 centromere, may be oriented on the mitotic spindle in such a way that both centromeres on a single chromatid are drawn into a single daughter cell, a situation presenting no mechanical problem. But it is also possible for the 2 centromeres on a single dicentric to be drawn each separately toward a different one of the 2 daughter cells, thus giving rise to a chromatid or chromosome anaphase bridge. Such bridges, which do not appear in human or other mammalian material, may result in the bridged chromosome or chromatid failing to be incorporated in either of the daughter nuclei, thus leading to the loss of the aberration. Furthermore, dicentric chromosomes in which there is a twist intervening between the 2 centromeres may become interlocked at anaphase, even though the centromeres on the same chromatid are drawn toward only one of the daughter cells. Similarly, not only are the acentric fragments resulting from ring formation lost, but the ring structure itself may be lost if exchanges occur between the daughter chromatid rings, a fairly frequent and apparently normal phenomenon. Odd numbers of such exchanges result in "double dicentric ring bridges", while even numbers of exchanges may result in interlocked rings which cannot separate at anaphase. Fig. 3.6 illustrates several sorts of anaphase bridge formations.

Completely rejoined symmetrical exchanges generate no acentric fragments and do not present any topological abnormality which would result in their loss through cell division. Thus "inversions" and "translocations" can persist over many cell divisions, usually just as well as the normal chromosomes in the complement. Because of this distinction, deletions and asymmetrical exchange aberrations have been referred to as "unstable", while inversions and translocations have been designated "stable" aberrations. It is also important

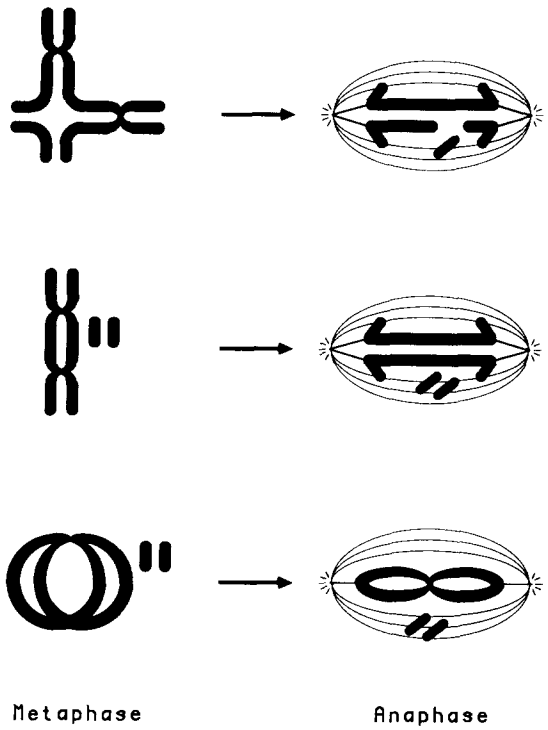

Fig. 3.6. Schematic diagram illustrating anaphase bridge formation.

to note that chromatid aberrations (with a few exceptions), if they survive their first anaphase, may be replicated in the succeeding interphase and will appear as the analogous chromosome type in subsequent divisions. Thus a dicentric chromatid, following replication, becomes a dicentric chromosome, although of course tending to lack its acentric fragment. Such aberrations are often described as "derived" chromosome aberrations.

4. Human peripheral blood lymphocytes and their utilization for radiation cytogenetics

4.1. Lymphocyte types and function

The white blood cells in peripheral blood used to study chromosomes in populations exposed to mutagens are small lymphocytes that are part of a population of cells that make up the lymphoid lineage of the immune system. These lymphoid cells play a vital role in the body's defense against foreign proteins (antigens) and react in a variety of ways on exposure to antigens. Some types of

lymphocytes become activated and secrete antibodies into blood and body fluids that combine specifically with the antigen that induced their activation — a process that results in the eventual destruction of the antigen. Other types destroy antigens directly or incite the activation of other cells, including lymphocytes, in the host defense system (Staines et al., 1985).

The small lymphocytes of the immune system are 2 main kinds which serve rather different functions: T cells which differentiate initially in the thymus and B cells which differentiate in the fetal liver, spleen, and adult bone marrow. During their development, both B and T lymphocytes acquire specific receptors for antigens which commit them to a single antigen specificity for the rest of their life span. There is also a third population of non-B or -T cells, which are referred to as null cells; these are larger and more granular than the typical small lymphocyte, although they may possess some of the characteristics of T cells, and they include the so-called NK (natural killer) cells that appear to be involved in the destruction of cancer cells.

B and T cells have different cell surface properties and they can be readily distinguished by the use of specific antibodies. B lymphocytes represent 5–15% of the circulating lymphoid pool, and they mature into plasma cells with the principal function of producing antibodies, which are located at their surfaces. With the exception of a few antibody responses, it appears that all immune responses depend upon T cells, of which there are various subclasses. T cells do not secrete antibody molecules but, like B cells, have surface receptors for antigens and become activated when exposed to appropriate antigens and may proliferate. T cells are classified into several functional subpopulations (known as subsets) each containing collections of cells reactive to different antigens. Two subsets of T cells, referred to as "T-helper (T_H) cells" and "T-suppressor (T_S) cells", perform a regulatory role and control the production of antibody by B cells. The T_H cells are those which promote the immune responses, and the T_S, those which suppress or inhibit such responses. These 2 regulatory cell types also modulate the generation and activity of a third class of T cells, the "cytotoxic T (T_C) cells" that primarily have the role of destroying cells of the body that have become infected with viruses. In functional terms there is also a fourth subset of T cells, referred to as "Tdth cells", which produce and secrete substances called "lymphokines" that stimulate and influence the activity of other lymphocyte types and of other cells such as macrophages (Roitt et al., 1985).

4.2. Lymphocyte distribution, availability, and life span

The primary lymphoid organs that produce lymphocytes are the thymus and adult bone marrow, with about 10^9 cells being produced per day in a normal healthy adult. Some of these cells migrate to the secondary lymphoid tissues, such as the spleen, lymph nodes, and unencapsulated lymphoid tissues. The average adult has about 10^{12} lymphoid cells, and the lymphoid tissues account for 2% of total body weight. At any given moment only a small proportion ($\sim$ 3%) are in the circulating blood. Lymphoid cells represent about 20% of the total white blood cells (leukocytes) in the adult circulation; they are relatively large in number, circulate throughout the tissues of the body, and are widespread in distribution. The lymphocytes in peripheral blood thus constitute a source for study of human cells with a widespread distribution in the body. They are readily accessible in large numbers (1 ml of peripheral blood contains approximately one million lymphocytes).

Although the production of many millions of "new" lymphocytes per day implies a continuous turnover of cells in the lymphocyte pools, many lymphoid cells are very long-lived and may persist as "memory cells" for many years. Studies on induced chromosome damage (see below) in lymphocytes of people exposed to radiation indicate an average lymphocyte half-life of around 3.5 years (i.e., one-half of the population is replaced on the average every 3.5 years) (Norman and Sasaki, 1966; Buckton et al., 1967b; Dolphin et al., 1973). Moreover, it is evident that a proportion of the cells may survive within the body for many decades without undergoing proliferation.

4.3. Lymphocyte activation in culture

The small lymphocytes in peripheral blood are normally present in a nondividing, or interphase

(G_0), state, but they become activated when they are presented with a relevant antigen, and their activation may result in their proliferation (Ling and Kay, 1975; Resch and Kirchner, 1981). Antigen-induced proliferation can be achieved in vitro by cultivation in the presence of a relevant antigen. It is this in vitro stimulation of lymphocytes to undergo mitosis and reveal their chromosomes while in culture that provides the basis of the methods used by cytogeneticists to study human chromosomes.

Exposure of small lymphocytes to an extract from beans (phytohemagglutinin, or PHA) results in the activation, primarily and initially, of T lymphocytes within minutes of coming into contact with the stimulant. B cells may also become activated later in time; agents that preferentially stimulate B cells to proliferate are available (Melchers and Andersson, 1984) but have not been utilized to any degree in studies on individuals exposed to radiation.

Activated T lymphocytes increase in size, and cells from the 2 major subsets, T_H and T_S cells, progress into and through a proliferative cycle (Perry and Thomson, in press). After around 24 h in culture some of the more rapidly progressing cells enter a DNA synthesis, or S, phase, and some 12 h later the first cells appear in mitosis. In cultures that are allowed to continue, cells may progress through further cell cycles, and at later times some B lymphocytes may enter into a proliferative cycle.

4.4. Cell kinetics and the importance of culture and sampling times

The level of response to PHA and the rate of development through a proliferative cycle are not uniform between cells. Progression through the cell cycle from G_0 to G_1 to S to G_2 and thence into mitosis is, however, partially synchronous so that a wave of mitosis is usually evident in cultures between 36 and 60 h. At around 48 h the majority of the dividing cells are in their first mitosis in culture, but the proportion of cells in their first mitosis in culture at this time is dependent upon a number of factors including the nature of the culture medium and temperature (Purrott et al., 1981a, b).

Although the bulk of the stimulated cells may take around 48 h or so to complete their first cell cycle in culture, the time taken to complete a second cycle may be less than 12 h. In consequence, some cells may be in their second mitosis in culture even in samples fixed after 48 h. In contrast, a proportion of cells progress much more slowly and may not arrive at their first mitosis until the third or fourth day, or even later, following exposure to PHA. The number of proliferative cycles completed by a cell in culture can be determined if cells are allowed to incorporate 5-bromodeoxyuridine (5-BrdU) into their DNA during culture (Tice et al., 1976). Distinctive staining patterns characterize cells with BrdU-substituted DNA in their 1st, 2nd, or 3rd mitosis in culture; a typical distribution of cells in these

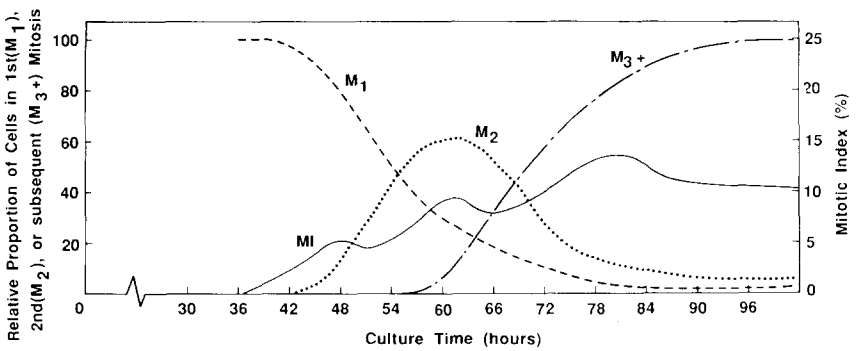

Fig. 4.1. Mitotic frequency (MI) of PHA-stimulated lymphocytes and proportion of cells in first (M_1), second (M_2), and later (M_{3+}) mitosis in relation to duration of blood culture.

stages is shown in Fig. 4.1.

In cytogenetic studies on induced chromosome damage, the importance of analyzing cells in their first mitosis in culture should be emphasized. As discussed in Ch. 3, asymmetrical ("unstable") chromosomal or chromatid aberrations (e.g., chromosomal fragments lacking centromeres [acentric fragments], or chromosomes with multiple centromeres [polycentrics], or chromosomes which have broken and rejoined to form ring structures) are unstable at mitosis and frequently give rise to micronuclei and are lost from the daughter cells. Unstable rearrangements and loss of sizeable amounts of chromosomal material may also result in impaired proliferative potential and may result in cell death. Symmetrical (stable) chromosome rearrangements (e.g., reciprocal translocations, inversions, and duplications) do not give rise to mechanical problems at mitosis but may result in genetic imbalance in daughter cells. It is evident therefore that for a given frequency of chromosomally abnormal cells in a blood sample, the proportion of aberrant relative to cytogenetically normal cells seen in their second, or later, mitosis in culture will be significantly reduced as compared with that seen at the first mitosis in culture. To obtain estimates of maximum aberration frequency, it is therefore essential to sample cells at their first mitosis in culture and, if possible, at their first mitosis following exposure of the cells to a known, or possible, mutagen (see below).

Although many small lymphocytes are long-lived and may indeed reside within the body for many years before being involved in clonal proliferation, there is a very considerable degree of turnover (Roitt et al., 1985). From what has been described above, it follows that the later a blood sample is taken from an individual following his or her exposure to a mutagen, the lower will be the aberration yield in cells observed.

The rate of decline in the yield of unstable aberrations with increasing time of blood sampling after mutagen exposure in cells from patients with ankylosing spondylitis who were treated with up to 1500 rad of partial body X-ray exposures is shown in Fig. 4.2 (Evans 1982, 1985). Detailed studies show that the yield of unstable aberrations begins to decline in the months following exposure, and the data in Fig. 4.2 show an

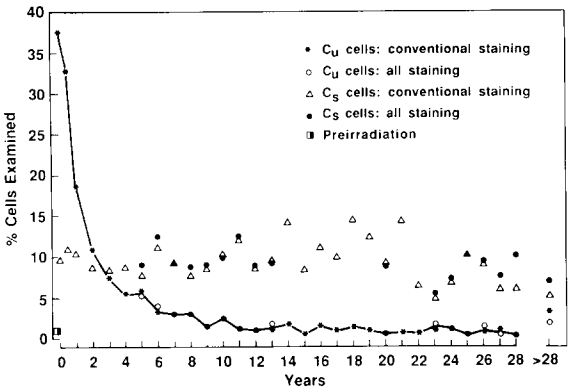

Fig. 4.2. Rates of decline in frequencies of unstable (C_u) and stable (C_s) aberrations in blood samples taken at various times after X-ray radiotherapy from patients treated with partial body irradiation to the spinal region (Evans, 1982, 1985).

approximate 50% reduction in unstable aberrations per year for the first 2–3 years post exposure with much smaller rates of decline thereafter up to 22 years post exposure. Even at this late stage following irradiation, a small proportion of blood cells induced to undergo mitosis in culture are in their first postirradiation mitosis. It is because of this longevity of mitotically inactive lymphocytes that it is possible to observe radiation-induced unstable aberrations in blood cells of, for example, survivors of Hiroshima and Nagasaki, who received high levels of radiation exposure 40 years prior to sampling (Awa et al., 1978; Sasaki, 1983).

The rate of decline in the yields of stable aberrations is less than that of unstable aberrations, and in some cases,clones of cells containing copies of the same aberration may be observed (Buckton et al., 1978). Stable and unstable aberrations are induced with equal frequency, but the former appear to be less frequent because they are more difficult to detect. Moreover, stable aberrations may be derived from chromatid-type damage induced by a very wide range of chemical or biological mutagens, whereas unstable aberrations, such as dicentric and ring chromosomes seen at the first mitosis following their induction, are hallmarks of exposure to ionizing radiations (and a very few chemical mutagens).

Thus, despite the fact that the yield of aberrations declines with increasing time of sampling of blood cells following exposure and with increasing

times of sampling of cells in culture, it may nevertheless be possible to detect the effects of exposure to a clastogenic mutagen many years after exposure. If the rate of decline in aberration frequency with time is known, then it is theoretically possible to deduce the induced aberration frequency at the time of exposure and hence the mutagen dose, and indeed this has been attempted in a few cases (Lloyd et al., 1980; Randolph and Brewen, 1980; Bender and Wong, 1982). However, the rate of decline in any one individual may not be the same as in another, for turnover rates of lymphocytes in the body are influenced by a number of factors including those that may induce the body to mount an immunological defense; so application of this method to individuals must be approached with caution.

4.5. Other confounding factors

A variety of confounding factors may influence the observed frequency of chromosomal aberrations in a cultured blood sample. The importance of time of sampling of blood and of cells in culture has already been emphasized, but there are other factors that should be taken into account. For example:

(1) The level of chromosome damage sustained by different individuals exposed to the same level of exposure to a given mutagen may differ because of differences in inherent sensitivities, and in some very specific instances (see Ch. 3), these differences may be quite large but will probably not confound for biological dosimetry.

(2) Chromosomal aberrations may arise as a consequence of "normal" internal cellular processes as well as following exposure to mutagens in the environment, so that a "background" or "spontaneous" level of aberrations is to be observed in all individuals. This spontaneous aberration frequency will be influenced by a variety of factors including an individual's inherent sensitivity, previous exposure to mutagens, and possibly age.

(3) In addition to chemical or physical mutagens, exposure to certain infectious agents, and in particular certain viruses, can also result in increased aberration frequency.

(4) Elevated aberration frequencies associated with ill health are also observed in certain noninfectious conditions, such as vitamin B deficiency (which at the extreme results in pernicious anemia), benign blood dyscrasias, and in association with certain neoplastic states.

(5) Because of the importance of time of sampling and proliferation rate in culture, any factors which influence the rate of cell progression in culture may influence the measurement of aberration frequencies. Blood cells from different individuals may respond differently to the stimulating effect of PHA, and different culture media and/or sera also give different cell progression rates.

(6) The accurate scoring of aberrations requires a specialized expertise; the degree of skill of an observer will have some influence on the aberration frequency observed in cells from a given blood culture.

5. Cytogenetic techniques

Although radiation-induced chromosomal aberrations had been studied earlier in cultured human cells (Bender, 1957) and in human bone marrow cells in vivo (Tough et al., 1960), it was the development of the short-term peripheral blood lymphocyte culture technique (Moorhead et al., 1960) that permitted the rapid development of human radiation cytogenetics and its application to biological radiation dosimetry. Peripheral blood samples are easily obtained and can be made to yield high-quality metaphases that represent the first in vitro division and often first postirradiation division. These metaphases are relatively easily scored for chromosomal aberrations, and so these cells are used to the virtual exclusion of any other cell types.

5.1. Lymphocyte culture methods

Many minor variations of the basic short-term peripheral lymphocyte culture method are in use, but the basic elements of all are the same. A sterile sample of a few milliliters of venous blood is collected into a syringe or vacuum tube containing heparin to prevent coagulation. Either whole blood, lymphocyte-rich serum obtained by allowing the red cells in the sample to settle, or a pure white cell, or even lymphocyte, fraction obtained by density gradient centrifugation is used to inoculate a suitable tissue culture medium, usually

containing 10–15% serum, often fetal calf serum, and (usually) antibiotics such as penicillin and streptomycin. Total culture volume may range from 1 to 10 or 15 ml, with the whole blood or lymphocyte inoculum adjusted accordingly. The inoculum may range from a few drops of whole blood or lymphocyte-rich serum to several milliliters. Sometimes the inoculum is adjusted to give on the order of one-half to one million leukocytes/ml of total culture volume.

Many different tissue culture media have been used for the short-term culture of human lymphocytes, including, for example, Eagle's Minimal Essential Medium, McCoy's 5A, TC 199, Ham's F-10, and RPMI 1640. Choice of medium appears to make little difference as to whether the lymphocytes can be stimulated to undergo mitoses. However, it has been shown that the richer media, such as RPMI 1640 or Ham's F-10, allow the lymphocytes to arrive at their first and subsequent mitoses more rapidly than do some of the other media (Purrott et al., 1981a). In view of the generally accepted importance of sampling as few second or later in vitro mitoses as possible for assessment of radiation exposure, this factor should be taken into account. In addition, some media, notably TC 199, are deficient in a folate source, thus allowing the expression of heritable chromosomal fragile sites (Hecht and Sutherland, 1984); their use is now generally avoided.

Whatever the tissue culture protocol, the lymphocytes must be stimulated to pass out of their G_0 state and enter active cell cycles. This is generally done with the plant lectin phytohemagglutinin, but a number of other agents may be used as well, ranging from old tuberculin to an extract from pokeweed. Most chromosomal aberration data has been collected using phytohemagglutinin, which preferentially stimulates the T lymphocytes. Differences in aberration yields between T and B cells have been suggested (Santos Mello et al., 1974), so mitogens which preferentially stimulate B cells, such as pokeweed mitogen, should probably be avoided.

Cultures are generally incubated at 37°C. Because the usual tissue culture media contain bicarbonate as a buffer, cultures are either sealed, or the incubator is provided with an atmosphere containing 5–7% carbon dioxide. Occasionally, organic buffers such as Hepes have been used to avoid having to control gas -phase carbon dioxide.

Prior to their fixation, cultures are treated with the spindle poison colcemid for a few hours to accumulate cells in metaphase that lack a spindle apparatus and produce well-spread fixed metaphase preparations. Colchicine or Vinca alkaloids such as Velban may be used as well.

5.1.1. The issue of fixation time. There is a large literature dealing with the choice of "proper" fixation times for studies in human radiation cytogenetics. During the past years a fixation time of 48 h has generally been recommended. It is, however, important to recognize that there is nothing "magic" about any particular fixation time. The objective is simply to have an adequate number of mitoses, while at the same time having as few second and later in vitro mitoses as possible. As already mentioned, there are many factors influencing the numbers of first in vitro divisions in cultures harvested at 48 h, including temperature, culture medium, and individual variation (Purrott et al., 1981a, b). Several groups have noted that harvest at 48 h does not ensure obtaining first in vitro mitoses only (Scott and Lyons, 1979; Purrott et al., 1981a). An ongoing survey involving several thousand cultures from about 500 people found that the frequency of first in vitro divisions in cultures made with RPMI 1640 and fixed at 48 h ranged from 100% to a low of 49% in 1 individual, with an average of 92.3% (Bender et al., 1986).

Several investigators have noted that the same technique, 5-bromodeoxyuridine incorporation followed by differential staining, which is used to determine the percentage of first and later in vitro divisions, may also provide a means of circumventing the problem of variations from culture to culture in the numbers of non-first mitoses (Bender, 1979; Scott and Lyons, 1979). These studies showed that addition of the analog itself does not change aberration yields (unless, of course, it has already been incorporated at the time of irradiation, in which case it greatly sensitizes the DNA), and its use has become standard technique in several laboratories. Another technique is to add colcemid to the cultures 24 h after initiation so that all mitoses are arrested, preventing the beginning of a second cell cycle.

5.2. Slide preparation and staining methods

Colcemid-treated cultures are centrifuged, the supernatant medium removed, and the cells resuspended in a hypotonic solution, most commonly 0.075 M KCl, for 10 or 15 min to swell the metaphase cells prior to fixation. They are then spun down, the supernatant hypotonic solution removed, and the remaining cells fixed with (usually) 3 : 1 absolute methyl alcohol : glacial acetic acid. Following several washes in fresh fixative, the cells are concentrated in a small volume of fresh fixative and the resulting suspension spread on glass microscope slides and dried.

There is a great deal of variation in the precise fixation and spreading methods recommended in the literature. For example, the fixative may be ice cold or at room temperature, the cells fixed as a pellet or as a resuspension in a drop or two of the hypotonic solution, and the spreading done with cold or warm, wet or dry slides and with or without flaming to enhance the spreading. Whatever method is adopted in a particular laboratory, the objective, of course, is ample numbers of well-spread metaphase plates without too much cell breakage which would give rise to an unacceptable frequency of incomplete metaphase spreads.

Though many stains have been used, the most commonly used is Giemsa, usually 5–10% in water or buffer for about 10 min. If cultures have incorporated 5-bromodeoxyuridine, differential staining is generally done by some variation in the "fluorescence-plus-Giemsa" technique (Perry and Wolff, 1974), first staining with the dye Hoechst 33258, exposing to ultraviolet light, and finally restaining with Giemsa.

5.3. Scoring methods

Slides are scanned under the low power of the light microscope (usually 80–125 ×), and suitable spreads scored with a high power oil-immersion objective (1000–1500 ×). The selection of suitable figures is made under low power in an effort to avoid selection bias for or against spreads with aberrations. Every effort is made not to reject spreads after they have been examined at high magnification. Selected metaphases (first division, if differentiated cells from 5-bromodeoxyuridine-containing cultures are used) are scored for all chromosome-type aberrations — including acentric fragments, rings, dicentrics, etc., as well as those inversions and translocations that are readily apparent to the scorer. Because the asymmetrical exchange types, dicentrics, and rings always generate at least 1 acentric fragment, total chromosome deletions in a cell equal the number of acentric fragments less the total number of rings and dicentrics, if any (i.e., 1 acentric fragment is assigned to each asymmetrical exchange and any remaining are counted as deletions). Counting the chromosomes (more properly, the centromeres) is important. Usually, metaphases outside of the range 46 ± 1 centromere are rejected. Furthermore, knowledge of the chromosome count is sometimes required to resolve questionable cases, particularly of possible small ring chromosomes.

Many scorers find doing a "visual karyotype" (checking to see if all of the more readily recognized chromosomes are present and accounted for) to be quite helpful. If the chromosome preparations are banded for more easy recognition of the stable aberrations, translocations, and inversions, each metaphase selected for scoring may be photographed and a cutout paste-up karyotype constructed. In the case of differentiated preparations from cultures that have incorporated 5-bromodeoxyuridine, scoring is of course restricted to those metaphase spreads exhibiting the first division staining pattern (i.e., both chromatids of all chromosomes darkly stained).

The numbers of metaphases required to be scored in any particular sample may vary a good deal depending on the statistical sensitivity desired. Generally speaking, however, samples of less than 100 to a few hundred metaphases are regarded as inadequate. Some laboratories routinely score 500 or more per sample.

5.4. Resource requirements

The cytogenetics laboratory must provide the usual facilities for the sterile culture of human cells, usually including a laminar flow, sterile work station and a CO_2 incubator. For scoring, compound research light microscopes with high-quality optics are required, and often some provision is also made for automatic photography. Clearly, however, the most important, and in the long run the most expensive, resource requirement for the

cytogenetics laboratory is the highly skilled and experienced personnel required to do the actual scoring. This is because of the highly time-consuming and demanding nature of the visual scoring process. While individual capacities vary, and average capacities may be exceeded briefly during emergencies, it is unusual for a trained cytogenetics technician to be able to score more than 100–200 metaphases in a working day. Even this figure may be significantly reduced if the quality of the material that must be scored is less than optimal. Thus, though the culture preparation, fixation, slide preparation, and staining phases do not require very much time, the scoring of a 500-cell sample from a single individual could easily consume 1 person-week, and scoring such samples from hundreds or thousands of persons takes years for even the largest laboratories currently doing radiation cytogenetic assays.

Naturally the cost of actually doing cytogenetic analyses varies between laboratories. Costs are often recovered at between 1 and 2 dollars per cell analyzed in the U.S.A., or depending of course on the number of cells included in the analysis, on the order of $500 per case.

5.5. Future developments

Much effort has been spent over the years in attempts to automate chromosomal analysis and circumvent the very large burden which the actual scoring constitutes. Hardware, though rather expensive, is readily available which will allow automatic slide scanning and microscope focusing and acquisition of digital images of chromosome spreads. Suitable software allows automatic metaphase finding and various image-processing and analysis functions, including rapid automatic display of metaphases selected for human operator analysis.

Two such devices have recently been tested for adequacy as automatic metaphase finders for human radiation cytogenetic analysis and were found to perform very well (Finnon et al., 1986; Shafer et al., in press). It seems clear that such devices can very substantially reduce the time required for aberration analysis, perhaps by a factor of 10 or more.

However, progress in the development of automatic analysis of metaphase chromosome images has been slow. It has been shown that there is no problem in designing an algorithm which will accurately score aberrations, given only that the image-processing system be able to accurately recognize chromosomal objects, their ends, and their centromeres (Bender et al., 1972). Unfortunately, accurate recognition of chromosomal objects and determination of ends and centromeres has turned out to be a singularly difficult pattern recognition problem, and no system currently is available that will perform accurate aberration analysis unaided by a human scorer. Nevertheless, it is possible that completely automatic chromosome-scoring devices will be developed in the future (Rutovitz, 1983).

6. Background frequency of chromosomal aberrations

6.1. Introduction

In the great majority of studies on the estimation of radiation dose by chromosomal aberration analysis for occupational, accidental, or medical exposures, the preirradiation (or background) frequency of aberrations is not available. For fairly acute exposures (> 10 rad received over minutes or a few hours) when blood samples are taken shortly after exposures (i.e., within a few days), this lack of knowledge of an individual's background frequency does not normally present a problem. This is because the estimation of dose will be based upon a total induced frequency, usually for dicentrics, that is obviously different from the upper range of reported background frequencies for individuals exposed only to background radiation.

For chronic or fractionated exposures, low-level acute doses, and in the case of delayed samples after acute exposures, the yields of aberrations will be low, and the estimation of a dose, if even possible, will depend heavily on the background aberration frequency. Information on the background frequency for the individuals who are exposed, or possibly exposed, would represent the ideal situation. However, since such data are usually unavailable, it is necessary to establish an estimated background for the individuals, attempting to take into account those factors that are reported to influence background frequency.

This chapter will consider the available litera-

ture on the analysis of chromosomal aberrations in control populations, where the studies were performed for a variety of different purposes, and where "control" will describe a range of different selected populations. The studies included in this discussion are those where samples from more than 20 individuals were analyzed, where there was no previous known radiation exposure other than background routine diagnostic radiation, and where the subjects had not been diagnosed as having a specific medical problem or disease. An exception is individuals with ankylosing spondylitis, who are included because aberration analysis has been obtained for many years after radiation therapy and preirradiation aberration frequencies were measured. The frequencies of reciprocal translocations (chromosome-type symmetrical exchanges) have been obtained by a variety of different scoring criteria, ranging from a rather superficial karyotype analysis to a more complete analysis by specific chromosome pairing on banded or nonbanded preparations. Thus, it must be noted that it is not feasible to make a meaningful comparison of reciprocal translocation frequencies.

The summarized data from the selected studies are shown in the accompanying tables. In several cases the data can be broken into subsets from comparisons of aberration frequency versus, for example, age, sex, race, or smoking history. However, the presentation of these data becomes rather unwieldly, and so general conclusions will be presented. The frequency of dicentrics per 1000 cells is presented in Table 6.1, simply because this was the only category which could readily be abstracted from all the studies. The range in dicentric frequencies is from 0.0 in a study of newborns, to 2.8/1000 cells in the control group for the Love Canal Study. This latter control group was selected from an area close to the Love Canal region and represents persons who reside in a region of relatively high environmental exposure to chemical agents from industrial sources. In a study of a group of employees at Brookhaven national Laboratory, performed by the same 2 laboratories that conducted the Love Canal analysis, the frequency of dicentrics was 1.7/1000 cells. This statistically significant difference in aberration frequency between 2 control populations highlights the complex considerations involved in combining data from several studies where the frequencies might be influenced by factors that were not considered in a particular study or by factors that were very differently represented in different studies.

An overall mean dicentric frequency can be calculated from the sample of studies in Table 6.1, although this disregards known or possible sources of variation that exist among the study populations (i.e., age, sex, race, smoking, occupation, and living environment); the unweighted mean is 1.3/1000 cells. This value could be considered acceptable as a background frequency when the induced frequency (or radiation dose) is sufficiently high that the increases or decreases in frequency that appear to be present in separate subpopulations of the general population would not be expected to confound an estimate of dose. However, in the case of radiation exposure to low doses, chronic exposures, or where samples are taken at long intervals after exposure, the observed induced frequency of aberrations will be low, and simply taking a mean background frequency without regard to possible sources of variation increases the uncertainties in the dose estimates.

The question then becomes, how legitimate is it to choose the background aberration frequency for a study group that approximates the epidemiological profiles of individuals for whom an estimate of radiation dose has been deemed to be necessary? The 2 major problems with such an approach would seem to be (1) the availability of adequate information for group matching and(2) the sizeable error in an estimate of dose based on mean values resulting from the range in aberration frequency for any selected study group. Perhaps the most appropriate approach, and one similar to that performed for atomic bomb survivors, is to attempt to determine dose estimation for groups rather than individuals (or a few individuals) where mean background frequencies for approximately matched groups could be used.

Another problem area is the handling of high-aberration-frequency cells, sometimes called "rogue" cells, that usually contain several chromosome-type exchanges and a wide range (2–> 10) of interstitial deletions ("double minutes"). The frequency of these cells varies from individual

TABLE 6.1

SUMMARY OF DATA ON ABERRATION FREQUENCIES IN CONTROL SUBJECTS

	Number of samples	Number of cells	Number of chromosome-type aberrations			Recip. trans.	Freq. dicentrics/ 1000 cells
			Dic.	Ring	Acentric		
Hiroshima (1968–69) (Awa, 1986)	263	24414	58	5	59	137	2.38
Hiroshima (1970–71) (Awa, 1986)	82	7897	18	2	24	62	2.28
ORAU (Littlefield, 1986)	81 Mean age 36.5	16215	26	7	17	63 [a]	1.6
Munich (Bauchinger, 1986)	68 Mean age 41.1	36000	15	4	126	19 [a]	0.42
Brookhaven/Oak Ridge (Bender and Preston, 1986)							
Love Canal Control	44	8800	25	3	– [b]	8 [a]	2.8
Harwell (Lloyd et al., 1980)	316	23300	30	– [c]	76	– [c]	1.3
Brookhaven/Oak Ridge (Bender and Preston, 1986)							
BNL employees	431	76900	134	16	150	45 [a]	1.74
Edinburgh (Buckton and Evans, 1986)							
Newborn	4501	15403	0	$C_u = 7$	$C_s = 17$		0.0
General Practice	736	7547	11	$C_u = 23$	$C_s = 64$		1.5
Ankyl. spondyl	59	2944	3	$C_u = 19$	$C_s = 21$		1.0
Rosyth dockyard pre-employment	79	7900	12	$C_u = 23$	$C_s = 10$		1.5
Mol (Leonard et al., 1984)							
Power plant workers	23	11500	13	0	27	7	1.1
Berlin (Obe and Beek, 1982)	83	14652	5	0	– [c]	– [c]	0.34
Sofia (Ivanov et al., 1978)	105	16267	11		116		0.7
Oxford (Blackwell et al., 1974)	156	17394	7		69		0.4
Ethylene oxide controls (Galloway et al., 1986)	304	31503	62	11	92		2.0

[a] Includes inversions.
[b] Acentric fragments included with isochromatid deletions.
[c] Data not presented.

to individual but, in those studies where they have been observed, is generally in the range of 1 in 2000–5000 cells (Awa and Neel, 1986). In the large study of Brookhaven national Laboratory employees (Bender et al., 1986), no "rogue" cells have yet been observed in a sample approaching 100000 cells from 500 subjects. The presence of such high-aberration-frequency cells does not appear to be related to a known exposure to a clastogen, and at present the mechanism whereby these multiple aberrations arise is not known. This makes the significance of such cells rather equivocal. However, their contribution to background frequencies of aberrations could be very significant. At this time, in view of the equivocal nature of the observation, it would seem to be most appropriate to record such high-frequency cells, but not to include them as part of the normal background aberration frequency.

6.2. Population variables that could influence background aberration frequency

There are a small number of factors that would clearly influence aberration frequency and cannot be considered as normal population variables. Most population studies omit certain individuals from the study to prevent such factors influencing the measure of background frequency. These in-

clude individuals with recent radiation exposures (other than routine diagnostic X-rays), certain medications (such as recent chemotherapy), known genetic disorders associated with chromosome fragility (such as ataxia telangiectasia), and certain specific neoplastic diseases (e.g., leukemia or lymphoma).

While the studies of the influence of inherent population variables on aberration frequency are by no means exhaustive, several have been considered with, in several cases, rather equivocal conclusions.

6.2.1. Age. In some studies that are large enough for several decades to be represented by 10 or more individuals, there is an increase in aberration frequency with increasing age (Galloway et al., 1986; Evans et al., 1979; Tonomura et al., 1983). The response is not necessarily linear, having an apparent plateau in the middle years. No significant change in frequency with age has been observed in the study of Bender et al. (1986), but data on the very young age group have not yet been completely reported. It is not known if the increase, if real, is due to the gradual accumulation of aberrant cells in the peripheral lymphocyte pool as a result of errors of normal cellular functions, or whether it is due to an increase in accumulated exposure to environmental clastogenic agents. Whatever the cause of the effect, it seems prudent to use a background frequency for an age group similar to that for any particular group for which a radiation dose estimate is to be attempted.

6.2.2. Sex. The majority of studies have not demonstrated a significant difference in aberration frequency between males and females over a fairly wide age range. However, the selection of a background frequency for males or for females would present little problem if sufficient data for males and females are available. Since the vast majority of the veterans in question are male, the most prudent approach would be to use data from males only.

6.2.3. Smoking history. Several studies have indicated an increase in aberration frequency in smokers compared to nonsmokers (Vijayalaxmi and Evans, 1982; Obe and Beek, 1982; Obe et al., 1982; Obe et al., 1982; Galloway et al., 1986). On the other hand, other large studies have found no significant difference between the two groups (Heath et al., 1984; Bender et al., 1986). The increases reported in smokers vary from an effect on all aberration classes (chromosome- and chromatid-type) to effects on exclusively chromatid-type or chromosome-type aberrations. It has not yet been established whether or not there are other confounding variables unevenly distributed among the smoking and nonsmoking groups which could influence the conclusion that there is an increase in aberrations in smokers. It might, nevertheless, be appropriate to calculate background aberration frequencies for smokers and nonsmokers separately from all studies where this was a known variable, irrespective of whether or not an increase was observed. In this way, all studies (positive and negative for smoking) would be given equal weight.

7. Dose–response relationships for external radiation

7.1. Introduction

All persons accumulate some level of radiation exposure during their lifetimes; for example, from naturally occurring background radiations, consumer goods, from dental or medical procedures used for diagnostic or therapeutic purposes, or very rarely, from inadvertent or accidental exposures. Such exposures may be to radiations emitted by sources outside the body (i.e., external radiation) or from certain types of radioactive elements or compounds, called "internal emitters", that may be inhaled, ingested, absorbed, or otherwise deposited in the body. Because special problems must be considered in interpreting dose–response data in situations involving internal contamination with radionuclides (see Ch. 8), it is convenient to separately summarize cytogenetic information derived from exposures of human or other mammalian cells to external radiations and internal emitters.

7.2. External radiation — linear energy transfer (LET)

All types of ionizing radiations induce the same kinds of chromosomal aberrations in exposed cells

(i.e., their effects are qualitatively identical at the chromosomal level). However, the numbers of aberrations induced depend on the level of radiation exposure (dose), which is directly related to the amount of energy deposited by independent radiation tracks within the cells of the body (in the context of chromosome damage, within critical sites or "targets" in the cell nucleus). The scientific term that describes the relative amounts and distributions of ionization and excitation energy released along the track of a photon or charged particle is "linear energy transfer", or "LET" (for detailed discussions see ICRU 16, 1970). LET represents an average and is usually expressed as the average along the track in units of thousands of electron volts generated per micron of track segment ("track average" LET).

Those types of sparsely ionizing radiations that deposit relatively small amounts of energy along segments of their paths, but have sufficient kinetic energy to travel considerable distances in tissue, are broadly classified as low-LET radiations. Examples of low-LET radiations are various types of X- and γ-radiations, having LETs of about 3–3.5 keV/μm or less. In contrast, high-LET radiations, such as fission spectrum neutrons of varying energies, and α particles emitted in the radioactive decay of certain radioisotopes, have LETs rangingfrom tens to hundreds of keV/μm. Typically, high-LET radiations deposit large amounts of energy in discrete packages and, relative to low-LET radiations, travel quite short distances in cells or tissues. As will be discussed in the following paragraphs, LET is an important parameter in determining the shape of aberration dose–response curves, the relative efficiency of differing types of radiations in inducing chromosomal aberrations, and the distributions of chromosome damage in affected cells.

7.3. Low-LET radiation dose–effect curves

Numerous laboratories have determined dose–response relationships for chromosomal aberration induction in human lymphocytes exposed to various low-LET radiations in vitro (for a recent review see Lloyd and Edwards, 1983). These in vitro calibration curves formally describe the relative numbers of chromosomal aberrations induced per unit dose of radiation (usually expressed in rad or centigray) and serve as reference standards for estimating dose in persons having external exposures to penetrating radiations of similar energy.

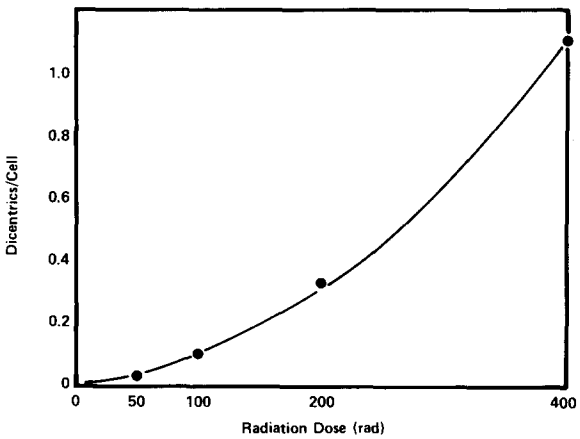

Fig. 7.1. Typical dose–response relationship for dicentric chromosomes observed in human lymphocyte metaphases after exposure of human whole blood to low-LET (γ) radiation (redrawn from data of DuFrain et al., 1980).

Following acute in vitro exposures to low-LET X- or γ-radiations, the frequencies of aberrations that result from single breaks in a chromosome segment (such as terminal deletions) increase, essentially as a linear function of radiation dose. In contrast, the frequencies of exchange-type aberrations (such as dicentrics) that are formed as the result of independently induced breakage and rejoining of 2 separate chromosomes or chromosome segments increase in proportion to a higher order function of dose (Fig. 7.1). Such a dose–response relationship is typically observed when the frequencies of exchange-type aberrations are plotted against dose of low-LET radiation.

Such data may be evaluated with statistical techniques to determine the slope of the dose–response curve, which represents a numerical measure of the relative effectiveness of the specific type of radiation in inducing chromosomal aberrations in human lymphocytes. These dicentric data can be fitted to various dose–response equations by analytical methods. Such analyses demonstrate that these low-LET dose–response data may be adequately described by what is often termed a "linear–quadratic" dose–response equation (more properly, simply a quadratic) which is described

by the formula $Y = c + \alpha D + \beta D^2$ (Lea, 1946). In this equation, Y = the yield of chromosomal aberrations (in this instance, dicentrics), D = radiation dose in rad, and c = the baseline, or background frequency, of aberrations that would be expected to be observed in lymphocytes from nonirradiated or control persons (as already discussed in Ch. 6). The numerical values of α and β are the slope terms, or coefficients, that are derived from the curve fitting.

The quadratic expression above is in fact modified at very high doses by a saturation effect. Such saturation effects are frequently observed in radiobiology and are often attributed to cell-killing effects. In the case of 2-break exchange production in human lymphocytes, however, Norman and Sasaki (1966) have clearly shown the saturation to result from the finite number of chromosomes available for recombination, so that at the higher doses, breaks resulting in exchange formation are more and more likely to undo an existing exchange in the process.

The biophysical and molecular processes involved in the formation of chromosomal aberrations following radiation exposures are quite complex and not totally understood. However, it is possible to interpret the low-LET dose–response equation in rather simple terms.

The linear–quadratic equation predicts that the total yield of dicentrics observed after exposure to low-LET radiation is actually the sum of the yields of aberrations induced by 2 separate dose–response functions. Some portion of the dicentrics (i.e., that defined by the αD term in the equation) varies as a linear function of radiation dose, whereas a second portion of the dicentrics (i.e., that defined by the βD^2 term in the equation) increases in relation to the square of the radiation dose. When dicentric formation is interpreted in the context of the linear–quadratic dose response model, the αD, or linear term, can be considered to describe the number of dicentrics induced by single low-LET radiation tracks, while the βD^2, or dose-squared term, can be considered to represent that portion of dicentrics (or other types of 2-break aberrations) induced by 2 or more independent radiation tracks.

It logically follows that at very low doses of sparsely ionizing X- or γ-radiation, only low numbers of dicentrics will be induced, and these will primarily be the result of single-track events. At high radiation doses, there is greater probability for interaction of DNA lesions induced by 2 or more independent tracks. In such instances, most dicentrics will result from 2-track events, and their yield will vary primarily as a function of the square of the radiation dose.

7.4. High-LET dose–response curves

In contrast to the pronounced curve in the shape of the dose response observed after exposures of human cells to low-LET radiation, all types of chromosomal aberrations vary predominantly as a linear function of dose after exposures of cells to high-LET radiations. An example of a typical high-LET dose–response curve is shown in Fig. 7.2, in which the frequency of dicentrics is plotted against dose of fission-spectrum neutrons. The dicentric dose–response function is adequately described by the equation $Y = c + \alpha D$, in which Y = yield of dicentrics, c = the baseline, and D = neutron dose.

Such a linearity in dose–response function might be expected, since fission neutrons (or more properly, the "knock on" protons produced by

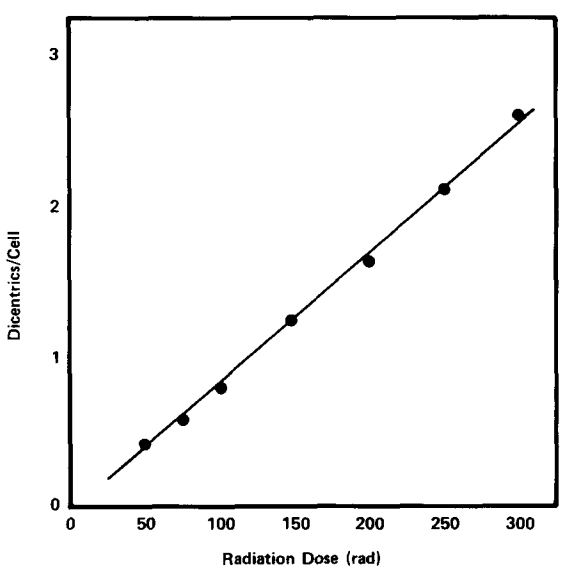

Fig. 7.2. Typical dose–response relationship for dicentric chromosomes observed in human lymphocyte metaphases after exposure of human whole blood to high-LET fission spectrum (0.7 MeV) neutrons (redrawn from data of Lloyd et al., 1976).

their collisions with hydrogen nuclei) deposit large amounts of energy along all segments of their paths, and it might be predicted that multiple sites of DNA damage would be induced within a cell nucleus after traversal by a single, statistically independent track. Thus, the majority of the chromosomal exchange-type aberrations result from the interaction of these single-hit DNA lesions. Because radiations having high track average LETs deposit sufficient energy along single tracks to induce multiple chromosome breaks, the dose-squared, or β, component (which predominates in the dose response observed for low-LET radiation) contributes insignificantly to aberration induction following exposures to most types of high-LET radiation.

7.5. RBE for high- and low-LET radiation

When one compares the relative numbers of dicentrics induced in lymphocytes exposed to equal doses of γ-radiation or of high-LET radiations such as fission neutrons, it is apparent that the high-LET radiations are much more efficient in inducing dicentric aberrations per unit dose than are the low-LET radiations (Fig. 7.3). Because the shapes of the dose–response curves for aberration induction in lymphocytes are predominantly linear following exposures to neutron radiations, but curvilinear following exposure to high doses of low-LET radiation, it follows that the relative efficiency of the high-LET radiation will vary inversely with radiation dose.

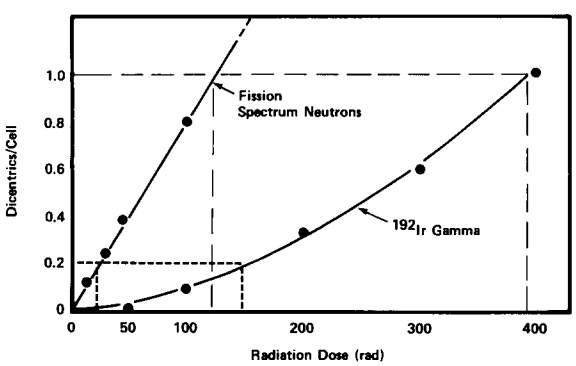

Fig. 7.3. Comparison of the relative biological effectiveness (RBE) of fission spectrum (1.0 MeV) neutrons vs. γ-rays in inducing dicentric aberrations in human lymphocytes. Note that the ratio of X-ray and neutron doses (RBE) required to yield 0.2 dicentrics/cell is about 6, whereas at higher doses (yield of 1.0 dicentric/cell) the RBE is about 3 (Littlefield, 1982).

The differences in the efficiency in the 2 types of radiations represent a measure of the differences in their "relative biological effectiveness", or RBE. In radiobiological terms, RBE is a ratio of the dose of a standard radiation (usually 250 keV X-rays or γ-rays) needed to produce a given magnitude of a certain effect to the dose of another radiation needed to produce the same magnitude of effect (for general discussion see Casarett, 1968). The RBE of various radiations depends on both the average rate of energy loss along the paths of individual ionizing particles or photons (LET) and the level of effect. In general, RBE values are observed to increase with increasing LET up to about 70–100 keV/μm and then to decrease as LET becomes larger (i.e., as energy in excess of that required to induce aberrations is "wasted" after being deposited in critical targets by the high-LET tracks).

It is also apparent from Fig. 7.3 that because of the difference in curve shapes, there is no single value for RBE and that the differences in RBE are most pronounced at low radiation doses when the majority of dicentrics induced by low-LET radiations results from single-track events. Thus, the maximum, or "limiting", RBE for any high-LET radiation may be estimated by calculating the ratio of linear coefficients of the high- (α_1) and low-LET (α_2) radiations (i.e., $\mathrm{RBE}_{max} = \alpha_1/\alpha_2$) (Neary et al., 1963). The limiting RBE represents a measure of the relative efficiency of single high- and low-LET radiation tracks in inducing aberrations.

7.6. Dose rate, fractionation effects

The dose–response relationships for high- and low-LET radiations presented in previous sections were derived following exposures to radiations delivered at high dose rates. When cells are exposed to low-LET radiations delivered at very low dose rates, or to high dose-rate radiations delivered in 2 or more fractions, reductions in the frequencies of chromosomal exchange-type aberrations are observed (Lea, 1946; for human lymphocyte examples see Brewen and Luippold, 1971; Purrott and Reeder, 1976).

The well-established dose rate (or fractionation) reduction factor results from the fact that sites of radiation-induced DNA damage that can interact to give rise to dicentric formation remain available for interaction for only a finite period fo time (i.e. an average repair time of about 2 h) (Schmid et al., 1976; Liniecki et al., 1977; Virsik and Harder, 1980; Lloyd et al., 1984). When cells are exposed to very low dose-rate radiation, or to doses delivered in multiple fractions, DNA damage induced by a single radiation track may be repaired before another potentially interacting DNA lesion is induced by a second traversal. This results in a reduction in the frequency of chromosomal exchange-type aberrations induced by the interaction of 2 independent radiation tracks and a concomitant decrease in the β or dose-squared coefficient in the dose–response equation. An example of dose rate effects in human lymphocytes is shown in Fig. 7.4.

Theoretically, it is possible to decrease the dose rate to the point at which no dicentric chromosomes at all would result from the interaction of 2 independent ionizing events. In such instances, the frequency of dicentrics would be proportional to a simple linear function of low-LET radiation dose. Because proportionally few exchange-type aberrations result from the interaction of damage induced by 2 separate high-LET tracks, dose rate or fractionation effects are not commonly seen for cells exposed to high-LET radiation.

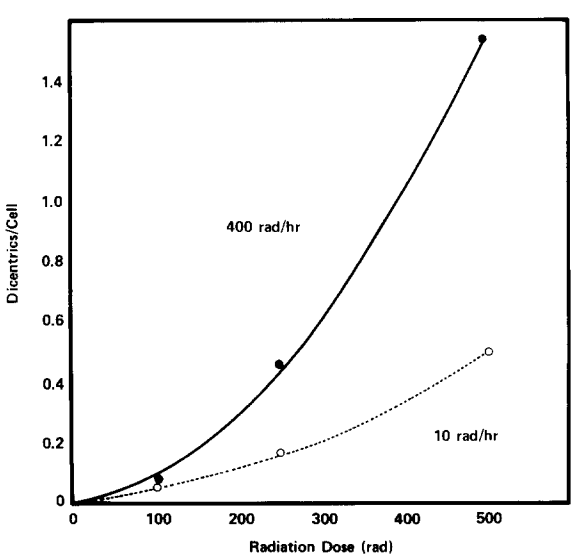

Fig. 7.4. Comparison of the number of dicentric chromosomes observed in human lymphocyte metaphases after exposure to low-LET radiation delivered at high vs. low dose rates (i.e., 400 vs. 10 rad/h). A pronounced reduction in dicentrics is observed at the low dose rate (redrawn from data of Purrott and Reeder, 1976).

7.7. Cellular distribution of aberrations

The relative amount of energy deposited within cell nuclei by radiations of differing LET is also important when considering the distribution of chromosomal aberrations observed in lymphocyte metaphases. Following exposures to evenly applied doses of penetrating low-LET radiations, all exposed lymphocytes are at equal and random risk for being traversed by the mean number of sparsely ionizing radiation tracks. In such situations the relative proportion of metaphases having 0, 1, 2, or more aberrations is usually modeled by the Poisson distribution. After exposures to similar total doses (but obviously fewer tracks) of some types of high-LET radiations, fewer cells are traversed by densely ionizing tracks, and those lymphocyte nuclei that are traversed at all are likely to receive larger and more variable depositions of energy. Such an uneven dose distribution among individual cells will result in an excess number of metaphases (as compared with expected) having multiple chromosomal aberrations as well as a compensatory excess number having no damage at all (i.e., aberrations will be "overdispersed" relative to the Poisson expectation). Such overdispersion is typically observed following exposures of human lymphocytes to densely ionizing α particles, such as those emitted in the radioactive decay of isotopes of plutonium.

Overdispersion of aberrations in lymphocyte metaphases is also observed in persons having nonuniform exposures to penetrating external radiations. In situations in which only a part of the body receives a radiation dose, only those lymphocytes that are in transit through the radiation field during the period of exposure will be irradiated. Others will not be exposed at all. Afterwards, the exposed and nonexposed lymphocytes will be mixed as the blood circulates, which results in an "averaging" of aberration yield. Using the degree of deviation from the expected Poisson

distribution as a basis for calculations, sophisticated mathematical models have been proposed for estimating the fraction of exposed lymphocytes and for applying "correction factors" in dose estimation (Sasaki, 1983; Lloyd, 1984). Although these approaches may be useful in resolving dose in some instances of partial-body exposures, nonuniformity of external radiation dose generally introduces significant complications in interpretation of cytogenetic data.

8. Internally deposited radioactive material

8.1. Special problems associated with internal emitters

Using chromosomal aberrations to evaluate radiation exposures resulting from internally deposited radioactive materials in people presents several unique problems. First, the deposition, distribution, and dose to individual cells are dependent on the radionuclides involved, the route of exposure, the metabolic state of the individual, and the chemical and physical form of the material. Second, because of individual differences, it is impossible to make a reliable estimate of the radiation dose that the individual received even if the exposure level, radionuclides involved, and their physical and chemical form are known. Finally, even if the individual is well studied after the exposure, it is difficult in humans to obtain samples of tissues with the highest dose and risk for radiation-induced disease. The biological response, in terms of radiation-induced chromosome aberrations, is often measured in blood lymphocytes even though the biologically significant radiation dose may be very nonuniformly distributed and concentrated in other targets in the body.

8.1.1. Types of exposure. To provide a background for this chapter, it is essential to briefly discuss the physical and biological kinetics of different types of radioactive materials in nuclear weapons fallout or nuclear industry accidents. For this discussion, the materials fall into 3 general classes. The first class are those radionuclides with short physical half-lives that cause brief radiation exposure when internally deposited. Most medical isotopes fall into this class, as well as many nuclides that are associated with fallout or a nuclear accident soon after the event. Some of the major nuclides of biological importance in this class are the isotopes of iodine. For example, iodine-131 has an 8-day half-life, but it moves very rapidly through the food chain and is concentrated in the thyroid gland. Deposition of ^{131}I can result in a large local dose to the thyroid, with little dose to the remainder of the body. For this isotope, ingestion is the main route of entry into the body.

The second class of radionuclides are those with a rather long physical half-life but a short retention time in the body. The best example of this is cesium-137, which has a 30-year physical half-life but is retained in the body with a biological half-life of only 130 days (Taylor et al., 1962). Soluble forms of this radionuclide are uniformly distributed throught he body, which results in a uniform whole-body exposure with a changing dose rate. Ingestion and inhalation are the primary routes of entry into humans.

The third class of compounds are most important from a radiological point of view. They have long physical half-lives and are retained in the body for long times due to their chemical nature or the physical matrix with which they are associated. A major route of entry into the body for many of these radionuclides is through inhalation of small particulate materials since they are not readily taken up through the gastrointestinal tract (Bair, 1979). For example, ^{137}Cs is soluble in the body, but if it is trapped in an insoluble particle matrix, it can be retained for a long time in the lungs and lung-associated lymph nodes with a rather slow translocation to other body organs (McClellan et al., 1979). Other radionuclides such as soluble strontium and radium are taken into the body by both ingestion and inhalation and are deposited primarily in the bone. They are retained in the bone matrix for extended periods of time and result in protracted exposure of the bone and bone marrow (Pool et al., 1973). Inhalation of many radionuclides in the plutonium and transplutonium series results in deposition in the lung with subsequent translocation to the liver and bone. This results in the lung, liver, and bone receiving the major radiation exposure from these elements (Durbin, 1973; Jee, 1976).

In addition to artificially produced radionuclides, there are several naturally occurring radionuclides which contribute to background dose. These have been reviewed by the National Council on Radiation Protection and Measurements (NCRP, 1975). The major internally deposited, β-emitting radionuclide is potassium-40 which contributes about 10 mrem/year to the soft tissue. The major α dose is contributed by the uranium series and the thorium series; calculated doses for α emitters range from 8 mrem/year in gonads to as high as 110 mrem/year to the cortical bone. Higher doses have been calculated for localized regions of the respiratory tract following inhalation.

8.1.2. Biological parameters for estimation of radiation dose. To estimate radiation dose from internally deposited radionuclides, it is essential to first define their uptake, deposition, distribution, and retention patterns. Extensive research has been conducted to define these parameters for many radionuclides. Models have been developed which use these parameters to calculate radiation dose to the organs of interest and to help set limits on the levels of uptake allowed for radiation workers (ICRP), 1979). It is essential to express exposure in terms of dose, since the time of exposure may be short or long, and, depending on the physical and chemical nature of the radionuclide involved, the retention time may also be either short or long. Combining these variables, time of exposure and retention time, may result in a wide difference in dose and dose rate patterns. Thus, deposition of radionuclides can provide a source of radiation dose long after the exposure to a contaminated environment and entry of the radionuclide(s) into the body has ended. The dose rate from internally deposited radioactive material is often low and changes as a function of time after the deposition. In addition to being nonuniformly distributed in the body, many radionuclides, especially α emitters and radionuclides trapped in particles, are nonuniformly distributed within various organs. This results in very nonuniform exposure of cells in organs where the radionuclides have been deposited. In some cases a small fraction of the total cells in an organ may receive rather large radiation doses, while the majority of the organ is subjected to little or no radiation dose.

8.1.3. Cytogenetic response. As described in more detail in Ch. 4 of this report, the frequency of chromosomal aberrations in peripheral blood lymphocytes can be used to estimate radiation dose. This is relatively straightforward in the cases of external exposure to penetrating radiations such as γ-rays or fast neutrons. For internal exposures, however, and particularly for particulates or for α particles, meaningful dosimetry is difficult if not impossible. For nonuniformly distributed internally deposited radionuclides, it is important to recognize that not only the radiation dose is nonuniform, as described above, but that the lymphocytes used to evaluate the exposure are also nonuniformly distributed throughout the body in the blood, organs, lymph nodes, and lymph follicles. During most of a lymphocyte's lifetime, it resides in lymph follicles and lymph nodes. To estimate radiation dose to lymphocytes from the measured distribution of the isotope and the distribution of lymphocytes relative to the isotope, must be known. This information must then be combined with estimates of the life span of the lymphocytes and exposure histories before dose–response relationships can be calculated and radiation dose to the lymphocytes estimated from aberration frequency measurements. In humans such information is seldom available. It is essential to keep this in mind when evaluating the usefulness of chromosomal aberrations measured in blood lymphocytes to predict dose from internally deposited radioactive material.

8.2. Human experience

Everyone has been exposed to internally deposited radioactive materials as a part of their normal background exposure. Many human populations have been further exposed in their work environment, in radiation accidents, or as part of medical therapeutic or diagnostic treatment. Cytogenetic data from some of these human populations are listed in Table 8.1. In these people the radioactive material was deposited in the body, and at different times after the deposition, the frequency of chromosomal aberrations was evaluated in the blood lymphocytes.

Several human populations have received occupational exposures that resulted in substantial body burdens of radioactive material. These include the radium-226 exposures of luminous-dial

TABLE 8.1

THE INDUCTION OF CHROMOSOME ABERRATIONS IN THE BLOOD LYMPHOCYTES OF HUMANS EXPOSED TO INTERNALLY DEPOSITED RADIOACTIVE MATERIAL

Group	Measure of exposure	Time in culture (h)	C_u aberrations/cell [a]		Rings and dicentrics/cell		Ref.
			Control	Exposed	Control	Exposed	
Uranium miners	10–5400 WLM [b]	72	0.07	0.16	0.0	0.003	Brandom et al., 1972
Radon spa workers	0.2–2.4 rad/year + ^{222}Rn	48–72	0.05	0.15	NR [g]	NR	Pohl-Ruling et al., 1976
Radium-226 dial painters	0.003–1.0 μCi ^{226}Ra + daughters	53	0.009	0.019	0.001	0.001	Hoegerman, 1976
Radium-226 dial painters	> 1.0 μCi ^{226}Ra + daughters	53	0.009	0.037	0.001	0.006	Hoegerman, 1976
Luminous dial painters	15 rem/year γ + ^{226}Ra	NR	0.016	0.024	NR	NR	Boyd et al., 1966
Luminous dial painters	^{90}Sr + ^{226}Ra	72	0.016	0.12	0	0.04	Tuscany and Muller, 1967
Plutonium workers	> 40 nCi ^{239}Pu	50	0.012	0.054	0.006	0.037	Brandom et al., 1978
Plutonium workers	^{239}Pu + 14 rad gamma	NR	NR	NR	0.003	0.005	Dolphin, 1971
Plutonium wound	^{239}Pu 14.2 μCi [c], 1.0 μCi [d], 0.59 μCi [e]	48	NR	0.042	0.001	0.030	Schofield et al., 1974
Monazite sands mill workers	40–80 rad/year γ + ^{232}Th + daugh.	72	0.02	0.03	0.002	0.004	Costa-Ribeiro et al., 1975
Thorotrast	25 ml Thorotrast + ^{233}Th + daughters (200–1200 rad/year) [f]	48	NR	0.16	NR	0.11	Buckton et al., 1967
Thorotrast	25 ml Thorotrast + ^{232}Th + daughters (200–1200 rad/year) [f]	72	NR	0.10	NR	0.03	Teixiera-Pinto et al., 1979
Thorotrast	25 ml Thorotrast + ^{232}Th + daughters (200–1200 rad/year) [f]	72	NR	0.19	NR	0.08	Fischer et al., 1966
Wound nuclear worker	^{241}Am 3000 μCi [c], 1000 μCi [e]	48–54	NR	NR	0.1–0.45	0.27	Littlefield et al., 1983

[a] Cu, unstable chromosomal aberrations.
[b] WLM, work level months.
[c] Amount in the wound site.
[d] Body burden after accident.
[e] Amount excreted in urine and feces.
[f] Calculated dose to lymph nodes (Steinstrassen, 1981).
[g] NR, not recorded.

painters (Boyd et al., 1966; Hoegerman, 1976), uranium, radon, and radon progeny exposure of uranium miners (Brandom et al., 1972), radon and radon progeny exposures to health-spa workers (Pohl-Ruling et al., 1976), and plutonium deposition in nuclear workers (Dolphin,1971; Hepplemann et al., 1973; Schofield et al., 1974; Brandom et al., 1978). In most of these populations, there was a small, but not always statistically significant, increase in the aberration frequency in the exposed population above that observed in the control population. Since the increase in aberration frequency was small and variable, it could not be used to estimate individual burdens of the radionuclides. There were also complicating factors in many of these human studies, such as exposure to external radiation from occupation or medical sources in addition to the internally deposited materials. This makes it impossible to relate the chromosomal aberration response directly to the level of internally deposited material or radiation dose from these materials. The only function that scoring aberrations in worker populations can serve is to indicate whether or not the population had a significant radiation exposure. The aberation frequencies alone cannot define the dose from internally deposited material to individuals within the population.

A few individuals have been accidentally exposed to internally deposited radioactive materials. One individual received a rather large body burden of plutonium-239 (14.2 μCi) and had a measurable increase in aberration frequency ().03 rings plus dicentrics/cell) above the background level reported (0.001 rings plus dicentrics/cell) (Schofield et al., 1974).

In another case, the frequency of chromosomal aberrations has been followed as a function of time after a radiation accident which resulted in deposition of a large burden of americium-241 (Littlefield et al., 1983). The chromosomal aberration frequency (rings plus dicentrics) is plotted as a function of time after the accident in Fig. 8.1. The aberration frequency in the blood lymphocytes of this individual were significantly elevated above the background level and fluctuated as a function of time after the exposure. The yield of aberrations decreased as a function of time for the first 39 months after the accident. The frequency

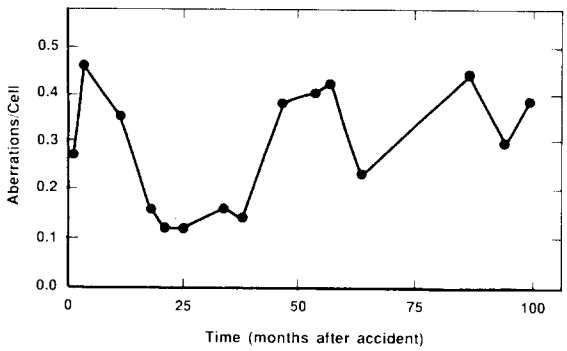

Fig. 8.1. The change in the frequency of rings and dicentrics after accidental ^{241}Am exposure (Littlefield et al., 1983).

then increased and reached a plateau between months 39 and 97 after the accident. The total cumulative radiation dose was, of course, increasing as a function of time. Thus, there was no direct relationship between cumulative radiation dose and observed aberrations in the blood lymphocytes. The dose rate and dose distribution pattern to the blood lymphocytes may also have been changing since the subject received chelation therapy after the accident. This example illustrates that even though the individual was carefully evaluated to determine the uptake, clearance, and translocation of the ^{241}Am as a function of time after the exposure, the lymphocyte response to that protracted exposure was very difficult to relate to such factors. The aberration frequency measured as a function of time is, of course, a measure of radiation damage to the lymphocyte population but is not a simple reflection of the cumulative dose to the blood or other body organs.

People injected with Thorotrast (thorium-232 dioxide and its daughter isotopes) represent a major human population with a large body burden of internally deposited radioactive material. A colloidal suspension of the thorium dioxide solution was injected as part of a medical diagnostic procedure. The medical consequence of this treatment has been determined and was summarized in a series of articles in *Health Physics* (Rundo et al., 1983). Many of the isotopes in the thorium decay chain are α emitters and represent a major health hazard to those exposed. The colloidal particles were taken up by the reticuloendothelial cells of the body and concentrated in the lymph nodes,

liver, spleen, bone marrow, and lung. This resulted in a very nonuniform distribution of α-irradiation as well as exposure to several γ-rays with different energies and produced a marked increase in the cancer incidence in these people, especially in the liver where the incidence of cancer has approached 40% in many of these populations (Grossner et al., 1986). The frequency of chromosomal aberrations in the blood lymphocytes of several different populations exposed to Thorotrast has been measured by several different investigators. In all these populations there was a marked increase in the frequency of ring and dicentric aberrations above historical control values. This increase must be viewed in light of the estimated radiation dose to the lymph nodes of 200–1200 rad/year of α-irradiation, with the exposure often extending over many years (Steinstrasser, 1981).

Early research (Fishcer et al., 1966) suggested that there was an increase in aberration frequency as a function of the product of the Thorotrast concentration and time after injection, which, of course, is one measure of radiation dose. Later research, however, indicated that the frequency of aberrations did not increase in any simple fashion with increased injected activity, radium-224 equivalents (a measure of injected activity), or time after injection (Buckton et al., 1967c; Teixeira-Pinto et al., 1979; Steinstrasser and Kemmer, 1981). The reason for the apparent lack of correlation with radiation dose may be related to sampling time and differences in radiation dose distribution. The problems in relating the chromosome response to the potential radiation dose have been reviewed (Steinstrasser, 1981).

It was noted that internally deposited radioactive material can result in an increase in the frequency of chromosomal aberrations in blood lymphocytes of exposed human populations. However, there are many physical variables associated with human studies, including the combined exposure to both internal and external radiation, the lack of accurate dosimetry, and the nonuniform distribution of the material in the body at the organ or cell level. When these variables are combined with biological variables such as sampling the proper cell population and estimating the fraction of the lymphocyte cell population exposed, it is not possible to derive dose–response relationships for internally deposited radioactive materials in humans. However, studies in animals have been conducted where some of the above physical and biological variables can and have been controlled.

8.3. Laboratory research

Some of the major questions generated by the human studies, such as the influence of LET on the induction of chromosomal aberrations, the influence of nonuniform dose distribution, the fraction of the cell population exposed, and the influence of cell proliferation on the aberration frequency, have been evaluated using animal and in vitro cellular models.

8.3.1. Dose–response relationships in vitro. The question of the radiation sensitivity of human lymphocytes following exposure to α emitters relative to their response to low-LET radiation has been addressed in vitro (Purrott et al., 1980; Edwards et al., 1980a, b; DuFrain et al., 1979). In these studies the expected linear dose–effect curves were generated. It was determined that the RBE for the induction of aberrations in human lymphocytes exposed to high-LET α emitters was 10–40 relative to the frequency produced by protracted γ-ray exposures (i.e., relative to the αD term for acute γ-rays) (Brewen and Luippold, 1971). The α coefficients reported were 3.8×10^{-3} dicentrics/cell/rad for ^{239}Pu, $2.9 \pm 1.5 \times 10^{-3}$ dicentrics/cell/rad for curium-242, and surprisingly, $49.0 \pm 4.2 \times 10^{-3}$ dicentrics/cell/rad for ^{241}Am. The rather high RBE observed for ^{241}Am seems to be related to dosimetric considerations associated with the nonuniform distribution of the americium in the cell cultures.

8.3.2. Influence of organ distribution. It is important to understand how differences in organ distribution, especially for α-emitting radionuclides, affect the frequency of chromosomal aberrations observed in the blood lymphocytes. Many accidental exposures involve radionuclides that are taken up and retained in a variety of different organs. By controlling exposure condition and the chemical form of the radionuclide, the relationship between body burden, organ dis-

tribution, exposure time, calculated dose to the blood lymphocytes, and aberration frequency in the blood lymphocytes has been evaluated.

Nonhuman primates were exposed by inhalation to either insoluble ^{239}Pu oxide particles (LaBauve et al., 1980) or a more soluble form of ^{239}Pu as a nitrate (Brooks et al., 1983) and the level of chromosome damage in the blood lymphocytes was evaluated. The ^{239}Pu oxide was retained in the lung and the lung-associated lymph nodes with very little activity translocated to other body organs. This distribution pattern resulted in a slight increase in the frequency of chromosomal aberrations as a function of total dose to the lung. The magnitude of the increase was small relative to the large body burdens that were utilized and the somatic effects observed such as lung disease and cancer.

A similar change in aberration frequency was seen following exposure to ^{239}Pu nitrate which was distributed in the lungs, liver, and bone. In both of these studies the radiation dose was protracted over several years. These data suggest that even with a large plutonium body burden of soluble isotope exposing the cells for a long period of time, with a rather wide distribution of the isotope throughout the body, there was only a small increase in the aberration frequency in the blood lymphocytes (Brooks et al., 1983). These studies demonstrate that in nonhuman primates the aberration frequency in blood lymphocytes had little relationship to dose distribution on an organ level.

Other studies have been conducted on the induction of chromosomal aberrations in blood lymphocytes of Chinese hamsters following the deposition of α-emitting radioactive materials in the lung (Brooks et al., 1974) and in the liver (Heinze and Steinstrasser, 1986). In both of these studies the aberration frequency increased as a function of the calculated radiation dose either to the lung or to the lymph nodes. The response in both of these studies was 10–20 times lower than those values reported for induction of aberrations following exposure of lymphocytes in vitro to acute low-LET radiation (Preston et al., 1972).

8.3.3. Influence of LET and cell proliferation. Studies have been conducted using aberrations induced in liver cells as a model system to determine the influence of LET on the induction of chromosomal aberrations following internally deposited radioactive materials. The nuclides selected for study are deposited and retained in the liver for rather long time periods. By using the liver as a model, many variables can be controlled. The distribution of the isotope throughout the cell population can be rather well defined, and the total radiation dose and dose rate to the liver cells can be calculated; the life span of the cells is long relative to the radiation exposure time. Liver cells can be stimulated to divide by partial hepatectomy. Thus, the liver cells, which remain in an undividing stage during the low dose rate exposure, can be stimulated to divide by partial hepatectomy and the cumulative chromosome damage measured. This exposure can be to internal emitters or to low or high dose rate external radiation. Fig. 8.2 presents the relationship between the chromosomal aberration frequency in liver cells exposed to a variety of radiation types including internal emitters (Brooks, 1975). In these studies it was determined that there was a linear increase in the frequency of chromosomal aberrations as a function of radiation dose to the liver cells for both protracted γ, β, and α exposure with little influence of dose rate on the response. The slopes of the dose–response curves were compared, and the relative effectiveness for the production of aberrations for low- vs. high-LET exposure was between 15 and 20. This is similar to

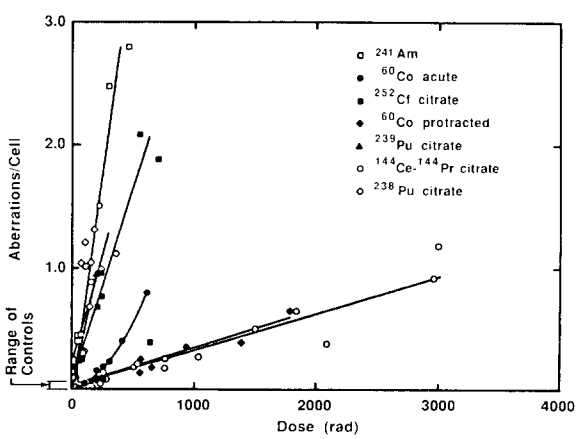

Fig. 8.2. Influence of LET on the production of chromosomal aberrations in the liver of the Chinese hamster.

the values observed when neutron (Lloyd et al., 1976) and protracted γ-ray exposures (Brewen and Luippold, 1971) are compared for human lymphocytes.

The liver cells were also evaluated for chromosomal aberrations to determine the influence of nonuniform localized distribution of radionuclides on the production of chromosomal aberrations. The aberration frequency was measured after injection with a monodisperse solution of plutonium citrate, which distributed rather uniformly in the liver, and was compared to the frequency and distribution of aberrations after injection with plutonium oxide particles of different sizes and specific activity (Brooks et al., 1976) or with Thorotrast (Brooks et al., 1986).

It was noted that the aberration frequency was similar for cells exposed to either ^{239}Pu citrate or ^{239}Pu oxide particles up to 0.84 μm real diameter. The aberration frequency decreased with increasing particle size for ^{239}Pu oxide because of very high local doses to cells around the particles which resulted in cell killing and wasted radiation. The aberration frequency for very nonuniformly distributed Thorotrast was similar to that produced by plutonium citrate if the proper corrections are made to estimate the dose to the liver parenchymal cells.

The influence of cell proliferation on the accumulation of damage after protracted exposure to deposition of radioactive materials has been studied in the liver, a slowly dividing cell system, and in the bone marrow, an example of rapidly proliferating cells (Brooks, 1980). In the bone marrow, it was observed that the frequency of aberrations increased as a function of radiation dose rate, as shown in Fig. 8.3 (Brooks, 1980). In rapidly dividing cells, the response seems to be related to the amount of dose to which the cells are exposed over a few cell cycles, with cell division selecting against badly damaged cells. In slowly proliferating cells the aberration frequency increases as a function of total radiation dose. Following exposure to internal emitters or other protracted radiation, then, the cells must not be dividing rapidly for the aberration frequency to be related to total radiation dose.

In summary, from the existing human data for occupational exposures to internally deposited radioactive material, there is a very small increase in the frequency of chromosomal aberrations in the blood lymphocytes. With this small response and the individual variability in the response observed, the sensitivity of aberrations in blood lymphocytes to detect deposition of radioactive material is very low and of little practical use. In the accidental exposure cases where very large doses were received or in medical evaluations where large doses were delivered (i.e., Thorotrast), the aberration frequency is markedly increased. The frequency of chromosomal aberrations may be related to the radiation dose that the blood lymphocytes have received. However, this increase in aberration frequency cannot be directly related to the body burden, time of exposure, or organ dose.

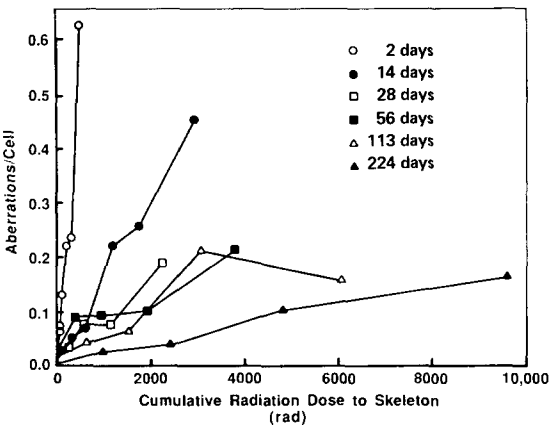

Fig. 8.3. Chromosomal aberration frequency in Chinese hamster bone marrow as a function of time after injection with ^{90}Sr/Y.

The relationship between the radiation dose and aberration frequency is dependent on the LET of the emission from the radionuclide. This has been well defined and is similar to that observed for protracted external radiation exposure. However, aberration frequency can be influenced by the retention pattern of the radionuclide, the organ and cellular distribution of the radionuclide, and the survival time of the damaged blood lymphocytes. These factors can often result in low doses to the blood lymphocytes relative to the dose to many of the other organs in the body where the material is retained preferentially because of its physical and chemical nature. Because of these complications, aberration frequency in

blood lymphocytes does not seem to be an accurate reflection of total body burden, of damage produced in other organs by the radioactive materials, or of the total risk for the development of late occurring disease.

9. Dose–response constants and their use in estimating exposure levels

The coefficients, α and β, for the general quadratic expression $Y = c + \alpha D + \beta D^2$ discussed in Section 7.3 have been determined for in vitro exposures by a number of laboratories around the world for both acute and chronic doses of various radiations of both low and high LET. Generally, whole blood samples or lymphocytes suspended in culture medium are irradiated in vitro, often at 37 °C so as to mimic conditions in vivo as closely as possible, prior to culture in precisely the same manner as is used for samples from persons whose radiation-induced aberration frequency is to be determined. Clearly, such dosimeter calibrations are best determined by the laboratory that will actually use them to make cytogenetic dose estimates. In this way interlaboratory differences in techniques, scoring criteria, etc., are eliminated. Nevertheless, there is a surprising degree of uniformity among the coefficients published by different laboratories, particularly during the last 15 years.

9.1. Coefficients for low-LET radiations

The first coefficients published were for 250-kV X-rays (Bender and Gooch, 1962). Such early calibrations were carried out with a limited range of doses and limited numbers of cells sampled, thus limiting accuracy. Furthermore, some authors fitted simple dose square relations to the 2-break ring and dicentric data, making no attempt to evaluate the linear αD contribution (Bender and Gooch, 1962, 1966; Bender and Barcinski, 1969). Nevertheless, it is surprising how well some of these early calibrations agree with those more recently published.

Table 9.1 gives a selected sample of coefficients of dicentric production published by groups at

TABLE 9.1

COEFFICIENTS OF DICENTRIC PRODUCTION (SELECTED EXAMPLES) ($Y = \alpha D + \beta D^2$) FOR ACUTE DOSES OF X-RAYS AND γ-RAYS

Authors	Date	Source	Dose range (rad)	$\alpha \pm$S.E. (10^{-4})	$\beta \pm$S.E. (10^{-6})
X-Rays					
Brewen and Luippold	1971	250 kV	50–400	9.1 ± 2.0	6.0 ± 0.7 [a]
Schmid et al.	1972	220 kVp	25–400	7.8 ± 1.3	4.2 ± 0.3
Lloyd et al.	1975	250 kVp	5–800	4.8 ± 0.5	6.2 ± 0.3
Schmid et al.	1976	220 kVp	25–400	7.9 ± 0.4	5.4 ± 0.2
Leonard et al.	1977	250 kVp	50–400	5.2 ± 3.0	7.2 ± 1.1
Muramatsu and Maruyama	1877	200 kVp	48–384	3.7 [b]	8.0 [b]
Barjaktarovic and Savage	1980	250 kVp	100–500	3.8 ± 2.9	7.2 ± 0.9
X-Rays					
Brewen et al.	1972	^{60}Co	50–400	3.9 ± 1.0	8.2 ± 0.4
Lloyd et al.	1975	^{60}Co	25–800	1.6 ± 0.3	5.0 ± 0.2
Bauchinger et al.	1979	^{60}Co	25–400	2.7 ± 0.7	4.8 ± 0.3
Littlefield	1986	^{60}Co	25–400	1.6 ± 0.7	5.7 ± 0.3
Means (unweighted): X-rays				$Y = (6.0 \pm 2.2) \times 10^{-4} D + (6.3 \pm 1.3) \times 10^{-6} D^2$	
γ-rays				$Y = (2.5 \pm 1.1) \times 10^{-4} D + (5.9 \pm 1.6) \times 10^{-6} D^2$	
Both				$Y = (4.7 \pm 2.6) \times 10^{-4} D + (6.2 \pm 1.3) \times 10^{-6} D^2$	

[a] Brewen's coefficients include rings as well as dicentrics.
[b] Information insufficient to allow calculation of S.E.

various laboratories (see Lloyd and Edwards, 1983, for a more complete review and reanalysis of published data). They agree rather well. The unweighted means for X-rays are $\alpha = (6.0 \pm 2.2) \times 10^{-4}$ and $\beta = (6.3 \pm 1.3) \times 10^{-6}$. For the representative γ-ray determinations, $\alpha = (2.5 \pm 1.1) \times 10^{-4}$ and $\beta = (5.9 \pm 1.6) \times 10^{-6}$. Though it appears that the value of α tends to be lower for the γ-ray determination, as their lower average LET suggests they should be, the difference is not statistically significant. Therefore, it seems reasonable to adopt the average values of $\alpha = (4.7 \pm 2.6) \times 10^{-4}$ and $\beta = (6.2 \pm 1.3) \times 10^{-6}$ as reasonable overall estimates for acute doses of low-LET X- or γ-rays.

As discussed in Ch. 7, it is expected that as dose rate decreases the βD^2 term for low-LET radiation will gradually disappear, so that at or below sufficiently low dose rates only the linear αD term will be left. This has been at least partially demonstrated with human lymphocytes irradiated in vitro, but technical difficulties with protracted exposures of lymphocytes have precluded reaching the point where no dose square component is left (Scott et al., 1970; Brewen and Luippold, 1971; Purrott and Reeder, 1976; Bauchinger, 1984). In a large collaborative study of aberration frequencies in lymphocytes given very low doses of X-rays. Pohl-Ruling et al. (1983) attempted to actually measure the α coefficient at doses low enough so that the contribution of the β term is negligible. Depending on the statistical methods of analyses used, they found α coefficients ranging from zero to $1.7 \pm 1.2 \times 10^{-4}$. For extremely protracted exposures to γ-rays, then, the best estimate is probably simply the $(2.5 \pm 1.1) \times 10^{-4}$ D derived from the acute γ-ray experiments.

9.2. *Coefficients for high-LET radiations*

For exposures of veterans during nuclear weapons testing, only 2 kinds of exposures to high-LET radiations appear to be of interest. One, possible radiation exposure from internally deposited α-emitting radionuclides, has already been discussed in Ch. 8; the other is possible exposure to fast neutrons having a fission spectrum of energies that could have resulted from nuclear weapons detonations. Again, a number of laboratories have carried out in vitro determinations of calibration curves for fission spectrum neutrons. All agree that for neutrons of this energy range the dose–effect relation is linear, as would be expected from their average LET. Table 9.2 presents a summary of the published αD coefficients for dicentric production. These agree rather well, yielding an unweighted mean of $(77.7 \pm 13.4) \times 10^{-4}$. One study (Bauchinger et al., 1984) not unexpectedly found that the shape of the dicentric dose–effect curve included a significant dose-squared βD^2 component when lymphocytes were irradiated with fission spectrum neutrons of average energy 1.6 MeV with a substantial admixture of γ-rays.

TABLE 9.2

COEFFICIENTS OF DICENTRIC PRODUCTION ($Y = \alpha D$) FOR FISSION NEUTRONS (SELECTED EXAMPLES)

Authors	Date	E (MeV)	Dose range	$\alpha \pm$S.E. (10^{-4})
Bender and Gooch	1966	~ 0.5	25–200	56.0 ± 6.0
Biola et al.	1974	Fission	68–317	90.1 ± 0.3
Biola et al.	1974	Fission	100–300	87.4 ± 0.4
Biola et al.	1974	Fission	22–142	64.8 ± 0.3
Lloyd et al.	1976	0.7	50–300	83.5 ± 1.0
Lloyd et al.	1976	0.9	6–265	72.8 ± 2.4
Vulpis et al.	1978	0.4	25– 50	89.6 ± 6.9
Mean (unweighted): $Y = (77.7 \pm 13.4) \times 10^{-4} D$				

9.3. *Sources of variability*

Lymphocytes (and cells generally) from individuals homozygous for the very rare recessive disorder, ataxia telangiectasia, are well known to be chromosomally radiosensitive (Higurachi and Conen, 1973; Taylor et al., 1976; Taylor, 1978; Natarajan and Meyers, 1979; Bender et al., 1985a, b), as also may be lymphocytes from homozygotes for several other rare human genetic diseases (Higurachi and Conen, 1971, 1973; Bigelow et al., 1979). These are very rare conditions, however, and affected individuals are recognized in childhood and thus would not have been included among the atomic veterans. Though the frequency of heterozygotes, who are not affected, is much higher than that of affected individuals, the heterozygotes do not appear to display any

increased chromosomal radiosensitivity (Bender et al., 1985a, b), at least of their lymphocytes. Because markedly sensitive individuals occur at all, it is natural to ask whether there may not be a distribution of lymphocyte chromosomal radiosensitivities among members of the general population. Although the time-consuming nature of the aberration scoring required has precluded the testing of large populations, the calibration experiments yielding the coefficients of aberration production (for example, those listed in Tables 9.1 and 9.2) have involved a fairly large number of individual subjects, but none has yet been found to be unequivocally radiosensitive (or radioresistant). Neither have the possibly confounding influences of demographic factors, such as age and race, been shown to affect radiation-induced aberration yields.

It is possible that individual lymphocytes from one person may differ somewhat in their chromosomal radiosensitivity, and indeed the overdispersion sometimes seen when chromosomal aberration yields from irradiated cells are tested for their goodness of fit to the Poisson distribution may in some cases reflect such a phenomenon. However, to the extent that such a distribution of sensitivities might characterize all human lymphocyte and lymphocyte precursor populations, it is "taken care of" in the coefficients of aberration production, since these experiments must have had the same chance of sampling sensitive cells as would samples from possibly irradiated persons.

There is some evidence for differential radiosensitivity between lymphocytes of the T and the B types. Several authors have presented evidence suggesting the existence of 2 subpopulations of lymphocytes with differing radiosensitivity (Bender and Brewen, 1969; Steffen and Michalowski, 1973; Beek and Obe, 1976). As described in Ch. 4, stimulation with phytohemagglutinin results in more activation of T lymphocytes than of B lymphocytes, with the T lymphocytes beginning to come into mitosis earlier in the culture life than the B lymphocytes. Both Santos Mello et al. (1974) and Schwartz and Gaulden (1980) also showed that when lymphocytes were irradiated, the B cells were eliminated from the responding population more rapidly than the T cells. Furthermore, Santos Mello et al. (1974) found evidence that both T and B cell populations are actually composed of 2 subpopulations, one of each pair surviving less well than the other. Schwartz and Gaulden (1980), however, found no evidence of different radiation-induced chromosomal aberration frequencies in T and B cells.

On the other hand, Scott and Lyons (1979), using the 5-bromodeoxyuridine incorporation-differential staining method to limit their scoring to unequivocal first postirradiation divisions, found no evidence of any difference in aberration yields in first division cells sampled over a fairly wide range of sampling times and therefore concluded that human lymphocytes are homogeneous in their chromosomal radiosensitivity. Nevertheless, since Bender and Brewen (1969) also limited their scoring to unequivocal first division cells (by using the more tedious tritiated thymidine incorporation and autoradiography technique), it appears that the question remains unresolved.

9.4. In vivo–in vitro comparisons

The whole concept of quantitative biological dosimetry using peripheral lymphocyte chromosomal aberration frequencies depends, of course, upon the assumption that the chromosomal aberration yields induced in vitro are the same as (or at least bear some constant relationship to) those induced in vivo. This question has been addressed in several ways. Before the peripheral lymphocyte culture system was available, tests were done with bone marrow irradiated in vivo and tissue cultures irradiated in vitro (Bender, 1957, 1960), which showed no significant difference. Since the lymphocyte culture technique became available, several groups have irradiated various species of experimental animals and also peripheral blood samples taken from them before their irradiation and compared aberration yields in short-term peripheral lymphocyte cultures (Clemenger and Scott, 1971; Brewen and Gengozian, 1971; McFee et al., 1972; Preston et al., 1972; Bajerska and Liniecki, 1975). No evidence for any difference between in vitro and in vivo radiation was found.

A number of human accident or therapeutic whole-body exposures for which there was reasonably accurate physical dosimetric information have also been investigated to test this question.

Sasaki et al. (1963), Norman et al. (1964), Buckton et al. (1967b), and Langlands et al. (1968) found no difference between the yields of aberrations in lymphocyte samples obtained promptly from cancer patients undergoing whole-body irradiation and those predicted on the basis of in vitro calibration curves. Sharpe et al. (1968) compared in vitro exposures with extracorporeal irradiation of the blood of a Hodgkin's patient and found similar dicentric yields. Such tests, however, suffer from the uncertainty as to whether the response of these patients in vivo would be the same as that of normal healthy people.

A number of groups have measured lymphocyte aberration frequencies in lymphocytes obtained from accidentally irradiated persons within a matter of days following their exposure. Among the early cases are Bender's (1964) investigation of 3 persons accidentally irradiated with γ-rays; Bender and Gooch's (1966) investigation of 3 men irradiated in the Recuplex critically accident at Hanford, Washington; LeGo's (1967) investigation of a man irradiated in the criticality accident at Mol; LeJeune et al.'s (1967) investigation of 4 accidental irradiations of several different kinds; and Schneider et al.'s (1969) and Brown and McNeill's (1971) investigations of 2 cases of accidental exposure to iridium-192 γ-rays. Though the total doses were low in each of these cases, leading to some variability, in no case did the biological dose estimates based on chromosomal aberration frequencies disagree seriously with the also somewhat uncertain physical dose estimates.

The cytogenetics group of the National Radiological Protection Board (NRPB) in the United Kingdom has routinely performed cytogenetic dosimetry on cases of actual or suspected accidental irradiation for many years (the results are summarized in a series of NRPB reports authored by Lloyd and co-workers and obtainable directly from NRPB or from Her Majesty's Stationary Office; see for example Lloyd et al., 1986). Again, in cases where physical dose estimates are available, their results are in good agreement with predictions based on in vitro calibration curves.

Perhaps the best demonstration of the ability of in vitro calibration curves to accurately estimate whole-body dose, however, is that of the study by Brewen et al. (1972) of aberration frequencies in peripheral blood samples from a man accidentally exposed to a homogeneous whole-body dose of cobalt-60 γ-rays estimated to have been 127 R at the midline. The biological dose estimate from prompt blood samples was 140 R $\pm$ 20 R, excellent agreement in a case where the dose was substantial and possible confounding sources of variability minimized.

9.5. Deducing exposure from delayed samples

As noted earlier, chromosomal aberrations may persist in peripheral lymphocytes and their precursors over long periods of time. Acentric fragments and asymmetrical exchange aberrations, often termed "unstable aberrations", tend to be lost at cell division. Symmetrical exchange aberrations, sometimes referred to as "stable aberrations", on the other hand, do not appear to be lost or selected against as cell populations proliferate. It would appear, then, that stable aberration frequency measurements would be the ideal means of assessing radiation exposures occurring a long time prior to sampling. Unfortunately, symmetrical exchanges are ascertained inefficiently (it is estimated that without banding only about one quarter of those induced are detected; Buckton et al., 1978), and because their detection is quite subjective, detection efficiency of different scorers varies widely. The detection of asymmetrical exchange aberrations, on the other hand, is much less subjective,t ending to make up for the problem of the loss of such aberrations as a function of increasing irradiation-sampling interval.

A number of authors have determined the rate of elimination of aberrations from lymphocyte samples from irradiated persons (Norman et al., 1965, 1966; Buckton et al., 1967d). The decrease appears exponential, with half-lives variously estimated between 530 and 1600 days (see Ch. 4). In addition to many early reports dealing with small populations, there have been 2 reports of cytogenetics studies of extensive populations of occupationally exposed persons. Evans et al. (1979) reported an extensive study of aberration frequencies in 197 nuclear dockyard workers who were followed over a 10-year period. Lloyd et al. (1980) studied aberration frequencies in a population of 146 radiation workers from U.K. nuclear establishments. Both studies found significant popula-

tion increases in the frequencies of rings and dicentrics and acentric fragments, though increases in individuals were not large enough to be statistically significant. In the dockyard study, aberration frequency was correlated with total cumulative radiation exposure; there was, however, much greater impact on aberration frequencies due to "recent" exposures. This was corrected for in somewhat different ways in the two reports; that of Evans et al. (1979) fitted different coefficients for the early and late components of total dose, while that of Lloyd et al. (1980) used a 3-year estimated half-life to weight individual increments of dose. Interestingly, the two groups obtained virtually identical coefficients for dicentric production by chronic occupational exposure; that of Evans et al. (1979) was $(2.32 \pm 1.01) \times 10^{-4}$/rad while that of Lloyd et al. (1980) was $(2.22 \pm 0.94) \times 10^{-4}$/rad. These values compare favorably with the mean α coefficient of $(2.5 \pm 1.1) \times 10^{-4}$ derived from the experiments listed in Table 9.1.

The accidental whole-body γ-irradiation case studied promptly by Brewen et al. (1972) has been resampled a number of times over the intervening years. The results, published (Preston et al., 1974) and unpublished (Littlefield, 1986), offer an interesting opportunity to derive lymphocyte aberration frequency decay parameters for a case uncomplicated by low and/or uncertain dose, dose inhomogeneity, or lack of prompt aberration frequency data. Calculations using these data are discussed in Appendix A.

The exposed populations irradiated during the bombings of Hiroshima and Nagasaki constitute by far the largest irradiated human population available for cytogenetic study. Unfortunately, the techniques which made it practical to study the survivors for chromosomal aberration frequencies did not become available until over 15 years after the exposures. Nevertheless, a great deal of data has been collected since (Awa, 1983; Awa et al., 1984). It was established early that not only did the survivors still show elevated aberration frequencies, but that the aberration frequencies were inversely related to distance from the hypocenter, and consequently, directly related to estimated dose (Sasaki and Miyata, 1968). More recently, attempts have been made to "back-extrapolate" from aberration frequencies observed in the survivors' lymphocytes to either their original aberration frequencies or the doses that produced them (Randolph and Brewen, 1980). In vitro derived coefficients of aberration production, together with ascertainment efficiencies for symmetrical exchanges and loss coefficients for asymmetrical aberrations were used to calculate deduced exposure from chromosomal aberration frequencies published by Awa et al. (1978). Later, Bender and Wong (1982) used the same methodology to calculate from 2 different sets of physical dose estimates for survivors at Hiroshima and Nagasaki what the chromosomal aberration frequencies would have been had the subjects been sampled promptly after the bombing (in a sense, doing the reverse of what had been done by Randolph and Brewen [1980]). Because the survivors' physical dose estimates have undergone recent revision (Loewe and Mendelsohn, 1981), Bender and Wong's calculations were made to see if the preliminary new dose estimates would help to eliminate the large difference in aberration yields between the two cities — which they did.

Two studies have been published of lymphocyte chromosomal aberration frequencies in small human populations accidentally exposed to fallout radiation from anuclear detonation at Bikini Atoll in 1954. One reports a study of 43 exposed Rongelap Islanders (Lisco and Conard, 1967) while the other is a study of 14 Japanese exposed aboard the fishing boat Fukuryu-maru (Ishihara and Kumatori, 1965; Kumatori, 1971). Internal dose estimates were high, ranging from 70 to 150 rad for the Rongelap Islanders and from 170 to 690 R for the Japanese fishermen. Nevertheless, in both cases the lymphocyte samples, obtained about 10 years after the exposures, showed only small elevations in chromosomal aberration frequencies, and only in some subjects, although there were statistically significant elevations when the exposed were compared as a group with suitable controls.

10. Estimation of doses from observed chromosomal aberrations

From what has been said in the previous chapters, it will be clear that it is not only possible, but common practice to estimate doses from

chromosomal aberration frequencies observed in promptly sampled peripheral lymphocytes of persons exposed to moderate-to-high doses of radiation. Also, it is clear that radiation exposure can be detected in *populations* when doses are very low (e.g., in radiation workers) or, when doses are higher, when lymphocytes are sampled many years after exposure (e.g., in atomic bomb survivors). The precision with which low doses can be detected in *individuals,* or even higher ones in individuals sampled long after their exposure, is less clear. Unfortunately, this is precisely the problem presented by the exposed veteran populations: their exposures occurred long ago and, from the physical evidence available (film badges, dose reconstructions, etc.), seem likely in most cases to fall in the low-dose category ($\leqslant 10$ rad). Thus the pertinent question is, with what precision can small exposures be detected in individuals whose lymphocytes are sampled decades afterward? Put another way, we may ask what confidence we should have in concluding that an individual was indeed exposed several decades earlier if we observe some particular number of aberrations in a sample of a certain number of cells.

10.1. Conventional analysis

To illustrate the problem, let us consider the observation of particular numbers of dicentrics in samples of 500 metaphases from lymphocyte samples obtained promptly after a possible radiation exposure. The background frequency of dicentrics in lymphocytes from unexposed persons is known; for illustration we will take the observation of 154 dicentrics in 90400 cells from a current study of about 500 people (Bender et al., 1986), which yields a mean relative frequency of 0.0017. Thus we would expect an average of 0.85 dicentrics in samples of 500 metaphases or roughly 1 in 500 metaphases would show a dicentric aberration. We cannot observe 0.85 dicentrics in any sample, of course, but only 0, 1, 2, 3, etc., and we may thus expect to see some samples with more than the mean number of dicentrics in any group of samples, even if it is from an unexposed population. We also have estimates of the yield of dicentrics per rad in human lymphocytes.

Taking the mean value of the linear coefficient for γ-rays of 2.5×10^{-4} D from Table 9.1, we might calculate that observing 2 dicentrics in a particular 500-metaphase sample, a frequency of 0.004, indicates an exposure estimate (ignoring the negligible contribution of the D^2 term) of 9.2 rad, but we clearly can have only little confidence in such a calculation, for the uncertainty of the estimate does not exclude the possibility that the dose was 0 rad, and indeed we know that samples in which 2 dicentrics are observed in a sample of 500 metaphases from unirradiated people do occur. Worse, the lapse of decades between the exposure and sampling will lead to reduction in the induced dicentric frequency, so that instead of expecting a mean of 2/500-metaphase sample following a 9.2-rad exposure, we could only expect a mean of perhaps 0.855 in 500-metaphase samples (0.850 spontaneous ones plus ~ 0.005 induced ones). This decrease of the relative frequency of dicentrics with time implies an increase of the uncertainty of the dose estimates.

10.2. Bayesian analysis

The only approach which permits a complete and quantitative description of uncertainty is the Bayesian approach. This approach to statistics uses probability as the measure of uncertainty (Lindley, 1978, 1984). Probability as used in this chapter is defined and comprehensively described by DeFinetti (1979). The appellation "Bayesian" comes from Thomas Bayes, who published a foundational paper in 1763 (later reprinted with a biographical note; Bayes, 1958). In this paper, Bayes describes for the first time a theorem which can be used to update the probability of uncertain events after new information about the events has become available.

The committee has used the Bayesian approach to dose estimation simply because it is the only approach which completely answers the questions with which we are faced. The committee was asked to study the feasibility of estimating the radiation dose which an individual received prior to the observation of the chromosomal aberrations in a sample of blood and to describe the remaining uncertainty about this dose given all the available information. The Bayesian approach is the only approach which admits the use of all available information and which describes quantitatively the remaining radiation dose with the help

of probability. More specifically, we can state the probability that the unknown "true" dose will fall in a certain interval by using probability densities for the radiation dose.

The following example, which uses a probability density derived in the appendix will clarify this. Fig. 10.1 shows a probability density (PD) for the radiation dose measured in rad. The abscissa of the plot gives the dose interval 0–500 rad which contains the true dose. The ordinates, labeled "predictive density", start at values close to zero, reach a maximum of 1.0 around 255 rad and decline rapidly to nearly zero for the remainder of the interval. The area under a PD like the one shown in Fig. 10.1 may be normalized to be equal to unity, that is, 1. This simply means that with probability 1, that is, certainly, the true dose will lie somewhere in the dose interval on the abscissa. The probability that the true dose is contained in a certain interval, say 250–300 rad, is given by the area bounded by the PD, the dose interval on the abscissa from 250 to 300 and 2 vertical lines (not shown) from the end points of the dose interval (250–300) to their points of intersection with the PD. The numerical values for this area and any other area corresponding to other finite dose intervals on the abscissa are always *less* than one. These numbers describe, on a probability scale, our confidence that the "true" dose lies in the specified interval.

It needs to be emphasized that the numerical values of the ordinate (e.g., 1.0 for about 255 rad in Fig. 10.1) have no direct interpretation and that it is *erroneous to conclude,* for example, that 255 rad as an estimate of the true dose has probability 1.0. Probability is always represented by the area under the graph and not by the ordinate of the graph corresponding to a certain value on the abscissa.

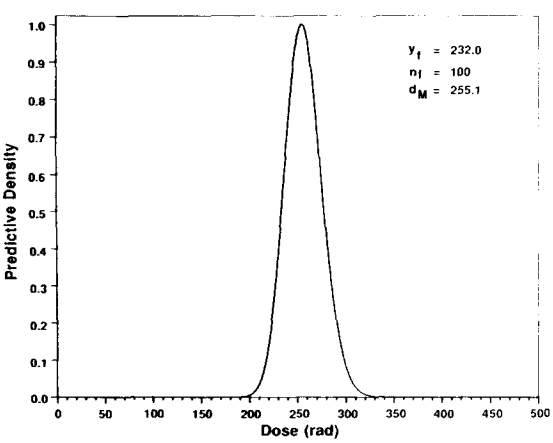

Fig. 10.1. Probability density for radiation dose (rad).

Another measure of uncertainty with a *totally different interpretation* is the "confidence interval". Without describing the underlying theory which leads to this concept, we refer the reader to a paper by Neyman (1977) for a detailed and authoritative discussion.

In this report we will not use confidence intervals in Neyman's sense. Instead uncertainty about a parameter will be expressed by probability. For the deeper reasons why probability and its calculus should be used for estimation, description of uncertainty, and as a basis for decision making under uncertainty we refer the interested reader again to the classical literature on this subject (DeFinetti, 1979; Raiffa and Schlaifer, 1968).

The problem facing us is to derive, by reference to calibration data, from chromosomal aberration yields observed 30 or more years later, not only dose estimates, but estimates of the confidence we may have in them. In addition to statistical uncertainties about both the observed chromosomal aberration yields and the calibration curves, we must also take into account the uncertainty of our knowledge of the disappearance rate of aberrations with time. Details of 3 worked examples are given in the Appendix. The first treats the entire problem of data recorded as ranges of aberration yield and the estimation of dose ranges. The other 2 tackle separately the problems of dose estimation from calibration data and the estimation of the correction for aberration disappearance with time.

The first example (in Section A.1) shows how one may estimate a dose range from the aberration yield range, given just general, though experienced, judgment regarding dose–effect relationships and disappearance rates for aberrations and a set of data on aberration frequencies from observations on Japanese atomic bomb survivors made 30 years later. The intent of Section A.1 is to illustrate how the Bayesian approach can be used to combine these two pieces of information

to provide more accurate dose range estimates. This is done by codifying the first piece of information, the cytogenetic judgment, as a prior distribution and modifying it by the second. The analysis shows that it is not possible to determine unequivocally the dose, even in broad ranges, for any specific subject.

In Section A.2 is illustrated in greater detail how one may use the Bayesian approach to estimate dose when an observed aberration yield is compared to calibration data without the added complication of any correction for time between irradiation and sampling. For simplicity the example uses a neutron calibration curve which is linear. The same methods are, however, applicable to the more complicated case of curvilinear ones such as those for acute doses of low-LET radiation. The probability distribution of dose here depends only on the probability distribution for the slope of the curve and on the uncertainty associated with the measured aberration yield. Section A.2 shows how these may be combined and illustrates, in principle, how additional uncertainties (like that surrounding the spontaneous aberration frequency) can be handled.

Our last example (Section A.3) considers the problem of the disappearance of aberrations with time after radiation exposure. Here the decay constant has a distribution, and this is combined with the uncertainties surrounding the measured aberration yield to provide, using the Bayesian approach, the posterior distribution of dose.

11. Genomic end points other than chromosomal aberrations that may reflect previous human exposure to ionizing radiations

Only a small proportion of genomic damage (essentially DNA damage) induced following exposure to ionizing radiations, and which is not of itself lethal, is reflected in permanent heritable alterations in the genomes of affected cells and their descendants. Most such damage is repaired shortly after exposure. Thus, although there are approaches which may be used to detect DNA damage at very short times (hours, days) after exposure, such as measurement of the incidence of DNA strand breakages, or the incorporation of new bases in the repair of DNA, or the use of specific antibodies to detect specific base alterations in DNA, these approaches are not applicable in terms of revealing a history of much earlier exposure. To detect effects of exposures that had occurred many months, or years, prior to tissue sampling, it is clearly necessary to utilize methods that detect more or less permanent genomic damage. This essentially implies permanent changes in DNA composition or structure which may or may not be expressed phenotypically as mutational changes.

11.1. DNA alterations detected as expressed mutation changes

Assays for determining mutation frequencies at specific loci in human somatic cells exposed to mutagens in vivo are very limited in number and are at an early stage in development. Two types of assays are available which utilize human peripheral blood cells. One of these is based on the detection of mutations in lymphocytes which render them resistant to the killing effects of 6-thioguanine in culture (Albertini, 1985). The background frequency of such resistant mutant cells in peripheral blood is on the order of 1 in a million, and their frequency is increased in blood samples of patients receiving treatments with mutagenic chemicals. Their frequency is also increased in blood cells exposed to ionizing radiations in vitro, but in view of the very limited in vivo studies there is no information on the relationship between frequency of mutant cells, mutagen dose levels, and time of sampling after exposure. Thus, although mutations of the type detected by this assay can be induced by ionizing radiation, it does not at present provide a practical means for detecting whether or not an individual had been subjected to a previous radiation exposure, even if such an exposure had involved large doses of the order of those used in cancer radiotherapy.

An alternative approach involves the use of fluorescence-tagged monospecific antibodies to detect the presence of mutant proteins in red blood cells (rbc) (Klasen et al., 1982). Antibodies to various abnormal hemoglobins (e.g., sickle cell hemoglobin [HbS]) have been used to detect the presence of presumed mutant cells in blood sample from normal individuals. HbS-positive rbc have a frequency of around 1 in 10 million in normal

individuals, and this frequency is increased in cancer patients receiving chemotherapy (Stamatoyannopoulos et al., 1980).

Mutant cells can be detected by flow cytometry which enables the analysis of some million cells per second, but there are 2 problems. The first is that of false positives detected by machine (Bigbee et al., 1981), and the second, the fact that the hemoglobin mutants detected by these antibodies involve single base substitutions or frameshift mutations. Although such mutations are probably induced by ionizing radiations, they may be very infrequent events relative to those radiation-induced mutations that involve the loss of a gene or its functional inactivation. This approach may therefore be more particularly relevant for the detection of exposure to chemical mutagens, and certainly at the present time, it is not applicable for the detection of previous exposure to radiation.

Another approach which will detect loss mutations in rbc is currently under development and involves the use of fluorescence-labeled monoclonal antibodies to human glycophorin A, the rbc protein responsible for the M and N blood serotypes (Jensen et al., 1984). The loss of either the M or the N allele can be detected in rbc of MN heterozygote individuals, and the frequency of variants ("mutants") is of the order of 8 per million rbc in normal individuals and is significantly increased in cancer patients undergoing cancer chemotherapy (Mendelsohn, 1985). Variant cells are identified and counted by flow cytometry, and the feasibility of this approach is currently being studied in atomic bomb survivors. However, we should emphasize that although this approach is promising, it is still as yet some way removed from being a proven technique to detect previous radiation exposures.

11.2. DNA alterations detected as changes in base composition or structure

Certain alterations of bases in the DNA of human cells exposed to certain chemical mutagens can be detected by the use of fluorescence-tagged antibodies that are specific for these changes. These antibody techniques are extremely sensitive and can be used, for example, to detect very small numbers of guanine adducts produced as a result of exposure of cells to alkylating agents (Adamkiewicz et al., 1982). Ionizing radiations produce a wide range of changes in the DNAs of exposed cells, many of which are short-lived, and none of which has as yet been shown to yield useful antibodies that can be used to monitor, or detect, radiation damage in the DNA of exposed individuals.

A major problem in detecting and measuring ionizing radiation-induced DNA changes in human somatic cells follows from the random distribution of induced damage within and between cells, so that at a given exposure level some cells may have little or no damage, and the heterogeneous nature of the damage induced. Thus, those techniques that are so efficient in detecting alterations in base sequence of a specific DNA segment, and which would enable the detection of progeny differences in the offspring of exposed individuals, are not immediately applicable to detecting the relatively rare changes that may be induced in a specific DNA sequence in somatic cells. Comparison of unique sequence reference DNAs with homologous DNAs extracted from cells of irradiated individuals by hybridization properties, or direct sequencing, may be theoretically possible, but are not immediately practical for such heterogeneous DNA samples.

Detection of changes at sites of DNA cleavage by restriction endonucleases may also provide a workable approach (Lo et al., 1982), particularly using certain repetitive sequence DNAs in the human genome, such as the alphoid DNAs where each cell has some 300 000 copies of well-defined 342-base-pair-length DNA fragments. Thus, although the molecular techniques that can be used to reveal changes in DNA structure and composition provide a promising avenue that may ultimately yield powerful methods for measuring ionizing radiation-induced DNA damage, at the present time none of these approaches has been sufficiently pursued to provide a workable system to yield information on the radiation history of cells of exposed individuals.

12. Recommendations

If cytogenetic analysis of chromosomal aberration frequencies in peripheral blood lymphocytes

of exposed veterans are undertaken, primary attention should be given to those veterans with the highest estimated doses. Based upon the results obtained, it will be possible to determine whether there is any merit in extending sampling to other personnel with smaller probable exposures.

Further research and analysis should be undertaken to extend application of the Bayesian approach to retrospective dose estimation from chromosomal aberration frequencies in peripheral blood lymphocytes as illustrated by the examples given in the Appendix. Also, further research should be undertaken to develop the image analysis technology that would allow more fully automated chromosomal analysis devices than are presently available, thus facilitating cytogenetic study of large population samples.

13. Acknowledgments

The committee is indebted to Dr. John Storer for his review of this report, and wishes to express its thanks to Louise Wyatt for her secretarial assistance in preparing this document.

Appendix. Examples of application of the Bayesian approach to dose estimation

A.1. Discrete analysis of stable chromosomal aberrations in A-bomb survivors

An unpublished data set for this analysis was provided to the committee by A. Awa. For the purpose of this analysis the data were rearranged into a 4×4 table showing 4 dose and 4 CA categories (see Table A.1). In this data set, doses for individuals are based on T65D estimates (Auxier, 1977). The numbers in parentheses give the total number of A-bomb survivors whose maximum number of CA and T65 doses fall in the appropriate category. If rings and/or dicentrics were observed, their total number was subtracted from the maximum number of total aberrations, so that only stable aberrations were used in the analysis. The number outside the parentheses in each cell represents the expert judgment of 2 cytogeneticists on the committee before the data given in parentheses were revealed to them. They were asked to distribute 30 "points" or less over the 4 dose categories depending on their degree of confidence that an individual's dose would fall in the respective dose categories given a certain number of chromosomal aberrations, for example, 0–4 or 11–19. The points, which can be interpreted as equivalent chromosomal aberrations, were then converted into so-called prior distributions as will be explained below.

TABLE A.1

PRIOR INFORMATION AND NUMBER OF OBSERVED STABLE CHROMOSOMAL ABERRATIONS IN A-BOMB SURVIVORS (HIROSHIMA)

Stable aberrations/ 100 cells	T65 dose (rad)			
	0–97	100–191	193–340	344–884
0– 4	24 (27)	4 (4)	1 (1)	1 (1)
5–10	3 (6)	10 (19)	5 (4)	1 (2)
11–19	1 (0)	5 (10)	10 (15)	3 (9)
20–56	1 (0)	4 (0)	16 (12)	16 (20)

Bayesian estimation of any parameter starts always with the description of the uncertainty about the parameter with a prior distribution. A prior distribution is a PD with the possible values of the parameter indicated on the abscissa. "Distribution" and "density" in PD are synonymous. The attribute "prior" means "before certain data are incorporated into the analysis". A prior distribution is interpreted just like a PD. An example of a PD was shown in Fig. 10.1 and its interpretation was discussed in Ch. 10. As new information in the form of data (e.g., the numbers in parentheses in Table A.1) arrives, the prior probability distribution is modified and we obtain a new PD, the "posterior distribution" with the help of Bayes' theorem. The detailed procedure of how this is accomplished is thoroughly and clearly discussed in Schmitt (1969). If further data become available, the posterior distribution becomes the new prior and so on. As Lindley put it: "Today's posterior is tomorrow's prior" (Lindley, 1984).

The analysis of the prior information and data in Table A.1 used the following mathematical fact discussed by Basu and Pereira (1982) and illustrated here with an example from Table A.1: If the prior distribution for $p(1|1)$, the probability of belonging to dose category 1 (0–97 rad) given a number of chromosomal aberrations belonging to category 1 (0–4), is Be(24, 6), then the posterior

distribution for $p(1|1)$ is Be(51, 12). (Be(a, b) means a β distribution with parameters a, b; see Lindley [1970] for a definition of this distribution.) Inspection of Table A.1 shows that 24 is the number of points in bin (1, 1) and that 6 is the sum of the points in the other bins in the first row. Similarly, $51 = 24 + 27$ is the sum of points and observed number of chromosomal aberrations in bin (1, 1) and $12 = 4 + 4 + 1 + 1 + 1 + 1$ is the sum of points and observed chromosomal aberrations in the remaining bins in row 1. The fact that $p(1|1)$ and analogously $p(i|j)$ for bin (i, j) has a β distribution with the parameters related to points and numbers of CAs in the remaining bins as demonstrated above will not be explained here. The interest reader may consult Basu and Pereira (1982) for the mathematical details.

Figs. A.1–A.3 show the prior (squares) and the posterior distributions for the $p(i|j)$s in the first row of Table A.1. For instance, Fig. A.2 shows the prior and posterior PDs [Be(4, 26) and Be(8, 55)] for $p(2|1)$ or in words, the distributions for the probability that the radiation dose to which the individual was exposed about 30 years ago was between 100 and 191 rad *given* that between 0 and 4 CAs were observed. If the number of chromosomal aberrations in a blood sample from a new individual with *unknown* dose are observed to be in category j, the probability that the unknown dose falls in category i is given by the mean of the posterior PD for $p(i|j)$.

For example, if the number of observed stable chromosomal aberrations was between 0 and 4, then the probability that the unknown dose falls in dose category 2 (100–191 rad) is given by the mean (expectation) of Be(8, 55) shown in Fig. A.2. This follows from probability calculus and the definition of expectation (see e.g., Schmitt, 1969):

$$\begin{aligned} \mathrm{p}(2|\mathrm{data}) &= \int_0^1 \mathrm{d}p_2\, p(2|p_2)\mathrm{Be}(8, 55) \\ &= \int_0^1 \mathrm{d}p_2\, p_2 \mathrm{Be}(8, 55) \\ &= E(p_2|\mathrm{data}) \end{aligned}$$

where "data" stands for the number of CA observed. It is a mathematical fact that the expectation of Be(a, b) is given by $a/(a+b)$. Therefore, $p(2|\mathrm{data}) = 8/(8+55) = 0.13$.

The means of the other β densities shown in Figs. A.1–A.3 can be calculated in the same fashion. Inspection of these means shows clearly what can be said about the unknown radiation dose to which a person was exposed about 30 years ago. For example, if between 0 and 4 stable aberrations were observed in a sample of 100 cells, we can state the probabilities that the unknown radiation dose belongs to the 4 dose categories shown in Table A.1. They are for the 4 categories respectively: 0.81, 0.13, 0.03, and 0.03. This demonstrates clearly that we can never say with absolute

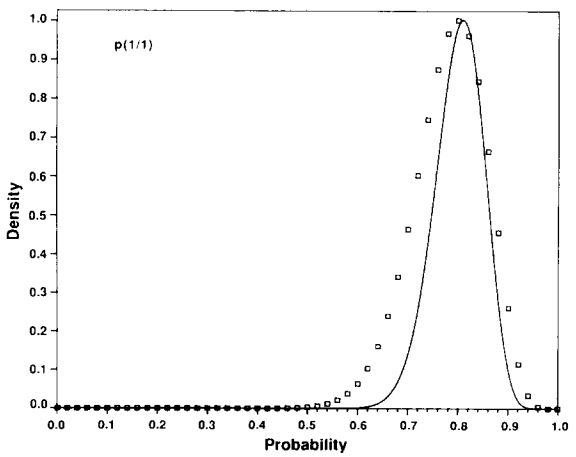

Fig. A.1. Prior (squares) and posterior density for $p(1|1)$.

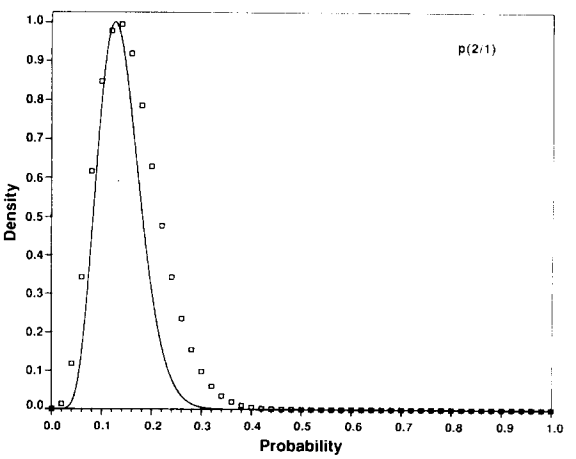

Fig. A.2. Prior (squares) and posterior density for $p(2|1)$.

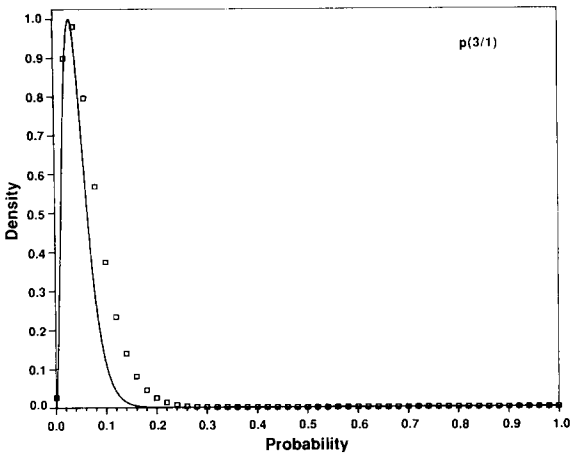

Fig. A.3. Prior (squares) and posterior density for $p(3\,|\,1)$ and $p(4\,|\,1)$.

certainty that an unknown dose falls into a certain dose category. In the example considered here it is 4 times as probable that the dose was less than about 100 rad, but a chance of about 20% remains that the dose was greater than 100 rad.

Using a portion of a published data set (Otake, 1979) we studied also the estimation of low doses by forming the dose and CA categories shown in Table A.2. Some of the results of this analysis are shown in Figs. A.4–A.6. Again prior and posterior distributions are shown. If 0–1 CA are observed, the expected probabilities that the unknown dose falls into the 3 dose categories ($\leqslant 9$, 10–100, $>$ 100) are 0.69, 0.19, and 0.12, respectively. For the second CA category (2–4), we found the following probabilities for the same dose categories in the same order: 0.49, 0.14, and 0.37. These results show that it is not possible to determine unequivocally the dose category for the unknown dose with the data sets shown in Tables A.1 and A.2.

A.2. Dose estimation with parametric models

In the first part of this section we are describing procedures for dose estimation which use a linear model of the form

$$Y = \alpha_1 + \alpha D$$

This model discussed in Ch. 7 is applicable to both low doses of X- or γ-radiation and to doses of high-LET radiations. Y is the yield or incidence

TABLE A.2

PRIOR INFORMATION AND NUMBER OF OBSERVED CHROMOSOMAL ABERRATIONS IN A-BOMB SURVIVORS (HIROSHIMA)

Number of total aberrations/100 cells	T65 dose (rad)		
	<9	10–100	>100
0–1	59 (181)	36 (28)	5 (37)
2–4	6 (89)	12 (15)	2 (69)

of aberrations per cell after exposure to radiation dose D. For our purposes, we need a mathematically more precise description of the statistical model. This description is given below:

$$E(Y\,|\,\alpha_1,\ \alpha,\ d,\ n) = n(\alpha_1 + \alpha d)$$

In words, this equation says,

The mean number of chromosomal aberrations (e.g., dicentrics), if the background rate α_1, the parameter α, the parameter α, the radiation dose d, and the number of metaphases scored n were known, is given by the right side of the equation.

In more succinct mathematical parlance,

The conditional mean of Y given α_1, α, d, n is $n(\alpha_1 + \alpha d)$.

It is conventional to use small letters for known quantities, capital letters for unknown (random) quantities, and small Greek letters for parameters to be estimated. E, as in Section A.1, is again used

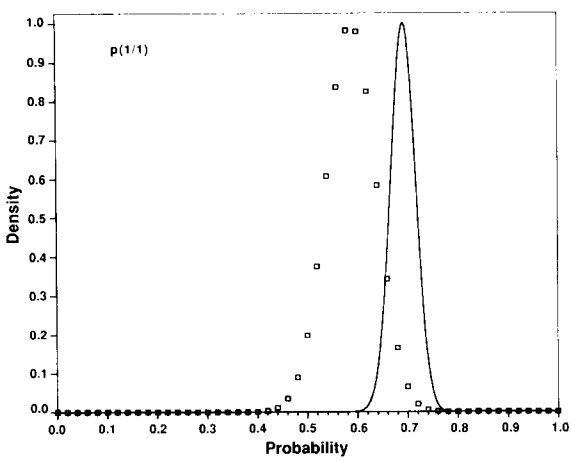

Fig. A.4. Prior (squares) and posterior distribution for $p(1\,|\,1)$

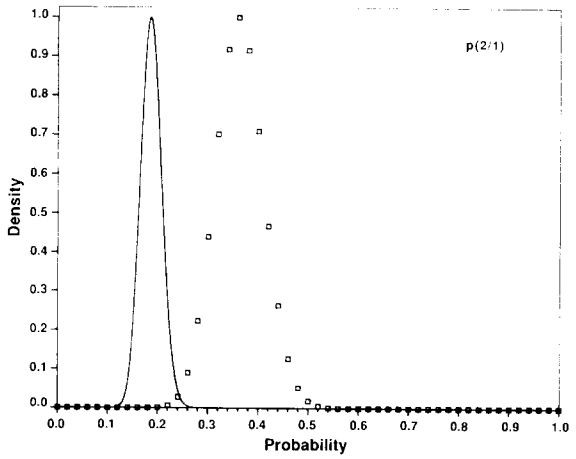

Fig. A.5. Prior (squares) and posterior density for $p(2\,|\,1)$.

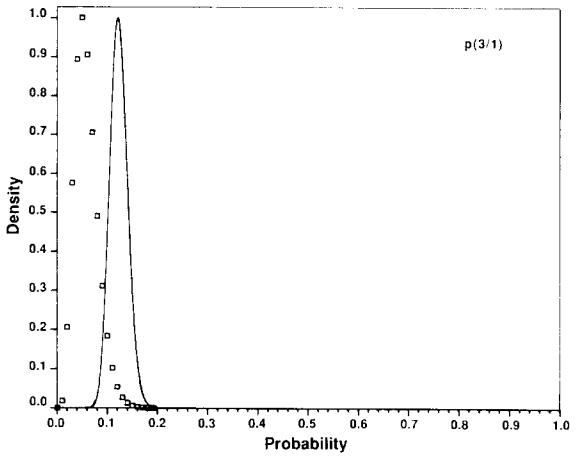

Fig. A.6. Prior (squares) and posterior density for $p(3\,|\,1)$.

to denote the expectation (mean) of a random quantity (rq). Using these conventions the statistical models used in the estimation procedures can be specified as follows:

$$Y^*\,|\,(\alpha_1,\ \alpha,\ n) \sim Y^*\,|\,(\alpha_1,\ n) \sim \mathrm{Po}(n\alpha_1)$$

and

$$Y\,|\,(\alpha_1,\ \alpha,\ d,\ n) \sim \mathrm{Po}\left[n(\alpha_1 + \alpha d)\right]$$

This is mathematical shorthand for the statements

The number of chromosomal aberrations, Y^*, in n unirradiated metaphases, if the background rate were α_1, has a Poisson distribution (for a definition see, e.g., Lindley, 1970) with mean $n\alpha_1$.

Given α_1 and α, the number of chromosomal aberrations, Y, in n metaphases which received a radiation dose d has a Poisson distribution with mean $n(\alpha_1 + \alpha d)$.

First we consider the simplest case:

$$Y = \alpha D$$

In this simplest dose-dependent model, the usually small background rate α_1 in unirradiated cells is neglected. This simple model is thought to be valid for high-LET radiation and approximately also for low doses (< 10 rad) of low-LET radiation. We will use this model to analyze in vitro calibration data for ^{210}PoBe neutrons reported by Edwards et al. (1979). In this experiment,different numbers of cells, n_i, were scored for dicentrics after exposure to several different radiation doses d_i. The data are shown in Table A.3. In addition we also observe $(y_f\,|\,n_f)$. In words: We observe y_f dicentrics in n_f cells of the individual whose radiation dose D_f we want to estimate. f is short for "future" and is meant to indicate that relative to the calibration data in Table A.3, y_f represents a later observation. All the data $\tilde{D}$ are, therefore, represented by

$$\tilde{D} = \{(y_1\,|\,d_1,\ n_1),\ \ldots\ (y_7\,|\,d_7,\ n_7);\quad (y_f\,|\,n_f)\}$$

The first portion of $\tilde{D}$ consists of the number of dicentrics observed at the 7 different dose levels

TABLE A.3

CALIBRATION EXPERIMENT WITH BePo FISSION NEUTRONS

Dose (rad)	Cells scored	Dicentrics observed
50	269	109
75	78	47
100	115	94
150	90	114
200	84	138
250	59	125
300	37	97

Source: Edwards et al., 1979.

in the corresponding number of metaphases shown in Table A.3. Doses and number of metaphases are shown to the right of the vertical stroke to indicate that they were fixed in the calibration experiment. The second portion of $\tilde{D}$ is the "future" observation of dicentrics in the blood sample from the individual whose dose is unknown to us. Notice that, therefore, only n_f is given. We are interested in the "predictive density" (Schmitt, 1969) for D_f. This PD describes the remaining uncertainty about the unknown dose D_f after all the data (i.e., $\tilde{D}$) have been used in the estimation procedure. An expression for the predictive density can be derived using Bayes' theorem and some standard results of the probability calculus. We will not give the details of this derivation and state only the final result:

$$f(d_f \mid \tilde{D}) \propto (d_f)^{\tilde{B}-1}(d_f + \tilde{A})^{-\tilde{b}} \exp(-Ad_f) \quad (1)$$

$f(d_f \mid \tilde{D})$ is proportional to the expression on the right side. If it is divided by its maximum value it will have a maximum ordinate equal to 1. An example for this density for the calibration data in Table A.3 was already shown in Fig. 10.1, with $y_f = 232$ dicentrics and $n_f = 100$ metaphases. Before we give the expression for the "mode" of $f(d_f \mid \tilde{D})$ and other examples, the symbols in Eqn. 1 need to be explained.

$\tilde{B} = B + y_f$, where B is one of the parameters for the prior γ density (Schmitt, 1969) of D_f. In the shorthand notation used earlier for the Poisson models,

$$D_f \sim \mathrm{Ga}(A, B) \quad (2)$$

B is increased by the number of dicentrics observed, y_f, to yield $\tilde{B}$.

$\tilde{A} = \tilde{a}/n_f$, where $\tilde{a} = a + \sum_{i=1}^{7} n_i d_i$. The sum goes over all 7 dose levels shown in Table A.3 and a is one of the parameters of the γ prior for the model parameter. In symbols,

$$\alpha \sim \mathrm{Ga}(a, b) \quad (3)$$

n_i stands for the number of metaphases scored at dose d_i (e.g., $n_6 = 59$ at $d_6 = 250$ rad). Since $n_f = 100$, $\tilde{A} = \tilde{a}/100$ for Fig. 10.1.

$\tilde{b} = \sum_{i=1}^{7} y_i + y_f = b$, where y_i is the number of dicentrics scored at dose d_i. For instance, $y_6 = 125$ at $d_6 = 250$ rad in Table A.3. y_f was defined above. b is the second parameter of the prior density for the model parameter α (see Eqn. 3). A is the second parameter in the prior γ density for D_f (see Eqn. 2). γ prior densities were used to obtain analytical expressions for $p(d_f \mid \tilde{D})$. The family of γ densities is sufficiently flexible to permit expression of the analyst's prior uncertainty about α or D_f by one family member with appropriate parameters, (a, b) or (A, B), respectively.

Prior information about α comes from other calibration experiments. Prior dose information can be derived from dosimeter readings or possibly from dose reconstruction efforts by health physicists after a radiation accident or after exposure to radiation from a nuclear blast. Whether such other information should be included is left for the decision maker.

The mode of a PD is the value on its abscissa for which it reaches a maximum. In a sense, the mode is the "best" estimate. For $p(d_f \mid D)$ given in Eqn. 1, the mode can be found by solving the quadratic equation:

$$AX^2 + (A\tilde{A} + \tilde{b} + 1 - \tilde{B})X - (\tilde{B} - 1)\tilde{A} = 0 \quad (4)$$

If we define the coefficient of the linear term in Eqn. 4 as

$$C = A\tilde{A} + \tilde{b} + 1 - \tilde{B}$$

then we can write for the mode d_m of $p(d_f \mid \tilde{D})$

$$d_M = -C + \sqrt{[C^2 + 4A\tilde{A}(\tilde{B} - 1)]}/2A$$

This is the only positive and therefore meaningful solution of Eqn. 4.

In Figs. A.7–A.12 we show $p(d_f \mid \tilde{D})$ for the y_fs and n_fs indicated on the graphs. For all these figures the same calibration experiment shown already in Table A.3 was used. Furthermore, the same prior distributions for D_f and α were used. D_f was assumed to be distributed as Ga(0.06, 1.5) and the prior for α is Ga(1000, 10). The prior and posterior distributions for α are shown in Fig. A.13. The Ga(1000, 10) prior for α describes the uncertainty about α before the update with the calibration data shown in Table A.3. The prior

parameters, a and b, were obtained using the procedure described in Martz and Waller (1982). To use this procedure, the expert analyst has to specify a lower (α_L) and upper limit (α_U) for α and a personal estimate of probability, p (e.g., here 80%), that the unknown true value of α falls in the interval $[\alpha_L, \alpha_U]$. From $\alpha_L = 0.005$, $\alpha_U = 0.01$ and $p = 80\%$, it is possible to derive a and b for the prior. Based on calibration experiments with other types of radiation, we derived $a = 1000$, $b = 10$, and, therefore, $\alpha \sim \text{Ga}(1000, 10)$.

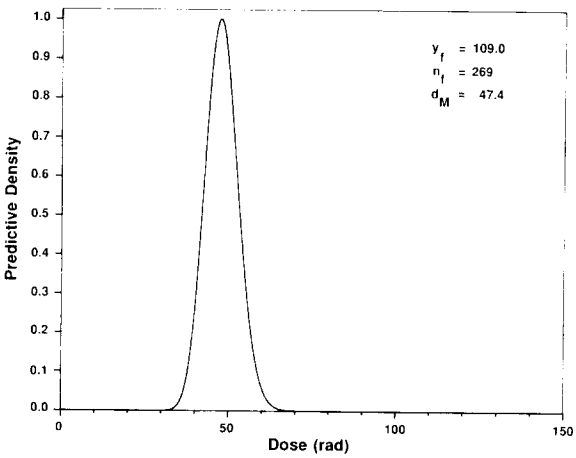

Fig. A.7. Predictive density for D_f (rad) (109 dicentrics in 269 metaphases).

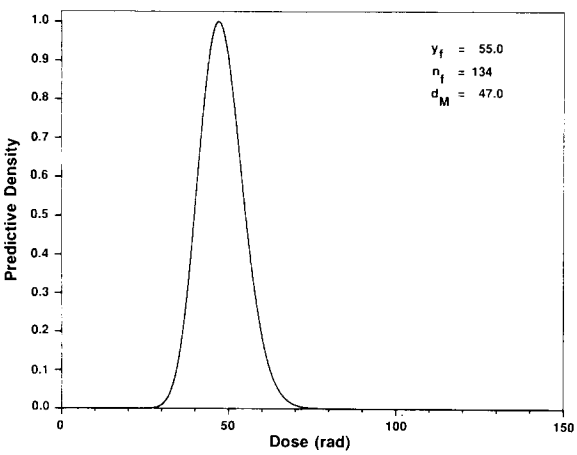

Fig. A.8. Predictive density for D_f (rad) (55 dicentrics in 134 metaphases).

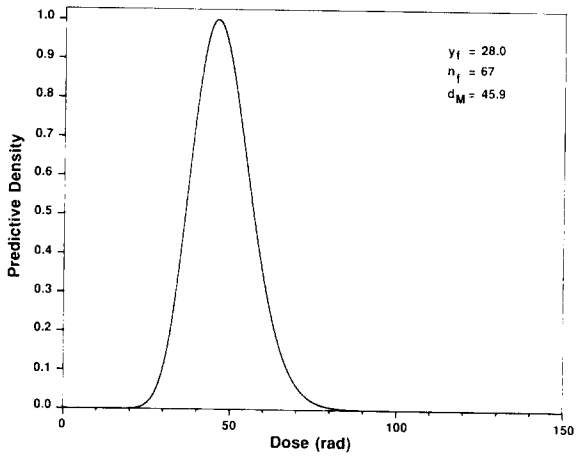

Fig. A.9. Predictive density for D_f (rad) (28 dicentrics in 67 metaphases).

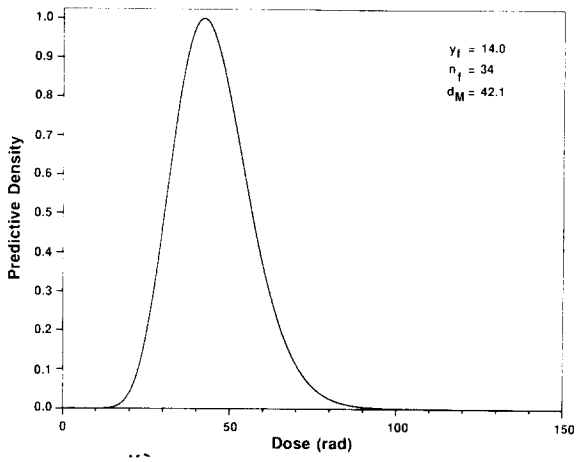

Fig. A.10. Predictive density for D_f (rad) (14 dicentrics in 34 metaphases).

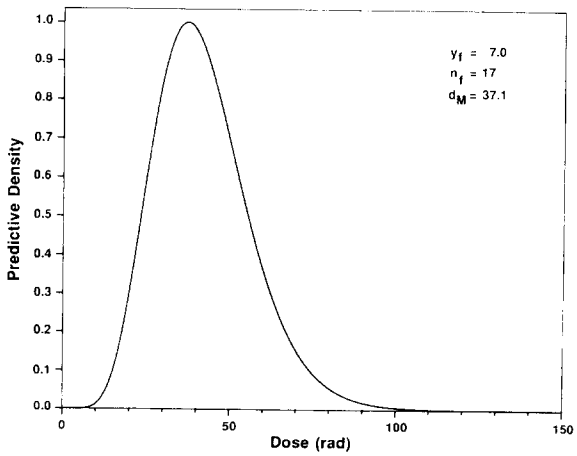

Fig. A.11. Predictive density for D_f (rad) (7 dicentrics in 17 metaphases).

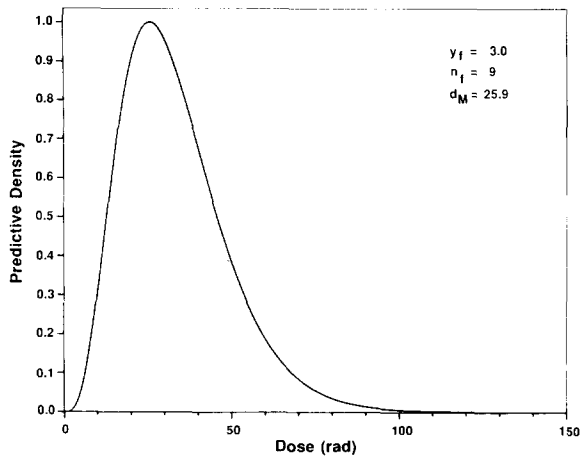

Fig. A.12. Predictive density for D_f (rad) (3 dicentrics in 9 metaphases).

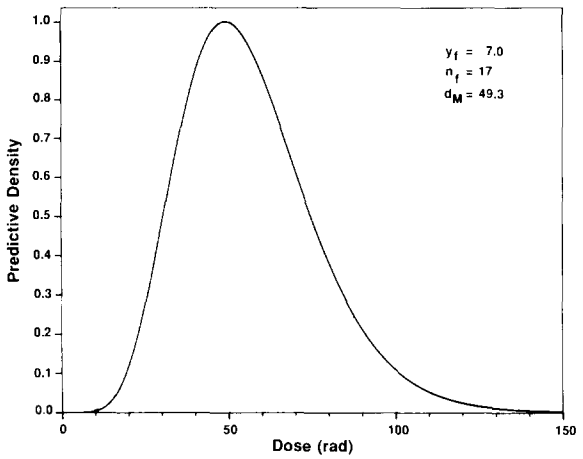

Fig. A.14. Predictive density for D_f (rad) with the same number of dicentrics as in Fig. A.11 but with a uniform prior for D_f.

Figs. A.6–A.12 show the increase in uncertainty as the number of cells scored (n_f) is decreased by roughly one half and the ratio y_f/n_f is held approximately constant. The increasing uncertainty expresses itself in a widening of the PDs. Figs. A.14 and A.15 show the influence of prior information on $p(d_f \mid \tilde{D})$ by comparison with Figs. A.11 and A.12. For the PDs in Figs. A.14 and A.15, a uniform prior over a large domain for D_f was assumed. These two PDs are "wider" than the corresponding PDs in Figs. A.11 and A.12 which incorporate a Ga(0.06, 1.5) prior for D_f in the analysis.

In Fig. A.16 we show the influence of the calibration experiment on the PD for D_f. For this figure, we used the calibration experiment with 14.7 MeV neutrons, a different type of high-LET radiation with a smaller LET but higher energy (Edwards et al., 1979). A comparison of Figs. A.16 and A.7 shows totally different results. The mode in Fig. A.16 is 98.8 rad and the mode in Fig. A.7 is only 47.4 rad. For both figures, y_f, n_f, and the prior density of D_f were the same.

So far, we have limited our discussion to the simplest case $Y = \alpha D$. A generalization of this model mentioned earlier is $Y = \alpha_1 + \alpha D$. This model contains a background term α_1. Under the

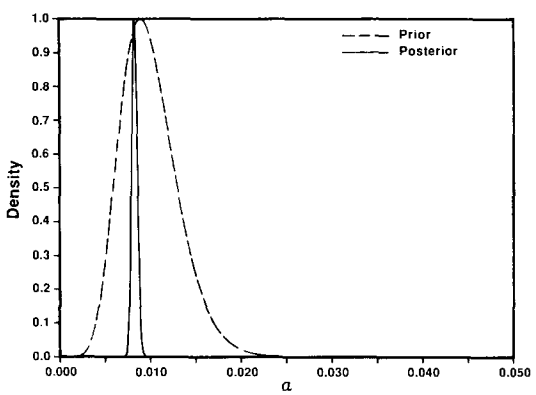

Fig. A.13. Prior and posterior distribution for α.

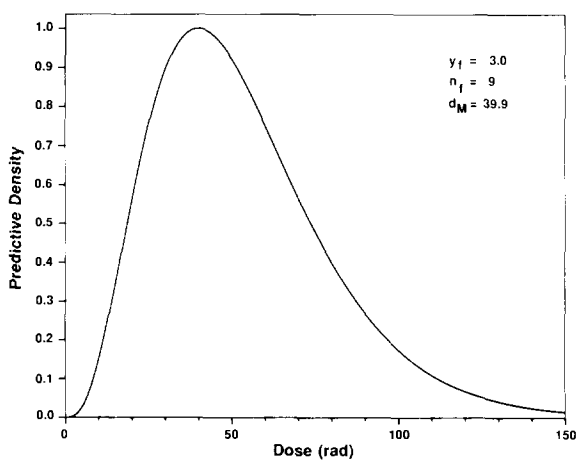

Fig. A.15. Predictive density for D_f (rad) with the same number of dicentrics as in Fig. A.12 but with a uniform prior for D_f.

following distributional assumptions for the parameters

$$\alpha_1 \sim \mathrm{Ga}(a_1, b_1), \quad \alpha \mid \alpha_1 \sim \alpha \sim \mathrm{Ga}(a, b)$$

$$D_f \mid (\alpha_1, \alpha) \sim D_f \sim \mathrm{Ga}(A, B)$$

and the Poisson models for Y^* and Y, given the parameters, mentioned at the beginning of Section A.2, it is again possible to derive an analytic expression for $f(d_f \mid \tilde{D})$. The total data $\tilde{D}$ consist now of

$$\tilde{D}_1 = \left\{(y_1 \mid n_1), \ \ldots \ (y_{N_1} \mid n_{N_1})\right\}$$

and

$$\tilde{D}_2 = \left\{\left(y_{N+1} \mid d_{N_1+1}, n_{N_1+1}\right), \ \ldots \ (y_N \mid d_N, n_N), \right.$$
$$\left. (y_f \mid n_f)\right\}$$

that is, $\tilde{D} = \tilde{D}_1 + \tilde{D}_2$. If $\tilde{D}_1$ is incorporated into the analysis through the posterior distribution of α_1, we obtain for $f(d_f \mid \tilde{D})$ the following expression:

$$f(d_f \mid \tilde{D}) i$$
$$\propto d_f^{B-1} \exp[-A d_f]$$
$$\times \int_0^\infty \left[\alpha_1^{b_1 - 1} \exp\left\{-\left(a_1 \tilde{N} + n_f\right)\alpha_1\right\}\right]$$
$$\cdot \left\{\int_0^\infty \left[(\alpha_1 + \alpha d_f)^{y_f}\right]\left[\sum_{i=1}^{N} (\alpha_1 + \alpha d_i)^{y_i}\right]\right.$$
$$\left. \times \left[\alpha^{b-1} \exp\left\{-a + \tilde{d} + n_f d_f\right)\alpha\right\}\right] d\alpha \Bigg\} d\alpha_1 \qquad (5)$$

where $\tilde{N} = \sum_{i=1}^{N} n_i$ and $\tilde{d} = \sum_{i=1}^{N} n_i d_i$. This integral has a closed form which is however quite lengthy and is therefore not given here. Eqn. 5 was not evaluated for a particular calibration experiment and for different sets of parameters (a_1, b_1, a, b, A, B) and y_fs. Incorporation of the background rate α_1 into the analysis will clearly result in a widening of the $f(d_f \mid \tilde{D})$. In other words, the uncertainty about D_f will be greater than for the case where α_1 was neglected.

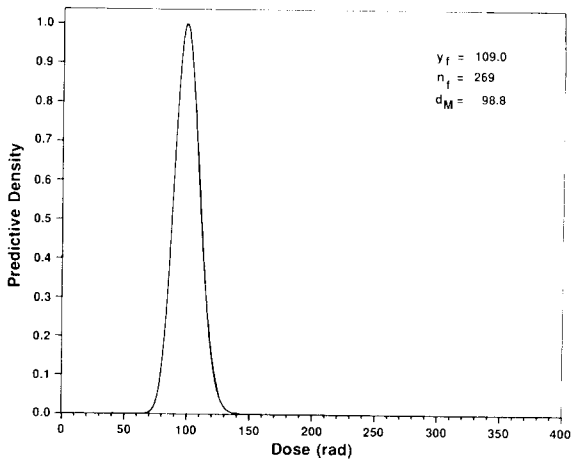

Fig. A.16. Predictive density for D_f (rad) with the same data as in Fig. A.7 but with a calibration experiment for 14.7 MeV neutrons.

The model for Y can be further generalized through inclusion of a term proportional to D^2 (i.e., $Y = \alpha_1 + \alpha D + \beta D^2$). This additional term expands the validity of the model to the full dose range for low-LET radiation. We stated earlier that the model for Y linear in dose is generally accepted for high-LET radiation and for the low dose domain for low-LET radiation. No analytic, closed expressions can be derived for this expanded model. The necessary intergration over the parameters α_1, α, and β have to be done numerically.

A.3. Disappearance of chromosomal aberrations and dose estimates many years after exposure

Asymmetrical CAs (e.g., dicentrics) disappear gradually as time since exposure increases. This disappearance is usually modeled with an exponential term of the form $\exp(-\lambda t)$ where λ is the rate fo disappearance and t is the time since exposure. We used data from a radiation accident victim (Brewen et al., 1972; Preston et al., 1974; Littlefield, 1986) to estimate λ. In vitro cobalt-60 calibration data appropriate for this individual permit more precise estimates of the dose–response parameters α and β for this person. We used the maximum likelihood estimates for α and

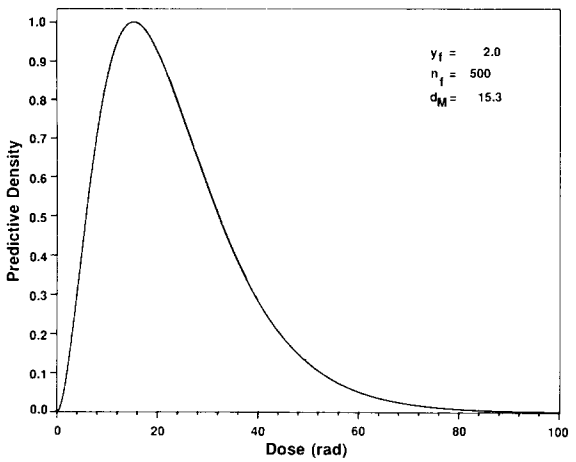

Fig. A.17. Predictive density for D_f (rad) (2 dicentrics in 500 metaphases) with a uniform prior for D_f and ^{60}Co calibration data.

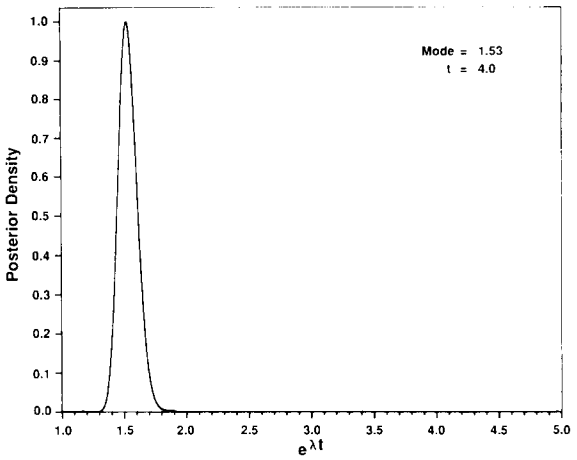

Fig. A.19. Posterior density of $e^{\lambda t}$ using the density for λ from Fig. A.18 and $t = 4$ years.

β in our estimation of λ. The uncertainty about λ is again described by a PD. This PD is conditional on the estimates for α and β from the in vitro data for this individual.

With this PD for λ and the exponential model it is possible to extrapolate backwards in time and to determine the PD, $f(d_0)$, for the dose at $t = 0$. If $f_t(d_f \mid \tilde{D})$ in Fig. A.17 is the PD for D_f at time t since exposure and $f(e^{\lambda t})$ the PD for $e^{\lambda t}$, then the PD for the rq $D_0 = D_f \cdot e^{\lambda t}$ follows from the "product rule" for rqs. $f(e^{\lambda t})$ can be calculated from the PD for λ, $f(\lambda)$ shown in Fig. A.18. Figs. A.19 and A.20 show $f(e^{\lambda t})$ for $t = 4$ and $t = 10$ years, respectively. From the graphs of $f(d_0)$ in Figs. A.21 and A.22, it is clear that the extrapolated dose estimate is quite uncertain. The PDs (Figs. A.21 and A.22) for D_0 after backwards extrapolation for 4 and 10 years are based on lognormal approximations for $f_t(d_f \mid \tilde{D})$ and $f(e^{\lambda t})$.

It is, of course, possible to calculate the PD of the product $D_f \cdot e^{\lambda t}$ with the exact distributions $f_t(d_f \mid \tilde{D})$ and $f(e^{\lambda t})$ using numerical integration. But it is mathematically much easier to determine

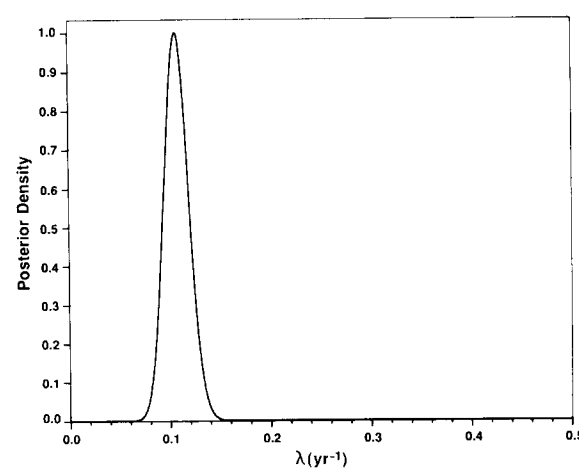

Fig. A.18. Posterior density for λ in a radiation accident victim.

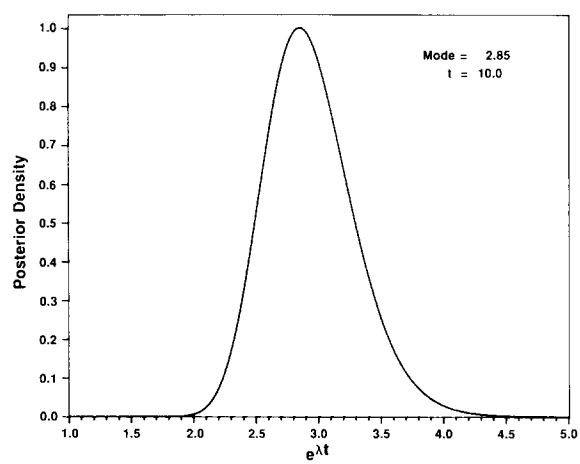

Fig. A.20. Posterior density of $e^{\lambda t}$ using the density for λ from Fig. A.18 and $t = 10$ years.

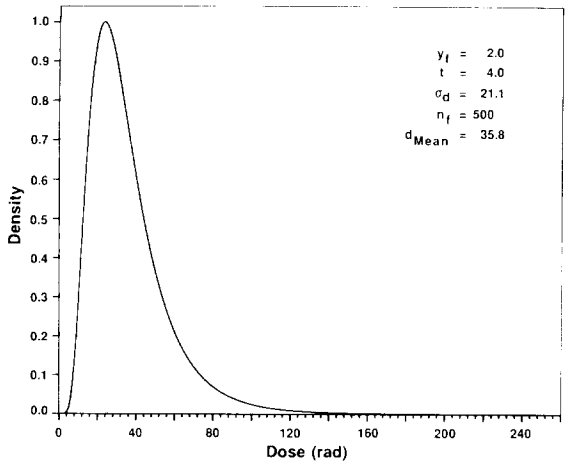

Fig. A.21. Density for D_0 based on Figs. A.17 and A.19.

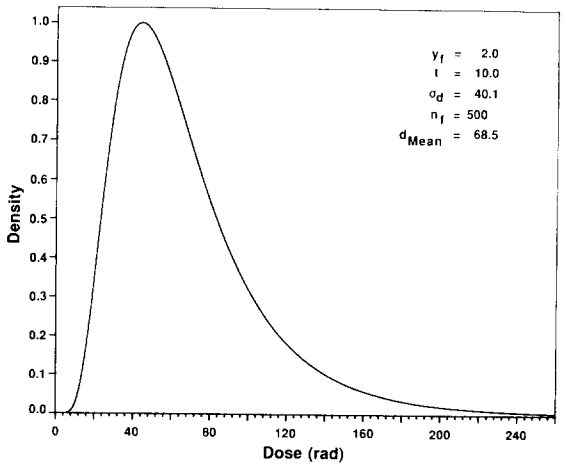

Fig. A.22. Density for D_0 based on Figs. A.17 and A.20.

the PD for D_0 with approximate distributions of the factors D_f and $e^{\lambda t}$. Since both PDs are skewed, it seemed reasonable to use γ or lognormal distributions as approximations. If $f_t(d_f \mid \tilde{D})$ and $f(e^{\lambda t})$ are both approximated by $\mathrm{Ga}(a_1, b_1)$ and $\mathrm{Ga}(a_2, b_2)$ distributions, respectively, then one finds

$$f(d_0) = \frac{2a_1^{b_1}a_2^{b_2}d_0^{b_2-1}}{\Gamma(b_1)\Gamma(b_2)}(a_2 d_0/a_1)^{(b_1-b_2)/2} \times K_{b_1-b_2}\left(2\sqrt{a_1 a_2 d_0}\right) \quad (6)$$

where $\Gamma(x)$ is the complete γ function. Eqn. 6 involves a Bessel function $K_\gamma(z)$ which is not tabulated. If lognormal approximations are used, the PD of the product D_0 is again lognormal. The parameters β and δ for the lognormal PD of the product expressed as functions of the parameters of the lognormal factors are

$$\beta = \beta_1 \cdot \beta_2, \quad \delta = \sqrt{\delta_1^2 + \delta_2^2}$$

β_1 and δ_1, the parameters of the lognormal approximation for $f(e^{\lambda t})$, were obtained by equating mean and variance of the exact distribution with the mean and variance of the lognormal. β_2 and δ_2, the parameters of the lognormal approximation for $f_t(d_f \mid \tilde{D})$, were calculated by equating lognormal mode and mean with the corresponding quantities of the exact distribution. Since the expectation (mean) of a product of 2 independent rqs X, Y $E(X \cdot Y) = E(X) \cdot E(Y)$, the lognormal density for D_0 has the same mean as the exact distribution. The variance of a product of independent rqs, X, Y, is given by

$$\mathrm{Var}(XY) = \mathrm{Var}(X) \cdot \mathrm{Var}(Y) + E^2(X) \cdot \mathrm{Var}(Y) + E^2(Y) \cdot \mathrm{Var}(X) \quad (7)$$

Since the variance of the lognormal approximation for $f_t(d_f \mid \tilde{D})$ is smaller than the variance of the exact distribution, the lognormal variance of the product $D_0 = D_f \cdot e^{\lambda t}$ will also be smaller than the exact product variance. This follows immediately from Eqn. 7 since $E(D_f)$ and $E(e^{\lambda t})$ of the lognormal approximations are equal to the corresponding expectations of the exact distributions. That means that the exact distributions for D_0 will be more spread out than the lognormal approximation. In other words, the uncertainty about D_0 will be somewhat greater than the lognormal approximation indicates.

Without "personal" in vitro calibration data and without estimates of λ for the person which received D_f, the situation is clearly worse; for example, the PD for $f(d_0)$ is "wider". The PD for λ shown in Fig. A.23 supports this claim. This PD shows the uncertainty about λ if different calibration data (Edwards et al., 1979) but the same data for the disappearance of dicentrics in the accident victim are used. The difference between Figs. A.18

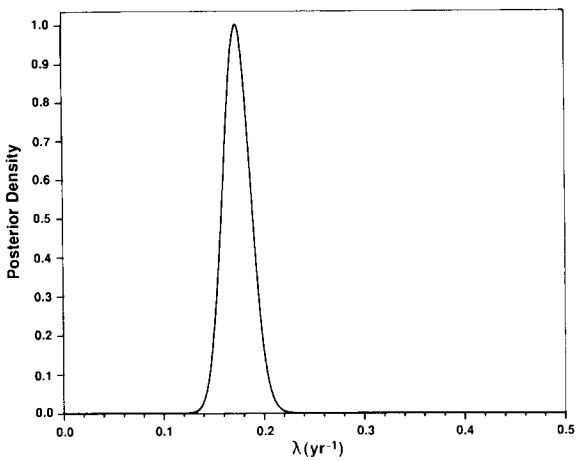

Fig. A.23. Posterior density for λ with different ^{60}Co calibration data.

and A.23 is due to the different αs and βs used. The uncertainty about α_1, α, β, and λ contributes to the overall uncertainty of the dose estimate at the time of exposure. The extrapolation procedure used here is a deterministic procedure which uses simply the factor $\exp(\lambda t)$ to go backwards in time. A full stochastic treatment of this problem would require

$$Y \mid (\alpha_1, \alpha, \beta, \lambda, t, n)$$

$$\sim \mathrm{Po}\left[n\left(\alpha_1 + \alpha d + \beta d^2\right) \exp(-\lambda t)\right]$$

as a model and extensive numerical work.

The discrete analysis of stable chromosomal aberrations in A-bomb survivors in Section A.1 and the extrapolated dose estimates based on information from a radiation accident victim show the considerable uncertainty of dose estimates if $y_f \mid n_f$ is obtained many years after the exposure to D_0.

Other models with greater variance (Ochi and Prentice, 1984) have been suggested for the analysis of chromosome aberration data. Such models would yield — ceteris paribus — wider distributions than models with smaller variance and would therefore only support our conclusions that dose estimates are plagued by considerable uncertainty.

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