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NOTE

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Annex C

RADIATION-INDUCED CHROMOSOME ABERRATIONS IN HUMAN CELLS

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I. Introduction

1. It has been known for a long time that aberrations of chromosome structure (chromosome aberrations or chromosome structural changes) and alterations in chromosome number arise spontaneously at a low rate in somatic and germ cells of plants and animals and that the frequency of such aberrations increases following exposure to ionizing radiations. These aberrations may, in fact, comprise the major component of the genetic damage resulting from radiation exposure, but in many instances the genetic consequences of certain kinds of aberrations are so disastrous as to result in the early death of the cells containing them.

2. Although a considerable fraction of induced chromosome aberrations may behave as dominant lethal events, all aberrations that do not result in an "immediate" loss of viability are mutational changes which may be transmitted to descendant cells and to the offspring of the irradiated individual. Chromosome aberrations are clearly, therefore, of great genetic importance, and a considerable amount of work has been devoted to studying the mechanisms of their induction by radiation, their behaviour at mitosis and meiosis and their genetic consequence.

3. Up until relatively recently, most of this work had been carried out on species particularly well suited to the demands of cytological study (that is, species having a relatively small number of rather large chromosomes) and on organisms amenable to use in breeding experiments (1958, 1962 and 1966 reports[1-3] of the Committee). This work has provided, and will continue to provide, fundamental knowledge on the actions of radiations on chromosomes and also information on the genetic hazards of radiation exposure in the particular species chosen for study. However, extrapolation from these species to man is beset with difficulties, particularly in the absence of any comparable information on the radio-sensitivity of human chromosomes that could serve as a reference point. Quantitative estimates of the radiation hazard to man's chromosomes have, therefore, been fraught with uncertainties.

4. In the late 1950s, routine cytogenetic studies on mammalian chromosomes became possible as a consequence of the development of simple and reliable methods of culturing mammalian cells *in vitro* and of techniques similar to those previously used in plant cytogenetics. Refinements of these techniques[4-7] opened the way for cytological studies on the response of mammalian chromosomes to radiation exposure.

5. In the original work carried out by Bender[8] and by various other authors[9-11] in the United States, studies were made on the effects of x radiation on chromosomes in human epitheloid and fibroblast cell populations cultured *in vitro*, and comparisons were later made between the responses of human cells and of cultured cells obtained from spider monkeys and Chinese hamsters.[12] At about the same time, Fliedner *et al.*[13] reported that chromosome aberrations could be detected in cells from bone-marrow samples taken from a number of persons accidentally exposed to a mixed neutron-gamma-ray beam.

6. Prior to all these observations, a large number of earlier reports had shown that the chromosome aberrations induced by the irradiation of mammalian cells were similar to the aberrations induced in other animal and plant cells.

7. At the same time that these developments in mammalian radiation cytogenetics were occurring, the general field of human cytogenetics was rapidly emerging. The initial work in this field soon confirmed that the kinds of chromosome aberrations already well known to occur in plants and animals also arose spontaneously in man, and demonstrated that, in man, these aberrations were responsible for a number of very important harmful traits (1962 and 1966 reports[2, 3] of the Committee). These advances in human cytogenetics were given further impetus by the development[14] in 1960 of a simple and reliable technique for obtaining preparations of mitotic cells from cultured peripheral blood leucocytes. As a result of these developments, information on the spontaneous frequency and on the general consequences of chromosome aberrations in man has been continually accruing.

8. The advent of the peripheral blood culture technique afforded an opportunity to examine, by means of a simple and painless procedure, the response of human chromosomes in individuals exposed to ionizing radiations. Moreover, since large numbers of mitotic cells could be obtained from only a few millilitres of blood, frequent cell samples could be taken from an individual at various time intervals after exposure. The first studies of this kind were carried out by the Edinburgh group[15] in the United Kingdom, and in the last nine years a great deal of information has been obtained on chromosome damage and the potential hazards of radiation to man's genetic materials.

9. Observations have been made on chromosome aberrations induced *in vivo* in persons x-rayed for diagnostic reasons, in personnel subjected to low-dose occupational exposure (either externally or internally, or to a mixture of both external and internal radiation), in patients exposed to therapeutic radiation and in individuals accidentally exposed to radiation. In addition to this, information is also available on members of the surviving populations at Hiroshima and Nagasaki. Much of the information on patients given therapeutic doses has come from partial-body irradiation studies, and here the data are somewhat difficult to interpret since accurate physical dosimetry, particularly in relation to the cells sampled, is difficult to obtain. More recently, a little information has been gathered from a few patients exposed to low doses of whole-body radiation.[16]

10. Although in terms of application of our knowledge we are clearly most interested in results obtained from *in vivo* studies, a great deal of information can be and is being obtained from *in vitro* studies. Here, cultured cells can be exposed to accurately measured radiation doses, and accurate information on dose-response kinetics, etc. can be obtained. Such knowledge forms an important background to the *in vivo* work, and it has been generally thought it may well prove possible to extrapolate directly from the *in vitro* state to the *in vivo* state, provided certain requirements are met.

11. In view of the developments in this field over the past few years, the Committee decided that an appraisal of the progress made in this area was necessary. The time seemed particularly opportune for two reasons. First, a number of laboratories have made use of chromosome aberration yields as a method of estimating absorbed dose in individuals accidentally exposed to radiations, and a considerable amount of information on the relation between dose and aberration yield from both *in vivo* and *in vitro* exposure has been accumulating. Second, as a consequence of the developments in human cytogenetics, there has been an increasing amount of information on the importance of certain aberrations as causal factors in human congenital abnormalities and also on the possible association between certain kinds of chromosome aberrations in somatic cells and the development of neoplastic disease.

12. As the Committee is primarily concerned with evaluating risks and with reviewing pertinent scientific data, information on the genetic consequences of chromosome aberrations in man and on the possibility of using the levels of chromosome-aberration yield following radiation exposure as a measure of dose is particularly relevant. In the present report, therefore, emphasis has been placed on somatic cell damage, and attention has been centered on methodology, the possible application of aberration yields in dosimetry, their biological significance and their possible use in the assessment of risk.

II. The types of aberrations produced, their structure and behaviour

A. The general pattern of response

13. The types of chromosome aberrations induced in human cells are identical in structure and behaviour with the aberrations induced in other animal and plant cells having similarly organized monocentric chromosomes. These aberrations are usually considered to be of two basic types—the simple deletion, which may be the result of a single break in the chromosome thread, and the exchange, which involves at least two breaks and an exchange of parts either between different chromosomes (interchange) or between different parts of the same chromosome (intrachange).

14. The detailed mechanisms of formation of the aberrations are not fully understood, and two hypotheses are currently in vogue (see reference 17). The more generally accepted classical theory, which was developed principally by Sax[18-20] and later by Lea and

Catcheside (see reference 21), proposes that x-ray induced simple deletions are a consequence of single breaks in the chromosome produced through the action of a single electron track, whereas exchange events are a consequence of the aberrant rejoining of breaks produced through the action of one or more (usually two) separate electron tracks.

15. On this classical theory, the evidence obtained from dose, dose-rate and dose-fractionation studies is interpreted to indicate that broken chromosome ends remain available for rejoining with themselves (thus restituting the original chromosome structure) or with other broken ends (thus giving rise to an exchange aberration) for only a limited time period (rejoining time) of around thirty minutes.[18, 21] This timing, however, is very dependent upon conditions.[23] Since the exchange aberrations can only be produced if the two breaks involved are closely associated spatially[21, 22] and are produced close together in time, it follows that, on this theory, the yield of simple deletions should increase linearly with increasing x-ray dose, and two-break exchanges should increase as approximately the square of the dose, when exposure times are short relative to the rejoining time (see references 17 and 21).

16. On the exchange hypothesis of Revell,[24] all aberrations, including the so-called simple chromatid deletions, are believed to be a consequence of exchange. On this hypothesis a proportion of the simple deletions could result from the interaction of the effects of two separate electron tracks. The deletions are believed to be the consequence of an incomplete exchange between two regions within a chromosome so that the deletion is associated with an inversion or duplication of a short length of the chromosome at the point of "failed union". Thus, on this hypothesis, simple deletions can show either a negligible or a significant "dose-squared" component in their rate of increase with increasing x-ray dose.[25] On both hypotheses, with high LET radiations all aberration types increase linearly with increase in dose.[17, 23]

17. In general, although there are certain exceptions, proliferating somatic cells spend by far the majority of their lifetimes in an interphase state and pass relatively rapidly through the division process of mitosis. The duration of interphase may range from the life span of the individual in a non-dividing differentiated cell, to a number of years in a mitotically quiescent cell or to a period of less than one day in the case of an actively proliferating cell. In all cases, however, the duration of the mitotic phase is usually, at most, an hour or two and is, therefore, short in relation to interphase. Thus, although the chromosome aberrations produced in irradiated cells are only observed when the chromosomes appear at mitosis (or at meiosis in the gonads), on the average almost all the aberrations produced are a consequence of damage sustained in an interphase state.

18. The aberrations observed in dividing cells are thus visible manifestations of radiation damage sustained at an earlier point in time. A number of cellular (enzymic) processes may, therefore, intervene between the initial radiation exposure and the final development of an aberration. Thus, for a given cell type, radiation dose, quality, etc., the final yield of aberrations may be modified by physiological as well as physical factors. The influence of such modifying factors was considered in some detail in the Committee's 1962 and 1966 reports.[2, 3]

19. The types of aberrations induced following radiation exposure fall into three groups according to the unit of breakage or exchange that is involved. Aberrations which involve both chromatids of a chromosome at identical loci are generally referred to as *chromosome-type* aberrations, whereas those in which the unit of aberration formation is the half-chromosome or chromatid are termed *chromatid-type* aberrations. The third category of aberrations known as *subchromatid-type* aberrations appear to involve breakage and exchange of subunits of a chromatid.

20. Which of the three basic types of chromosome aberrations are observed at mitosis (or at meiosis) depends upon the stage of development of the cell at the time of irradiation. In a mitotically proliferating cell, the interphase period of the cell cycle can be partitioned into three phases:[26] the pre-DNA synthesis or G_1 phase of early interphase; the DNA synthesis or S phase; and the post-DNA synthesis or G_2 phase of late interphase. With few exceptions, cells not actively proceeding through a mitotic cycle usually rest in the G_1 phase (for example, the peripheral blood small lymphocyte in normal healthy individuals), and such cells are sometimes referred to as being in a G_0 state.[27] However, there are exceptions to this general rule, and certain types of mitotically inactive cells (for example, certain epidermal cells in the mouse ear)[28] may rest in a G_2 phase.

21. Irradiation of all resting cells and of the majority of proliferating cells in a G_1 phase results in the production of chromosome-type aberrations. At the very end of the G_1 phase[29, 30] there is a transition from the chromosome-type aberration to the chromatid-type, and this transitional phase extends from late G_1 into early S (figure 1). Thus, most of the cells irradiated while in S and all the cells exposed while in G_2 yield chromatid-type aberrations. Subchromatid-type aberrations are only produced in cells irradiated in the early prophase of mitosis (or mid-prophase of meiosis), and cells exposed to radiation at the metaphase or later stages of mitosis yield chromosome-type aberrations at their next mitosis (see figure 1 and reference 17).

22. In addition to aberrations involving changes in chromosome structure, certain kinds of damage may also result in alterations in chromosome number and yield aneuploid or polyploid cells (paragraphs 77-89). Such changes in chromosome number are the result of errors (non-disjunction) in chromosome or chromatid segregation at meiosis or mitosis, and these errors are often, although not invariably, a consequence of the presence of chromosome structural changes.

23. It should be emphasized here that all of the varieties of chromosome structural changes and of changes in chromosome number that are to be observed in irradiated cells are also to be found in cells exposed only to natural background irradiation. The frequency of such spontaneous aberrations in unirradiated cells from normal healthy individuals is, of course, extremely low (table I), but the kinds of changes found are precisely the same as those induced as a consequence of radiation exposure.

B. Chromosome-type aberrations

24. Chromosome-type aberrations, in which both chromatids of a chromosome are broken or exchanged at the same locus and in an identical fashion, are the aberrations that have been most frequently studied in human cells. This is because most of the work on man

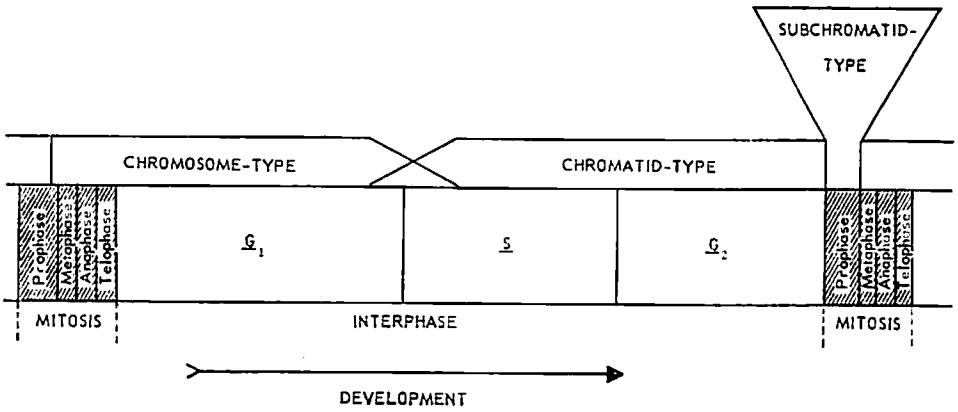

Figure 1. Relation between type of aberration induced by radiation and stage in cell cycle at time of irradiation

has been carried out on peripheral blood leucocytes that were irradiated while in a resting G_1 phase and examined at a mitotic metaphase following the stimulation of development of these cells in culture.

25. Studies on metaphase somatic cells reveal that seven kinds of chromosome-type aberrations can be distinguished cytologically (figure 2). Aberration types (i) to (v) are produced within single chromosomes

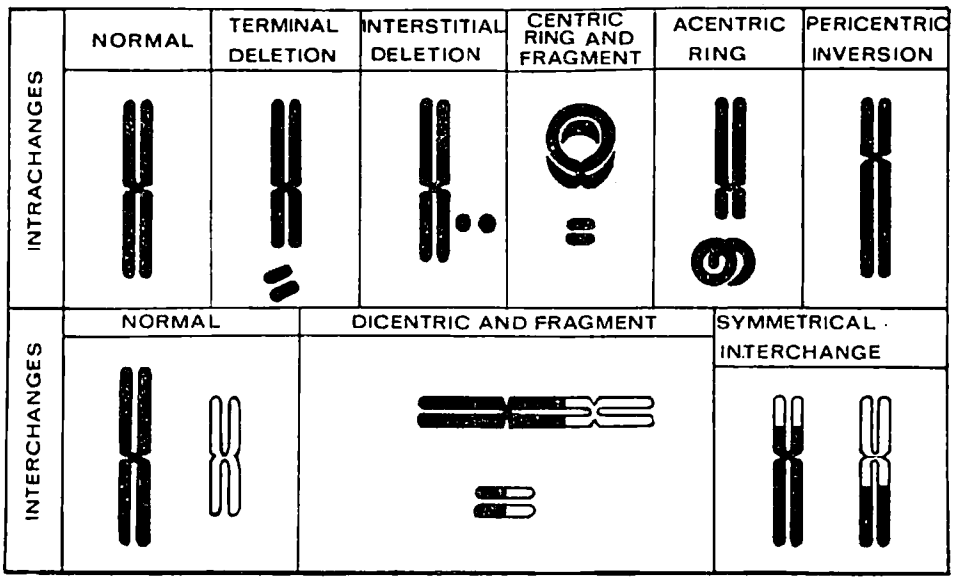

Figure 2. Chromosome-type aberrations that can be distinguished cytologically at mitosis

and are referred to as *intrachanges,* whereas types (vi) and (vii) involve an exchange of parts between different chromosomes and are, therefore, *interchanges.*

26. (i) *Terminal deletions* are paired acentric fragments which have the appearance of resulting from a simple break across the chromosome and are not associated with an exchange aberration such as a ring or interchange (paragraphs 28, 33-34). Some authors refer to terminal deletions simply as free acentric fragments.

27. (ii) *Minutes (interstitial, isodiametric or dot deletions)* are pairs of acentric fragments, smaller in size than terminal deletions, characteristically appearing as paired spheres of chromatin, hence the terms, dot or isodiametric deletions. These deletions are usually not terminal but intercalary and are the consequence of two closely juxtaposed transverse breaks across the chromosome.

28. (iii) *Acentric rings* are the result of two transverse breaks and an exchange within the chromosome. The linear separation between the two breaks is greater than in the case of minutes so that the excised paired fragments are larger and are ring-shaped. The distinction between minutes and rings is often arbitrary, since it is based purely on the size of the interstitial region of the chromosome that is deleted.

29. (iv) *Centric rings* are ring-shaped chromosomes resulting from an exchange between two breaks occurring on either side of the centromere. The centric-ring aberration is clearly distinguished from its acentric counterpart and is accompanied by one (rarely two) acentric fragments.

30. (v) *Pericentric inversions* result from two breaks, one on each side of the centromere, followed by the inversion of the centromeric segment and its reincorporation into the chromosome. If the two breaks (or points of exchange) are not equidistant from the centromere, then the pericentric inversion is clearly characterized by the altered location of the centromere within the chromosome. However, if, as is probably most often the case, the exchange points are approximately equidistant from the centromere, then the in-

version cannot be detected in mitotic cells but could be detected in meiotic cells following chromosome pairing.

31. Paracentric inversions, where both points of exchange lie on the same side of the centromere, cannot be cytologically detected in mitotic cells but could be identified at meiosis.

32. In types (ii) to (v), the exchange event may, in a small proportion of cases, be incomplete, only two of the four free ends involved in the exchange actually undergoing rejoining. Thus, an incomplete paracentric inversion will be scored as a terminal deletion, and a ring chromosome may be accompanied by two, rather than one, acentric fragments.

33. (vi) *Symmetrical interchanges (reciprocal translocations)* are exchange aberrations resulting from a breakage in each of two chromosomes followed by aberrant rejoining such that the distal regions of the two chromosomes are transferred (translocated) from one to the other. The aberrations are described as being symmetrical since they do not result in the formation of a dicentric structure (paragraph 34). Occasionally, if the exchange is incomplete, an acentric fragment may result. If the exchange is equal, that is, if an equal length of chromatin is translocated from one chromosome to the other, then the exchange could be detected at meiosis. Such an exchange could not be detected in somatic cells, however, except when incomplete, in which case it would appear as a simple terminal deletion. Symmetrical interchanges between acrocentric chromosomes are sometimes referred to as centric fusions. They result from the translocation of entire chromosome arms, the exchange occurring in the region of the centromeres of the chromosomes involved.

34. (vii) *Asymmetrical interchanges (dicentric aberrations or more complex polycentrics)* are exchange aberrations due to a breakage in each of two or more chromosomes followed by aberrant rejoining such that the proximal regions of the chromosomes become united, thus forming a dicentric or polycentric structure and an associated acentric fragment (rarely two fragments, when the exchange is incomplete and the two distal regions do not unite).

35. As mentioned above, incomplete rejoining in exchange results in an increased frequency of free fragments associated with an aberration. It should be stressed, however, that the fragment associated with an exchange aberration, such as a dicentric or centric ring, is part of the exchange aberration and is not scored as a separate fragment, that is, as a terminal deletion. The presence of a dicentric- or centric-ring structure with no accompanying fragment is an almost certain indication that the aberrant cell has proceeded through at least one mitotic division after irradiation and prior to observation.

36. Mention has been made of the difficulties in detecting certain forms of pericentric inversions (inter-arm intrachanges) and of symmetrical interchanges in somatic cells so that not all seven chromosome-type aberrations considered can be scored with equal efficiency. It is to be expected that pericentric inversions that do not result in a change in the relative arm lengths of a chromosome must comprise a significant proportion of the total of such aberration types. This is so because, as a result of the V-shaped arrangement of the chromosomes following anaphase separation and of the restrictions on chromosome movements in interphase,[17, 31] there must be a much higher probability of exchange between points equidistant from the centromere than between points at different distances. Similarly, in the case of symmetrical interchange aberrations, exchanges between points equidistant from the centromeres of the two chromosomes involved will be very frequent. Thus, if the chromosome complement contains a number of chromosomes having arms of equal or similar lengths, as is the case in the human complement, then a symmetrical exchange often will not result in an altered morphology of the exchanged chromosome(s), and the aberration will pass undetected.

37. The inefficiency of scoring symmetrical and equal inter- and intrachange chromosome-type aberrations is not encountered in the comparable chromatid-type aberrations. At the chromatid-type level, because of the close pairing between sister chromatids, asymmetrical and symmetrical aberrations can all be scored with equal efficiency. Studies on chromatid-type aberrations in plants and animals have shown that the symmetrical and asymmetrical variants of any given aberration type occur at approximately equal frequencies,[17, 21] and it has generally been assumed that this approximate equality must also obtain at the chromosome-type level. This assumption has recently been confirmed in studies on the morphologically well-marked large chromosomes of the plant *Vicia faba*, where the frequency of involvement of these chromosomes in symmetrical (reciprocal translocation) and asymmetrical (dicentric) chromosome interchange was shown to be equal.[32]

38. Since it is to be expected that asymmetrical and symmetrical interchange events occur with equal frequency in irradiated human cells, it is possible to estimate from published data[16, 33-35] that the efficiency of scoring symmetrical events in man's chromosomes is not more than 20 per cent. This follows from the fact that the frequency of dicentric- plus centric-ring aberrations should equal the frequency of reciprocal translocations plus pericentric inversions, whereas, in the data available, dicentric and ring chromosomes are approximately five times as frequent as abnormal monocentric chromosomes. Similarly, the frequency of acentric-ring plus minute aberrations should correspond to the frequency of paracentric inversions. As noted earlier, paracentric inversions cannot be detected in mitotic cells.

39. In addition to the general difficulties in detecting symmetrical aberrations, it should be noted that the efficiency of their detection varies between different observers. However, these problems do not arise in the case of the asymmetrical aberrations. It is for these reasons that it has long been the practice of radiation cytogeneticists working on plant and animal cells to classify chromosome-type aberrations into terminal deletions, minutes, acentric rings, centric rings and dicentrics (polycentrics) and to use data on these aberrations, but not data on pericentric inversions and symmetrical interchanges, for quantitative studies.

40. It should be stressed that the aberrations described under the above five headings form the bulk of the structural alterations that can be observed. They can be scored efficiently and there is little variation due to subjective differences between different observers. In the case of the possible use of chromosome aberration yields as indicators of absorbed dose in man, there is little doubt that the classification of the

aberrations into these categories is essential. However, it is important to note that the aberrations that are simplest and least ambiguous to score include many of those that may result in cell death.

41. For instance, at the anaphase stage of mitosis, a proportion of the dicentric aberrations form chromatin bridges linking the two anaphase groups and interfering with the mechanical separation of the two daughter cells. Such an interference frequently results in the death of the cells. In addition, chromosome fragments lacking centromeres may be excluded from the daughter nuclei produced as a result of mitosis, and, depending upon the gene content and amount of material lost, such genetically deficient nuclei may be inviable. In general, therefore, a considerable proportion of the asymmetrical aberrations constitute a short-term hazard in the sense that the cells that carry them have a very much reduced potential for survival. However, those cells that can survive will still be mutant and constitute a long-term hazard both to the individual and, if present in the germ line, to his offspring.

42. On the other hand, symmetrical aberrations, which are simply a consequence of the rearrangement of chromosome material either within or between the chromosomes in the complement, will not result in any chromatin deficiency when induced in somatic cells. Cells carrying such aberrations will encounter no mechanical difficulties in proceeding through the mitotic process and may be perfectly viable. However, if such changes are present in the germ line, then, as a consequence of chromosome pairing and segregation at meiosis, they may result in sterility and in the production of unbalanced gametes. For example, a number of spontaneous translocations are known to exist in man[3] and these are, in certain cases, responsible for a reduced fertility and, in others, for the production of viable offspring with harmful traits, for example, Down's syndrome.

43. It should, therefore, be re-emphasized here that the criteria for scoring aberrations are not based on their particular biological importance. Thus, although the symmetrical aberrations which result in little or no change in chromosome morphology are difficult to score objectively, they nevertheless may constitute a very important long-term hazard, since they result in transpositions and rearrangements of chromatin between non-homologous chromosomes and in duplications and inversions of the genetic materials. Moreover, these aberrations are equally as frequent as their asymmetrical counterparts. At present, the scoring of such symmetrical aberrations in somatic cells is both tedious and extremely inefficient, but their detection might well be improved with the advent of mechanization and computer techniques in cytogenetics.[36-42]

44. The method of classification of the aberrations that has been outlined is that generally in use by radiation cytogeneticists and is based on the structure of the aberrations. This method of scoring does not involve any assumptions as to the precise mechanism of formation of any particular aberration and does not group together aberrations that are structurally different but which may have similar mechanical consequences at mitosis.

45. An alternative system, which does involve assumptions as to the mechanism of formation of aberrations, was originally proposed by Darlington and Upcott[43] in their early work on chromosome aberrations in plant cells. This system, however, was superseded by the descriptive classification. In more recent years, an additional and more general classification has been introduced.[44, 45] This classification places emphasis on the cells carrying the aberrations, cell types being defined, as indicated in the subsequent paragraphs, on the basis of the kinds of aberrations that they contain.

46. *Type A Cells* have no apparent evidence of a structural chromosome abnormality. They may be divided into "modal A cells" that are apparently normal diploid cells and "non-modal A cells" that are aneuploid, that is, that contain fewer or more chromosomes than the normal diploid number.

47. *Type B cells*, as originally defined,[44] included two sorts of cells, those containing chromatid gaps or isochromatid gaps (non-staining regions of the chromosome either affecting one or both chromatids), which are not discontinuities and do not result in the formation of acentric fragments, and cells containing simple chromatid breaks (chromatid terminal deletions) but not isochromatid aberrations nor presumably chromatid minutes. In other words, type B cells, as originally defined, contain either non-staining gaps or one of the many possible types of chromatid-type aberrations. Fortunately, however, the phrase, "type B cells", has come to be used to describe cells containing chromatid-type aberrations of any kind (see reference 33) as opposed to cells carrying chromosome-type aberrations (type C cells).

48. *Type C cells* contain chromosome-type aberrations. Type C cells were originally put into three categories, C_1, C_2, C_3,[44] but later[45] they were reclassified into two categories, C_u and C_s. C_u cells contain asymmetrical aberrations or incomplete symmetrical aberrations, that is, dicentrics (polycentrics), ring chromosomes or fragments. The suffix, u, in C_u denotes the fact that the cell contains an "unstable" aberration which will either result in mechanical difficulties at mitosis or result in a loss of chromosome material in the form of an acentric fragment. C_s cells contain "stable" aberrations, that is, complete symmetrical aberrations (symmetrical interchanges or pericentric inversions), which can only be detected if the exchange events result in a change in the position of the centromere or in the lengths of the chromosomes involved in a rearrangement (paragraph 36).

49. It has already been stressed that the C_s cells can only be detected with very low efficiency. It should also be pointed out that certain types of C_u cells (for example, those containing small terminal or intercalary deletions) may be unambiguously scored as clear C_u cells at the first mitosis after irradiation, but that, if viable, their descendants could well be scored at the second or subsequent mitosis following irradiation as either normal A cells or as C_s cells.

50. The use of the C_s and C_u form of classification may be useful as a short-hand system, particularly when considering the fate of cells carrying aberrations over very long periods of time after irradiation; this is precisely where this general scoring system has been largely employed. Nevertheless, there can be no question that the maximum information can only be obtained when aberrations are categorized on the basis of their detailed structure. The Committee, therefore, strongly recommends that the detailed system of scoring be employed, particularly in those cases where attempts are made to obtain information on dose-response relationships.

C. Chromatid-type aberrations

51. Chromatid-type aberrations occur if damage is sustained either at the time of, or following, chromosome splitting and replication in late G_1 and S of the cell cycle[17, 29, 30] (paragraphs 19-21). In the case of unsplit G_1 chromosomes, any radiation damage sustained is itself replicated when the cell proceeds into S so that the whole chromosome (both chromatids) is involved in a chromosome-type aberration, both sister chromatids being affected in exactly the same way and at identical loci. Chromatid-type aberrations are thus distinguished by the fact that the unit of breakage or exchange is the single chromatid.

52. Chromatid-type aberrations are, therefore, induced in cells irradiated while in the DNA synthesis (S) and post-DNA synthesis (G_2) stages of interphase. Such aberrations also arise spontaneously, presumably as a consequence of replicating errors. They can be readily induced by exposure of cells *in vivo* and *in vitro* to a wide variety of chemical agents and have been found in peripheral blood leucocytes taken from individuals suffering from certain virus infections and in cultured fibroblasts exposed to viral and other infectious agents.

53. A large proportion of the chemical agents that produce mutations in micro-organisms, insects and plants induce chromatid-type aberrations in plant[46-48] and mammalian[47, 49] cells exposed *in vivo* or while in continuous culture *in vitro*. A number of these agents, particularly those known to interact with DNA or to interfere with DNA synthesis (including carcinogenic hydrocarbons such as dibenzanthracene),[50] have now been tested in human cells and shown to produce chromatid-type aberrations in peripheral blood leucocytes and in fibroblasts exposed *in vitro*.[51-70] Similar aberrations are also to be found in leucocytes taken from patients treated for certain clinical conditions with potent mutagens, for example, nitrogen mustard.[71-75]

54. Considerable interest has recently been aroused in the possibility that the hallucinatory drug, lysergic acid diethylamide (LSD-25), might act as a mutagenic agent in man. Cohen *et al.*[76] originally presented evidence indicating that exposure of peripheral blood leucocytes in culture to LSD resulted in the production of chromosome aberrations; in addition, a low, but significantly increased, aberration yield was found in cultured peripheral blood cells taken from a patient extensively treated with this drug over a period of four years.

55. More recent studies on LSD users (which frequently include individuals taking other drugs in addition to LSD) have yielded conflicting results. Some authors[77-79] have reported small, but significantly increased over normal, aberration yields in cultured leucocytes of LSD users, whereas others have found no evidence of a change in aberration yield in LSD users[80, 81] or in patients treated with LSD.[81, 82] Studies on the mouse[83] have yielded suggestive evidence of a slight effect on high doses of LSD on meiotic chromosomes, whereas mutation studies in *Drosophila* have either shown no effects[84] at does levels comparable to those used in the mouse work or significant increases in the yield of recessive lethals when massive, highly toxic, doses were used.[85]

56. The demonstration by a number of workers[86-108] that viral infections may produce chromatid-type aberrations in human and in other mammalian cells and the possible implications of virus infection in relation to carcinogenesis (paragraphs 286-288) has prompted a variety of studies on this aspect of aberration production. A wide variety of both DNA and RNA viruses has been reported as being responsible for the production of chromatid aberrations in human peripheral blood leucocytes and in human and other mammalian fibroblast cells maintained in continuous culture. The viruses that have been claimed to induce aberrations include Sendai virus,[86] chicken pox virus,[87] measles virus,[99, 100, 102] yellow fever,[93] vaccinia,[109] poliomyelitis,[88] the Schmidt-Ruppin strain of the Rous sarcoma virus,[94, 99, 103] herpes simplex,[104, 107] cytomegalovirus,[105] infectious hepatitis virus[87, 88, 95, 96] and various human and simian adenoviruses.[106]

57. Studies on human embryo cells exposed to avian pseudo-plague virus have shown that infection with viable virus results in the formation of chromosome structural changes but that no such changes are produced following exposure of the cells to heat-inactivated virus.[110] In addition, chromatid aberrations have been reported in cultured human fibroblasts infected with *Mycoplasma*[111, 112] and similar aberrations reported[113] in *Drosophila* exposed to Rous sarcoma virus and in other arthropods infected with a "Rickettsia-like" organism.[114]

58. Although there are conflicting reports on the presence or absence of aberrations in cultures of peripheral blood leucocytes from patients suffering from various virus infections,[91, 93, 108] there is no doubt that, under certain conditions, viral and other infectious agents can induce chromatid-type aberrations in human cells. The general conclusion arrived at by many workers in this field is that the effects of these agents on chromosomes are very similar to the effects of chemical mutagens that interfere with DNA synthesis. This conclusion is supported by the observations of Nichols *et al.*[68] of a synergistic action of the Schmidt-Ruppin strain of the Rous sarcoma virus and of cytidine triphosphate (a nucleoside triphosphate that induces chromatid-type aberrations in human cells) in producing chromatid aberrations in human leucocytes treated *in vitro*. Moreover, the aberrations induced by nucleosides and by viral agents are both localized to particular chromosomes and chromosome regions[67, 68] and thus differ from radiation-induced aberrations which are more randomly distributed.

59. It is important to note that Stich and Yohn[106] have recently obtained evidence that, at least in the case of certain types of adenovirus, aberrations are only produced by viruses which initiate but do not complete a full replication cycle. Moreover, it should be pointed out here that chromatid aberrations observed in peripheral blood leucocytes of patients suffering from virus infection are actually produced when the cells are in culture. (Chromatid-type aberrations produced *in vivo* would appear, if the cells were viable, in the first mitosis observed in culture as "derived" chromosome-type changes.) Indeed, it has been suggested[115] that the conflicting reports from different individuals and laboratories may be simply due to positive results being obtained more often when cells are allowed to proceed through more than one cell cycle in culture prior to observation.

60. It should be strongly emphasized that, in the case of the viruses, of the alkylating agents and of the majority of the other chemical mutagens that have been studied, *only* chromatid-type structural changes

have been seen at the *first mitosis* following treatment. The aberrations produced by these agents presumably arise as a consequence of misreplication,[116, 117] so that aberrations are not directly produced in cells exposed while in the G_1 phase of interphase. On the other hand, exposure of such G_1 cells to ionizing radiations results in the formation of typical chromosome-type aberrations.

61. The kinds of chromatid-type aberrations produced by ionizing radiations in S and G_2 cells and by a variety of mutagens, including ultra-violet and the chemical and infectious agents referred to above, are all basically similar and are similar to those that arise spontaneously in culture. However, because the unit of aberration formation is the chromatid, and because sister chromatids remain closely paired at mitosis, these aberrations exhibit a greater variety and are more efficiently detected at mitosis than their chromosome-type counterparts.

62. The variety of possible chromatid-type aberrations has been discussed in detail by a number of authors,[17, 118] and a detailed description and illustration of these aberrations will not be given here. In brief, the aberrations include terminal deletions, intercalary deletions (chromatid minutes), acentric rings, isochromatid deletions, duplications, inversions, interarm asymmetrical intrachanges (centric rings), interarm symmetrical intrachanges (equivalent to pericentric inversions) and symmetrical (reciprocal translocations) and asymmetrical (chromatid dicentrics) interchanges.

63. In addition to these chromatid-type structural changes, cells irradiated in the S or G_2 phases of interphase, or subjected to infectious agents or to certain chemical mutagens, may also contain achromatic lesions that are usually referred to as gaps or erosion zones. These gaps do not represent transverse breaks across the chromatid thread but are simply unstained regions similar in appearance to normal secondary constrictions or nucleolar organizing regions.[24, 119] There is evidence from studies on plant chromosomes that these gaps are reparable lesions that do not result in a permanent structural change in the chromosome.[120, 121].

64. Information on the spontaneous yield of chromatid-type aberrations in human cells has been obtained from studies on peripheral blood leucocytes. Although a number of reports (paragraphs 65-66) have indicated that the frequency of spontaneous chromatid aberrations (more particularly chromatid deletions) in these cells may be somewhat variable (averaging around 0.05 per cell), there is no doubt that much of this variability is due to cells being allowed to proceed through more than one cell cycle in culture before sampling. The aberrations are, therefore, produced in culture (probably as a consequence of misreplication). Culture conditions are extremely important in this connexion, particularly since the cells and cell products themselves contribute to changing conditions.

65. Mouriquand *et al.*[122] in a study of 1,000 leucocytes taken from ninety individuals and cultured for seventy-two hours prior to observation, reported a chromatid-deletion frequency of 0.057 per cell and a chromatid-gap frequency of 0.077 per cell. In a somewhat larger study carried out by Court Brown *et al.*,[123] the frequency of chromatid aberrations, including gaps, in 12,000 leucocytes cultured for seventy-two hours was around 1 per cent. More recently, in a survey[124] in which care was taken to sample cells at their first mitosis in culture, 1,200 leucocytes from 400 individuals were examined, and it was found that the frequency of chromatid-type aberrations in these cells was very low (0.033 aberrations per cell) and did not vary with the age of the donor. Moreover, in this latter survey, it was clearly shown that the frequency of these aberrations increased with increasing duration of the period of leucocyte culture.

66. A number of workers have noted the presence of chromatid-type aberrations in peripheral blood leucocytes irradiated *in vitro* during the G_1 phase and in cells obtained from individuals exposed to ionizing radiations. The frequency of these aberration types in irradiated individuals is very low (for example, around 0.02 aberrations per cell)[125] and is usually similar to the frequency of these aberrations in blood cells of unirradiated personnel. Indeed, studies on patients following radio-therapy treatment,[16,126,127] accidental or occupational radiation exposure[125,127-132] and exposure to radiation following a nuclear explosion[133-135] have revealed no significant differences in yields of chromatid-type aberrations between irradiated and control personnel.

67. On the other hand, there have been some suggestions of a slightly increased chromatid-type aberration yield in irradiated personnel.[136] and, in two instances, reports of much higher yields in cells irradiated *in vitro* and sampled seventy-two to ninety-six hours after exposure.[137,138]

68. Since chromatid-type aberrations cannot be directly induced by the irradiation of unstimulated leucocytes, their presence in such cells is generally agreed to be almost certainly due in part to a possible secondary effect giving rise to these aberrations in culture (and this is particularly true in *in vitro* radiation experiments) and largely to effects occurring in culture that may have no connexion whatsoever with a radiation exposure. It should also be pointed out that, when relatively high yields of "spontaneous" chromatid aberrations are observed, very few exchange aberrations are noted, virtually all the aberrations being simple deletions (see reference 139). This very low frequency of exchange suggests that some of the chromatid breaks that are observed may well be consequences of the mechanical forces operating when the cells are being dried during cytological processing.

69. Chromatid-type aberrations can, of course, be induced by radiation *in vitro* if the radiation is delivered during late interphase.[11, 140-146] Moreover, such aberrations are induced if the cells are exposed to radio-activity labelled DNA precursors.[147, 148] Similarly, these aberration types will be produced *in vivo* in those cells that are actively engaged in proliferation at the time of radiation exposure. However, these chromatid-type aberrations will be lost before cells develop into circulating lymphocytes, or, if the aberrations are symmetrical and therefore do not result in mechanical difficulties in the separation of chromatids at anaphase, they will pass into the daughter cells, proceed through a replication phase and reappear as "derived" chromosome-type changes at the following mitosis (paragraph 74 and figure 3).

70. Since chromatid-type aberrations can only be induced in cells irradiated while in the S or G_2 phases

of the cell cycle, they are clearly quite useless as indicators of dose in cells, such as the peripheral blood leucocytes of man, exposed to radiation while in the G_1 phase. However, these aberrations can be used as dose indicators in normally proliferating cell populations, although it should be noted that the yield of chromatid-type aberrations at any given dose level is very much dependent upon the exact stage of development of the cell at the time of exposure (see reference 149).

71. Detailed studies on cells of the plant *Vicia faba*[121] have shown that the chromatid-type aberration yield induced by x rays in mid-G_2 cells may be three or four times higher than the yield induced in early G_2 cells. Moreover, G_2 cells are more sensitive than S cells, and variations also occur within the S phase. Similar variations have also been observed in mammalian cells irradiated either *in vivo* or *in vitro*. For instance, the yield of chromatid-type aberrations in Chinese hamster fibroblasts receiving doses of 250 rads from cobalt-60 gamma rays *in vitro* was found to be three times higher in cells exposed while in G_2 than in cells exposed while in S.[150] Similarly, data on bone-marrow cells taken from Chinese hamsters that had received 100 rads from x rays[151] (240 kV, 15mA, HVL = 2mm of Cu) or 100 rads from cobalt-60 gamma rays[151] *in vivo* also show that cells in G_2 are much more sensitive than cells irradiated in earlier phases of the cycle.

72. The limited number of studies that have been carried out with human cells on change in aberration yield with change in cell phase all accord with the earlier observations made on plant and other animal cells in showing that changes in response occur with changes in development phase. Most of the studies on radiation-induced chromatid-type aberrations in human cells have either been made on samples observed at only one fixation time after x irradiation[141, 143, 144] or many hours after exposure,[8] or on samples fixed at unspecified times after irradiation. The four studies on peripheral blood leucocytes[142, 144, 146] and on "fibroblast-type" cells in culture,[11] where samples were fixed at various times after irradiation, indicate that, for a given x-ray exposure, the yield of chromatid-type aberrations is higher in G_2 than in S cells. This change in response both within and between cell phases at the chromatid-type level underlines one of the difficulties inherent in the use of these particular aberration types as indicators of dose.

73. The intraphase variation in sensitivity that has been observed for chromatid-type aberrations is not apparent with the chromosome-type aberrations. Studies on plant cells[18, 22, 153, 154] and human peripheral blood leucocytes[34, 115, 155-157] (paragraphs 124-137) indicate that the yield of chromosome-type aberrations is constant throughout the G_1 phase. In the plant and animal studies referred to above, it has generally been found that, at a given dose level, the yield of chromosome-type aberrations is less than the yield of chromatid-type aberrations. The limited comparisons that have been made between the frequencies of those two kinds of aberrations in human peripheral blood leucocytes suggest that a similar pattern exists, the maximum sensitivity occurring in cells irradiated while in G_2.[141, 142, 144]

74. It should be emphasized here that symmetrical chromatid intrachanges (including duplication, deficiencies and pericentric inversions) and symmetrical interchanges will all result in an abnormal monocentric chromosome in one (or in both in the case of interchange) of the daughter cells produced as a result of mitosis. The replication of these abnormal chromosomes will result in the appearance of "derived" symmetrical chromosome-type aberrations at the second mitosis following their induction. Similarly, if asymmetrical chromatid interchanges and chromatid fragments are included in the daughter nuclei, then these also will result in "derived" chromosome-type aberrations appearing at the second mitosis. Such asymmetrical chromatid aberrations (for example, dicentric chromatids) have a finite probability (up to P = 0.5) of being transferred intact to one of the daughter nuclei so that up to one-half of them will inevitably result in "derived" chromosome-type aberrations (figure 3).

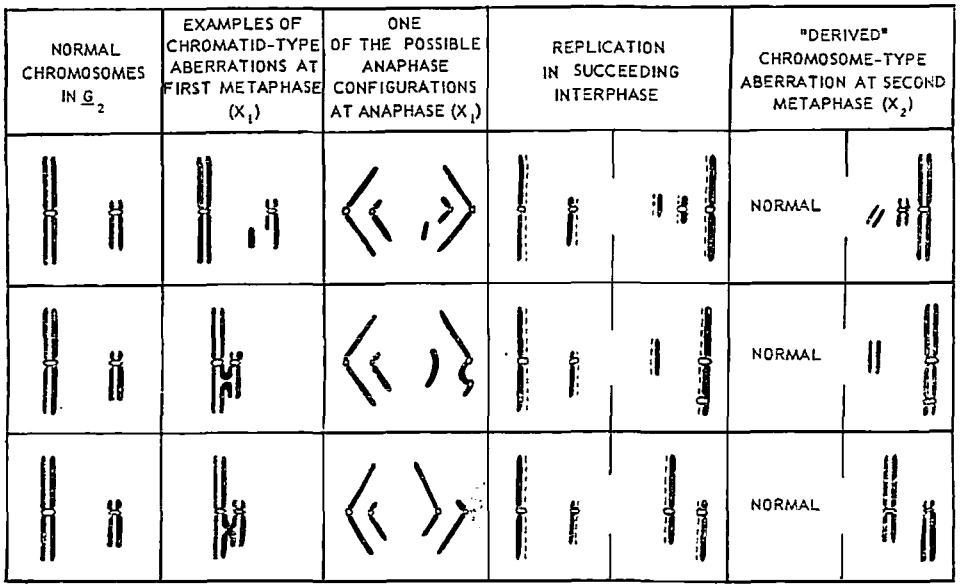

Figure 3. Examples of "derived" chromosome-type aberrations at the second (X_2) mitosis after irradiation[a]

[a] These aberrations have been derived from aberrations that were of the chromatid-type at the first mitosis after irradiation. Note that only a limited number of the possible anaphase configurations are shown and that in many instances an acentric fragment will be lost and may not, for example, be found in association with the chromosome(s) from which it is derived.

D. Subchromatid-type aberrations

75. Subchromatid-type aberrations are exchanges within or between chromosomes which appear to involve a subunit of the chromatid.[17] These aberrations arise spontaneously in meiotic prophase cells of a wide variety of plant and animal species[14] but have not so far been recorded in man. They can be induced by chemical agents, ultra-violet light and ionizing radiations,[17, 46, 48] but they are produced only in cells exposed while in early prophase of mitosis or meiosis.

76. Because subchromatid-type aberrations are relatively infrequent and because they cannot be induced by irradiation of unstimulated peripheral blood leucocytes (these cells being in early G_1), no information on their frequency in irradiated human cells is available. They will, therefore, not be considered further in this review.

E. Aneuploidy

77. In cytological preparations of cells from normal diploid individuals, a small percentage of the cells appears to be deficient for one or more chromosomes (i.e., are hypodiploid) and an even smaller percentage may contain one or possibly more extra chromosomes (i.e., are hyperdiploid). The frequency of such aneuploid cells may vary between individuals as well as between samples taken concurrently from the same individual. A certain proportion of the aneuploid cells is certainly an artefact resulting from cell breakage during cytological processing. However, there is reason to believe that aneuploidy is a natural phenomenon in peripheral blood leucocytes of standard diploid individuals and that its frequency may be related to age and sex of the individual.

78. Cytogenetic surveys on human populations[122, 124, 158-164] have shown that aneuploidy is slightly more frequent in peripheral blood leucocytes of females than of males. In females, this aneuploidy is largely a consequence of the loss of a chromosome in groups 6 to 12 (possibly an X chromosome, presumably the inactive X), whereas, in males, it is largely a consequence of the loss of the Y chromosome. Aneuploidy in blood leucocytes of the new-born may be less than 3 per cent but may reach a value as high as about 13 per cent in adult females and 7 per cent in males. In males, this increase with age is not clearly apparent until around age sixty-five, but, in females, it is apparent a decade earlier. Kerkis *et al.*[162] have suggested that this increase of aneuploidy with age may be a consequence of differences in the response of cells to hypotonic treatment in culture.

79. It has been shown[122] that the incidence of aneuploidy in any given cell culture increases with increasing culture time as a consequence of cells undergoing more than one mitotic division in culture. Reference has already been made to the fact that the presence of chromosome structural changes in cells will frequently lead to chromosome loss, and this is to be expected particularly with aberrations that may result in bridges at anaphase. Thus, the presence of even a low frequency of spontaneous or radiation-induced aberrations at the first division of the cells in culture will lead to an increase in hypodiploidy in the daughter cells observed at the second and subsequent mitoses.

80. In addition to the increasing frequency of hypodiploidy with increasing age, it should be mentioned that, in rare instances, certain individuals may contain two or more cell lines differing in chromosome number as a result of chromosome loss or gain occurring in the early stages of development of the individual. These particular instances of chromosome mosaicism are not entirely relevant here, since the majority of such cases are to be found in individuals having a cell line possessing one or more chromosome additional to the normal diploid chromosome number or, in abnormal females, containing a proportion of cells lacking an X chromosome.

81. A number of workers have reported an increased incidence of aneuploidy (hypodiploidy) in peripheral blood leucocyte cells obtained from individuals exposed to radiation,[44, 128, 129, 165-168] and similar increases have been reported in *in vitro* studies.[136, 138, 169-172] Thus, two groups of workers[136,138] suggest, on the basis of their *in vitro* data, that the incidence of aneuploidy increases linearly with increasing x-ray exposure, at least up to a certain dose. It should be pointed out, however, that in all these studies, the cells sampled had been allowed to grow in culture for seventy-two hours or more so that in many cases cells in their second and third division in culture were being sampled (paragraphs 124-137).

82. In some of the earlier[12, 173, 174] and in most of the more recent studies,[16, 127, 132, 133, 135] no differences have been found in the incidence of aneuploidy in blood leucocytes from irradiated individuals or in cells irradiated *in vitro* as compared with controls. Buckton *et al.*[16] quote a mean value of 3.8 per cent aneuploidy for fifty-three patients treated with x rays for ankylosing spondylitis and a value of 3.7 per cent in unirradiated control patients. Similar values of 3 per cent were noted by Ishihara and Kumatori[175] in their Thorotrast patients and in controls, although rather higher values of around 10 per cent in both control and irradiated personnel were found by Visfeldt.[127]

83. There seems little question that many of the hypodiploid cells that have been noted in radiation studies were either cells that were carrying chromosome structural changes, such as dicentrics, or were more probably cells that had contained aberrations but had proceeded through more than one mitosis in culture and had lost aberrant chromosomes (for example, dicentrics or centric rings) at their first division. The incidence of aneuploidy cannot, therefore, be simply correlated with a previous radiation history and cannot be used in a quantitative manner as an indicator of radiation absorbed dose.

84. In genetic terms, the loss of a normally genetically active chromosome from the complement is serious, and, if it does not result in the death of the cell, clearly constitutes a mutation. The presence of aneuploid cells in the germ line would lead to the formation of inviable zygotes, except in the case of the loss of one sex chromosome (as discussed in the 1966 report[3] of the Committee) or in very rare instances of the loss of a chromosome in group G.[176-178]

F. Polyploidy

85. Many workers have noted that irradiation *in vivo*[15, 44, 126, 179, 180] or *in vitro*[136, 140, 173] may result in an increased incidence of polyploidy in peripheral blood leucocytes. Thus, Kelly and Brown[136] reported that, with x-ray doses of up to 400 rads *in vitro*, the incidence of polyploidy increased in proportion to the square of the x-ray dose; these authors used culture times of

seventy-two to ninety-six hours. Other authors, for example, Fischer *et al.*[179] in their work with Thorotrast patients, although noting an increase in polyploidy in blood cells of irradiated patients, have found no quantitative relationship between the frequency of polyploidy and radiation dose. On the other hand, in some of the other early work, both *in vivo*[128, 129] and *in vitro*.[125] there appeared to be no association whatsoever between radiation exposure and an increase in polyploidy.

86. More recent work has shown that few or, quite often, no polyploid cells are observed following *in vivo*[45, 181, 182] or *in vitro*[115, 183] irradiation, provided the leucocytes are cultured for no more than forty-eight to fifty-four hours. However, if cells are cultured for longer periods of time, then polyploid cells appear (even in unirradiated samples), and their incidence increases with increasing culture time up to sixty-eight hours.[115]

87. It has been noted[45, 182, 183] that a very high proportion of polyploid cells contain aberrations, particularly chromosome dicentrics and centric rings, in pairs (i.e., the aberrations have been duplicated). In the studies of Ishihara and Kumatori[182] on the incidence of polyploidy in leucocyte cultures irradiated *in vitro* and sampled seventy-two hours or ninety-six hours later (that is, cells in their second, third or fourth mitosis after irradiation—paragraphs 124-137), it was found that the tetraploid and octoploid cells consistently contained a higher frequency of chromosome aberrations than the diploid cells in the cultures: that is, the number of pairs of identical aberrations in the polyploid cells was greater than the number of single aberrations found in an equivalent number of diploid cells.

88. In leucocyte cultures sampled ninety-six hours after an exposure to 350 roentgens of gamma rays, the same authors found that all the polyploid cells contained pairs of aberrations (mainly dicentrics, tricentrics, and rings), whereas only one-third of the diploid cells contained aberrations of any sort. This kind of observation suggests that polyploidy in these cases is largely a consequence of the presence of chromosome aberrations. Asymmetrical aberrations will interfere with the separation of sister chromatids at mitosis, and interlocked chromosomes and chromosome bridges will prevent a clean separation of the anaphase groups. Thus, the nucleus may not be allowed to complete its division but passes into interphase in its doubled state and re-emerges as a polyploid nucleus at the next division.

89. It is clear from these considerations that the bulk of the observed polyploidy is probably a consequence of the mechanical difficulties arising from the presence of asymmetrical aberrations in cells that are allowed to proceed through a number of divisions in culture. Polyploidy is, therefore, a secondary phenomenon, and, since its incidence will vary not only with dose but also with the number of mitotic cycles completed *in vitro*, it cannot be used as a reliable indicator of absorbed radiation dose. It is probable that polyploidy in somatic cells may be of little significance in terms of somatic hazards (for instance, polyploidy exists as a natural phenomenon in a proportion of normal adult liver cells), although there is no direct information on this point. Polyploidy in primitive germ cells (meiocytes) will, however, result in the formation of unbalanced gametes.

G. Endoreduplication

90. The presence of cells containing endoreduplicated chromosomes has, on occasion, been noted in peripheral blood leucocyte cultures exposed to x irradiation.[140, 146, 184, 185] As in the case of polyploidy, cells showing endoreduplication are sometimes found in untreated leucocyte cultures, and the process of endoreduplication can be facilitated by exposure of the cells to certain spindle inhibitors, such as colcemid.

91. No relationship has been found between radiation dose and the frequency of endoreduplication.[140, 146, 184, 185] Moreover, it is clear that endoreduplication necessitates two or more cycles of chromosome replication in culture and is, therefore, rarely observed in leucocytes cultured for forty-eight hours, whereas it increases in frequency with increasing culture time.[140, 186] The incidence of endoreduplication has no real merit as an indicator of absorbed radiation dose.

H. Conclusions

92. The aberrations that have been described must now be considered from the two viewpoints of their importance in relation to genetic hazards, particularly in somatic cells, and of their use in providing a measure of absorbed dose. These aspects will, of course, be considered in detail in the later sections, but there are a number of both general and specific points that can best be made at this time.

93. It is clear from the description of the aberrations that all result in some kind of genetic change and that the majority result in genetic deficiencies. If the deficiencies are small, they may be tolerated. The amount of loss that can be tolerated will depend both upon the nature of the genetic information lost and upon the normal destined role of the cell in the body. Genetic deficiencies in stem cells will, in general, be of far greater importance than similar deficiencies in cells that were undergoing differentiation.

94. Many of the asymmetrical aberrations will be cell-lethal so that their consequences are more or less immediate. Cell death may follow, either as a direct consequence of the loss of genetic information in the form of acentric fragments or even whole chromosomes, etc., or as a result of the mechanical difficulties that occur at mitosis. Aberrations in resting cells may play no role until the cells are stimulated to undergo mitosis. Only in those tissues that normally contain proliferating cells, therefore, will chromosome aberrations be a significant contributory cause of the cell depletion that occurs very shortly after radiation exposure. However, only a proportion of the aberrations will result in early cell death, since the presence of certain structural changes, such as the symmetrical aberrations described, does not result in a rapid lowering of cell viability. If symmetrical aberrations are produced in germ-cell precursors, they may result in genetic imbalance in the gametes, leading either to dominant lethality in the embryo or to drastic effects in the resultant offspring. It is clear, therefore, that the aberrations that will contribute to long-term hazards in both somatic cells and germ cells are essentially the small deficiencies and symmetrical changes (duplications, inversions and reciprocal translocations) that are difficult to score efficiently and are not cell-lethal.

95. In considering the various kinds of chromosome aberrations produced by ionizing radiations (and by other agents), it has already been indicated that certain types of aberrations may offer a more useful index than others in the context of their possible application as biological indicators of absorbed dose. Aneuploid and polyploid cells produced following radiation exposure have been shown to arise largely as a secondary consequence of the presence of chromosome structural changes. Moreover, the presence of these abnormal cell types depends upon the fact that cell division must intervene between the time of radiation exposure and the time of observation. These particular anomalies are, therefore, not only less frequent than the chromosome structural changes that give rise to them, but also highly variable in their frequency, and must be considered as very inferior biological endpoints relative to the chromosome structural changes.

96. A vast amount of information exists on the relationship between radiation dose and the yield of the two kinds of chromosome structural changes (i.e., chromosome-type and chromatid-type aberrations) in a variety of plant and animal cells. This has shown[17, 22] that, for a given quality of radiation, there exists a strict relationship between aberration yield and absorbed dose and that, for certain aberration types, the yield is markedly dependent upon the dose rate and the stage in development of the cell at the time of irradiation. Detailed studies, particularly with plant materials, have shown that, for a given cell type and known sampling time after irradiation, the variation between individuals is small and that different observers score similar aberration yields when materials are exposed under similar conditions. Indeed, trained observers using materials such as *Tradescantia* microspores and *Vicia faba* root-tip cells can, through determining aberration yield, estimate doses to within a few per cent.

97. The experience and information obtained on chromosome damage in species other than man suggest that a similar strict relationship between radiation dose and chromosome aberration yield should also apply to human cells, and *in vitro* radiation studies strongly indicate that such a relationship, in fact, exists. Moreover, there is every prospect that, in man, the variation in response at the chromosome level between different individuals will be of the same order as the small variation observed between individual plants, and preliminary studies indicate that this is, in fact, the case.

98. It has been stressed earlier that chromatid-type aberrations are only induced by radiation when cells are exposed while in the S or G_2 phases of interphase. These aberrations are, therefore, of little use as dose indicators in the case of peripheral blood leucocyte cells existing in a normal G_1 state, but they can be used in normally proliferating cells. However, it should be emphasized that great care must be exercised in using such aberrations, since, even within the confines of the G_2 (or S) period, the yield of chromatid-type changes is very markedly influenced by the degree of development of a cell *within* a given interphase stage. No such dependence has been noted in relation to chromosome-type aberrations induced in G_1 cells.

99. In addition to the constant sensitivity of G_1 cells to chromosome-type aberration induction by radiation, these particular aberration types have an added advantage as possible indicators of radiation-absorbed dose since their spontaneous frequency is extremely low. A number of cytogenetic surveys on human populations, in which chromosome analysis has been carried out on peripheral blood leucocytes taken from hundreds of individuals, have shown that the presence of an asymmetrical chromosome-type aberration is an extremely rare event. For instance, an analysis of some of the available data (table I) indicates that a dicentric aberration occurs, at most, about once in a sample of about 2,000 cells, and possibly its frequency may be even less than one in 8,000 cells.

100. It is evident from the foregoing considerations that, on general cytological grounds, chromosome-type aberrations are superior to other forms of chromosome aberrations in terms assessing absorbed dose. It should also be noted that the simplest and most convenient source of human material for studying aberration yield following *in vivo* exposure are the peripheral blood leucocytes and that the aberrations that are induced in these cells by ionizing radiations are chromosome-type aberrations.

101. It has already been concluded that the frequency of chromatid-type aberrations in cultured peripheral blood leucocytes may bear no relationship to the radiation exposure of an individual, and it has been pointed out that these aberrations may arise spontaneously in culture or be produced in culture following virus attack or exposure to certain chemical agents. It should be added here that the aberrations that may be induced by virus attack will not influence the yield of chromosome-type aberrations, provided that only cells in their first division in culture are sampled. One can feel fairly sure on this point, since the frequency of chromosome-type aberrations in blood cells of individuals previously exposed to a virus infection is no higher than in unexposed individuals.

102. The fact that (paragraph 74) chromatid-type aberrations seen at the first mitosis following irradiation can result in "derived" chromosome-type changes at the second division will be of importance if cells are allowed to proceed through more than one division in culture. The relative importance of such "derived" changes will depend markedly on dose and will be small when the yield of true chromosome-type aberrations is high but will be very important when the yield of true chromosome-type changes is low.

103. Although the possible complications introduced by chromatid-type aberrations and their "derived" chromosome-type counterparts is obviated if only cells in their first mitosis after irradiation are sampled (paragraphs 124-137), we should note that the exposure of individuals (as opposed to cells *in vitro*) to certain chemical agents[187] may well result in increased frequency of chromosome-type aberrations in their peripheral blood leucocytes. It is important, however, to recall that leucocytes (small lymphocytes) carrying chromosome-type aberrations may survive in the body for long periods (up to many years). This complication of *in vivo* effects of chemical mutagens may only be important in relation to individuals that have been treated for certain clinical conditions with potent chemical mutagenic agents,[71, 75] for example, nitrogen mustard.

104. Finally, it should be stressed that, when attempts are made to obtain information on absorbed dose through scoring chromosome-type aberration yields, it would be valuable if the aberrations were

classified in detail in the form described in paragraphs 25 to 34.

III. Materials and methods of study

A. Introduction

105. Any tissues containing cells that are normally involved in proliferation or cells that can be made to proliferate by various means can be utilized for chromosome analysis. Chromosome studies on mammalian cells have been carried out on a variety of proliferating tissues, including skin, intestinal epithelium, corneal epithelium, bone marrow, various lymph nodes, spleen, thymus and gonads (particularly testis). In addition, certain cells from tissues that do not normally proliferate in the adult animal can be made to undergo mitosis; this has been done mainly with liver tissue and blood. Mitotic divisions occur in liver cells when the liver regenerates following partial hepatectomy, and blood leucocytes can be stimulated by various means to proceed into a mitotic phase in short-term *in vitro* cultures.

106. All the above cells or tissues have been used by various workers in studies on radiation-induced chromosome aberrations. In laboratory mammals, bone marrow, lymphatic tissues, corneal epithelium, liver cells, peripheral blood leucocytes and gonads have been the principal tissues used. In man, the great majority of the work has been carried out with peripheral blood leucocytes, and some information has been obtained using bone marrow and skin.

107. Chromosome preparations can be made from cells that are proliferating *in vivo* without the necessity for *in vitro* culture, and direct preparations made in this way exclude the possibility of adverse effects arising during *in vitro* culture. However, in the case of man, the only tissue from which a sufficient number of dividing cells can be directly obtained without recourse to surgery is the bone marrow. Mitotic cells can, of course, be obtained from skin, but to obtain sufficient cells of good cytological quality from small samples of skin requires *in vitro* culture.

108. Excellent techniques[188, 189] exist for obtaining direct chromosome preparations for human bone-marrow cells, but, to obtain good quality spreads, the cells must in all cases be exposed to certain pre-fixation treatments, including treatment with colchicine and hypotonic saline so that some handling of the living cells *in vitro* is required. In the case of human skin, the standard technique (see reference 190) involves setting up primary cultures and making cytological preparations from outgrowing fibroblasts some days after culture initiation.

109. One major disadvantage of the use of bone marrow and skin cells in terms of their possible use in "aberration dosimetry" is the fact that these tissues consist of populations of asynchronously developing cells. Thus, when an individual is exposed to radiation, bone marrow and skin cells in all stages of development in the mitotic cycle will be irradiated. This means that both the type and yield of chromosome aberrations will change quite rapidly with time even in the first few hours after exposure.

110. Information on the sensitivity of man's proliferating cells (both somatic and gonadal) to radiation-induced chromosome damage is of the utmost importance in relation to assessing hazards. However, it is clear that, except under very exceptional circumstances, for example, where serial-marrow aspirates can be taken shortly after irradiation and when the time of exposure is accurately known, the idea of using chromosome damage in these particular cell systems as a general means of estimating absorbed dose cannot be entertained.

111. Because of the change in radiation response with cell-development phase, cells that are exposed to radiation while in a resting phase and are then stimulated under controlled conditions to proceed into mitosis offer the best possible system for radiation dosimetry from the cytological viewpoint. Although regenerating liver cells fall into this category, clearly their use in man must be ruled out. However, the development of a technique (paragraphs 113-123) for culturing peripheral blood leucocytes and stimulating them to undergo mitosis in short-term culture has provided a most suitable cytological system. The peripheral blood leucocyte culture technique is simple and reliable. By using this technique, large quantities of mitotically active cells can be obtained quickly and painlessly and, if required, large numbers of samples can be taken from any one individual without causing bodily injury or suffering.

112. Peripheral blood leucocytes from normal healthy individuals do not usually undergo mitosis in peripheral blood vessels, and exposure of these cells to tritium-labelled thymidine[191-193] reveals that less than one cell in 1,000 undergoes DNA synthesis. It has been established [193-195] that these leucocytes rest in an early interphase or G_1 state so that, following radiation exposure, they contain chromosome-type aberrations if and when they appear at mitosis. Moreover, since these cells are all in the same stage of development, the variation in response between cells should be minimal. Since most of the work on radiation damage in man's chromosomes has been carried out with these cells, and because some differences between results obtained in different laboratories have emerged, some of the details and various modifications of the techniques used will now be briefly considered.

B. The peripheral blood culture techniques

113. A number of recent articles[196, 197] have detailed the basic principles of the leucocyte culture technique and have described the earlier developments by Osgood and his colleagues[198, 199] and by Nowell[200, 201] that culminated in the successful use of this system for human cytogenetics.[14, 202] In this brief account, therefore, we shall be concerned only with the general principles and with certain specific variations in methodology that have been used in the studies on radiation-induced chromosome damage to be considered in the succeeding sections.

114. The culture of peripheral blood leucocytes involves the introduction of leucocytes, either following their separation from the other blood elements or simply in whole heparinized blood, into a tissue-culture medium containing a mitotic stimulant. The tissue-culture media used contain a standard, defined, synthetic medium (such as TC medium 199) with antibiotics plus serum (or plasma), the serum making up from 10 to 40 per cent of the total volume (usually about 6-10 ml). The serum used may be autologous or homologous (usually AB) human serum, or fœtal or adult bovine serum.

115. The mitotic stimulating agent (or mitogen) normally employed is the plant mucoprotein phytohemagglutinin (PHA), and it is clear that the cells that are stimulated to transform into blast cell types under the action of PHA and then to proceed into mitosis are the small lymphocytes.[192, 203, 204] Although PHA is the mitogen that has been used in almost all radiation work, another plant extract from the pokeweed (*Phytolacca americana*) is also effective.[205]

116. The fact that PHA stimulation appeared to be restricted to the immunologically competent cell was partly responsible for the work that led to the finding that small lymphocytes from donors sensitive to a particular antigen may be stimulated to transform into blast cells and divide in the presence of this antigen.[206-208] In fact, this conversion to blast cells also occurs when lymphocytes from different donors are mixed in culture in the absence of any plant mitogen.[208, 209]

117. The amount of heparinized blood used for a single culture may be as little as a fraction of a millilitre ($\sim$ ¼ ml) if whole blood is inoculated into a culture vessel containing around 5 millilitres of culture medium, giving a so-called microculture; or, leucocytes may be separated from samples of around 5 to 10 millilitres of whole blood and approximately 107 cells inoculated into a culture bottle containing 5 to 10 millilitres of culture medium.

118. In cultures containing PHA and incubated at 37°C, the small lymphocytes are stimulated to undergo RNA and protein synthesis, enlarge in size and proceed through a DNA-synthesis phase and thence pass into mitosis. The first cells to reach mitosis do so after thirty-six to forty hours in culture. At forty-eight hours, a considerable number of cells are in their first division in culture (paragraphs 124-137). We should note, however, that temperature is, of course, a very important factor in influencing the rate of cell development and that fluctuations of as little as 1°C have marked effects. Moreover, there may be other, as yet undefined, factors that may influence the cell development rate.

119. The cells that are stimulated to pass into mitosis may go through a series of cell cycles so that the maximum number of cells in division in the culture are usually to be found approximately seventy-two hours after culture initiation. The cultures, however, are strictly short-term and cannot be maintained indefinitely, since there is a continued decline in the number of viable mitotic cells after the first few days. In a large proportion of the laboratories that carry out human cytogenetic studies, peripheral blood leucocytes are cultured for seventy-two hours so as to obtain the maximum number of divisions. This practice has also, unfortunately, been the custom in much of the work in radiation cytogenetics.

120. Up to four hours (or more in certain cases) prior to termination of a culture, a small amount of colchicine or diacetylmethylcolchicine ("Colcemid" —around 0.05 μg ml^{-1} of medium) is added to accumulate cells at the metaphase stage of mitosis. After an appropriate time, the culture medium is removed, following centrifugation (5 or 10 minutes) at about 25 to 100 G, and the cells resuspended in a hypotonic solution such as 1 per cent sodium citrate[3] or a 0.75 molar solution of KCl for a few minutes before they are fixed in acetic alcohol.[210] Suspensions of the cells in acetic alcohol are dispensed as drops onto clean microscope slides and allowed to dry, the cells flattening out during the drying process. Most laboratories have their own variations on the general cytological technique outlined here, but the end result is the production of a number of slides containing scores of well spread metaphase cells from each culture. These cells are usually stained with acetic orcein or Giemsa, etc., according to individual preferences.

121. Although first class preparations are required for accurate scoring, the variations between laboratories in their detailed cytological procedure after fixation may not be of great importance in the context of influencing the frequency of the chromosome aberrations that are eventually scored in metaphase cells. However, it has been suggested [211, 212] that variations in the techniques of culture (for example, the question of type of serum used and the use of whole blood or leucocytes separated by sedimentation or by centrifugation) may be important, since different aberration yields have been obtained in *in vitro* studies carried out by laboratories using slightly different techniques (paragraphs 154-170).

122. In most of the work on chromosome aberrations induced following *in vivo* radiation exposures, leucocytes have been separated from blood samples either in buffy coat, namely, following fairly high speed centrifugation ($\sim$ 3,000 rpm in a clinical bench centrifuge, or around 1,800 G), or by low-speed centrifugation ($<$500 rpm or about 25 G) and/or gravity sedimentation with or without the presence of an agglutinating agent. Separated leucocytes have also been used in many of the *in vitro* studies, whereas in other *in vitro* work small samples ($\sim$ 0.3 ml) of whole blood have been used to set up microcultures.[213]

123. It is not yet known whether there is any selective loss of cells suffering from radiation damage when high speed centrifugation is used. At equivalent dose levels, however, aberration yields are significantly higher in laboratories using the microculture technique following *in vitro* irradiation of whole blood than in laboratories using cells obtained following buffy coat separation (paragraphs 154-183). Recent comparisons[214, 215] have indicated that there is no difference in aberration yield between leucocytes separated by gravity sedimentation and leucocytes cultured as part of a whole blood inoculum. Direct comparisons between each of these techniques carried out within the different laboratories are certainly required.

C. Culture sampling time

124. It has already been indicated that chromosome-type aberrations in peripheral blood leucocyte cells offer the best cytological combination, if aberrations are to be used for biological dosimetry. Consideration should, therefore, now be given to the influence of culture sampling time on the yield of these aberrations, since in recent years it has been clearly shown that the yield of chromosome-type aberrations declines, as is, of course, to be expected, with increasing leucocyte culture time.

125. Most of the published data on chromosome-type aberration frequencies in human leucocytes cultured *in vitro* have been obtained from cells that were allowed to grow in culture for seventy-two hours before fixation and slide preparation. As has already been mentioned, however, it has been known for some

time that a number of cells enter into mitosis as early as thirty-six hours after culture initiation and that a high proportion of the cells can be seen in division at forty-eight hours. The presence of mitotic cells in culture at forty-eight hours after culture initiation was noted in some of the early studies by Nowell[201] and also by Bender and Prescott.[195] More recent work has now made it quite clear that, at seventy-two hours after initiation, a majority of the mitotic cells may be in their second or third division in culture. It is, in fact, now evident that the cell-cycle time between successive mitotic divisions in culture is about twenty to twenty-four hours. [62, 115, 216, 217]

126. It was established by Buckton and Pike[45, 181] from their studies on patients exposed to x rays for treatment for ankylosing spondylitis that the frequency of chromosome-type aberrations in the blood cells of these patients varied according to the duration for which the cells were allowed to grow in culture. Cells observed after seventy-two hours were found to contain fewer aberrations than cells from the same blood sample that were allowed to grow in culture for only forty-eight hours. In cultures fixed after seventy-two hours, tetraploid cells were present, and these cells contained duplicated aberrations. Such duplicated aberrations were not observed at the earlier times, tetraploid cells being rare or absent in cultures that were allowed to grow for only forty-eight hours.

127. Observations similar to those of Buckton and Pike were reported by Ishihara and Kumatori[175] from their studies on blood cells obtained from Thorotrast patients and from *in vitro* studies on x-irradiated blood samples obtained from normal individuals. These latter authors observed that the yields of aberrations in both *in vivo* and *in vitro* studies were twice as high in cells harvested after forty-eight hours as they were after seventy-two hours in culture and roughly four times as high as the aberration yields in the same cell populations after ninety-six hours in culture. Moreover, their later studies[182] on the incidence of polyploidy are also in accord with the observations of Buckton and Pike. Similar observations of a decline in aberration yield with increasing culture time were made by Nowell,[218] although in his studies the longer culture times of seventy-two and 120 hours were used.

128. In summary, the work at Edinburgh in the United Kingdom and at Chiba in Japan showed (*a*) a reduction in the yield of chromosome-type aberrations with increasing period of culture from forty-eight to seventy-two hours; (*b*) the virtual absence of polyploid cells at forty-eight hours but their presence in high frequency at seventy-two and ninety-six hours; and (*c*) the presence of duplicated aberrations in many of the polyploid cells. These observations led inescapably to the conclusion that a considerable proportion of the cells observed in samples cultured for seventy-two hours and ninety-six hours were cells in their second (X_2) or later (X_3, X_4, etc.) divisions in culture. This conclusion was later confirmed by workers in the Soviet Union[217] who showed that many of the cells observed at metaphase after seventy-two hours in culture at 37°C were in their third mitosis.

129. The reduction in the frequency of chromosome-type aberrations with increasing culture time follows from the fact that the aberrant chromosome structure may be lost at anaphase of mitosis and that a proportion of the cells carrying aberrations will, therefore, be unable to participate in any further mitotic activity. For instance, acentric fragments tend to be excluded from anaphase groups at mitosis so that both daughter cells, if viable, may be difficult to distinguish from normal cells when they divide at the second division in culture, particularly if the fragments are very small.

130. In the case of dicentric- and centric-ring aberrations, a proportion of these structural changes will result in anaphase bridges so that the aberrations and, in most instances, the cells carrying them, will be lost from the dividing population. Recent studies by Norman and his colleagues[34, 218] on aberration yields in cells cultured for fifty hours and seventy-two hours indicate that the probability of loss of a dicentric is 0.5 per division. Although aberrations and cells carrying aberrations may be lost, undamaged cells will proliferate normally and will, therefore, comprise an ever-increasing proportion of the cell population as culture time increases.

131. Attention was earlier drawn to the fact that by far the majority of workers have used seventy-two-hour culture periods in their *in vivo* and *in vitro* studies and that most of these quote the autoradiographic data of Bender and Prescott[194] as demonstrating that cells observed at mitosis after seventy-two-hours in culture are cells in their first mitosis in culture. In fact, Bender and Prescott stated that "the cells are in their first post-labelling division at seventy-two hours" when the cells were exposed to tritium-labelled thymidine for thirty minutes after forty-eight hours in culture. In their experiment, it was clearly pointed out that "numerous mitoses accumulated (by colchicine) between forty-two and forty-eight hours in culture", and the authors refer to "the first wave of mitoses" occurring at this time. From the recent extensive data of Sasaki and Norman[216] and of Heddle, Evans and Scott,[115] it would appear that the first post-labelled mitoses seen at seventy-two hours may well have been cells in their second mitosis in culture, the majority of the cells being exposed to label in the interphase period following the first mitosis in culture.

132. In studies of Sasaki and Norman,[216] cultures of separated leucocytes were exposed to tritium-labelled thymidine after various times during culture and then sampled at various fixation times to determine the frequency of labelled mitotic figures and the patterns of label over the chromosomes. In addition, cells were also x irradiated, and the frequencies of polyploid cells and of cells containing doubled sets of acentric fragments were studied after culturing for either fifty hours or seventy-two hours. The results obtained with these four different parameters showed that, at seventy-two hours, 70-80 per cent of the cells were in their second mitosis in culture, whereas there were no indications of second division cells being present after fifty hours in culture.

133. In the work of Heddle *et al.*,[115] the mitotic index, the incidence of polyploidy and the yield of chromosome-type aberrations were studied using a whole blood microculture technique. These three parameters were scored in a series of cultures grown for periods of from thirty-six to 100 hours. The cultures were terminated at successive four-hour intervals throughout this period, and the cells were subjected to a four-hour colchicine treatment prior to fixation. This technique made possible an effectively continuous sampling of all cells from the time of first appearance of mitosis in culture up to sixty-four hours later. The results showed that first division cells were observed

up to fifty-two hours and that a small proportion of second divisions appeared at around sixty hours. At approximately sixty-four hours, a significant proportion of the cells were in their second division, and, at seventy-two to seventy-six hours, by far the majority of cells were in their second or even third divisions with only a few first division cells present.

134. The data of Heddle *et al.*[115] show that, in cultures irradiated *in vitro* with x rays (150 rad) and sampled at seventy-two hours, the yield of dicentric and ring aberrations was approximately half the yield found in similar cultures grown for up to fifty-six hours. Furthermore, it was shown that this culture time of seventy-two hours was at a transition point between a peak of mitotic activity (due to second divisions) occurring at sixty to sixty-four hours and a later peak (due to third divisions) occurring at seventy-six hours.

135. One of the reasons contributing to the use of seventy-two hours as a standard culture time was the possibility that irradiated cells were delayed in their progression through the cell cycle. It is well known that irradiation can result in mitotic delay in proliferating cells but that the amount of delay depends, amongst other things, on the stage of development of the cells at the time of irradiation. For instance, it has been reported[219] that x irradiation of human fibroblast-type cells in tissue culture results in virtually no delay at the first post-irradiation mitosis of cells irradiated in early G_1 but in a considerable delay in the development of cells irradiated while in late G_1, S or G_2. The recent data of Sasaki and Norman[216] on blood cells given a dose *in vitro* of 500 rads from x rays and of Heddle *et al.*[115] on microcultures given a dose *in vitro* of 150 or 300 rads from x rays have indicated that little or no mitotic delay occurs at these dose levels.

136. The data of Evans[155, 156] show that the response of the peripheral blood leucocytes to x-ray-induced chromosome damage does not change with development of the cells through the G_1 phase in culture. But these data, obtained from cells sampled at one fixation time, do not preclude the possibility that more than one cell population with differing radiosensitivities may be present in culture. There is not a great deal of information available on this point, but the data of Norman[34] and of Heddle *et al.*[115] show that there is no difference in aberration yield between cells that undergo an early, as opposed to a late, transformation to blast cell types. The available observations, therefore, suggest that, if there is a variation in the average rate of development of blood cells in culture between different individuals, and if there is some indication that certain blood donors may be "slow growers",[115] then this may be of little consequence provided that only first division cells are sampled for aberration yield.

137. This point of cell cycle times has been considered at some length, since, for comparing quantitative data on aberration yield, it is clearly of the utmost importance to ensure that aberration frequencies are determined using only first division cells. On the information that is at present available, this would necessitate the use of cultures grown for around forty-eight hours at 37°C. We should note, however, that at least one laboratory[45] has reported the presence of a proportion of cells in their second mitosis in cultures exposed to colcemid in their final three hours and terminated at forty-four to fifty-two and a half hours. This has led other workers[216, 219, 221] to expose fifty-hour cultures to colcemid for twenty-four hours prior to fixation in order to prevent cells from proceeding into a second mitosis in culture. The duration of colcemid or colchicine prefixation treatment may, therefore, be an additional factor to consider in conjunction with duration and other conditions of culture.

D. Conclusions

138. Chromosome analysis in man can be carried out quite readily on cells from three sources, namely, skin, bone marrow and peripheral blood. For qualitative work, cells from each of these three sources can be used, and a number of studies have, in fact, been made on the persistence and proliferation of cells containing symmetrical aberrations in bone marrow and in blood. In these studies, the presence of clones of cells derived from an original single cell containing a radiation-induced symmetrical aberration have been noted in individuals studied many years after exposure to ionizing radiations.[134, 222-224] The possible importance of such persistent symmetrical aberrations as long-term somatic hazards is considered later.

139. At the quantitative level, where the question of using the aberrations produced in proliferating cells to estimate absorbed dose is concerned, various physical and general biological problems arise. These problems will be considered later, whereas this section has been confined to the relative cytological merits of the various proliferating cell systems. From this discussion, it is clear that, from the cytological viewpoint, as well as because of the ease and simplicity of obtaining single or repeated cell samples, the peripheral blood lymphocytes are far superior to bone-marrow or skin cells for quantitative work.

140. These peripheral blood lymphocytes exist in a uniform stage of development in G_1 so that only chromosome-type aberrations are produced in them following radiation exposure. The more complex chromatid and subchromatid-type aberrations that will be induced in a proportion of the skin and bone-marrow cells are, therefore, normally absent. However, some chromatid-type aberrations are observed in irradiated lymphocytes, but these have been shown to arise during cell development in culture, and their relatively low frequency does not raise complications in the scoring of the chromosome-type changes if only cells in their first post-irradiation mitosis are scored.

141. Despite the apparent simplicity of the peripheral blood leucocyte system, certain differences in *in vitro* response have been observed between different laboratories (paragraphs 154-183). Some of these differences are a consequence of the use of radiations of differing qualities (paragraphs 154-177) but, from the discussion of the methods of leucocyte culture used in various laboratories, it is evident that there are at least two other factors of importance.

142. First, it is clear that different aberration frequencies are observed if cultures are allowed to grow for various periods of time in excess of fifty-four hours and that this is almost certainly a consequence of the appearance in culture of second and subsequent mitoses which result in increasing the proportion of undamaged cells. The usual standard fixation time of seventy-two hours used in many laboratories is not only too late to find many first division cells but may also be on the border-line between waves of second and third mitoses in culture so that small differences in timing

and in culture conditions can be expected to have exaggerated effects on the aberration frequency. Information on the rate of decline in aberration yield with increasing culture time suggests that estimates of aberration yield made at this late time of seventy-two hours may be too low by as much as a factor of two.

143. Second, it has been suggested that differences in the methods used to handle the cells (and, in general, differences in culture techniques) might conceivably contribute to variation in aberration yield. This suggestion needs to be explored, and the various techniques must be compared within laboratories. Moreover, because of the present lack of knowledge concerning possible subtle effects of minor variations in technique, it is of the utmost importance for workers in this field to define clearly the conditions of culture being used, including temperature, centrifugation methods and cytological techniques.

144. Finally, it cannot be over-emphasized that laboratories should endeavour to standardize scoring methods and presentation of data, giving, where possible, the maximum amount of information on the frequencies of all the various aberration types as outlined in paragraphs 24 to 34.

IV. The relationship between aberration yield and dose

A. Introduction

145. In considering the relationship between radiation dose and aberration yield and the significance of the aberrations in terms of their potential hazard, it is important to note that a statistically significant increase in chromosome-type aberrations is observed in the peripheral blood leucocytes of individuals exposed to low doses of diagnostic radiation. The human chromosome complement is, therefore, sensitive to aberration induction.

146. Reference was made earlier (paragraph 96) to the relationship that exists between absorbed dose and aberration yield in a wide variety of organisms and cell types. A similar relationship between aberration yield and dose must also exist in the case of man's cells, and it is this relationship, coupled with the high sensitivity of the human chromosome complement, that forms the basis of the possible use of aberration yield in dosimetry.

147. Interest in the potential application of aberration yields to estimate dose was largely stimulated by early observations that the peripheral blood leucocytes of persons exposed to radiations, either accidentally or for therapeutic purposes, contained chromosome aberrations.[3] It was generally suggested that, in the case of accidental exposure, determining chromosome aberration yields in an exposed individual might provide not only a simple but also a much more valid alternative to dose estimates based on physical measurements and would make possible some sort of direct estimate of the degree of biological damage incurred.

148. The merits and possible disadvantages of "chromosome aberration dosimetry" are considered in paragraphs 326 to 340. The present section is more concerned with the kinds of data that have been obtained from experiments, from radio-therapy treatments and from incidents where aberration frequencies were determined under conditions where some kind of physical estimate of dose was available. The available data can be separated into two categories, namely, those that have been obtained in *in vitro* experiments where accurate physical estimates of dose were available and those obtained following *in vivo* (whole-body or partial-body) exposure where physical dose estimates were, in general, rather less accurate.

149. There are three principal reasons why studies on the effects of irradiation *in vitro* are very important in the context of any biological dosimetry technique that involves using aberrations produced *in vivo*. They are as follows:

(*a*) Chromosome damage sustained by leucocytes *in vivo* can only be readily observed following short-term *in vitro* culturing of the cells. As has already been seen, studies on aberrations induced *in vitro* are providing a means for determining the optimum conditions for sampling and for defining the types of observations that are required and the conditions under which they should be made.

(*b*) Since many of the fundamental aspects of aberration induction, such as the kinetics of response and the influence of radiation quality and of exposure time, etc., are similar in different species and in *in vitro* and *in vivo* exposures in organisms other than man, the response of human cells exposed *in vitro* is not expected to be different from the response obtained *in vivo*.

(*c*) Various authors[130, 225] have concluded that the x-ray-induced aberration rates in mammalian cells *in vitro* are very similar to those seen *in vivo*. There is, thus, a strong possibility that the sensitivity of the peripheral blood leucocytes *in vitro* may be similar to that *in vivo*.

150. Bender's conclusion was arrived at following comparisons between the response of chromosomes in Chinese hamster bone-marrow cells[151] and corneal epithelium cells[226] and, more particularly, between *in vivo* exposure of bone-marrow cells of the spider monkey (*Ateles* spp.) and *in vitro* exposure of continuously cultured kidney cells of the same species.[12]

151. It might be expected that the *in vitro* irradiation of freshly drawn human whole blood, as opposed to irradiation studies on established human cell culture lines, might closely approximate the irradiation of these cells while they are circulating in the peripheral blood system. As yet there is only a small amount of direct information on this point.[227]

152. In line with this suggestion are the recent preliminary and unpublished studies of Cleminger[228] on rabbits. In this work, blood was taken from animals and given a total gamma-ray dose of 300 or 500 rads from cobalt-60 following which the animals themselves were given either of these doses. Within each dose level, the aberration yields in blood cells exposed *in vitro* and in cells from blood sampled ten minutes after whole-body irradiation were found to be closely similar.

153. In the *in vivo* radiation studies made on man, a considerable amount of data has been accumulating on aberration yields in peripheral blood lymphocytes of individuals exposed to radiations, although a great deal of these data, as is the case in much of the *in vitro* work, comes from cultures grown for seventy-two hours or more. Since the conditions of *in vivo* exposure are so diverse and the information obtained is so varied, the *in vivo* studies will be considered separately according to type of exposure.

B. *In vitro* STUDIES

1. *X rays and gamma rays*

154. Dose-response data from *in vitro* x-irradiation studies on peripheral blood leucocytes have been obtained by fourteen groups of workers. These data appear in twenty-three separate publications,[34, 35, 125, 137, 138, 140, 156, 211, 229, 238] only thirteen of which report data on cells cultured for less than fifty-four hours. In assessing these data, there are at least three important differences in experimental conditions that are of importance. These are (*a*) the use of different culture times; (*b*) the irradiation of whole blood prior to culture as opposed to the irradiation of blood in culture; and (*c*) the use of different qualities of x rays.

155. In the original data of Bell and Baker,[140] terminal deletions and exchange aberrations both increased approximately linearly with increasing x-ray exposure, and the yield of exchange aberrations was dose-rate dependent. For instance, at 200 roentgens, 2.1 exchanges per cell were recorded when the exposure rate was 160 roentgens per minute but only 1.0 exchange per cell when the exposure rate was 1.6 roentgens per minute. In these experiments, however, the cells were cultured for 100 hours, and, in addition, in some of the experiments, radiation was given at various times after culture initiation. No firm conclusions on response with dose can, therefore, be drawn from these data.

156. The data of Bender and his colleagues[125, 229] were obtained by irradiating whole blood with up to 200 roentgens (250 kV x rays, HVL 2 mm Cu) and culturing separated leucocytes by using the buffy-coat technique. In Bender's laboratory, cells were sampled at seventy-two hours so that the aberration yields reported may be under-estimates. Coefficients of aberration production were given as yield per cell-roentgen in the case of deletions and yield per cell-roentgen squared in the case of dicentrics and rings. In two experiments, values of 0.9 and 1.1 10^{-3} deletions per cell-roentgen and 5.2 and 6.0 10^{-6} dicentrics + rings per cell-roentgen squared were obtained.

157. It has been claimed[156, 211] that Bender's dicentric + ring data give a best fit to the relationship $y = kD^{1.4}$ rather than to $y = kD^2$, where y = yield, k = a constant and D = dose. A very relevant point of interest in these data is the fact that, in the first experiment of Bender and Gooch, a culture was grown for fifty-four hours in addition to parallel cultures grown for seventy-two hours. In the fifty-four-hour culture, the aberration yield was 50 per cent higher than in similarly irradiated cells grown for seventy-two hours. This is in accord with the expectation that the coefficient for aberration production obtained from seventy-two-hour samples may be too low.

158. Kelly and Brown[137] irradiated whole blood (200 kV x rays, HVL 1.5 mm Cu) and cultured separated leucocytes for seventy to ninety-six hours after exposures of from 100 to 1,600 roentgens. The data were analysed according to the equation $y = kD^2$, but they were not uniform. Over the full dose range the coefficient for the yield of dicentrics was 0.9 10^{-6} per cell-roentgen squared and was considerably lower than that obtained by Bender and his colleagues. If the data obtained at exposure above 200 roentgens were omitted, a coefficient of 5.6 10^{-6} per cell-roentgen squared (i.e., similar to that obtained by Bender) was obtained. Clearly, culture time played a very important part in these experiments, and no firm conclusion on the relationship between dose and yield can be drawn from these data.

159. The original data of Norman and his colleagues[239] were obtained from lymphocytes that were irradiated in whole blood (100 kV to 1.9 MeV x rays) and then cultured, after separation by centrifugation, for seventy-two to ninety hours. Doses of up to 1,200 rads were used, and dose rates ranged from 10 to 200 rads per minute. No effect of dose rate was observed, and a coefficient for the production of dicentrics of 2.7 10^{-6} per cell-roentgen squared was obtained. These data for dicentrics gave an excellent fit to the equation $y = kD^2$, and the coefficients obtained in these data are approximately one-half of those obtained by Bender's group. No significant differences were observed between the effects of these two radiations of different qualities.

160. In the very recent publications from Norman's group,[34, 240] cells have been cultured for fifty hours as well as seventy-two hours, and doses of up to 5,000 rads have been used. In these experiments, higher aberration yields were observed at fifty hours, and the coefficient for dicentrics and rings in these shorter term cultures was 5.7 10^{-6} per cell-roentgen squared, almost identical with that obtained by Bender and his colleagues. These data were obtained with x rays from a linear accelerator giving a mean photon energy of 1.9 MeV, although it should be noted that Bender's data were obtained with the more efficient 240 kVp x radiation.[241]

161. Visfeldt[242] in his *in vitro* work has used only three dose levels of up to 200 rads of cobalt-60 gamma radiation and has reported higher aberration yields than Norman *et al.* and Bender *et al.* In Visfeldt's work, leucocytes were separated without resort to centrifugation, and cultures rather than whole blood samples were irradiated. Cells were sampled after forty-eight hours in culture, and it is of interest to note that these gamma-ray data from irradiated cultures approach the high yields obtained with x-irradiated whole blood microcultures. It is important to note here also that the RBE for chromosome-aberration production is about 0.8 for cobalt-60 gamma rays relative to 250 kV x rays.[243, 244]

162. Mouriquand *et al.*[138] have exposed separated leucocytes (gravity sedimentation) in autologous serum to x irradiation (160 kV, 7.5 mA, 100 R per min) prior to culture and have sampled cells seventy-two hours later. The yields of dicentric aberrations obtained by these workers were higher (coefficient of 8.2 10^{-6} per cell R^2) than those obtained by other authors who irradiated whole blood. The data of Mouriquand *et al.*[138] were very probably obtained from a mixture of first and second division cells, and their yields closely approach those obtained in x-irradiated microcultures sampled at seventy-two hours[115] and are about 25 per cent lower than the yields obtained in microcultures sampled at fifty-four hours.

163. Evans[156, 211, 212] has reported data from five experiments on whole blood x-irradiated (250 kV HVL 1.2 mm Cu) prior to or during culture, using doses of up to 460 rads and dose rates of from 17.5 to 230.5 rads per minute. These experiments differ from the others in that whole blood microcultures were used. The cells were sampled at fifty-four hours, and no differences were observed between cultures exposed at different dose rates. In these experiments, the dicentric

and ring aberrations did not increase in proportion to the square of the dose but give a best fit to the equation $y = kD^{1.2}$. The pooled data from all experiments analysed as a quadratic, i.e., $y = k + \alpha D + \beta D^2$, give $\alpha = 3.42\ 10^{-3}$ and $\beta = 3.5\ 10^{-6}$.

164. Bajerska and Liniecki[231] have recently reported experiments on x-irradiated cultures (180 kV with 1.05 or 1.8 mm Cu filtration) and have obtained results somewhat similar to Evans. In these experiments, using a dose range of up to 415 rads given at dose rates of around 100 rads per minute, the yields of dicentric aberrations best fit the equation $y = kD^{1.3}$, and the total yields are similar to those reported in the other published data on x-irradiated cultures (figure 4).

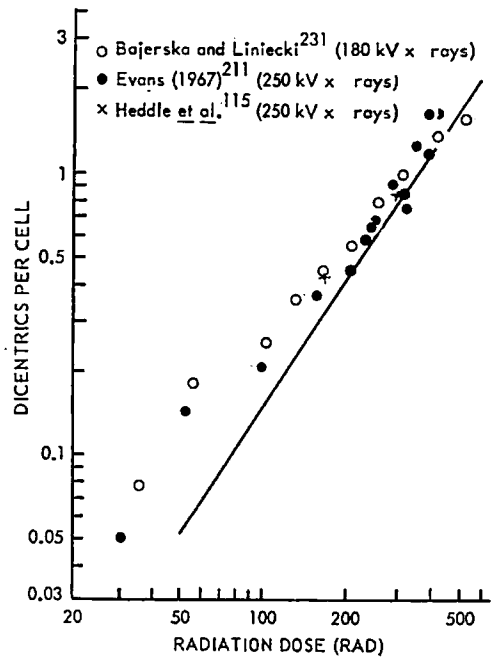

Figure 4. Dose-effect relationship for dicentric aberrations (irradiation with 180 and 250 kV x rays *after* PHA stimulation)[a]

[a] Whole blood x-irradiated *in culture*. Cultures grown at 37° C for *fifty* to *fifty-four hours*. Regression line fitted to data obtained from blood cells irradiated with 180 to 300 kV x rays *prior* to culture (figure 8).

165. Up until very recently the x-ray *in vitro* data appeared to be very confusing. Within individual laboratories, consistent and repeatable results were obtained, but little uniformity in the form of the relationship between aberration yield and dose existed between laboratories. Recently acquired experimental data have markedly improved the picture, however, particularly if separate consideration is given to data obtained using different techniques and radiations of differing quality.

166. Studies by a number of workers have now clearly demonstrated that 2 MeV x rays are less efficient than 180 to 300 kVp x rays, the RBE being 0.8[232, 234-236] when comparisons are made between samples handled in the same way with regard to irradiation technique and culture sampling time. The data of Sasaki[234] on the dose-response relationship of dicentrics + rings for radiations of differing qualities are shown in figure 5. These data were obtained using irradiated whole blood cultured for fifty hours prior to sampling, and the constants and dose exponents for the

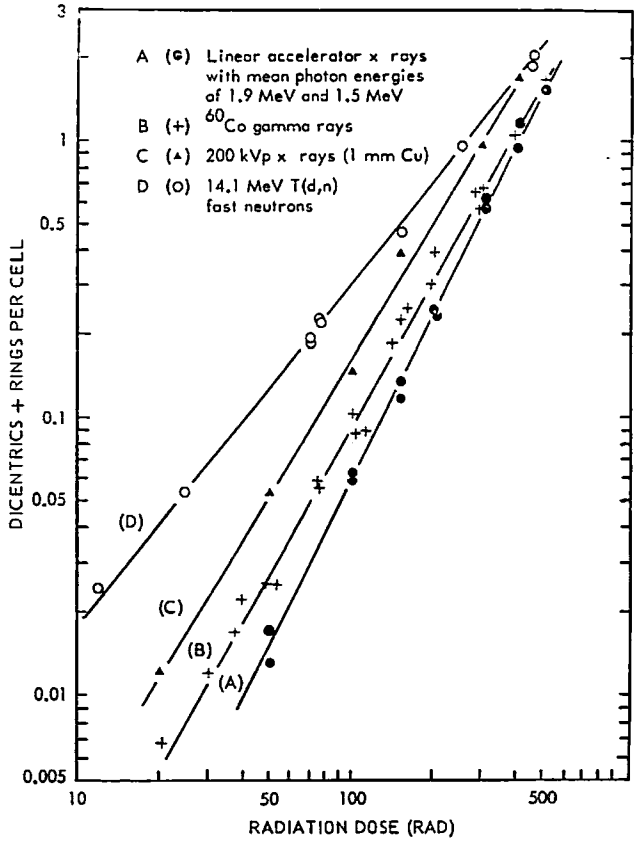

Figure 5. Dose-response relationship for dicentrics plus rings for different qualities of radiation[a]

[a] Cells irradiated *prior* to culture and cultured for *fifty* hours.[234]

fitted lines are as follows: 1.9 MeV and 1.5 MeV x ray, $y = 8.50\ 10^{-6}\ D^{1.94}$; cobalt-60 gamma rays, $y = 25.5\ 10^{-6}\ D^{1.78}$; 200 kV x rays (HVL 1 mm Cu), $y = 81.14\ 10^{-6}\ D^{1.60}$; 14.1 MeV T(d,n) fast neutrons, $y = 1.039\ 10^{-6}\ D^{1.24}$. The D^2 relationship for the 1.9 MeV x rays confirms the earlier studies[34, 240] using radiation of this quality.

167. Sasaki's data on the cobalt-60 gamma rays are very similar, both in terms of absolute yield of dicentrics and of dose kinetics, to recent data obtained with this radiation, under similar culture conditions, by Sevankayev and Bochkov[237] (figure 6). Both these sets of data differ, however, from Visfeldt's[242] results and from some recent data of Scott *et al.*[234] In these latter studies, cells were sampled after forty-eight to fifty-four hours in culture, but the cultures themselves (stimulated cells) rather than the freshly drawn whole blood (unstimulated cells) were irradiated. In this context, it is of interest to note that all the data on x- or gamma-irradiated cultures give lower dose exponents than do the data on irradiated whole blood, the difference being largely a consequence of higher yields at low doses in the irradiated cultures (figures 4 and 7).

168. Recent data from five different laboratories on the yields of dicentric aberrations in cells irradiated in whole blood prior to culture for up to fifty-four hours, with x rays of peak kilovoltage ranging from 180 kV to 300 kV, all give consistent results. These data are shown in figure 8, and the slope of the fitted line gives a dose exponent of 1.53. The aberration yields in these five sets of data are higher than the yields reported by

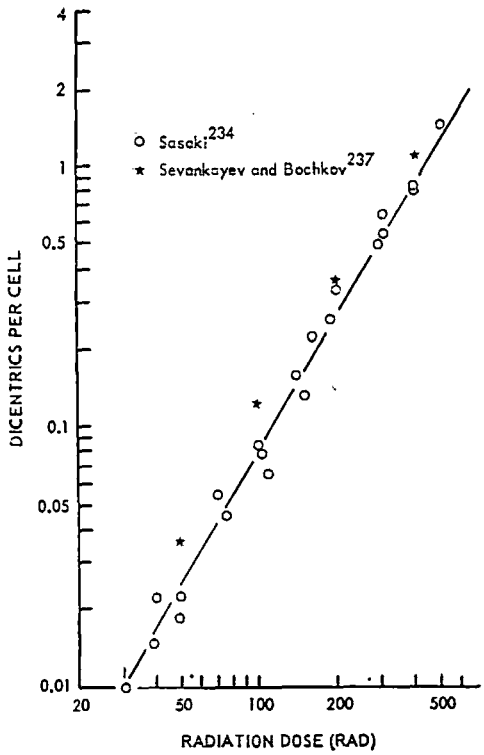

Figure 6. Dose-effect relationship for dicentric aberrations (irradiation with ^{60}Co gamma rays *before* PHA stimulation)[a]

[a] Whole blood irradiated *prior* to culture and cultured for *fifty* to *fifty-four* hours.

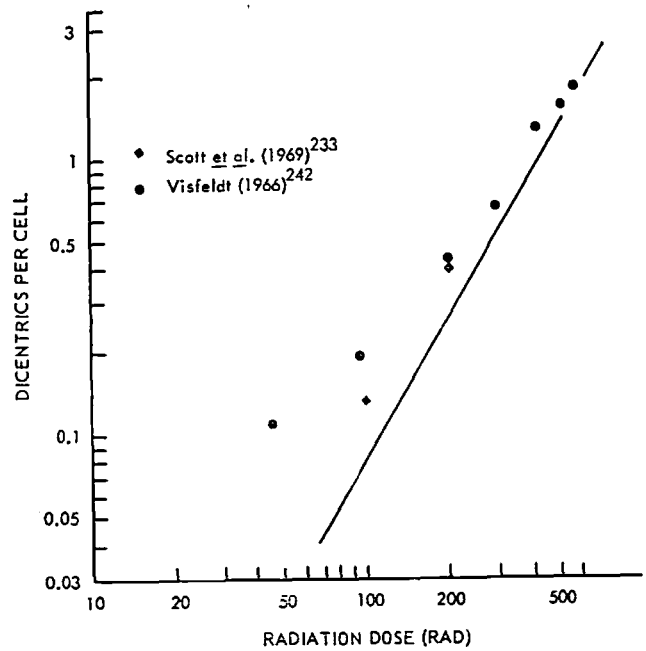

Figure 7. Dose-effect relationship for dicentric aberrations (irradiation with ^{60}Co gamma rays *after* PHA stimulation)[a]

[a] Whole blood irradiated *in culture*. Cultures grown at 37° C for *forty-eight* to *fifty* hours. Regression line fitted to data obtained from blood cells irradiated with ^{60}Co gamma rays *prior* to culture (figure 6).

other authors using longer culture periods; for the purpose of comparison, some of these latter data are plotted in figure 9.

169. It would appear, therefore, that, in the case of irradiated whole blood sampled after fifty-four hours

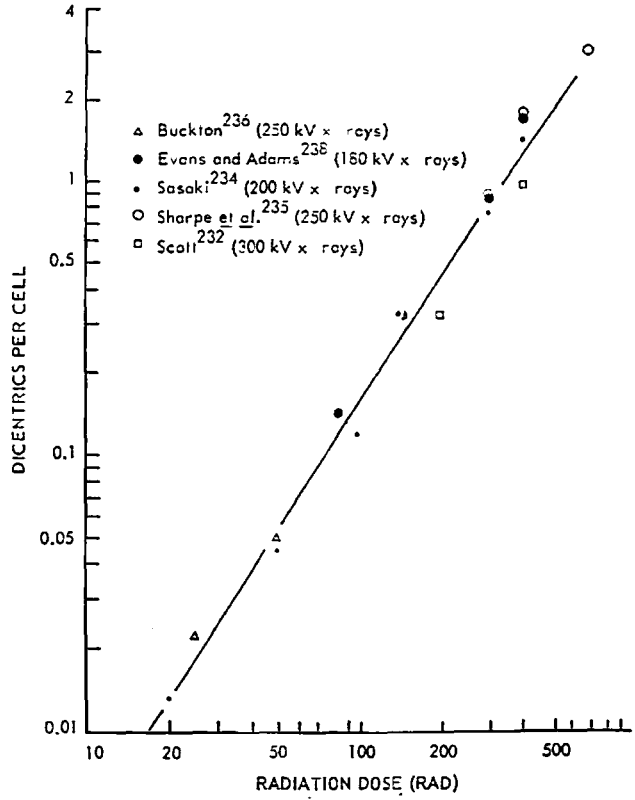

Figure 8. Dose-response relationship for dicentric aberrations (irradiation with 180 to 300 kV x rays *before* PHA stimulation)[a]

[a] Whole blood x-irradiated *prior* to culture and cultured for *fifty* to *fifty-four* hours.

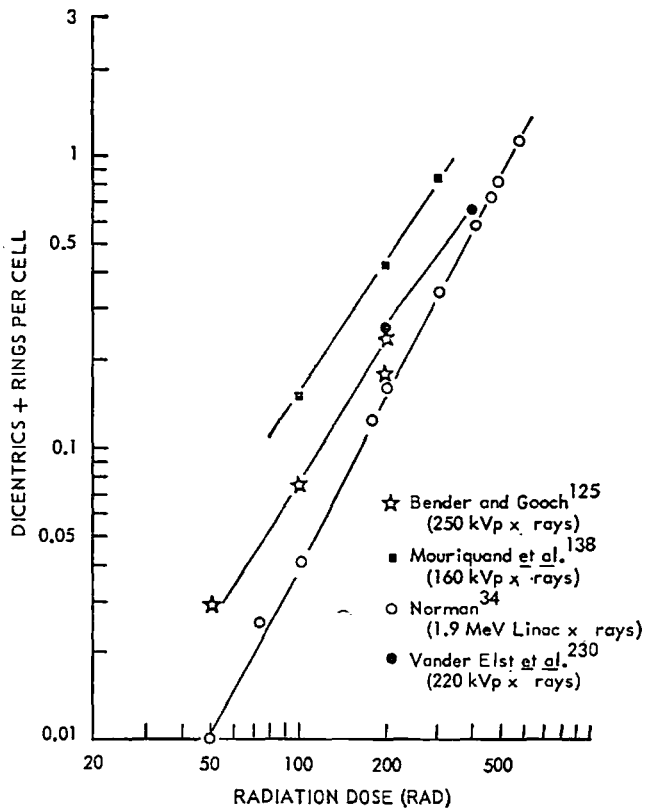

Figure 9. Dose-response relationship for dicentrics plus rings[a]

[a] Cells irradiated with various qualities of x rays *prior* to culture and cultured at 37° C for *seventy-two* hours.

in culture or less, the interchange aberrations induced by high-energy x rays are predominantly a consequence of the interaction between two lesions produced by independent tracks. However, over the same aberration yield range (up to about two dicentrics per cell) a significant fraction of the exchange aberrations induced by conventional x rays (150-300 kVp) are the result of the interaction between two lesions produced by a single track. The importance of this one-track contribution will, of course, decrease with increasing dose level.

170. Despite the excellent agreement between sets of data recently obtained quite independently in different laboratories, the difference in response, particularly at low doses, between irradiated cultures and irradiated whole blood requires further investigation.

2. *Fast neutrons*

171. Dose-response data from peripheral blood leucocytes exposed to fast neutrons *in vitro* have been reported by three groups of workers, namely, in the United States, the United Kingdom and Japan.

172. Gooch *et al.*[229] in the United States irradiated whole blood with 14.1 MeV DT and 2.5 MeV DD fast neutrons with doses of up to 200 rads, and the separated leucocytes were cultured for seventy-two hours before determining the induced aberration yields. With the 14.1 MeV neutron dose delivered at 6 rads per minute, it was found that "chromosome breaks" (terminal deletions + intercalary deletions?) increased slightly more than with the first power of the dose and that dicentrics plus rings increased as approximately the square of the dose (apparently the best fit to the equation $y = kD^n$, for dicentrics + rings gives a value[245] of $n = 1.42$). The coefficient of aberration production for deletions was 2.0 10^{-3} deletions per cell-rad and for dicentric and rings 12.1 10^{-6} aberrations per cell-rad squared.

173. It has been argued[156, 211] that the curvilinearity of these dicentric and ring data with 14.1 MeV neutrons might be due to the sampling of predominantly second and third division cells at low doses and, as a result of mitotic delay, to sampling of an increasing proportion of first division cells with increasing dose. There is no direct information on this possibility, but previous experiments with this quality of radiation on chromosome-aberration induction in plant cells have shown that all the aberration types increase approximately linearly with increasing dose.[246-249] However, it is important to note that, in the more recent studies of Sasaki[234] in Japan, using 14.1 MeV neutrons but with the cells sampled after fifty hours in culture, a dose exponent for dicentric aberrations of 1.24 was obtained.

174. Gooch *et al.*[229] compared their 14 MeV fast neutron data with those obtained with 250 kV x rays and obtained an RBE for these neutrons of approximately two. Preliminary data with 2.5 MeV DD neutrons yielded a linear dose response for all aberration types and an RBE of approximately four to five for deletions. It is of interest to note here that these authors derived an estimated RBE from *in vivo* exposure of three men to fission spectrum neutrons during a criticality accident. These estimates were arrived at after making certain assumptions, and an RBE value of the order of five was obtained (figure 10).

175. Scott *et al.*[245] in the United Kingdom exposed whole blood to fast neutrons of 0.7 MeV mean energy using doses of up to 150 rads. The cells were exposed prior to or following their introduction into whole blood microcultures. Continuous irradiations were given over periods of up to twenty-four hours and involved dose rates of 6.75 rads per hour and 3.41 rads per hour, and short-term irradiations were given at the approximately thousandfold higher dose rate of 50 rads per minute. No differences in efficiency between chronic and short-term exposures were found, and in both cases all aberration types increased linearly with increasing neutron dose. Comparisons with 250 kV x-ray data (doses of up to 500 rad) gave an RBE value for these 0.7 MeV fission neutrons of around three (figure 10).

176. In two of the five sets of 0.7 MeV neutron-dose-response data, some indication of a saturation in aberration yield was indicated at doses of above 100 rads in the chronic low dose-rate experiments. In these two experiments, the data also indicated a higher yield (of up to 10-20 per cent) in those cells irradiated while in the G_1 phase in culture (PHA-stimulated blood cells), as opposed to those cells irradiated prior to culture (i.e., unstimulated blood). The possibility of differences between the response to irradiation of stimulated and unstimulated cells has already been commented on (paragraph 171).

177. It has been suggested that the saturation effect results from a preferential loss of damaged cells due to "interphase death" under conditions of continuous irradiation in culture. This possibility again raises the question of preferential cell loss both prior to and during culture. It is of interest to note here that, in parallel to the higher yields of x-ray-induced aberrations observed by Scott *et al.* relative to those observed by Gooch *et al.*, similar, but rather more pronounced, increases in yield have been observed with the neutron data. This difference must, of course, partly be the consequence of differences in culture times and partly of differences in radiation quality. The difference is such that, at equivalent dose levels, the yields of dicentrics and rings with the 0.7 MeV neutrons is some ten times higher than the yields reported with the 14.1 MeV neutrons (figure 10). Further work is most certainly necessary here.

3. *The variation in response between blood samples obtained from different individuals*

178. Perhaps the only consistency among laboratories which has emerged from the *in vitro* studies is the indication that the variation between the *in vitro* response of blood cells obtained from different adult donors of both sexes is very small.[125, 131, 143, 229, 250] Five donors have been used by the Oak Ridge group, and data have been presented in two publications.[125, 229] In no case did the responses to the same dose level of cells obtained from different donors differ significantly.

179. In the Harwell studies,[250] seven donors were used, and all yielded closely similar aberration yields when exposed to similar doses. Moreover, cells from one donor were used for much of the x-ray dose-response work, and cells were, therefore, sampled at various times throughout a three-year period. Throughout this period, there was no significant change in the aberration yield at any given dose.[211]

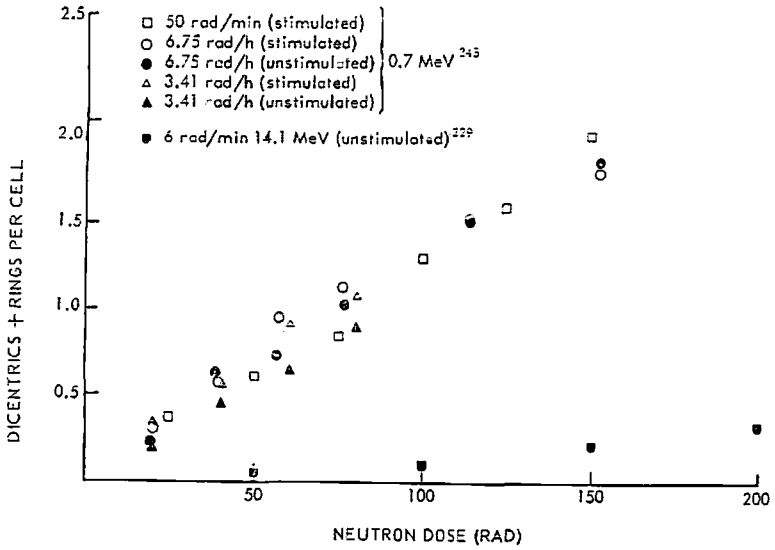

Figure 10. Dose-response data for *in vitro* fast neutron irradiation[245]

180. Recent work in the Soviet Union[251, 252] has indicated that the yield of x-ray-induced chromosome aberrations in peripheral blood lymphocytes irradiated *in vitro* may be slightly higher in cells taken from infants and from elderly people than in cells taken from other healthy adults. However, Migeon and Merz[253] had previously reported no significant differences between the responses of lymphocytes from infants and adults. Bochkov *et al.*,[254] in a study of fifty-nine individuals, reported that there was an influence of age on the frequency of spontaneous structural rearrangements in these individuals. The possibility of an influence of age of donor on radiation response clearly merits further study.

181. Studies on plant and animal cells (see references 17 and 255) have shown that, in a given species, the presence of an extra chromosome or chromosomes over and above the normal diploid complement results in an increased aberration frequency in these cells. The induced aberration frequency at any given dose level is, therefore, closely correlated with chromosome number as well as with chromosome size and chromosome morphology.[255]

182. Because of the disparity in size between the X and Y chromosomes in man,[256] it might be expected that, at a given dose, the frequency of chromosome aberrations in normal females might be very slightly greater than the aberration frequency in normal males. This expected very small difference between sexes has not yet been demonstrated, but, in line with expectation, a small increase in aberration yield in *in vitro* x-irradiated leucocytes from individuals trisomic for chromosome 21 (Down's syndrome), as compared with normal individuals, has been reported by three groups of workers.[143, 145, 257]

183. The constancy between individuals, and within individuals over a period of a few years, in the response of their blood cells to *in vitro* radiation exposure is heartening. Nevertheless, it must be strongly emphasized that the number of donors that have been compared to date is extremely small. Bearing in mind the influence of age and of sex on aneuploidy (paragraph 78), it is clear that more information is required from a larger number of donors of both sexes encompassing a wide age range. Such studies are currently in progress in a number of laboratories.

C. *In vivo* STUDIES

184. Quantitative studies on aberrations induced *in vivo* are beset with a number of difficulties additional to those considered in the *in vitro* work. These difficulties will be considered in some detail in this section as well as in paragraphs 341 to 343. Nevertheless, a discussion of the *in vivo* data cannot be initiated without emphasizing that difficulties with regard to physical dosimetry and to biological sampling are inherent in all the *in vivo* work. For instance, many studies have involved partial-body irradiation, and a variety of qualities of radiation has been used. Estimates of absorbed dose in such cases may not be very meaningful, and, in fact, difficulties with regard to the non-uniformity of absorbed dose exist even in the case of so-called uniform whole-body irradiation. Sampling problems are present because of the distribution, life span and mobility of the small lymphocyte within the body. These difficulties should, therefore, be borne in mind throughout the following discussion.

1. *Clinical exposure*

185. A number of instances have been recorded where an increased frequency of chromosome-type aberrations has been observed in peripheral blood leucocytes of individuals following the exposure of these individuals to diagnostic x rays. Although, to date, all the observations have been made on cultures grown for seventy-two hours or more (and, hence, some of the chromosome-type aberrations observed could have been derived following duplication of chromatid-type aberrations produced in culture—paragraphs 67 and 93), control data, where they exist, were also obtained from cultures grown for a similar period. The increases, therefore, must be a real consequence of radiation exposure.

186. The first observation of a possible effect of diagnostic x rays in inducing aberrations was made by Stewart and Sanderson[258] who reported the presence of two cells containing a dicentric out of a total of thirty-one cells scored in a patient with Klinefelter's syndrome. This patient was subjected to a skeletal survey involving a skin dose of less than 2 rads from 60 kV x rays, and blood samples were taken eight hours after exposure. Unpublished evidence[259, 260] on

the yield of spontaneous aberrations in Klinefelter patients who have not been exposed to diagnostic x rays shortly prior to study has indicated that the spontaneous yield in these individuals is no higher than in normal individuals (table I).

187. Observations similar to those of Stewart were made by Conen *et al.*[261, 262] who found two dicentric aberrations in 121 blood cells of an infant examined one week after exposure to a series of diagnostic x rays giving a total dose of 0.8 rad. Bloom and Tjio[263] did not detect any dicentric aberrations in blood cells from six patients given diagnostic chest x rays involving exposures of from 20 to 80 milliroentgens, but four dicentric aberrations were observed in 300 cells of five patients subjected to gastro-intestinal examination using fluoroscopy. The exposures of these five patients ranged from 12 to 35 roentgens, and blood samples were in all cases taken thirty minutes after irradiation.

188. Further indications of what appears to be a significant elevation in aberration yield are seen in the data of Court Brown[264] on ankylosing spondylitis patients subjected to diagnostic x rays (columns *b* and *c* in table I). Moreover, Sasaki *et al.*[265] have reported that, in a scan of over 7,000 cells taken from a total of eleven individuals, dicentrics were only found in one man who had received a number of lumbar spinal x-ray examinations some five years previously.

189. These observations suggest that very low-dose partial-body x irradiation at low (diagnostic) kilovoltage is capable of inducing a detectable frequency of chromosome-type aberration. The fact that we can detect the effects of such small doses of x rays is a consequence, first, of the relatively high sensitivity of human peripheral blood leucocytes to the induction of chromosome damage by radiation, and, second, of the extremely low frequency with which chromosome-type aberrations are found in individuals not exposed to ionizing radiations.

190. More recently, information has become available from patients treated with Thorotrast, a stabilized colloidal suspension of the dioxide of thorium-232. Thorotrast is taken up by the reticulo-endothelial cells and deposited in liver and spleen, and, to a lesser extent, in bone marrow and lymph nodes. Only minute quantities are excreted so that these tissues are subjected to continuous irradiation, much of which is due to densely ionizing alpha particles.

191. Ishihara and Kumatori[182, 266] reported that a significant yield of aberrations was to be found in blood leucocytes of persons given Thorotrast injections some twenty-five years prior to observation. The residual body burdens of these persons were estimated by whole-body counting, but no definite correlation was found between body burden and aberration yield.[267] Similarly, Buckton *et al.*,[224] in a cytogenetic study of thirty-six patients who received intra-arterial injections of Thorotrast some eleven to thirty-one years prior to study, reported a marked increase in aberration yields in the leucocytes of these patients as compared with those of control individuals. The cells in this latter study were cultured from forty-eight to fifty-two hours, and 9.2 per cent of the cells were found to contain unstable (asymmetrical) aberrations and 5.7 per cent stable (symmetrical) aberrations. It is of interest to note that, in this work, a very high frequency of tricentric aberrations was found (3.8 per 100 cells) and that many cells contained more than one aberration. This high aberration frequency in damaged cells is typical of damage induced by high LET radiation, such as the alpha particles of thorium.

192. The high aberration yields obtained by Buckton *et al.*[224] in their Thorotrast patients are considerably greater than the yields obtained by these authors[16] in patients receiving a whole-body dose of 50 rads of x rays or a partial-body dose of 300 rads. However, in the Thorotrast work, although the volume of Thorotrast administered and the time interval between treatment and observation were known, no relationship between these parameters and aberration yield could be demonstrated.

193. Fischer *et al.*[179, 268] examined blood cells from twenty individuals who had received Thorotrast from nineteen to twenty-seven years prior to sampling, and in these patients estimates of residual body burden were made through whole-body counting. Nineteen of these cases showed a significant increase in aberration frequencies above the background level; the only one that did not show such an increase had undergone a retrograde pyelography and had, thus, retained little radio-activity as was confirmed by the extremely low burden registered by the whole-body counter.

194. In this work, it was shown that, with an increasing amount of Thorotrast (estimated from whole-body gamma-ray counting), there was an increasing amount of chromosome damage and a significant linear correlation between these parameters when the data were weighted by the time interval elapsing between administration and observation. However, we should note that there are several difficulties in assessing these dose-response relationships, since considerable variation exists in the distribution of Thorotrast within the reticulo-endothelial system,[224] and estimates of dose from thorium and its decay products, based on gamma-ray measurements, require that allowance be made for the self-absorption of alpha rays; this is of particular importance if the thorium is not uniformly distributed. Because of these difficulties in physical dosimetry and since, in the work of Fischer *et al.*,[179] the leucocytes were cultured for seventy-two hours, it is difficult to arrive at any meaningful coefficients for aberration production.

195. Since the original paper of Tough *et al.*[15] who reported gross chromosome damage in cells from blood cultures of two patients after x-ray therapy for ankylosing spondylitis, a number of publications dealing with aberrations induced in patients following radiotherapy have appeared. Most of this work has been concerned with aspects of aberration induction (such as the question of the longevity of the small lymphocyte — paragraphs 252-263) other than the quantitative correlation between induced chromosome damage and absorbed dose. However, a limited amount of data on dose response *in vivo* has been obtained, and more should be available in the near future.

196. Norman *et al.*[239] obtained a limited amount of data on two patients receiving doses of 300 rads from 250 kV x rays, using blood samples that were collected immediately after irradiation. No details of the method of exposure were given, and the geometric mean of the aberration yields from the two rather different samples was nearly equal to the yield obtained from normal blood irradiated *in vitro* and receiving a dose of 300 rads. In these cases, all the cultures were grown for seventy-two hours.

197. A more recent paper[34] reports aberration yield data from six patients treated with (partial-body?) radio-therapy for malignant disease. Cultures were grown for fifty hours and seventy-two hours, but no estimate of dose is given. In considering such partial-body exposures, the question, of course, arises as to what significance can be attached to a partial-body dose if such a dose is reported.

198. The aberration yield observed in peripheral blood leucocytes sampled after a partial-body irradiation will depend upon a number of variables including the following: the physical characteristics of the radiation; the region of the body and the volume of tissue exposed; the absorbed dose in this volume and the duration of the radiation exposure; the proportion of the total body lymphocytes that were resident in this volume during irradiation; the proportion of blood lymphocytes that traversed this region during irradiation; the amount of exchange of lymphocytes between the lymphatic tissues and peripheral blood and the time of sampling after irradiation.

199. The question of dosimetry in cases of partial-body exposure is, therefore, complex and will be considered in some detail later (paragraphs 264-273). However, it is pertinent to note here the recent studies by Winkelstein *et al.*[227] on chromosome aberrations in leucocytes of three patients whose blood was exposed to extracorporeal irradiation (ECI) prior to renal transplantation.

200. In this work, blood was passed through a teflon loop outside the body, using a standard Quinton-Scribner shunt, and was subjected to ECI by exposure to beta-emiting ^{90}Sr-^{90}Y sources. Exposure times of up to four to eight hours were used, and the frequency of dicentric aberrations in leucocytes cultured with PHA immediately after the termination of the ECI period was determined. In addition, the frequency of dicentric aberrations in *in vitro* studies on blood put through the radiation applicator in a single passage was also determined.

201. In these ECI studies, physical dose estimates to the blood cells were made on the basis of patient blood volume, flow rate through the applicator and duration of irradiation exposure. Although no detailed data on aberration yields were given, the relationship between calculated physical doses (integrated over the whole blood volume) and the doses estimated on the basis of dicentric aberration yields in sampled blood leucocytes were compared. The dose was estimated from the aberration yield through the use of the proportionality constant (paragraph 160) of dicentric yield being equal to $5.7 \pm 0.5\ 10^{-6}$ per cell-rad squared as determined from previous *in vitro* studies[34, 240] of this group. A very close correspondence between physically and biological estimated dose was found (table II), provided that samples were taken after no more than a four- to eight-hour ECI exposure so that blood leucocytes were not replaced by populations of leucocytes from the unirradiated lymphoid tissues.

202. In an ECI study carried out by Sharpe *et al.*[243] on a patient with reticulum cell sarcoma, it was found that the relationship between estimates of dose based on blood flow rate and on total blood volume of a patient and of those based on the yield of dicentric aberrations differed by a factor of 2.7. The data obtained indicated that, in a treatment lasting three and a half hours, several cells made many transits through the irradiator and that there was a fairly rapid exchange between leucocytes of peripheral blood and those in much larger pools in extravascular sites.

203. Sharpe *et al.*[269] have recently reported on some further ECI studies made on a patient with Hodgkins disease. This work has confirmed and very much extended their earlier findings, and the results are somewhat at variance with the conclusions of Winkelstein *et al.*[227] In this recent study,[269] it was found, as previously shown by others,[227] that the yield of dicentric aberrations (0.83 per cell), in a sample of the patient's blood taken prior to ECI treatment and receiving an *in vitro* dose of 300 rads from 2 MeV x rays, was closely similar to the dicentric yield (0.87 per cell) obtained from blood allowed to proceed through one transit of the radiation coil (320 rad from a caesium-137 source) over an exposure period of four seconds. However, in blood samples taken from the patient after one and a half, three and twenty-four hours, continuous ECI-treatment, dicentric yields of less than 0.09 per cell were obtained.

204. From these data and from studies on the distribution of aberrations between cells, Sharpe *et al.*[269] concluded that there is a rapid exchange between lymphocytes in blood and lymphocytes in the extravascular pool. It was estimated that the peripheral blood contained 3 grammes of lymphocytes, whereas the extravascular pool contained between 800 and 1,070 grammes. Two independent estimates of the mean residence time of lymphocytes in blood gave values of 4.7 and 7.5 minutes.

205. The results of the ECI studies by these two groups of workers, although somewhat conflicting, are most interesting, and further work in this field will be particularly rewarding both from the point of view of yielding information on the population structure and movements of the leucocytes and in providing information applicable to the possible use of chromosome aberrations in dosimetry.

206. Following up on its original studies[15] on aberrations induced by x irradiation of spondylitis patients, the Edinburgh group has recently reported[16] data on the relationship between aberration yield and radiation dose in these patients and in patients suffering from neoplastic disease. With the spondylitis patients, single partial-body doses of 100 to 700 rads (250 kV x rays, HVL = 2.7 mm of Cu) were given and the cells cultured for forty-two to fifty hours. At all doses, blood samples were taken twenty-four hours after exposure, but, in some instances, samples were also taken at earlier and later times. The data indicate a slightly lower aberration yield in cells cultured immediately after exposure than in cells sampled for culture twenty-four hours later. A summary of the data obtained is given in table III.

207. Although we cannot define the absorbed doses in these cases for whom data are given in table III, there is evidently a clear relationship between skin dose and aberration yield. The data are somewhat variable, and for dicentric and ring aberrations the aberrations appear to increase in proportion to the 1.5 to 2.4 power of dose, at least for doses up to 300 rads. If the data from one patient given a partial-body dose of 700 rads is included in the kinetic analysis, then this high dose yield reduces the dose-squared component considerably. Extrapolation from the data shown in table III shows that these yields are higher

than those obtained by Millard[126] from patients exposed to partial-body (lower abdomen) radiation following orchidectomy. In Millard's data (2 MeV Van der Graff x rays), 20 per cent of the cells showed aberrations after doses between 925 and 1,550 rads and 32 per cent after doses between 3,100 and 4,330 rads. These latter data, however, were obtained from peripheral blood cells that were allowed to grow for seventy-two hours, and, moreover, the radiation treatment was spread over a period of thirty-six to seventy-four days.

208. Similar observations to those of Millard have been made by Dubrova[270] on two myeloma patients receiving radiation therapy. In this work, patients were treated with an accumulated partial-body exposure of 9,000 roentgens, and up to 42 per cent of the leucocytes cultured for forty-eight hours were found to contain chromosome aberrations. High aberration yields were also observed in blood cells of a similar patient sampled thirty-two months after the completion of a similar course of treatment.

209. Spondylitis patients were also given ten partial-body x-ray dose fractions over a period of twelve to fourteen days;[16] blood samples were collected immediately after each treatment. Again, there was a clear relationship between radiation dose and aberration yield, the data giving a very good fit to a linear relationship (figure 11). In these data, each fraction

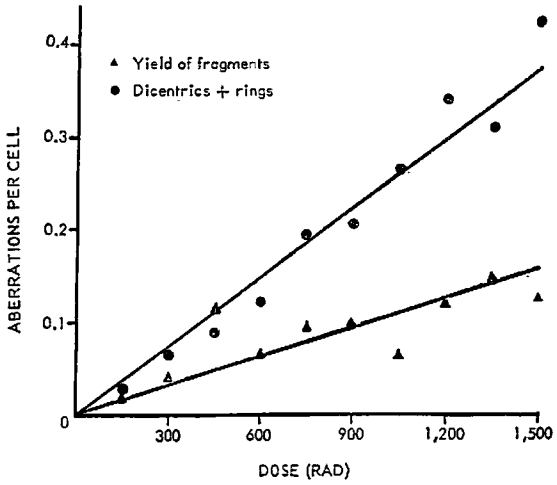

Figure 11. Yield of fragments and dicentrics plus rings in ankylosing spondylitis patients exposed to doses of up to 1,500 rads (250 kV x rays) given as a series of fractions of 150 rads[16]

of 150 rads (partial-body dose) gave an average yield of 3.6 dicentrics and rings per 100 cells analysed.

210. Buckton *et al.*[16] also reported preliminary data, summarized in table IV, on aberration yield in seven men suffering from bronchial carcinoma who received low doses (25 or 50 rad) from whole-body x irradiation (2 MeV Van der Graff). In contrast to the data obtained from patients exposed to partial-body irradiation (table III), no differences in aberration yields were found between bloods sampled immediately after exposure and twenty-four hours later (however, see paragraph 211). Moreover, the dose response for dicentrics and rings after whole-body irradiation was linear (n in the equation $y = c + aD^n$ being equal to 0.92 with 90 per cent confidence limits of 0.5 to 1.4). From the data presented, it would seem that the aberration yield obtained following a whole-body dose of 50 rads to the cancer patients was equivalent to the yield obtained with a partial-body dose of 250 rads to the ankylosing spondylitis patients.

211. The Edinburgh group has recently extended its studies on whole-body irradiation of patients with bronchial carcinoma and has now reported[271] data obtained from a further nine patients exposed within the dose range 17 to 50 rads. The dicentric and ring aberration frequencies in fifty-three-hour cultures of blood cells sampled immediately after irradiation and twenty-four hours later are summarized in table V for each of the sixteen patients.

212. In these data, there is a significant increase in aberration yield in bloods sampled twenty-four hours post-treatment as opposed to bloods sampled immediately after exposure in patients receiving doses of 50 rads. This observation is entirely in line with the earlier observations made by this group of workers on ankylosing spondylitis patients exposed to partial-body irradiation (paragraphs 206 and 210).

213. Analysis of the data obtained from these whole-body exposures revealed that the yield of dicentric and ring aberrations in blood sampled immediately after irradiation increased as the 1.13 power of dose ($n = 1.13$ with 95 per cent confidence limits of 0.52 to 1.74), whereas, in blood sampled twenty-four hours later, the yield increased as the 1.88 power of dose ($n = 1.88$ with 95 per cent confidence limit of 1.24 to 2.50). These two sets of data are shown in figures 12 and 13.

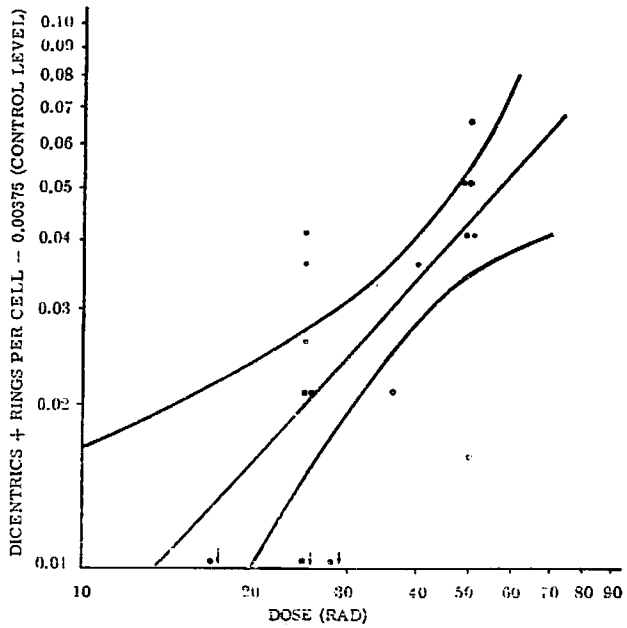

Figure 12. Relationship between yield of decentrics plus rings per cell and uniform whole-body dose in patients exposed to 2 MeV x rays[271 a]

[a] Data from blood samples taken immediately after radiation exposure. Points are from data given in table V, and curved lines represent the 95 per cent confidence limits of the regression line.

214. It is of interest to compare the 25-rad and 50-rad whole-body irradiation data with data obtained using similar doses (but with various qualities of radiation) by other authors in *in vitro* studies. These

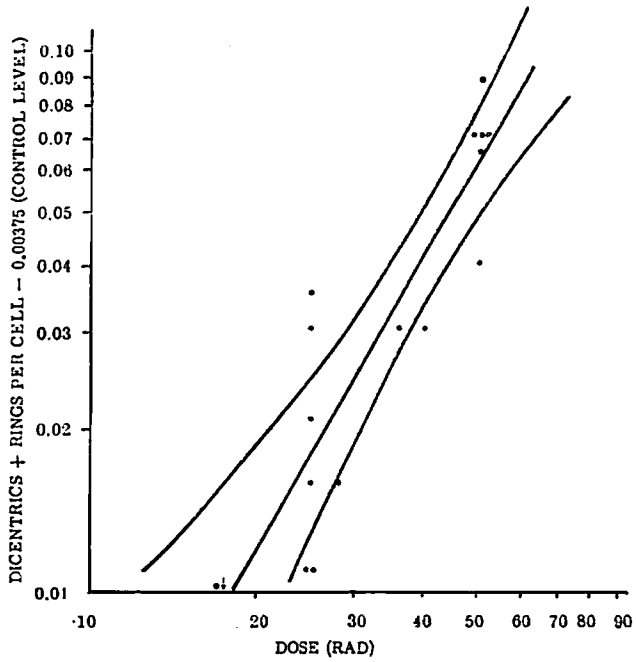

Figure 13. Relationship between yield of dicentrics plus rings per cell and uniform whole-body dose in patients exposed to 2 MeV x rays[271 a]

[a] Data from blood samples taken twenty-four hours after radiation exposure. Points are from data given in table V, and curved lines represent the 95 per cent confidence limits of the regression line.

comparisons are set out in table VI where it may be seen that scores obtained in the *in vivo* work fall in between the lowest and highest yields reported for the same absorbed doses in the *in vitro* studies. It is disappointing to note that, in the only *in vitro* work where a radiation quality similar to that used in the *in vivo* studies was used and where the cells were cultured for fifty hours, the aberration yield at a dose of 50 rads was four times lower than that found in the *in vivo* studies. However, the observations were made in different laboratories where techniques were not entirely comparable.

215. In addition to the data on therapeutic exposure to external radiation sources, there is some information on aberration yields in patients to whom radio-active materials had been administered internally for therapeutic purposes. Boyd *et al.*[272] initially reported that types of chromosome damage similar to those reported by Tough *et al.*[15] were to be found in blood cells of patients treated with radio-active iodine. It was suggested that, in quantitative terms, the effects of 100 millicuries of radio-iodine were similar to the effects of a partial-body dose of 250 rads of x rays and that a 10-millicurie dose of radio-iodine was probably sufficient to produce recognizable chromosome damage. Similar findings with radio-active iodine were also reported by other authors,[172, 273-275] and, in one report,[274] a significant yield of aberrations was observed fourteen years after completion of treatment.

2. *Occupational exposure*

216. A number of workers have reported the presence of chromosome-type aberrations in individuals receiving chronic low doses from external sources.[127, 132, 136, 165, 168, 239, 263, 265, 268, 276-280] Norman and his colleagues[265] observed seven dicentrics in 5,138 cells from ten hospital radiation workers who had received dose equivalents of up to 88 rems accumulated at an average rate of from 1 to 3 rems per year, whereas in ten control individuals no dicentrics were observed in 4,219 cells. Later studies[167] were carried out on thirty-six radiation workers who had received cumulative doses of from 10 to 98 rads with a median annual dose rate of 1.45 rads per year. In this later work, fourteen dicentrics were observed in 14,839 cells, whereas in twenty-three control individuals no dicentrics were observed in 5,784 cells. Observations similar to these have recently been made by Lisco and Lisco[277] and by Gorizontova.[278]

217. Studies somewhat similar to those of Norman and his colleagues have been reported by Court Brown *et al.*[276] and Buckton *et al.*[132] These authors studied sixty-seven adult males working in atomic energy establishments and divided their sample into five groups: (*a*) a control group that had received a cumulative dose of less than 1 rad; (*b*) a group with an average accumulated dose of 3.8 rads, with a range from 1 to 10 rads; (*c*) a group with an average accumulated dose of 27 rads, with a range from 23 to 34 rads; (*d*) a group with an average accumulated dose of 24 rads, with a range from 15 to 37 rads; (*e*) a group with an average accumulated dose of 84 rads, with a range from 75 to 98 rads. The irradiated groups differed not only in the doses received but also with respect to the time over which the exposures occurred. The control group did not differ cytologically from a control population drawn from outside atomic energy establishments, but all the irradiated groups showed a highly significant increase in aberration yield. Although yields of dicentric and ring aberrations of as high as eight per 1,000 cells were observed, no correlation between dose and yield could be discerned.

218. Visfeldt[127] studied aberration yields in peripheral blood cells of ten members of the staff of the Copenhagen Radium Institute who had received cumulative doses ranging from 1 to 116 rads over a period of ten years. Again, a clear increase in aberration yield was observed in irradiated (thirteen dicentrics plus rings in 950 cells) personnel as opposed to control (zero dicentrics plus rings in 300 cells) personnel, but the data are once more too meagre to show any correlation with the dose received.

219. El-Alfi *et al.*[279] analysed blood cells from twelve radiation workers, exposed over periods as long as four years, who received cumulative dose equivalents of up to 1,110 millirems of x, gamma or beta rays or up to 9,722 millirems of neutrons. No details on the quality of the radiation nor on the kinds of exposures were given, but significant increases in aberration yields were observed in the six individuals exposed to neutrons when compared with nine control individuals.

220. Data somewhat similar to those of Visfeldt's have recently been reported by Wald *et al.*[136] These authors studied aberration yields in six nuclear industry workers who had received external body dose equivalents ranging from 25 to 55 rems at an average accumulation rate of 4.3 rems per year. A significant increase in the frequency of stable and unstable aberrations in irradiated as opposed to control personnel was noted, but no relationship with the various dose levels could be discerned, and no detailed cytogenetic data are given.

221. A number of studies have been carried out on persons who have worked in the luminizing industry

and who have, as a consequence, high body contents of radium-226. These studies also have shown a significant increase in aberration yields in exposed versus unexposed individuals, even in individuals having body burdens well below the maximum permissible level.[167, 281] With these internal emitters, there is some evidence of a consistent gradient of increasing aberration yield with increasing radium body burden.[282] In the data of Boyd *et al.*[282] on individuals who accumulated body burdens between 0.10 and 0.56 microcuries of radium-226 eighteen years prior to study, some 3.2 per cent of the cells were classed as "unstable" and found to contain asymmetrical aberrations. In this work, the total occupational dose equivalent from external gamma rays averaged about 90 rems. There was no association between these low-level external exposures and aberration yield.

222. These data on occupationally exposed individuals all show significant increases in aberration yield in persons exposed to very low dose levels. This is, of course, in line with the earlier observations on the effects of low doses of diagnostic radiation. Moreover, it should be emphasized that, in those cases where accurate physical dosimetry has been carried out, it is possible to state that significant aberration yields have been observed in individuals receiving doses below the permissible levels.

3. *Accidental exposure*

223. Bender and Gooch[128, 129] studied aberration yields in peripheral blood cells of eight men exposed accidentally to mixed gamma and fast neutron radiation. The doses were estimated to range from 23 rads to 365 rads, with the neutrons comprising some 26 per cent of the total dose. No chromosome-type aberrations were found in five control individuals (total of 458 cells), but dicentrics and rings were present in all five individuals exposed to doses calculated to be over 200 rads. Blood samples were first collected twenty-nine months after the original exposure and then a year and a half later. Aberrations were present in all individuals except the person exposed at the lowest dose level. In one individual who received an estimated 339 rads, the frequency of dicentric and ring aberrations was 0.166 per cell (table VII). All the cultures in these cases were grown for seventy-two hours. Goh[283] followed up these observations and examined cells from blood samples, cultured for seventy-two hours, taken from six of these men seven years after the original accident. Cells from bone marrow were also sampled. Aberrations were observed both in cells from the marrow and in cells from peripheral blood, but dicentric aberrations were absent in marrow cells. Although the over-all frequencies of aberrations in peripheral blood cells had declined with time after exposure, significant yields of dicentric and ring aberrations were observed on each of the three occasions when samples were taken. The published data on dicentric and ring aberrations in these studies are summarized in table VII.

224. In a later criticality accident,[131, 229] three men received estimated doses of 12, 22.5 and 47 rads of mixed radiation (gamma and fission neutrons), the neutrons contributing, in the different individuals, 25-50 per cent of the total dose. In these cases, blood was sampled from four hours up to two years after exposure, and the cultures were grown for seventy-two hours. Dicentric aberrations were observed in all three individuals, their frequency showing a clear increase with increasing dose. A reasonable correlation between estimated physical dose and aberration yield was observed with some 3 per cent of the cells being affected at the highest dose level. Using these data, previous *in vitro* information and certain assumptions, it was suggested that the RBE for fission-spectrum neutrons versus gamma rays was of the order of five to one.

225. Biola and Le Go[284] have described studies on blood samples taken from an individual four days after a highly non-uniform exposure to mixed gamma and neutron radiation in an incident at Mol, Belgium. Physical estimates of dose suggested that the individual had received a mid-line exposure of around 500 rads. In parallel with the studies on the blood sample from the irradiated individual, studies were also made on blood cells taken from a normal individual and then exposed to cobalt-60 gamma rays with doses of 400 and 600 rads. For comparison with the data of Gooch and Bender,[229] all cultures were harvested at seventy-two hours or ninety-six hours, although it was evident that at seventy-two hours at least 10 per cent of the cells were in their second mitosis in culture. The aberration yields observed, therefore, were clearly an under-estimate of the true yield, but, since cultures of the blood of the irradiated individual and of the cells irradiated *in vitro* were handled in exactly the same way, valid comparisons could be made. The actual yields observed *in vitro* were similar to those obtained by Gooch and Bender[229] at the lower dose levels and to those obtained by Kelly and Brown[137] at the higher doses. The *in vitro* yield at a dose of 450 rads was equivalent to the yield obtained in the cultures from the irradiated individual so that a good correlation existed between physically estimated and biologically estimated dose.

226. More recently, Buckton *et al.*[132] analysed cells from two men who accidentally received whole-body doses of 17 and 18 rads, the men having additionally accumulated 10 and 9 rads, respectively, as an occupational exposure over several years of routine employment. Dicentric and ring aberrations were present in the blood cells of both men at levels up to a maximum of 3 per cent, depending on whether blood was taken at forty-eight hours or at one or three months following exposure. One of the two controls who had received occupational exposures of approximately 2 to 3 roentgens had a dicentric and ring frequency of approximately 1 per cent on two out of the three occasions on which his blood was sampled. The data here are too scanty to draw any conclusions on dose relationship.

227. Sugahara *et al.*[285] have reported data obtained from two men exposed to external irradiation from 250 kV x rays and cobalt-60 gamma rays, with estimated exposures of, respectively, 66 and 40 roentgens, and studied ten and twelve months after exposure. In addition, data were obtained from a further three men who inhaled uranyl fluoride and from whom blood cells were taken forty days after the accident. The amounts of uranyl fluoride (estimated from urine excretion) taken up ranged from 2.2 milligrammes to 3.9 milligrammes, representing an inhalation of between 2.6 and 4.6 10^{-3} microcuries.

228. In blood cells obtained from all five men in this study,[285] significant increases in aberration yields, as compared with those in cells obtained from control individuals, were noted, and dicentric and ring aberra-

tions were present in cultures from all but one of the individuals. The authors point out that the frequency of aberrations in the x- and gamma-irradiated individuals were in the range expected from earlier observations of other workers[128, 131] but that almost equivalent yields were obtained in two of the three men who had inhaled uranium (3.2 per cent enriched uranium). The cumulative, external, occupational dose equivalents of these men were small, ranging from 128 to 936 millirems. The observations made on these three men were comparable with those reported by Boyd *et al.*[282] on luminous dial painters (paragraph 219).

229. Wald *et al.*[136] have carried out cytogenetic studies on a group of seven workers who accidentally inhaled iodine-125 and whose body burdens were measured by direct counting methods. Body burdens ranging from 1.2 to 111 microcuries were determined. These workers had also been exposed to external sources and had accumulated dose equivalents ranging from 1 to 18.8 rems at an average rate of 1.4 rems per year. No details of the qualities of the external radiations were given. The data clearly show a significant increase in the frequency of cells carrying unstable aberrations over controls, but no breakdown of the aberration data is given.

230. Observations similar to those reported above have been made by Lejeune and his colleagues[286] on four individuals, one of whom received an estimated maximum dose of 33 rads of neutron and gamma rays (following an accidental exposure to a proton beam), and the others were exposed to unknown quantities of gamma rays, although in one individual the estimated dose was between 35 and 50 rads. Significantly increased aberration yields relative to controls were observed in samples taken at various intervals up to one year after irradiation. The aberrations included dicentrics and rings as well as a number of symmetrical changes, and the cells were cultured for seventy-two hours prior to preparation. In the individuals in whom physical estimates of dose were available, it was shown that the aberration yields observed were reasonably consistent with those predicted on the basis of the aberration-yield coefficient quoted by Bender and Gooch[229] (paragraph 156). The authors were careful to point out, however, that the data were insufficient to draw any firm conclusions on the relation between aberration yield and dose in these individuals.

231. Lisco and Lisco[287] have recently examined peripheral blood leucocytes (forty-eight-hour and seventy-two-hour cultures) of two radiation workers who exposed their right hands to mixed gamma-beta radiation from an iridium-192 source. The exposure was for a ten-minute period, and the physically estimated dose to the hands was 3,000 rads, 10 per cent of which was from gamma radiation. Eleven days after exposure, the yield of dicentric and ring aberrations in cells of both individuals was around 0.05 per cell (equivalent to that observed with a 50-rad whole-body dose from x rays), and high aberration yields were noted in each of the follow-up studies carried out at intervals up to three years after the accident. No aberrations were observed in bone-marrow cells.

232. The rather sparse data obtained from, fortunately rare, accidents underline the earlier statement that one of the complications in accidental exposure is the difficulty of obtaining good physical estimates of dose, particularly in those cases of mixed radiation exposure. In general, the cytological data that have been obtained are not inconsistent, but they are still too scanty to draw any firm conclusions on the usefulness of aberration yield for biological dosimetry in these particular cases.

4. *Nuclear explosion*

233. A number of studies[133-135, 168, 175, 182, 221] have been made on survivors at Hiroshima and Nagasaki who were exposed to radiation from nuclear explosion in 1945. The data of Ishihara and Kumatori,[135, 175] obtained from blood cells of persons who were between 500 and 2,000 metres from the hypocentre and who were studied nineteen years later, show a significant increase over control individuals in the yield of asymmetrical and symmetrical aberrations.

234. Bloom *et al.*[133, 134, 288, 289] carried out surveys on survivors in different age groups. In the first study[133] on ninety-four exposed individuals and ninety-four matched controls, all the individuals sampled were under the age of thirty years at the time of the bombings in 1945. Chromosome aberrations were found in 0.6 per cent of the peripheral blood leucocyte cells in the exposed individuals, whereas only 0.01 per cent of the cells contained aberrations in control individuals. In the control individuals, no dicentric aberrations were observed in the 8,847 cells scored, but nine such aberrations were found in 8,283 cells from the irradiated population sampled twenty years after exposure.

235. In the second survey by Bloom *et al.*[134] observations were made on seventy-seven heavily exposed (estimated dose greater than 200 rad of mixed gamma-neutron radiation) survivors and eighty control individuals, all of whom were over the age of thirty years at the time of the bombings. Sixty-one per cent of the heavily exposed survivors and 16 per cent of the controls were found to contain aberrations at a frequency of 1.5 per cent in cells of exposed individuals as opposed to 0.3 per cent in cells of control individuals. One dicentric aberration was detected in the 7,188 cells scored from controls, and this was observed in a cell from an eighty-year-old male. In the irradiated individuals, eight dicentrics were found in the 6,778 cells studied.

236. The relative frequencies of the asymmetrical dicentric, ring and fragment aberrations in the survivors of the two different age groups were very similar. However, the symmetrical translocations and inversions were found to be more frequent in the older exposed survivors than in the younger ones. In parallel with this latter observation, it was noted that symmetrical aberrations were also more frequent in the older of the two control groups. These observations once more raised the question of whether the sensitivity to aberration induction by radiation might be related to age, a problem that requires urgent attention.

237. The most frequent aberrations observed by Bloom *et al.*[134] in the exposed older survivors were translocations which were present in seventy-two of the 6,778 cells scored. It was noted, however, that twenty of these cells containing a translocation were detected in four individuals, and these represented five different types of translocation or five possible cell clones.

238. Estimates of the dose sustained by the exposed older survivors ranged from 204 to 991 rads of mixed gamma and neutron radiation. A preliminary attempt

to correlate aberration frequency with physically estimated dose suggested that the aberrations increased approximately linearly over the dose range studied, the over-all aberration frequency being about 1 per cent at 200 rads and increasing by approximately 0.5 per cent per 100-rad increment. It should be noted, however, that, in this work, the leucocytes were cultured for a period ranging from 66 to 70 hours, and, of course, samples were studied twenty years after an original radiation exposure.

239. Workers at the Atomic Bomb Casualty Commission [289] have analysed the karyotypes of 128 individuals who were born after at least one of their parents had received a minimum exposure of 100 rads as a result of the atomic bombings at Hiroshima and Nagasaki. Control studies were carried out on fifty-seven sibs of these individuals who were born before the time of the bombings. Particular attention was devoted to the 103 individuals who were born in the first five years after parental exposure, but no significant increase in chromosomally abnormal individuals could be detected. However, a detailed study of thirty-eight *in utero* exposed survivors,[288] whose mothers had been exposed to more than 100 rads (estimated range 104-477 rad) at the time of the bombings, revealed a small but significant increase in the frequency of lymphocytes with complex chromosomal rearrangements (0.52 per cent) as compared with matched control individuals (0.04 per cent).

240. Sasaki and Miyata[221] re-opened the question of the relationship between aberration yield and estimated physical dose in the atomic bomb survivors and presented a considerable amount of detailed data obtained from the scoring of over 80,000 cells from exposed and control individuals. Chromosome analysis was carried out on fifty-one Hiroshima survivors and eleven controls twenty-two years after the original exposure. Care was taken to score only cells dividing for the first time in culture, and the cultures were terminated after fifty hours. The aberrations were classified as outlined in paragraphs 24 to 34, and it was found that the mean number of dicentrics and rings in the exposed individuals (201 in 73,996 cells) was 0.0024 per cell as compared with 0.0002 per cell (2 in 9,510 cells) in the controls. It was also shown that the frequency of cells carrying stable symmetrical rearrangements (largely reciprocal translocations) was 0.40 per cent in exposed individuals as compared with 0.07 per cent in controls.

241. A significant yield of aberrations was observed among nineteen survivors who were more than 2.4 kilometres from the hypocentre and who, as was estimated on the basis of physical considerations, had received a dose of the order of 1 rad. Eleven of these individuals entered the bombed area within three days after the bombing, and the frequency of dicentrics and rings in these individuals was 0.0013 per cell as compared with a frequency of 0.0006 per cell in the eight individuals who did not enter the bombed zone.

242. The exposed individuals were divided into four groups based on distance from the hypocentre at the time of the bombing and on whether they were directly exposed or shielded by wood or by concrete. Proportionally more cells with aberrations appeared in survivors exposed at the shortest distance from the hypocentre, and, at a given distance, the aberration yield was highest in those directly exposed, intermediate in those shielded by wood and lowest in those shielded by concrete.

243. Since the observations were made twenty-two years after the original exposure, Sasaki and Miyata[221] used two different methods in an attempt to obtain dose estimates. Studies by other workers[33, 44, 290] have shown that the proportion of peripheral blood lymphocytes carrying stable chromosome rearrangements (C_s cells) observed many years after an irradiation exposure remains unchanged from the proportion observed shortly after exposure. The ratio of C_s cells to normal cells could, therefore, be used as an end-point in these Hiroshima survivors, and the relationship between this end-point and distance from the hypocentre is shown in figure 14.

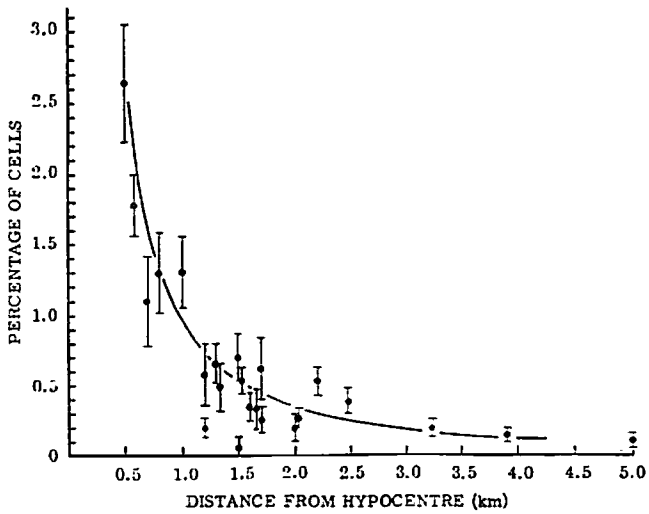

Figure 14. Frequency of C_s cells as a portion of all (except C_u) cells plotted against distance from hypocentre[221]

244. Asymmetrical aberrations would, of course, have been largely eliminated over the twenty-two years since exposure so that the total frequencies of dicentric and ring aberrations in individuals at different distances from the hypocentre would not offer a good method of relating aberration yield to absorbed dose. However, the distribution of chromosome aberrations within X_1C_u cells which had not divided since exposure should be the same as that existing immediately after exposure. In figure 15 are shown the number of dicentric and ring aberrations in C_u cells in relation to distance from the hypocentre.

245. Using the proportion of C_s cells to normal cells and the number of dicentric-plus-ring aberrations in C_u cells, Sasaki and Miyata[221] obtained absorbed-dose estimates simply by relating the values given in figures 14 and 15 to equivalent yields obtained with measured doses of 2 MeV x rays in *in vitro* studies. Figure 16 shows the dose estimates obtained in this way (without attempting to make corrections for shielding or for quality of radiation, etc.) plotted against distance from the hypocentre. The dashed line in this figure shows a recent[201] indirect physical estimate of air dose for comparison with the dose arrived at by using the biological methods.

246. The estimates based on chromosome aberration yields compared with the physical estimates (figure 16) are low in the survivors exposed close to the hypocentre and high in the remotely exposed people. The authors[221] suggest that these differences may reflect a selective mortality over the twenty-two-year period in the population exposed near the hypocentre and that

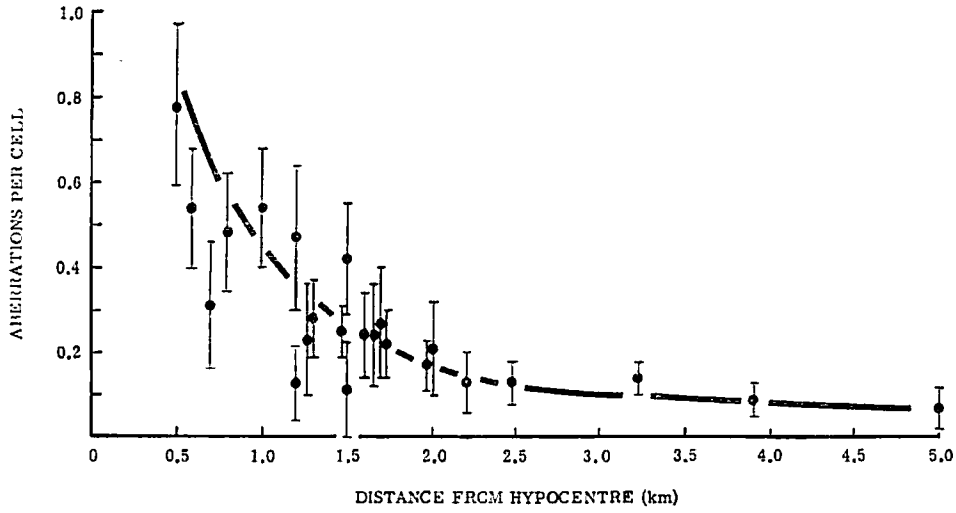

Figure 15. Number of dicentrics plus rings per X_1C_u cell versus distance from hypocentre[221]

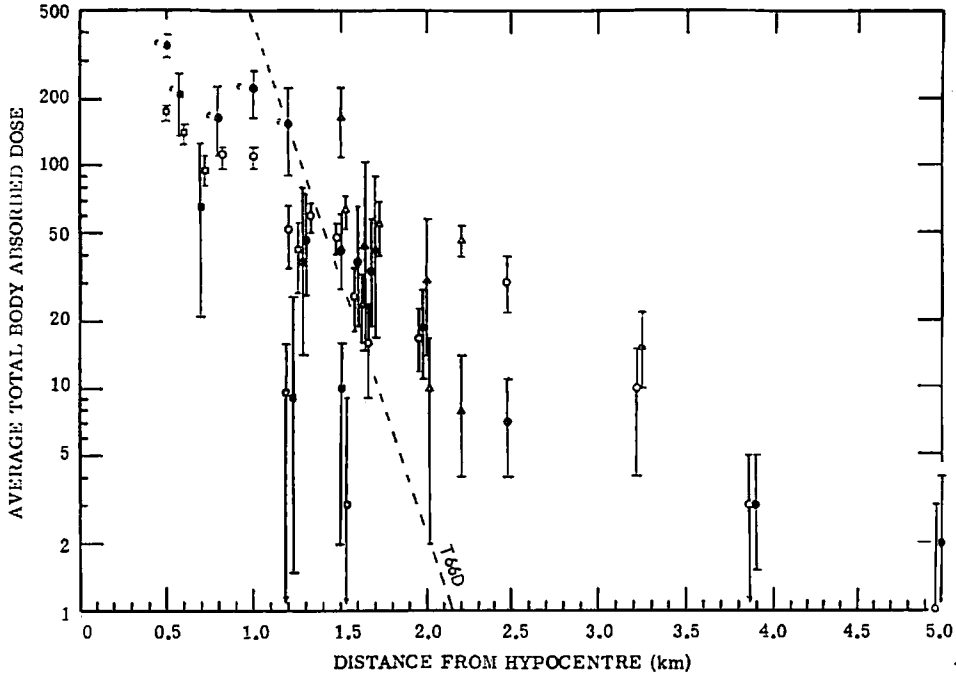

Figure 16. Average total-body absorbed dose estimated from the frequency of chromosome aberrations in Hiroshima survivors[221] [a]

[a] Vertical lines through points represent 50 per cent confidence limits, and dashed line, T66D, gives estimate of air dose derived from physical considerations. Estimates of absorbed dose based on the C_1 cell data (figure 14) are those given as open symbols, and estimates based on dicentric and ring aberrations in X_1C_u cells (figure 15) are given as closed symbols. Triangles, circles and squares represent directly exposed survivors and survivors shielded by wooden or concrete houses, respectively. Subscript *e* indicates those survivors who had epilation.

individuals who were more than 2.4 kilometres away might have received radiations from sources other than the primary rays.

247. Three papers describe observations on peripheral blood chromosomes of individuals accidentally exposed to radiation from radio-active fall-out due to the explosion of a thermonuclear device at Bikini in 1954.

248. Ishihara and Kumatori[135, 292, 293] studied cells obtained from eighteen of the twenty-two fishermen whose external exposure resulted in doses estimated to range from 220 to 660 rads and who had also received an unknown contribution from internally deposited radio-active material. Samples were first taken ten years after exposure and repeated sampling has continued since that time. In the original study[135] it was found that the aberration yield in the irradiated individuals was significantly above controls and that dicentric aberrations were present, but it was not possible to correlate aberration yields with physically estimated doses. However, when the individuals were divided into three groups according to the degree of damage indicated by the lowest neutrophil levels reached shortly after exposure, it was found that the mean frequencies of cells containing aberrations in these three groups were correlated with the extent of damage indicated by the original haematological findings. Follow-

up studies showed that three individuals possessed clones of cells with chromosome abnormalities in their bone marrow, and it was noted that these three persons were, in fact, in the group who had the lowest neutrophil counts shortly after exposure.

249. Lisco and Conard[294] have recently studied blood cells obtained from fifty-one Marshallese, of whom thirty had received an estimated whole-body gamma-ray dose of 175 rads, thirteen had received approximately 70 rads, and eight had not been exposed and served as controls. The results are curious in that more acentric fragments were found in controls than in irradiated individuals. However, if we consider only the dicentric and ring aberrations, a difference between exposed and unexposed individuals is observed. In the controls, no asymmetrical exchange aberrations were found in the 400 cells analysed, but three dicentrics and rings were found in 650 cells from the 70-rad group and six in 1,500 cells in the 175-rad group. A similar difference between control and exposed individuals was found for symmetrical exchange aberrations.

250. These data from individuals exposed to radiation following nuclear explosions all show significant aberration yields in the survivors studied. Moreover, the observations confirm earlier studies which showed that radiation-induced chromosome aberrations can be detected in leucocytes of individuals exposed to radiation for clinical reasons up to twenty-two years prior to observation. Most of these earlier studies did not permit quantitative conclusions relating aberration yield to absorbed dose, but the recent extensive data and analyses on Hiroshima survivors suggest that a fair measure of agreement exists between dose estimates based on the yields of chromosome aberrations and indirect estimates of air dose arrived at through the use of physical methods.

251. From the data discussed thus far it is evident that the aberration yield must decline with increasing time interval between irradiation and blood sampling, and the influence of this factor on aberration yield should now be considered.

5. *Time of sampling after radiation exposure*

252. Since the first publications[44, 128] demonstrating that a significant yield of aberrations could be observed in blood cells of individuals exposed to radiation many years earlier, virtually all of the publications on *in vivo* exposure present data confirming these observations.

253. In the original work of Buckton *et al.*[44] it was shown that the frequency of cells carrying asymmetrical aberrations ("unstable" C_u cells) showed an approximately exponential decline with increasing time after exposure, whereas the frequency of cells with symmetrical changes ("stable" C_s cells) stayed roughly constant with time (paragraph 243). Cytological evidence indicated that many of these aberrant cells with asymmetrical changes were in their first post-irradiation mitosis when sampled so that these data offered a means of determining the average *in vivo* life span of the dormant non-dividing leucocytes (small lymphocytes). Further studies by Buckton *et al.*[33, 295] and by Norman *et al.*[290, 421] have extended and refined the analysis and led to the conclusion that the mean life span of this cell lies somewhere between 500 and 1,500 days in accordance with the tritium-labelled thymidine data on these cells obtained by Little *et al.*[193]

254. Goh[283] has suggested that not all the aberrations observed in peripheral blood leucocytes many years after exposure are aberrations induced at the time of irradiation. The evidence in support of this suggestion is meagre and is open to an alternative interpretation. It is known, however,[296] that chromatid-type aberrations can be induced in human fibroblasts by exposure to extracts of allogenic lymphocytes, and Goh presents evidence[297] for a small, but significant, effect of irradiated human plasma in inducing aberrations in peripheral blood leucocytes, an observation supported by the independent work of Hollowell and Littlefield.[298] In Goh's experiments, the cultures were incubated for seventy-two hours and, from the figures in the published account, it is evident that most of the aberrations observed were chromatid-type changes with some "derived" (paragraph 74) chromosome-type fragments; no chromosome-type dicentrics or rings were noted. Hollowell and Littlefield[298] used plasma from patients given doses up to 4,500 rads from 2 MeV x rays and observed chromatid-type and chromosome-type changes in blood cells from normal individuals cultured in the presence of such plasma. Five dicentrics and rings were observed in 476 cells, but these aberrations were probably of the "derived" type. Unpublished works of other observers[299-301] have shown either no discernible effect or a small increase in chromatid aberration yields in first divisions of cultured cells exposed to irradiated plasma.

255. It is of interest to note here reports[302, 303] indicating that the long persistence of chromosomally damaged lymphocytes so clearly demonstrated in man and Rhesus monkey may not occur in certain other mammals. Studies on PHA-stimulated blood cells from monkey, rat, guinea pig and pig have shown that unstable chromosome aberrations are not observed for more than a few hours after irradiation except in the monkey where C_u cells were observed for as long as the animals were observed (seven months). This rapid loss of C_u cells in these animals is of particular interest, since, at least in the rat, *in vivo* studies with tritium-labelled thymidine[304, 305] have clearly demonstrated the presence of long-lived lymphocytes in unirradiated animals.

256. From the point of view of biological dosimetry, there is normally little interest in samples of blood cells taken from irradiated individuals many years after radiation exposure. However, follow-up studies will, of course, be of importance in connexion with the possible somatic risks that may be associated with the presence of aberrations. Chromosome studies on bone-marrow cells in particular will be of paramount importance with regard to leukæmia risks. In the case of the peripheral blood leucocyte (small lymphocyte), the composition of this cell population in peripheral blood (as well as in extravascular areas) will change with time simply because of the role played by this cell in immunological response.[306] The work of Nowell[218, 302] suggests that the aberration yields observed in leucocytes many weeks, months or years after exposure will be dependent upon the number and type of antigenic stimuli received by the individual between the time of exposure and the time of sampling.

257. Although these small lymphocytes are involved in immune responses, they are, nevertheless, normally non-dividing so that, in man, there should be no preferential loss of cells carrying chromosome damage in samples taken many hours, days or possibly weeks after exposure, provided the cells are not involved in

an immune response. Support for this concept has come from the *in vitro* studies of Scott *et al.*[245] and of Kozlov[307] who have compared aberration yields in peripheral blood leucocytes placed in a culture medium containing PHA immediately after irradiation with cells held for twenty-four hours post-irradiation or less in a culture medium devoid of PHA. In both these studies, using fast neutrons[245] (paragraphs 176-177) and cobalt-60 gamma rays,[307] the general finding was that an increased time between irradiation and mitotic stimulation had little or no influence on the aberration yield.

258. There is little information, however, on the variation in life span of the small lymphocyte and on the multiplicity of populations present throughout the body. Nevertheless, on the basis of information available, it would seem that, within any particular defined subpopulation, the aberration yield should remain constant until that population becomes involved in an immunological response. The difficulty is, of course, that of defining a subpopulation or compartment in a mixed population of morphologically identical but functionally diverse cells which migrate throughout the body.

259. In terms of biological dosimetry, the location, distribution and population density of lymphocytes throughout the body is a problem of no importance if an individual receives a uniform whole-body exposure to a radiation of high penetrating power. This problem could, however, be of great importance in the case of an individual receiving partial-body exposure or when the exposure is to radiation of low penetrating power or of mixed quality. The problem may be minimized if a sufficient cell mixing within the population occurs between the time of irradiation and sampling.

260. When blood is sampled *immediately* after an acute exposure to radiation, then leucocytes that were in the peripheral blood vessels at the time of exposure will presumably be the only population that is sampled. However, if sampling is delayed for a sufficient but unknown time, then leucocytes that were in various lymphopoietic centres at the time of irradiation may have been mobilized into the blood stream. The data obtained in the extracorporeal studies[227, 243, 244] suggest that this time interval is not greater than eight hours and may, in fact, be of the order of a few minutes (paragraphs 199-204). At later sampling times, some of the leucocytes observed in peripheral blood will not have been directly exposed to radiation but will have been derived from irradiated stem cells.

261. To date, there is not a great deal of information on the change in aberration yield in blood samples obtained at frequent intervals throughout the first two or three days following exposure. In the data of Bender and Gooch[131] obtained from three men receiving up to 47 rads of mixed gamma and neutron radiations in a criticality accident, there appeared to be a little difference in aberration yield in samples taken four hours, two weeks and four weeks after exposure.

262. In some more recent work of Buckton *et al.*[16] which involved the partial-body exposure of ankylosing spondylitis patients to various doses of x rays, it was observed that higher aberration yields were obtained from blood samples taken twenty-four hours after exposure than from samples obtained immediately after exposure (table III). More detailed studies were then carried out by these authors on patients who had received a partial-body exposure of 300 roentgens. Samples were taken from these patients at zero, three, six, twelve, twenty-four and forty-eight hours after exposure. The aberration yield increased with sampling time up to twenty-four hours and then declined at forty-eight hours. At zero hours and forty-eight hours, the frequency of "unstable" cells was approximately one-half the maximum yield that was observed in the twenty-four-hour samples. There was no significant difference between yields observed in the six-, twelve- and twenty-four-hour samples.

263. The very limited data on whole-body exposure in the criticality accidents, often involving uneven exposure, are insufficient to make any pronouncements on change in aberration yield in the first few hours following exposure. What few data exist do not contradict the expectation of no change in yield with time of sampling with this kind of exposure. However, the recent whole-body x-ray studies[271] support the observations made in the earlier partial-body x-ray work (paragraph 212 and table V). In the partial-body and in the extracorporeal blood exposures, there is clear evidence that the aberration yield changes with time even in the first twenty-four hours following exposure. There is but little information on the amount and rate of change and no information on whether these parameters are influenced by dose level, site of exposure, age and health of the exposed individual.

D. Conclusions

264. It is evident from the studies carried out on patients receiving low doses from diagnostic x rays and on individuals receiving chronic low doses as a consequence of their occupation that doses of the order of a few rads from x or gamma rays result in a significant increase in the yield of aberrations in blood leucocyte cells. This increase is particularly impressive when dicentric and ring aberrations, that are extremely rare occurrences in the blood cells of unexposed individuals, are considered. Thus, in terms of its possible use in biological dosimetry, it is clear that this particular system is a very sensitive one.

265. A knowledge of the form of the relationship between aberration yield and radiation dose is essential in any attempt to extrapolate from one to the other. Until very recently, the *in vitro* data that were available seemed rather disappointing, since considerable differences were evident when data obtained in different laboratories were compared. Nevertheless, within any one laboratory, when the same techniques were employed, the results were consistent and highly repeatable (paragraphs 178-179). The more recently available data on the response to radiations of differing quality reveal a high degree of consistency between laboratories when similar conditions of irradiation and of duration of culture are used.

266. The main factors contributing to differences in results between laboratories are (*a*) the use of different culture times; (*b*) the irradiation of whole blood prior to culture as opposed to the irradiation of blood in culture; and (*c*) the use of different qualities of radiation. It is evident that the aberration yields obtained in cultures maintained from forty-eight to fifty-four hours are generally higher than in cells irradiated and cultured under the same conditions but sampled at seventy-two hours. Higher aberration yields are also reported, particularly at dose levels below approximately 150 rads of x or gamma rays, if blood cells are irradiated after culturing rather than as whole

blood prior to culture. As a consequence of this, the dose exponents from experiments with irradiated cultures are somewhat lower than from experiments using cells irradiated prior to culture. The reason for these differences is not clear, and further work is certainly necessary in this area. Large differences in response are observed between radiations of differing quality, and the RBE for 2 MeV x rays as compared with 250 kVp x rays is 0.8.

267. Studies on the yields of dicentric aberrations in whole blood irradiated with 2 MeV x rays, cobalt-60 gamma rays or 150 to 300 kVp x rays, and cultured from forty-eight to fifty-four hours, show that the dose exponent, n, in the equation $y = kD^n$, is 1.9 to 2, 1.7 to 1.8 and 1.5 to 1.6, respectively. For blood cells irradiated in culture with 180 to 250 kVp x rays and cobalt-60 gamma rays, the dose exponents are reduced to around 1.2 to 1.3. These data indicate that exchange aberrations induced *in vitro* by 2 MeV x rays are predominantly a consequence of the effects of two separate tracks. With conventional x rays (150-300 kVp), however, up to dose levels yielding less than two dicentrics per cell, a considerable proportion of the exchange aberrations is the consequence of single track events. Studies with 2 to 5 MeV DD neutrons and 0.7 MeV (mean energy) fission neutrons indicate that with these radiations there is a linear relationship between dose and aberration yield over a wide range of doses. In the case of 14.1 MeV neutrons, the relationship is not linear, the dose exponent lying between 1.2 and 1.4. Further information on the effects of fast neutrons, particularly in relation to the influence of irradiation and culture conditions and technique, is required.

268. In the *in vivo* studies on the relationship between aberration yield and dose, much of the data have been obtained from individuals exposed to partial-body doses. Here, numerous complications arise, and most of the data indicate that the aberration yield changes with time within the first day or so after exposure. These changes reflect alterations in the number, distribution and mobility of the different leucocytes within and between the exposed and unexposed regions of the body at the time of exposure and the degree of mixing that occurs between the time of irradiation and the time of sampling from peripheral blood. It is clearly difficult to derive a measure of absorbed dose under these circumstances.

269. In view of the complications referred to above, there can, of course, be no simple standard dose-response relation for aberration yield in cases of partial-body irradiation. In those cases where defined areas of the body have been exposed to radiation for therapy purposes and blood samples taken at short defined intervals after exposure, an increase in aberration yield with increasing skin dose has been noted. In these cases, the relation between the yield of dicentric and ring aberrations and "skin dose" may be almost linear ($y = kD^{1.2}$) or approximately follow a dose-squared ($y = kD^2$) relationship.

270. In the whole-body studies in which patients received from 17 to 50 rads from 2 MeV x rays, the yield of dicentric and ring aberrations increased approximately linearly with dose in blood samples taken immediately after exposure ($y = kD^{1.13}$) but increased approximately in proportion to the square of the dose in samples taken twenty-four hours later ($y = kD^{1.68}$). This increased dose exponent in the later samples was a consequence of significant increases in the yields obtained in two of the patients given a dose of 50 rads.

271. A number of laboratories are now studying aberration yields in patients exposed to uniform whole-body irradiation so that more data on the relationship between aberration yield and radiation dose and quality should become available in the near future.

272. In the criticality accidents in which individuals were non-uniformly exposed to mixed gamma-neutron radiation (paragraph 225), the data are naturally difficult to evaluate because of the complications of dose distribution, radiation composition and RBE. However, in the individual that received a physically estimated dose of 47 rads (47 per cent of which was estimated to be due to gamma rays) the yield of dicentrics and rings was 0.033 per cell in cells sampled between four hours and four weeks after exposure and cultured for seventy-two hours. This yield is somewhat lower than that (0.056 dicentrics and rings per cell) observed following forty-eight-hour cultures of blood from individuals exposed to whole-body irradiation of 2 MeV x rays with a dose of 50 rads (paragraphs 210-213, and table V.)

273. The limited data on whole-body exposure indicate that the aberration yield could serve a useful purpose in dosimetry in such cases, but more information is certainly required. In this respect, we have pointed out that results from samples taken shortly after (within the first few hours or days) uniform whole-body irradiation may not be influenced by the distribution, etc. of the leucocytes at the time of irradiation or sampling. On the other hand, in the case of follow-up studies or where the first samples are taken many weeks or months after an original exposure, the aberrations will be studied in a selected population of long-lived cells. The primary aberration yields in surviving long-lived damaged cells will, however, be a reflection of the dose received in the original exposure (see the Hiroshima data referred to in paragraphs 240-246), and it may still be possible to make estimates of absorbed dose from such cells. However, much more work in this area is necessary, and a great deal more information is required on the rate of decline in aberration frequency with time and possibly, under various conditions, in the months or years following exposure.

V. Possible biological significance of the aberrations

A. Introduction

274. The possible biological significance of chromosome aberrations, particularly with reference to their presence in germ cells, has been the subject of continued review by the Committee.[2,3] There are no direct data on the genetic consequences of radiation-induced chromosome aberrations in the germ cells of man, although information on the genetic consequences of radiation-induced chromosome anomalies in laboratory mammals and on constitutional chromosome anomalies in man is available. Most of this information was reviewed in detail in the 1966 report[3] so that only a very brief consideration of the little new material will be attempted here.

275. The significance of chromosome aberrations in somatic cells has been the subject of much speculation. The idea that aberrations might be causal factors in certain somatic disease states in man has been considered since the early suggestion by Boveri[308] that abnormalities of chromosome constitution might be

significant causal factors in neoplasia. The fact that neoplasms can be induced by ultra-violet, ionizing radiations and chemical carcinogens, that these agents induce chromosome aberrations and that such aberrations are to be observed in many tumours has naturally provided a good deal of incentive to examine the possible relationships involved. This has been further stimulated by the more recent studies on virus-induced neoplasia in mammals and on virus-induced chromosome aberrations and also by the discovery of a specific chromosome change in bone-marrow cells of humans suffering from a particular form of leukæmia.

276. This section will, therefore, examine the mutational importance of chromosome aberrations in relation to metabolic effects, cell killing, life-shortening, neoplasia and immunological deficiency, effects which have all been suggested to be a consequence, at least in part, of genetic imbalance.

B. Aberrations in germ cells

277. The varieties and incidence of constitutional aneuploidy in man were considered in detail in the 1966 report[3] of the Committee, and there is only little new information of relevance to be considered here. In that report, reference was made to two well characterized syndromes causally related to a loss of autosomal chromosome material, the "cri du chat" syndrome (a deficiency in the short arm of chromosome 5)[309] and a congenital anomaly resulting from a deficiency of the long arm of chromosome 18 (18q —).[310, 311] Another deletion syndrome, first reported by Lejeune *et al.* in 1964,[312] was shown to be associated with a deletion of chromosome 21 (giving a partial monosomy), the syndrome showing many signs that appeared to be the reverse of those characteristic of Down's syndrome. Since that time, a number of cases of a syndrome[313] have been reported in which the affected individuals are completely monosomic for a group G chromosome[176-178] (generally considered to be chromosome 21). Further evidence is, therefore, accumulating that indicates that certain deficiencies in certain chromosomes and even the loss of a group G chromosome (presumably chromosome 21) may not be incompatible with life, although all appear to be associated with gross physical and mental abnormality.

278. Consideration was also given in the 1966 report[3] to the incidence of chromosome anomalies in spontaneous abortion and still-born, and reference was specifically made to the work of Carr.[314, 315] In this work, it had been shown that the incidence of 45X zygotes in man occurs at a frequency of about 8.3 per 1,000 conceptions; the majority of such XO conceptions abort,[316] one estimate indicating that only one in forty reach full term.[317] In the mouse, XO individuals are viable fertile females, and the average incidence of this condition in the mouse is around seven per 1,000 births[318, 319] (annex C, table VI, of the 1966 report[3]). The probable incidence of the XO condition at the time of conception in man and mouse may, therefore, be very similar, and it is of interest to note that, in the mouse, depending on the stage of development of the germ cells at the time of irradiation, it has now been shown that the incidence of XO offspring may be increased up to three or four times following an exposure of 100 roentgens of x rays.[318, 321]

279. In the last three years, considerable interest has been aroused in individuals having an XYY chromosome constitution, since they have been reported to occur with relatively high frequency in patients with "dangerous, violent and criminal propensities" in state hospitals[322] and in prison populations.[323, 324] Jacobs *et al.*[322] reported that 3 per cent of 314 patients in a state hospital in Scotland for the mentally sub-normal had an XYY chromosome constitution and that the mean height of such patients was significantly in excess of the mean height of men in the hospital with an XY chromosome constitution. It had previously been reported[325] that XYY individuals were fertile.

280. The possible significance of an extra Y chromosome with regard to criminal behaviour has not yet been clarified, as a number of conflicting reports[326, 327] have appeared since the original suggestion of this association.[322] Good estimates of the incidence of XYY males in "normal" populations are only just becoming available. It is reported[328] that only one XYY male was discovered in a survey of over 2,000 Edinburgh males examined prior to the state hospital and prison surveys. Recent studies in Canada[329] indicate a frequency of XYY males among new-born of two in 1,000, and a frequency between one and two in 1,000 in the general population is suggested by a survey carried out in France.[330]

281. Five sexually abnormal patients have been reported[331-334] to contain chromosome complements with an apparent dicentric Y chromosome, and it has been suggested[335] that some isochromosomes for the long arm of the X chromosome are dicentrics. These are referred to here simply to point out that, in those rare instances when the two centromeres of a dicentric chromosome are very clearly juxtaposed, the dicentric may behave in a manner functionally similar to a normal monocentric chromosome at anaphase of mitosis and, hence, be transmitted to all the descendant cells.

282. It was concluded in the 1966 report[3] that chromosome anomalies are to be observed in the somatic cells of about ten per 1,000 live new-born infants and that half of these abnormalities are accounted for by translocations. This estimate of five individuals with translocation per 1,000 live new-born is certainly a minimum estimate, and better estimates will not be available until considerably more work on meiotic cells is carried out. For this reason, it is relevant to consider here some new data[331] obtained from a highly selected population comprising fifty males attending a subfertility clinic.

283. These individuals were selected on the basis of being chromatin-negative and having a sperm count of less than 20 10^6 per millilitre; a proportion were azoospermic. The observation of interest here is that four of the men were found to be translocation heterozygotes on the basis of meiotic studies, but in only two of these men could the translocations be discerned at mitosis in somatic cells. This result serves to underline the inefficiency of translocation detection in somatic cells (see paragraph 38 where it is concluded that only approximately 20 per cent of the radiation-induced translocations can be detected in somatic cells) and points to a very high incidence of translocation heterozygosity in subfertile men. This observation is in itself significant, since it is usually assumed that the semi-sterility that is to be found in insects or laboratory mammals that are translocation heterozygotes will be of little consequence in man because family-size falls short of the fecundity of the species.

284. A study has recently been reported [338] on the incidence of constitutional chromosome anomalies in offspring of mothers exposed to abdominal diagnostic radiation (estimated maximum gonadal dose up to 7 rad) prior to conception, and the authors interpret their results as indicating an increased risk in such offspring. However, in this study, the frequency of trisomic offspring in matched-control unirradiated mothers was unexpectedly low, and, moreover, the incidence of still births in this control group was significantly higher than in the irradiated group. The Committee is continually reassessing this problem, but these new data do not alter its opinions stated in the 1962 and 1966 reports,[2, 3] that is, that exposure to ionizing radiations might result in an increase in the prevalence of developmental congenital malformations but that no quantitative estimates can be made at this time.

C. Aberrations in somatic cells

285. There has been much speculation concerning possible relationships between radiation-induced chromosome aberrations in somatic cells and various diseases in man. To date, however, almost no data have been obtained which permit precise statements relating a particular chromosome aberration to a particular lesion or a given yield of radiation-induced aberrations to a predictable incidence of a specific disease in man or other mammals. Nonetheless, the postulated relationships between chromosome changes and such disorders as neoplasia, auto-immune disease, and non-specific aging are worth reviewing if only to stimulate further work.

1. *Somatic mutation and metabolic effects*

286. One general hypothesis has suggested that radiation-induced chromosome aberrations may be an important mutational mechanism by which cellular alteration or depletion takes place in mammalian organs with increasing age leading to disease or death, but few corroborative data are available.[339, 340] Deleterious metabolic changes in mammalian parenchymal cells surviving radiation injury have not been directly related to induced chromosome changes. Material for such studies is possibly available in the clones of cells with chromosome aberrations which repopulate the peripheral blood and the hæmatopoietic tissues of humans and rodents surviving large doses of radiation.[131, 135, 222, 341-344] To date, however, the alterations of specific functional capacity of the enzyme activity of these chromosomally abnormal cells have not been compared to those of non-irradiated populations, although superficially, at least, these cells appear to be functioning normally.

287. In human chronic granulocytic leukæmia, including those cases believed to be radiation-induced, alkaline phosphatase levels are consistently reduced in leukæmic leucocytes which also carry an abnormal chromosome 21 (the Philadelphia chromosome).[345] Whether this enzymatic deficiency has any deleterious effect on the cells involved is not known.

288. Only very sketchy data are available on enzyme changes associated with constitutional chromosome abnormalities in man (these conditions were reviewed by the Committee in its 1966 report).[3] Trisomy 21 (Down's syndrome) is associated with increased levels of a number of leucocyte enzymes[346-348] as well as with alterations in tryptophane metabolism,[349] but these may reflect a general alteration in control mechanisms for RNA or protein synthesis rather than involvement of specific structural loci.[350] Abnormalities in hæmoglobin and haptoglobin synthesis have been reported in constitutional anomalies involving group D chromosomes (chromosomes 13-15), but again it is not clear whether structural genes or regulatory mechanisms are involved.[351, 353] In abnormalities of the human X chromosome, specific metabolic alteration in affected cells has not been recognized; apparently the abnormal X is consistently inactive genetically in such cells.[354] The metabolic effects of specific chromosome abnormalities in human cells thus remain almost totally unknown.

2. *Somatic mutation and cell killing*

289. With respect to the relationship between radiation-induced chromosome aberrations and cell killing, more extensive information is available. These data largely involve reproductive cell death *in vitro* (for example, inability to complete mitosis or to proliferate sufficiently to produce a viable clone) as opposed to interphase death (for example, death of non-proliferating cells), and this may be of importance in considering the relevance of such studies to the effects of radiation in the human body. In general, the x-ray dose-response curves have been similar for both cell reproductive survival and production of chromosome abnormalities in a number of cell systems,[149, 355] and there is good evidence that certain types of chromosome aberrations may result in cell killing (paragraphs 24-50).

290. However, discrepancies have been observed in some experiments, both in the shape of the curves, with consequent considerations of recovery and repair mechanisms, and in the effect of modifying factors. Thus, in some instances, mechanisms not involving chromosome aberrations can apparently cause reproductive cell death following radiation injury.[149, 197] Both somatic mutation and cell killing may underlie various somatic effects of ionizing radiation in the mammalian organism, and chromosomal damage may be visible evidence of the primary site of injury, but any quantitative statements, based on presently available data, attempting to relate these phenomena may subsequently prove to be oversimplified.

3. *Somatic mutation and life-shortening*

291. There has been considerable speculation about the role of chromosome aberrations as mediators of the aging process. The fact that radiation results in life-shortening has long been well documented in experimental animals with extensive consideration of a variety of radiation parameters,[356] but whether reduced life span results simply from radiation-induced increases in certain specific diseases or from acceleration of some generalized non-specific aging process has been debated. Where radiation-induced life-shortening is clearly related to increased tumour incidence, the same concepts of the role of chromosome aberrations would apply, as will be discussed in the next section. If some non-specific aging phenomenon is to be postulated, the possible significance of chromosome aberrations depends on the particular senescence theory being proposed. If radiation-induced aging of tissues and organs is considered to result from mutational events leading to death or diminished function of non-replaceable cells or to reproductive death of cells needed for renewal,

chromosome aberrations might obviously play a central role. Similarly, aging attributed to auto-immune mechanisms mediated by "forbidden clones" arising through somatic mutation (paragraph 317) could also have a chromosomal basis.[357, 359] On the other hand, theories of aging based on extracellular, degenerative changes, such as increased cross-linkage of collagen,[360] would seem unrelated to chromosome alterations.

292. Attempts to approach these questions experimentally have been relatively few. Curtis, in a series of studies in mice,[340] extending concepts and techniques of earlier workers,[339, 361, 362] has demonstrated a direct correlation between life-shortening and frequency of chromosome aberrations in liver cells. This relationship was observed after various types of radiation exposure and also in strains of mice differing naturally in life span. Curtis has cited these data to support the somatic mutation theory of aging, postulating radiation-induced life-shortening as the direct result of radiation-induced chromosome aberrations. This conclusion has been challenged on various grounds, including the lack of correlation between liver chromosome change and hepatic dysfunction and the failure of chemicals which produce liver chromosome aberrations in mice to induce life-shortening.[363] Kohn[360] has suggested that the observed aberrations might be the consequence, rather than the cause, of altered metabolic states or of diseases associated with life-shortening.

293. Comparable data are not available in man, although an increased incidence of aneuploidy with age has been observed in peripheral blood lymphocyte cultures from a large human population (paragraph 78). The incidence of such alterations is always low, however, and no increase in structural aberrations has been reported. In the absence of a generally accepted definition of biological aging, the relationship between radiation-induced life-shortening in experimental animals and age-related processes in the human population is difficult to assess. Even if such a relationship is accepted as valid, additional experimental evidence is required that specifically relates radiation-induced aging to chromosome aberrations as opposed to other possible mechanisms.

4. *Somatic mutation and neoplasia*

294. The most extensive evidence relating radiation-induced chromosome changes in somatic cells to significant biological effects in man would appear to be in the area of neoplasia. It has been recognized for many years that ionizing radiation produces chromosome aberrations and also tumours in both man and animals. Since chromosome changes have been demonstrated in nearly all tumours studied by modern cytogenetic techniques, older concepts concerning the causal role of chromosome aberrations in neoplasia[308] have been revived, and it has been tempting to speculate that radiation-induced tumours result directly from radiation-induced chromosome aberrations. Much evidence has accumulated which at least indirectly supports this hypothesis, but, at the same time, it has proved extremely difficult to demonstrate precise quantitative relationships, following radiation, between aberration frequencies and subsequent tumour incidence. In the following paragraphs, the evidence relating chromosome aberrations and tumours is briefly summarized, and some limitations of available data and concepts are indicated.

295. It is certainly well documented that many agents and conditions which produce chromosome aberrations also cause tumours. Not only ionizing radiation, but also ultra-violet light, a number of oncogenic viruses and several carcinogenic chemicals have been shown to have this capacity (paragraphs 53 and 56).

296. Both DNA and RNA tumour viruses, including Rous virus, adeno-viruses, SV40 and polyoma,[92, 98, 101, 364] have produced chromatid aberrations in human and animal cell cultures (paragraph 56). It is of interest that, while the Schmidt-Ruppin strain of the Rous virus causes chromatid abnormalities in human leucocyte cultures and also tumours in experimental animals, the Bryan strain of the Rous virus, under similar circumstances, produces neither chromosome changes nor neoplasia.[103]

297. Benzene, perhaps the best documented leukæmogenic chemical in man, also appears capable of causing chromosome changes in human cells.[365, 367] Carbon tetrachloride and other hepatic carcinogens have been shown to produce both tumours and chromosome aberrations in the rodent liver.[368, 370] Although a number of other mutagenic chemicals have recently been shown to yield chromatid alterations in human cells (paragraph 53), their carcinogenicity remains to be demonstrated.

298. It is also now clear that there is an increased incidence of leukæmia and lymphoma in several rare human diseases (Bloom's syndrome, Fanconi's syndrome, and perhaps ataxia-telangiectasia and xeroderma pigmentosum) in which there is a constitutional propensity for increased spontaneous chromosome aberrations observed as chromatid aberrations in leucocyte cultures.[340, 371, 372] Such data, involving a variety of agents and conditions, have suggested that genetic damage, as indicated by chromosome aberrations, might be a common mechanism by which most, if not all, carcinogens act.

299. The frequency of chromosome abnormalities in tumours has been used to support this argument. Certainly most neoplasms, radiation-induced or not, do show chromosome aberrations. Numerous studies in recent years[373, 381] have demonstrated that, except for some human acute leukæmias and some virus-induced rodent leukæmias, nearly every mammalian tumour, by the time it reaches macroscopic size, is characterized by chromosome changes.

300. Furthermore, the neoplastic cells bearing these abnormalities frequently appear as stemlines or clones, particularly in the leukæmias, but in many solid tumours as well. The entire neoplasm often consists of a single clone with all cells showing the same alteration in karyotype, or of a small number of clones, usually with related chromosome changes. This clonal phenomenon has suggested that a tumour may consist entirely of the progeny of a single aberrant cell,[344, 382] a cell having a proliferative advantage as a result of its altered karyotype. Subsequently, additional clones may appear and even come to predominate as further karyotypic changes confer additional selective advantages. Sequential studies in human neoplasms have supported this concept of the important role which chromosome alterations play in the progressive development of tumours.[382, 386]

301. However, it is still debatable whether the chromosome changes observed in mammalian neoplasms are primary or secondary phenomena and whether they

are involved in the initiation of the tumour or only in its subsequent progression. The alteration observed in one tumour is usually different from that observed in the next even when the two neoplasms are clinically and histologically identical.[373, 381] In general, each type of mammalian neoplasm has *not* been characterized by a specific chromosome abnormality. This, plus the occurrence of some leukæmias and even a few solid malignancies[373, 380, 387] without any demonstrable chromosome changes, has led many investigators, but not all,[373, 379] to conclude that the chromosome changes seen in most tumours are secondary phenomena superimposed on an already neoplastic process. This conclusion does not rule out genetic alteration, or alterations,[388] as the initiating event in neoplasia; it simply suggests that it may be submicroscopic.

302. The first example of a neoplasm *with* a specific chromosome change is human chronic granulocytic leukæmia, where, in 90-95 per cent of the typical cases, the karyotype is characterized by the same abnormality, namely, a chromosome 21 lacking approximately half of its long arm, the so-called Philadelphia chromosome (Ph^1). This is not an inborn change but rather an acquired abnormality,[405] ordinarily limited to the neoplastic hæmopoietic cells (myeloid, magakaryocytic and erythroid) and not present in lymphocytes or other tissues of the body.[374] (In irradiated individuals without leukæmia, the Ph^1 chromosome has occasionally been observed in extramedullary tissues (paragraph 309), and in two incompletely documented instances it may have occurred as a familiar abnormality).[389, 390]

303. When present in neoplastic cells, the Philadelphia chromosome is associated with chronic granulocytic leukæmia, although it has been, on rare occasions, observed in related myeloproliferative disorders such as polycythaemia vera.[389] It persists throughout the course of the disease with additional karyotypic changes frequently superimposed in the late stages. During remission, when immature myeloid cells disappear from the peripheral blood, the Ph^1 may not be demonstrable in peripheral leucocyte cultures, but it is still observable in dividing marrow cells.[375]

304. The constancy of the association between the Philadelphia chromosome and chronic granulocytic leukæmia has suggested that, in this instance, a chromosome change is a primary phenomenon and that the occurrence of this aberration in a marrow stem cell is directly involved in the initiation of the neoplasm.

305. A similar suggestion might also be made for the relatively few other human and animal tumours in which some of the cases have apparently demonstrated a characteristic chromosome change.[393, 397] These include such neoplasms as Waldenstrom's macroglobulinemia, Burkitt lymphoma, multiple myeloma, certain human ovarian and testicular tumours and several leukæmias in rats and mice. In the first three instances, for example, a number of cases have shown a large abnormal "marker" chromosome, similar to a group A chromosome (chromosome 1-3) in the Burkitt and Waldenstrom's tumours, and similar to a large D chromosome (chromosome 13-15) in multiple myeloma.

306. In none of these various human and animal tumours, however, has the constancy of the particular chromosome change noted in each instance approached that of the Ph^1 in chronic granulocytic leukæmia. For most of these tumours, the abnormality characteristic for the particular neoplasm has been found in less than half of the cases examined. In these disorders, therefore, it is much more difficult to postulate a primary role for the chromosome alteration observed.

307. In truth, of course, one cannot currently make an absolute statement about the primary nature of any chromosome change in any tumour. Only when specific chromosome changes can be related to specific metabolic abnormalities (and we also know what metabolic changes are critical in the initiation of neoplasia) will such a statement be made with assurance. In our present state of knowledge, one may perhaps only suggest that the changes observed in most neoplasms, because of their apparent inconstancy from case to case, seem likely to be evolutionary phenomena important in progression, while the Philadelphia chromosome, because of its constant and specific association with chronic granulocytic leukæmia, is probably involved in the initiation of that disease.

308. Radiation-induced tumours have proved to be no exception to these general concepts of the significance of chromosome alterations in neoplastic cells. Apparently all, or nearly all, radiation-induced tumours show chromosome changes, but, as with most other tumours, these vary from case to case.[344, 345, 366, 376, 398, 399] In addition, not all cell clones with radiation-induced chromosome aberrations are necessarily neoplastic. Heavily irradiated humans and animals have been found to have clones of cells marked by radiation-induced chromosome aberrations which are apparently non-neoplastic and functionally normal and persist in their hæmatopoietic tissues for long periods of time after recovery.[131, 135, 222, 341-344] The frequency of such non-leukæmic clones has made it impossible to use chromosome studies to predict which irradiated individuals will eventually develop leukæmia, although in non-irradiated individuals a clone of marrow cells with a chromosome abnormality appears to be a good indication that a preleukæmic disorder is in transition to a frank leukæmia.[341]

309. The Philadelphia chromosome is present in those cases of chronic granulocytic leukæmia which appear to be radiation-induced,[345, 400-404] as well as in those with no radiation history. In irradiated individuals *without* leukæmia, the Ph^1 chromosome has been observed (both in single cells and in clones) in peripheral lymphocytes and in skin cells,[135, 406] but, with one possible exception,[223] it has not been reported in the bone marrow. Since only in a marrow stem cell does the Ph^1 appear to have a role in initiating a malignancy, those individuals in which it is found in such a cell will be of particular interest to follow to determine if leukæmia subsequently develops.

310. In addition to these considerations, lack of quantitative conclusions on the role of chromosome changes in radiation carcinogenesis has also resulted from the difficulty of obtaining precise dose-response curves for either aberrations or tumours, although, in general, both show an increasing incidence with increasing dose. The induction of tumours by ionizing radiation is a complex problem which was extensively reviewed by the Committee in its 1964 report.[392] In addition to the important modifying effects on tumour incidence of such variables as radiation quality, dose rate, and non-uniform dose distribution over the body, one must also consider possible indirect mechanisms involved in radiation carcinogenesis.[407-410] Activation of oncogenic virus, depression of the immune response,

alteration of hormone levels and non-specific cell killing and regenerative stimulation may all be radiation-induced effects in the body which play an important role in carcinogenesis.

311. At the level of the individual cell, one must, of course, consider not only direct genetic damage by radiation but also the possibility of the cell previously damaged by radiation being more susceptible to attack by an oncogenic virus.[408] Such a radiation-damaged cell may also have a greater propensity for subsequent "spontaneous" chromosomal rearrangements during mitosis or other "spontaneous" genetic alterations[286] and may as well be more liable to reproductive cell death.[409] The evidence incriminating viruses in many radiation-induced tumours has recently become particularly strong,[410] but all of these various factors have contributed to make it extremely difficult to predict with confidence the tumour incidence subsequent to a given radiation exposure.

312. Similarly, radiation quality and dose rate are important modifying factors, although some *in vivo* data and many *in vitro* data now make it possible to relate quantitatively radiation doses to chromosome-aberration yields in human, as well as in animal, cells. In addition, the quantitative results of *in vivo* studies may also be affected by the frequency of cell division in the irradiated host of the particular cells studied.[218, 302]

313. Taking these variables together, it is perhaps not surprising that no data, either animal or human, are yet available on which to establish precise quantitative relationships involving a given radiation exposure, the resultant aberration yield and the number or kind of tumours to be subsequently expected. It is of interest that the RBE for neutrons versus x rays appears to be comparable for tumours[407] and chromosome aberrations, at least in some circumstances. Also, several studies have indicated that Thorotrast, through its primary localization in the reticulo-endothelial system, produces both leukæmias and hemangioendotheliomas as well as demonstrable chromosome alterations in lymphocytes, whereas radium, localizing in bone, produces osteogenic sarcomas more frequently than leukæmias and yields fewer chromosome changes in lymphocytes.[179, 224, 268, 411, 412]

314. However, very few experiments have approached directly the quantitative relationships between chromosome aberrations and tumour incidence. In one study that compared the effects of high and low dose radiation on the mouse liver,[413] the chromosome aberration yield was *higher*, but the subsequent incidence of hepatomas *lower*, after exposure to a high dose rate than it was after exposure to a low dose rate. The authors postulated a cell-killing effect of the high-dose-rate radiation, associated with visible chromosome aberrations, which removed potentially neoplastic cells from the surviving population.

315. *Summary.* In both man and animals, radiation-induced chromosome aberrations and radiation-induced neoplasms regularly appear together. The chromosome changes may represent visible evidence of intracellular alterations involved in the neoplastic process. However, the mechanism of radiation carcinogenesis is still far from clear, and the number or type of radiation-induced chromosome aberrations observed in an irradiated individual cannot at present be confidently used to predict the risk of his later developing a neoplasm, except perhaps in the case of chronic granulocytic leukæmia.

5. *Somatic mutation and immunological deficiency*

316. Both mutational events and cell depletion have been cited as possible mechanisms for immunological disorders and deficiencies, and both could result from radiation-induced chromosome aberrations.

317. Although radiation can produce acute immunological deficiency through its cell-killing effects on the lymphoid system,[414] how much of this effect is mediated through chromosome aberrations is not known. Nor is it known if the tendency towards increasing immunological deficiency with advancing age is due to either cell depletion or genetic alterations in the immune system. In a system, however, in which cell division is apparently required for the initiation of its specific functions, deleterious effects of chromosome aberrations can be readily visualized.

318. It has been postulated that human auto-immune disorders might stem from somatic mutations in the lymphoid system. For instance, statistical study of the age and sex distribution of rheumatoid arthritis, lupus erythematosus, multiple sclerosis and other possible auto-immune diseases has led Burch,[357] extending the concepts of Burnet[415] and others, to suggest somatic mutation as a source of "forbidden clones" of lymphoid cells capable of reacting against "self" and producing clinical disease. Although such theories do not necessarily require either radiation as the mutagen or visible chromosome aberration as the form of genetic change, such possibilities obviously exist.

319. Among the group of rare human diseases which have recently been shown to be characterized by increased chromosome fragility,[366, 371] it is of interest that at least one of them, ataxia-telangiectasia, is also associated with immunological deficiency.[416] Immune defects have not been prominent in either Fanconi's anæmia or Bloom's syndrome, the other two disorders showing excessive spontaneous chromosome breakage in lymphocyte cultures, but neither disease has been extensively studied from this standpoint.[417]

320. It must be stated, in summary, that at present there is neither epidemiological nor experimental evidence directly relating immunological disorders or deficiencies in man or animals to chromosome aberrations known to be induced by radiation.

D. Conclusions

321. In relation to aberrations in germs cells, there is little to add to the conclusions arrived at by the Committee in its 1966 report.[3] The only point worthy of extra emphasis here is the need for more information on human meiotic cells so that better estimates of the spontaneous level of translocations in man and a better understanding of their genetic consequences can be obtained.

322. At the somatic cell level, although attempts at relating certain constitutional chromosome abnormalities in man with specific metabolic deviations from the normal are continually being made, as yet there is little direct information relating alteration or loss of gene function with alteration or loss of a particular chromosome or chromosome segment. The demonstrations of the existence of clones of cells containing abnormal karyotypes in the peripheral blood leucocytes of individuals previously exposed to radiation now offer an opportunity for detailed metabolic studies on a wide variety of chromosomally aberrant cells. No

such studies have yet been reported, but work in this area will not only clarify the variety of detrimental effects that are to be expected to result from the presence of aberrations in somatic cells but will also provide information for the genetic mapping of the human chromosome complement.

323. It is to be expected on *a priori* grounds that certain kinds of chromosome aberrations will be cell-lethal and so will contribute to cell depletion. However, it is not possible to state in quantitative terms the relative importance of the variety of chromosome aberrations in contributing to cell killing in human somatic cells.

324. If one attempts to relate specific somatic effects, such as immunological deficiency or life-shortening, to chromosome changes, the problem becomes even more complex. Hypotheses have been advanced relating these disorders to radiation-induced somatic mutations, but strong, supportive, experimental evidence is lacking, and non-mutational mechanisms have also been advocated.

325. The significance of the role played by chromosome aberrations in the aetiology of neoplastic disease is also far from clear. In the case of chronic myeloid leukæmia, the evidence strongly implicates a specific chromosome aberration (the Ph^1 chromosome) as playing a significant role in the initiation of this disease. Although the possibility remains open that other specific chromosome abnormalities may be involved with other types of neoplastic change, the presence of a wide variety of chromosome aberrations in most tumours, and their complete absence in a few, militates against the notion of a simple causal relationship. The inconsistencies that have been observed may well be a consequence of there being many different pathways that lead to a common biological end-point. If neoplasia is a multi-step process, the possibility exists that a radiation-induced alteration of the genome may provide a more favourable environment for the development of additional essential alterations, through increased susceptibility to an oncogenic virus, or greater liability to "spontaneous" mitotic errors, or through some other mechanism. Until the basic mechanisms of radiation carcinogenesis are better understood, it will be difficult to define more clearly the role of chromosome aberrations in this process.

VI. Conclusions

A. Application of aberration yields for biological dosimetry

326. The possibilities of estimating the absorbed dose received by individuals through biological rather than (or where possible, in addition to) physical methods have an obvious interest, particularly in those cases where accidental exposure has occurred. Attempts to make rapid estimates of dose based on film badges worn by radiation workers are liable to error, especially if assumptions have to be made regarding shielding by objects intervening between the exposed individual and the radiation source. Moreover, the degree of radiation exposure is frequently not uniform over the whole body, and this leads to complications if the dosimetric device is in essence a point receiver.

327. In cases of criticality accidents, physical measurements made at a later time usually involve an experimentally contrived incident in an attempt to obtain a reasonable approximation of the dose received during the accident. Alternative biological techniques that have been studied from the point of view of their use as dosimeters have so far proved disappointing. A number of possibilities have been investigated.[276, 118] including studies of metabolic products excreted in the urine and of the frequency of lymphocytes with bilobed or double nuclei or of neutrophils carrying particles with specific staining properties. However, none of these biological parameters has been shown to vary consistently with radiation dose.

328. Considered in qualitative terms, there is no doubt that the presence of a significant number of chromosome aberrations, more particularly of the dicentric- and ring-type, may be indicative of a previous radiation exposure. This follows, since it has been shown that the spontaneous *in vivo* occurrence of a dicentric or ring aberration is an extremely rare event in the blood cells of the many hundreds of individuals that have been examined from this aspect. Such aberrations occur, at most, once in every 2,000 cells taken from unirradiated subjects, whereas 20 ± 5 such aberrations would be expected in 2,000 cells from an individual, or individuals, that had received a whole-body x-ray dose of 10 rads or its equivalent. The frequency of these aberrations would appear to be uninfluenced by previous exposure to infectious agents or (except in certain specific cases) to chemical agents. The frequency of dicentric and ring aberrations in a population or in an individual is, therefore, a good qualitative screening test for a previous radiation history. Moreover, because of the long life span of at least a proportion of the lymphocyte population, aberrations can be observed in these cells many years after the original radiation exposure.

329. A good example of the use of chromosome-aberration yield in this qualitative context is afforded by a recent report[16] of an examination of an individual who, on the basis of film badge measurements, was assumed to have received a dose of 300 rads. It was possible to state after chromosome analysis that a significant dose of radiation had not, in fact, been received by this individual, since no chromosome abnormalities were seen in cultured blood or even in direct preparations of bone-marrow cells. The badge was, therefore, assumed to be faulty or to have been exposed independently of the individual. In circumstances such as this, where there is no way of reconstructing the conditions under which the exposure occurred and thereby of checking the validity of the film badge information, information from chromosome analysis can be of great value.

330. The main advantages of a biological method as opposed to physical methods of measuring absorbed dose in man would follow from the directness and permanent availability of the biological method, from the fact that information on and extrapolation from the relative biological efficiencies of a spectrum of radiations received in a mixed exposure is not required and because a biological dosimeter is in itself one step nearer to the problem of assessing immediate damage and future risk. In the particular case of a chromosome-aberration dosimeter, it may be added that chromosome aberrations are believed to be responsible for a certain proportion of the cell killing following radiation exposure; they are mutational events and they are generally believed to play some part at least in the development of late somatic effects. However, it should be emphasized here that, in the current state of our

knowledge, aberration yields cannot be related to a biological effect and that the presence of a low frequency of aberrations in the peripheral blood cells of an individual can, therefore, in no way be regarded as constituting a medical risk.

331. The starting points for considering the possible use of chromosome aberrations as opposed to other possible biological systems as indicators of dose are (*a*) the high sensitivity of the human chromosome complement and the high resolution of the method, since the effects of doses of around 5 rads or less can be detected, and (*b*) the fact that the yield of chromosome aberrations induced is closely correlated in a specific manner with the dose of radiation received.

332. It was concluded (paragraphs 138-144) that, from the cytological viewpoint, by far the best system for use in chromosome-aberration dosimetry was the peripheral blood leucocyte system, provided chromosome-type aberrations were scored, preferably at the first mitosis following radiation exposure. Moreover, there is little doubt that a measure of the radiation damage incurred by leucocytes (more particularly the small lymphocytes) that are widely distributed throughout most tissues and areas of the body should be a good indicator of the effect of radiation on the individual as a whole. There are, however, a number of disadvantages to the system, although it should be noted that many of these disadvantages also apply to physical dosimetric systems.

333. Dosimeters require calibration, and the calibration with the peripheral blood leucocyte system will almost certainly require an accurate correlation between the response of these cells *in vitro* and their response when exposed *in vivo*. This cross reference to the *in vitro* system seems necessary, since opportunities for analysing cells from individuals exposed to various levels of whole-body radiation are fortunately rare. Thus, good *in vivo* dose-response curves, particularly from healthy individuals accidentally exposed to radiation, will be difficult to obtain. However, recent studies (paragraphs 199-205), including the use of the technique of extracorporeal irradiation, indicate that the response *in vitro* is equivalent to that obtained *in vivo*, although further work here is clearly necessary.

334. In the *in vitro* work, despite the fact that accurate physical estimates of dose are available and that, within most laboratories, a repeatable quantitative relationship between aberration yield and dose is always found, differences in results are, nevertheless, to be found between laboratories in both the absolute aberration yields at given dose levels and in the shape of the dose-response curves. Some of the explanations for these differences have now become clear. When factors such as radiation quality, methods of irradiation and duration of culture are taken into account, good interlaboratory agreement is obtained. Further studies on these factors are, however, necessary before the prospect of obtaining standard sets of coefficients relating aberration yield to radiation dose can be realized.

335. *In vitro* studies on the influence of dose rate on aberration yields induced by x rays have been carried out by a number of laboratories, and it can be concluded from the results obtained that there is little dependence of aberration yield on exposure time over times ranging from one to thirty minutes. This is, of course, a feature of some importance in attempting to relate aberration yields with dose. Similar studies with fission neutrons show that the yield of aberrations induced by these particles is, as expected, independent of dose rate and exposure time, and that fission neutrons (mean energy 0.7 MeV) and fast neutrons (2.2 MeV and 14 MeV) are two to five times as effective per rad as 250 kV x rays in inducing aberrations.

336. In the *in vivo* work, in only one study have individuals been exposed to accurately measured uniform whole-body radiation (2 MeV x rays). In this study, the yield of dicentric and ring aberrations in peripheral blood cells was shown to increase approximately linearly with exposure over the dose range studied (0-50 rad) if samples were taken immediately after radiation exposure, the coefficient of yield for these aberrations being 0.001 per cell-rad. Uniform whole-body exposure to a penetrating radiation, so that all cells receive a similar dose, is the ideal state for the purpose of biological dosimetry. Such a state is, of course, rarely encountered, and most of the *in vivo* work that has been done relates to partial-body or non-uniform exposures, and it is here that the greatest dosimetric problems arise.

337. In the case of acute partial-body exposure, the aberration yield in peripheral blood leucocytes will depend upon a variety of factors. These include (*a*) the amount of radiation energy deposited in that area of the body that is exposed and the duration of the exposure; (*b*) the area and volume of the body that is irradiated; (*c*) the proportion of peripheral blood leucocytes that are exposed to radiation and the time that they spend in the irradiated area during exposure; (*d*) the proportion of leucocytes (lymphocytes) in extravascular areas within the exposed region; (*e*) the amount of exchange of lymphocytes between peripheral blood and the extravascular pools; and (*f*) the time at which blood is sampled after the radiation exposure. Since there is evidence indicating that there is an appreciable exchange of lymphocytes between the blood vessels and extravascular sites in the first few hours following irradiation (paragraph 204), it is not yet possible to make any reasonable estimates of physical dose from an analysis of chromosome-aberration yields in partial-body exposures.

338. The question of what is meant by dose, in terms of biological effect or biological consequence, is itself not very meaningful in the case of partial-body irradiation. This follows, not only because aberration yields may be very much dependent upon the regions of the body that are exposed, but also because aberration frequency cannot be related to any given somatic effect. It should be noted, however, that the difficulties encountered with the type of biological dosimeter under discussion may be far less than the difficulties encountered with a point receiver measuring physical dose. Studies on individuals who received partial-body exposures at varying dose levels over similar regions (and areas) of the body have, in all cases, revealed a strict proportionality between physically measured skin dose and aberration yield. However, it is not possible simply to relate the aberration yield to skin dose and area or region exposed, since, because of cell mixing, the measured aberration yield varies with the time at which blood is sampled in the first twenty-four hours after radiation exposure.

339. It can only be concluded from this discussion that, in the case of partial-body exposure, a great deal more knowledge is required about the structure of the

populations (and subpopulations) of small lymphocytes and about the distribution, mobility and longevity of the cells before it is possible to equate an aberration yield observed at any given time to the dose absorbed in the lymphocytes. It should be noted that, in any case, within the limitations of existing knowledge, any given aberration yield in these cells can only be related in physical terms to an "equivalent whole-body dose".

340. It should be re-emphasized here that the difficulties that confront us in the case of partial-body exposure do not exist in the case of uniform whole-body exposure. With uniform whole-body exposure, there is no doubt that the yield of chromosome-type aberrations in peripheral blood leucocytes can be used as an accurate measure of dose received. In the case of accidental, non-uniform, whole-body exposure, the aberration frequency in peripheral blood cells can yield but little information on the degree of non-uniformity of the exposure but may more readily provide an estimate of an "equivalent whole-body dose".[423] Care should be taken to note, however, that dose estimates require the use of dose-yield kinetics obtained in *in vitro* studies and possibly also information on the form of the distribution of aberrations between cells.

B. Assessment of risks

341. There is no new information about the estimated frequency of aberrations induced in germ cells by radiations and the consequent risk to individuals and to offspring. These risks have been fully discussed in the 1966 report,[3] and the only new point to add is the preliminary observation of a possible association between translocation and subfertility in the human male. In this connexion, however, further data are required before any real assessment can be made.

342. In somatic cells, information on the yields and types of chromosome aberration does not as yet provide either a new approach to or a better estimate of risk, except in one specific case. With existing information, knowledge of chromosome-aberration yield in peripheral blood leucocytes does not enable us to make any quantitative statement regarding the risk of developing neoplastic disease, immunological defects and shortening of life span, etc. As a consequence, no information of clinical significance can be obtained from the presence of aberrations. Little can, therefore, be added to the statements on assessments of risk of somatic disease that have already been made by the Committee in its earlier reports. From the point of view of assessing risks, the only direct use of the aberration yield is when this is the only parameter from which a "physical" estimate of the dose can be obtained. Clearly, where physical dose estimates can be made, the aberration yield may serve as a valid supplement to the physical data.

343. The exception mentioned in the above paragraph relates to the association between chronic granulocytic leukæmia and the presence of the Ph^1 chromosome in cells of the bone marrow. The presence of such an abnormal chromosome in bone-marrow cells is, apart from one possible exception,[223] always associated with a blood dyscrasia—in almost all the cases with chronic granulocytic leukæmia and, in the others, with a disorder such as polycythaemia vera. It has not yet been shown whether a Ph^1 chromosome can be observed in bone-marrow cells prior to the development of overt hæmatological disease. However, its presence in a bone-marrow cell of any irradiated individual must, on present knowledge, be taken as indicating an extremely high risk for the individual of developing leukæmia. On the other hand, it should be stressed that Ph^1-like chromosomes have been observed in single cells and in clones in peripheral blood lymphocytes and skin cells of irradiated individuals, but their presence in such cells has not, to date, been associated with any kind of neoplastic change.

C. Recommendations for further study

344. Human cytogenetics is still a relatively new and vigorously developing field, and it is evident that there are large gaps in our knowledge both of the response of human chromosomes to radiation and of the consequences of radiation-induced chromosome aberrations in human germ cells and somatic cells. Some of the more immediate requirements and questions that need answering and some of the more general longer-term problems that require further attention are outlined in the following paragraphs.

345. Further studies should be undertaken on human meiotic cells, particularly from the point of getting better information on the frequency and possible genetic consequences of symmetrical spontaneous aberrations that cannot be detected in somatic cells.

346. A better understanding of the effects of culture conditions on aberration yield in peripheral blood leucocytes should be achieved, the aim being to develop a standardized technique for interlaboratory use so that standard coefficients for the yields of the various aberration types can be obtained.

347. Further *in vitro* studies on the effects of dose rate and exposure time with radiations of low LET and on the relative efficiencies with which aberrations are produced by radiations of different quality should be made.

348. Very much more information is required to define to what extent there exists a range of sensitivity to aberration induction in a human population and regarding the influence of age on response. These studies can be carried out by measuring the response of blood cells to *in vitro* exposures.

349. Further work should be undertaken on the relation between *in vivo* and *in vitro* responses, including studies on laboratory mammals and, where possible, on humans exposed to extracorporeal radiation treatments.

350. Further information is needed on the influence of various patterns of non-uniform radiation exposure and a better understanding required of the lymphocyte populations in the body, their distribution, age structure and turn-over.

351. Further data on the rate of decline of aberration yield with time following exposure under a variety of radiation régimes should be acquired. Attention should be devoted, in this respect, not merely to long-term changes occurring over periods of weeks, months or years after exposure, but to changes that occur in the first few hours and days. Consideration should also be given to the importance of immune response and other possible factors in such work.

352. Where possible, the utmost effort should be made to obtain data from individuals undergoing uniform whole-body irradiation.

353. Further work on the qualitative and quantitative differences between aberrations induced by chemical and infectious agents in relation to ionizing radiations and studies on the response of peripheral blood leucocytes (and bone-marrow cells) to radiation whilst in the S and G_2 phases of the cell cycle should also be undertaken, since current information on chromatid-type aberrations induced in these cells is minimal.

354. Future advances in all aspects of population cytogenetics will be greatly facilitated by the introduction of automation into the processes involved in cytogenetic analysis. In the case of surveys of populations exposed to mutagens that induce chromosome abnormalities, although a considerable amount of valuable information will continue to be obtained through conventional methods, automatic systems should provide powerful tools for the cytologist, and their development should be fostered.

355. A continued effort should be directed at attempts to relate both yields and types of chromosome aberrations to specific somatic diseases. In this connexion, follow-up studies on individuals exposed to radiations are desirable, and continued studies of irradiated individuals possessing Ph^1-like chromosomes and other clonal changes in their proliferating cells are essential.

356. Further studies on the possible relationship between the incidence of aberrations on the one hand and neoplasia on the other, both in man and in experimental animals, are required. In this connexion, it should be pointed out that there is no direct information whatsoever on a possible synergistic interaction between oncogenic viruses and radiation-induced chromosome damage. Experimental attack on this problem is now possible.

357. There is an absolute dearth of information relating radiation-induced aberrations to biological endpoints. It should, therefore, be emphasized that the existence of clones of cells containing abnormal karyotypes in the skin, bone marrow and peripheral blood leucocytes of individuals previously exposed to radiation now offers an opportunity for detailed metabolic study on a wide variety of chromosomally aberrant cells. In this context, human-animal hybrid cells[419, 420] may provide a useful tool. Such studies will provide valuable genetic information as well as information on the detrimental effects to be expected from certain kinds of aberrations.

358. A follow-up of the studies indicating a possible difference in the *in vivo* response of lymphocytes to chromosome-aberration induction in different laboratory mammals should be made.

359. Further studies on the radiation response in different tissues and the radio-sensitivity of different cell types, together with the possibility of utilizing materials other than blood cells to measure aberration yields, should be carried out.

360. Studies on the frequency, types and consequences of constitutional chromosome anomalies in man should be continued.

TABLE I. FREQUENCY OF DICENTRIC ABERRATIONS IN "NORMAL" SUBJECTS NOT EXPOSED TO RADIATION OTHER THAN ROUTINE DIAGNOSTIC EXPOSURE

The forty-seven individuals in column *c* were patients with ankylosing spondylitis, and the samples were taken prior to any therapy, but *shortly after* the individuals had received diagnostic radiation exposures. The thirty-eight individuals in column *b* were individuals from a general population and served as controls for population *c*

Authors	*Norman* et al.[165]	*Ishihara and Kumatori*[175]	*Evans and Speed*[422]	*Norman*[24]	*Bloom* et al.[183]	*Court Brown*[201]			*Sasaki and Miyata*[221]
						a	*b*	*c*	
Number of individuals in sample	23	20	200	?	94	438	38	47	11
Number of cells scored	5,784	2,875	2,400	2,295	8,847	12,420	1,060	2,269	9,510
Number of dicentrics observed	0	0	0	0	0	7	0	3	2
Frequency of dicentrics per cell	<1 in 5.8×10^3	<1 in 2.8×10^3	<1 in 2.4×10^3	<1 in 2.3×10^3	<1 in 8.8×10^3	<1 in 1.8×10^3	<1 in 1×10^3	<1 in 0.76×10^3	<1 in 4.7×10^3

TABLE II. PHYSICAL DOSES AND ESTIMATED DOSES BASED ON YIELD OF DICENTRIC ABERRATIONS IN PERIPHERAL LEUCOCYTES IN BLOOD CELLS OF PATIENTS[227]

A—Exposed to one passage through a ^{90}Sr-^{90}Y extracorporeal irradiator

B—After several passages through the irradiator

	Total calculated physical dose (rad)	*Total dose estimated from dicentric aberration frequency (80 per cent confidence)*
A *In vitro* studies: blood sampled after a single passage at a flow rate of 3.0 to 15.7 ml-min	450[a]	440–490
	565[a]	510–565
	295[a]	245–310
	148[a]	115–175
B *In vivo* studies: blood sampled immediately after a four- to eight-hour extracorporeal irradiation	120[b]	145[c]
	120[b]	180[c]
	240[b]	230[c]

[a] Physical dose calculated on the basis of flow rate and volume of blood passed through irradiator.

[b] Physical dose estimated on the basis of blood volume, flow rate and duration of irradiation.

[c] Estimated dose based on frequency of dicentric aberrations in 200-300 metaphase figures in blood leucocytes immediately after completion of irradiation.

TABLE III. ABERRATION YIELDS FOLLOWING A SINGLE PARTIAL-BODY EXPOSURE OF ANKYLOSING SPONDYLITIS PATIENTS TO X RAYS (250 KV)[16]

A cells—Undamaged cells
B cells—Cells containing chromatid-type aberrations
C_u cells—Cells with unstable chromosome-type aberrations (rings, dicentrics, fragments)
C_s cells—Cells with stable chromosome-type aberrations

Skin dose in rads	Time of sampling post-exposure in hours	Total cells analysed	A cells			B cells		C_u cells		C_s cells		Ring plus dicentrics		Fragments	
			Modal	Non-modal											
			No.	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
100	0	300	256	13	4.3	22	7.3	6	2.0	3	1.0	5	1.7	2	0.7
	24	300	266	8	2.7	14	4.7	5	1.7	7	2.3	3	1.0	2	0.7
150	0	400	349	14	3.5	16	4.0	13	3.3	8	2.0	8	2.0	7	1.8
	24	500	408	18	3.6	30	6.0	32	6.4	12	2.4	20	4.0	13	2.6
200	0	250	205	12	4.8	11	4.4	17	6.8	5	2.0	15	6.0	5	2.0
	24	300	240	7	2.3	21	7.0	27	9.0	5	1.7	16	5.3	13	4.3
250	0	300	218	8	2.7	33	11.0	24	8.0	17	5.7	11	3.7	14	4.7
	24	300	221	7	2.3	30	10.0	32	10.7	10	3.3	20	6.7	20	6.7
300	0	400	319	14	3.5	14	3.5	41	10.3	12	3.0	28	7.0	19	4.8
	24	350	240	23	6.6	13	3.7	67	19.1	7	2.0	55	15.7	27	7.7
700	24	100						29	29.0			30	30.0	18	18.0

TABLE IV. ABERRATION YIELDS FROM SEVEN PATIENTS EXPOSED TO SINGLE WHOLE-BODY DOSES OF X RAYS (2 MEV)[16]

A cells—Undamaged cells
B cells—Cells containing chromatid-type aberrations
C_u cells—Cells with unstable chromosome-type aberrations (rings, dicentrics, fragments)
C_s cells—Cells with stable chromosome-type aberrations

Dose in rads	Time of sampling post-exposure in hours	Total cells analysed	A cells			B cells		C_u cells		C_s cells		Rings plus dicentrics		Fragments	
			Modal	Non-modal											
			No.	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
"O" control		700	618	45	6.4	27	3.9	5	0.7	5	0.7	2	0.3	3	0.4
25	0	600	489	38	6.3	27	4.5	31	5.2	15	2.5	16	2.7	18	3.0
	24	600	499	31	5.1	30	5.0	23	3.8	17	2.8	16	2.7	11	1.8
50	0	800	632	56	7.0	26	3.3	61	7.6	25	3.1	37	4.6	27	3.4
	24	800	637	34	4.3	28	3.5	76	9.5	25	3.1	53	6.6	29	3.6

TABLE V. FREQUENCIES OF DICENTRICS PLUS RINGS IN PERIPHERAL BLOOD CULTURES TAKEN IMMEDIATELY, OR TWENTY-FOUR HOURS AFTER, WHOLE-BODY EXPOSURE OF PATIENTS TO 2 MeV X RAYS AT THE DOSES INDICATED[271]

Case number	*Dose received (rad)*	*Control (190 cells)*	*Immediately after treatment (200 cells)*	*24 hours treatment (200 cells)*
1	25	1	5	3
2	25	0	2	8
3	25	0	9	5
4	25	0	6	7
5	25	0	8	4
6	25	0	5	3
7	50	0	4	15
8	50	0	14	14
9	50	3[a]	10	15
10	50	1	9	9
11	50	0	11	15
12	50	1	9	7[b]
13	17	0	2	1
14	28	0	1	4
15	36	0	5	7
16	40	0	8	7

[a] One cell, containing a dicentric and a tricentric, has been scored as three dicentrics.
[b] Seven rings and dicentrics in seventy-five cells analysed.

TABLE VI. DICENTRICS PLUS RINGS FOLLOWING *in vitro* AND *in vivo* (WHOLE-BODY) IRRADIATION

Authors	*Radiation quality*	*Dose in rads*	*Irradiation*	*Sampling time in hours*	*Culture time in hours*	*Dicentric plus rings per cell*
Evans[212]	240 kV x rays	25	*in vitro*		54	0.065
		50				0.15
Gooch *et al.*[229]	250 kV x rays	25	*in vitro*		72	0.003
		50				0.017
Langlands *et al.*[271]	2 MeV x rays	25	*in vivo*	0	53	0.029
		25		24		0.025
		50		0		0.05
		50		24		0.07
Mouriquand *et al.*[138]	160 kV x rays	25	*in vitro*		72	0.025
		50				0.05
Norman and Sasaki[240]	1.9 MeV x rays	50	*in vitro*		50	0.014
Vander Elst *et al.*[230]	220 kV x rays	25	*in vitro*		72	0.009
		50				0.021
Visfeldt[242]	^{60}Co λ rays	50	*in vitro*		48	0.02

TABLE VII. FREQUENCIES OF DICENTRICS PLUS RINGS PER CELL IN PERIPHERAL BLOOD LEUCOCYTES AT VARIOUS INTERVALS AFTER EXPOSURE OF INDIVIDUALS TO MIXED GAMMA AND FAST NEUTRON RADIATION (CULTURES GROWN FOR SEVENTY-TWO HOURS; OBSERVED NUMBERS IN PARENTHESES)

Case	*Estimated dose (rad)*	*Time after exposure*		
		29 months[128]	*42 months*[129]	*7 years*[283]
A	365	0.01 (1)	0.04 (4)	0.02 (2)
B	270	0.01 (1)	0.02 (2)	0.03 (3)
C	339	0.166 (24)	0.02 (2)	0.018 (2)
D	327	0.04 (4)	0.023 (2)	0.07 (2)
E	236	0.013 (1)	0.03 (3)	0.05 (5)
F	68.5	0	0	0
G	68.5	0	0	—
H	22.8	0	0.01 (1)	—

No dicentrics or rings were observed in 900 cells from control individuals[129]

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