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SUMMARY

Chromosome-type aberrations, such as dicentric and translocation chromosomes, are biomarkers of exposure to ionizing radiation. So far, analysis of dicentric chromosomes in peripheral blood lymphocytes has been the only method routinely used in biological dose assessment. During division of T cell precursors, proliferative death of cells containing dicentric chromosomes reduces the number of such lymphocytes in peripheral blood. Dicentrics are thus suitable for dose calculations during a reasonably short period after exposure to radiation. Unlike dicentrics, translocations persist in cell division and enable dose estimation over long time periods following exposure. A recent development in molecular biology, the FISH (fluorescence *in situ* hybridization) chromosome-painting technique, has opened the possibility for accurate recognition of translocations and thus retrospective determination of dose. The purpose of the present study was to evaluate the applicability of translocation analysis by means of FISH chromosome painting for retrospective dosimetry.

In the construction of acute dose-effect curves for ^{60}Co γ-ray-induced chromosomal aberrations using FISH chromosome painting, translocations showed a linear-quadratic relationship to dose, similar to that seen in dicentrics. Donor-dependent translocation frequencies at control level and at low doses were observed. The linear part of the calibration curve for two-way translocations, i.e. both translocation chromosomes present, proved to be more reliable than the comparable low-dose response for total translocations, which include both two- and one-way translocations. The results indicate that the linear part of the curve requires precise

determination, particularly since application of the technique will probably cover mainly chronically exposed subjects.

An opportunity to gain direct information on translocation persistence over time was opened by obtaining repeated samples from subjects accidentally exposed to ionizing radiation in Estonia in 1994. Dose estimation applying solid-stained dicentrics from 18 persons analysed shortly after the accident revealed considerable doses, both protracted and partial-body, to five of the subjects. In FISH analysis, equal yields of translocations and dicentrics were seen in the first sample. During a two-year postexposure period of repeated sampling, translocations remained relatively stable, supporting their use in dose assessment of past exposures. Dicentrics, on the other hand, declined rapidly during this time. A decrease in translocations was, however, observed in one subject who had been exposed to a high-dose γ-radiation with a heterogeneous exposure pattern of both protracted and partial-body exposure that led to severe aplasia. This finding implies that retrospective dosimetry using the FISH technique may not be informative in cases of nonuniform distribution of dose. Follow-up of the accident victims is continuing to determine the long-term persistence of translocations.

In a study comprising 84 individuals living in dwellings with low, medium and high concentrations of radon, no relationship between chronic exposure to high radon levels and chromosomal aberrations in FISH chromosome-painting analysis was obtained. The result was valid for both translocations and unstable aberrations. The findings implied that even at high concentrations, the dose from chronic exposure to radon-derived α-particles was too low to induce chromosomal damage that could be detected from peripheral blood lymphocytes using this technique.

The capability of the FISH technique for revealing chronic exposure to low-LET radiation was evaluated by comparing twenty nuclear power plant workers with a mean cumulative dose of about 100 mSv with twenty matched controls. Regression analysis showed significant association between documented cumulative dose and translocation frequency. However, workers with similar recorded doses displayed large interindividual variability in translocation yields. The results indicate that it is possible to detect chronic exposure to low-LET radiation at the group level.

The present study showed that translocation analysis using FISH chromosome painting is superior to standard dicentric analysis in retrospective dosimetry. A significant age-dependence of translocations was observed, supporting the view that the age effect is one of the most important factors used in evaluating the sensitivity of the FISH technique. Moreover, the data suggested that several years after exposure, a dose with uniform distribution in the body can be determined more accurately than a partial-body dose. FISH chromosome painting also showed its potential in assessing chronic exposure to low-LET radiation at the group level, whereas the detection of high-LET radiation may be more restricted. The importance of the linear part of the calibration curve became evident in the course of the study. Although the results assisted in defining the feasibilities and limitations of the FISH technique in retrospective dosimetry, it is clear that continued effort is still demanded for comprehensive understanding of its applicability.

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Avainsanat biologinen annosmääritys, translokaatio, kromosomimaalaus-tekniikka.

YHTEENVETO

Ionisoiva säteily aiheuttaa solun tumassa kromosomityypin vaurioita, joista kromosomien väliset vaihdokset eli niin sanotut disentriset ja translokaatiot, ovat säteilylle kaikkein tyypillisimpiä. Disentristen kromosomien analyysi ääreisverenkierron lymfosyyteistä on toistaiseksi ollut ainoa biologisessa säteilyannoksen määrityksessä rutiininomaisesti käytetty menetelmä. Lymfosyyttien elinkaari on rajallinen, joten niiden esiasteet tuottavat täydennystä jakautumalla. Samalla kuitenkin disentristen määrä vähenee, sillä tämä vauriotyyppi aiheuttaa epävakaisuutta solunjakautumisessa, jolloin osa soluista kuolee. Disentrisiin perustuvaa annosarviointia voidaan näin ollen käyttää luotettavasti vain suhteellisen lyhyellä aikavälillä säteilyaltistuksen jälkeen. Translokaatiot sen sijaan ovat pysyviä ja mahdollistavat säteily-annoksen määrittämisen silloin, kun altistuksesta on kulunut hyvinkin pitkä aika. Viime vuosina molekyylibiologian alalla kehitetty menetelmä FISH (fluoresenssi *in situ* hybridisaatio) eli kromosomimaalaus-tekniikka on luonut työvälineen translokaatioiden tarkkaan havainnoimiseen ja siten taanneh-tivasti tapahtuvaan annosmääritykseen. Tämän työn tarkoituksena oli tutkia kromosomimaalaus-tekniikalla tapahtuvan translokaatioanalyysin soveltuvuutta retrospektiiviseen säteilyannoksen arviointiin.

Säteilyttämällä veren lymfosyyttejä kokeellisesti ^{60}Co γ-säteillä kromosomimaalaus-tekniikan avulla havaittujen translokaatioiden todettiin noudattavan lineaaris-kvadraattista annosvastekäyrää. Annosvastekokeeseen verta luovut-taneiden yksilöiden välillä havaittiin eroja translokaatioiden frekvenssissä kontrollitasolla ja pienillä säteilyannoksilla. Annosvastekäyrän lineaarinen osa osoittautui luotettavammaksi, kun analyysiin otettiin mukaan vain resiprookkiset

translokaatiot, joissa translokaation molemmat osapuolet ovat läsnä, kuin jos kaikki translokaatiotyypit otettiin huomioon. Tulokset viittaavat siihen, että käyrän lineaarisen osan tarkka määrittäminen on hyvin tärkeää, etenkin kun kyseistä menetelmää tullaan todennäköisesti käyttämään enimmäkseen kroonisen altistuksen annosarvioinnissa.

Translokaatioiden pysyvyyttä on voitu seurata tutkimalla Virossa 1994 tapahtuneen säteilyonnettomuuden uhreista pari kertaa vuodessa otettuja verinäytteitä. Perinteinen, disentrisiin kromosomeihin perustuva annosmääritys tehtiin pian onnettomuuden jälkeen 18:lle henkilölle. Viiden ihmisen arvioitiin saaneen huomattavan säteilyannoksen, joka muodostui vaihtelevasti joko viikkoja tai tunteja kestäneen altistuksen seurauksena ja joissain tapauksissa lyhytaikaisesta, osaan kehoa kohdistuneesta altistuksesta. Pian onnettomuuden jälkeen suoritetussa kromosomimaalaus-analyysissä todettiin yhtä paljon translokaatioita ja disentrisiä kromosomeja. Kun kromosomi-vaurioita seurattiin kahden vuoden ajan onnettomuuden jälkeen, translokaatiofrekvenssin havaittiin pääpiirteittäin säilyvän samalla tasolla. Tämä havainto tukee translokaatioiden käyttöä kauan sitten tapahtuneiden altistusten annosarvioinnissa. Disentristen kromosomien määrä putosi jyrkästi näiden kahden vuoden aikana. Näkyvää translokaatioiden vähene-mistä tapahtui kuitenkin yhdellä onnettomuus-uhrilla, joka oli altistunut hyvin suurelle, pitkään jatkuneelle ja epätasaisesti jakautuneelle säteilyannokselle. Tästä syystä osakehoon useita vuosia aikaisemmin kohdistunutta annosta ei mahdollisesti pystytä määrittämään luotettavasti kromosomimaalaus-tekniikan avulla. Onnettomuusuhrien seurantaa jatketaan translokaatioiden stabiilisuuden selvittämiseksi pitkällä aikavälillä.

Korkeissa huoneilman radonpitoisuuksissa tapahtuneen kroonisen altistuksen ja kromosomimaalaus-analyysissä havaittujen kromosomi-vaurioiden määrän välillä ei havaittu yhteyttä tutkimuksessa, joka käsitti 84 matalassa, kohtalaisessa tai korkeassa radon-pitoisuudessa asunutta henkilöä. Kyseinen havainto koski sekä translokaatioita että epästabiileja kromosomi-vaurioita. Tulos osoittaa, että korkeillakaan pitoisuuksilla krooninen altistus radonista peräisin olevalle α-säteilylle ei ole riittävän suuri aiheuttaakseen kromosomimaalaus-menetelmällä havaittavan nousun kromosomivaurioiden tasossa perifeerisen veren lymfosyyteissä.

Kromosomimaalaus-tekniikan kykyä havaita krooninen altistus matalan LET:n (energiansiirtokyky) omaavalle säteilylle pyrittiin selvittämään määrittämällä kromosomivauriot 20:stä ydinvoimalatyöntekijästä, joiden

keskimääräinen kumulatiivinen annos oli 100 mSv, ja vertailemalla niitä 20:stä kontrollihenkilöstä saatuihin tuloksiin. Kirjattujen kumulatiivisten annosten ja translokaatiofrekvenssien välillä havaittiin regressioanalyysissä merkitsevä riippuvuus. Työntekijöillä, joilla oli yhtäläiset rekisteröidyt annokset, oli kuitenkin hyvinkin suuria yksilöllisiä eroja translokaatioiden määrässä. Tulokset viittaavat siihen, että krooninen altistus matalan LET:n säteilylle voidaan kromosomimaalaustekniikan avulla todeta ryhmätasolla.

Tutkimuksen tulokset osoittivat, että translokaatioiden määrän mittaaminen kromosomimaalaus-menetelmällä on ylivoimainen retrospektiivisenä annos-mittarina perinteiseen disentristen kromosomien analyysiin verrattuna. Translokaatioiden voimakas ikä-riippuvuus tuli myös esille tässä tutki-muksessa. Ikä-efekti onkin yksi tärkeimmistä tekijöistä kromosomimaalaus-tekniikan herkkyyttä arvioitaessa. Aineisto antoi myös selviä viitteitä siitä, että tasaisesti jakautunut säteilyannos voidaan monen vuoden kuluttua määrittää tarkemmin kuin osakehoon kohdistunut annos. Kromosomimaalaus-tekniikka osoittautui potentiaaliseksi menetelmäksi arvioitaessa kroonisia säteilyannoksia ryhmätasolla. Merkkejä tekniikan herkkyyseroista eri säteilylaatujen välillä oli kuitenkin nähtävissä. Annosvasteen lineaarisen osan tarkka määritys nousi tutkimuksen kuluessa hyvin tärkeäksi. Vaikka tulokset selvensivät käsityksiä kromosomimaalaus-tekniikan mahdollisuuksista ja rajoituksista, menetelmän soveltuvuutta on tutkittava edelleen.

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V Lindholm C. Stable and unstable chromosomal aberrations among Finnish nuclear power plant workers. Submitted for publication.

LIST OF ABBREVIATIONS

AMCA	7-amino-4-methylcoumarin-3-acetic acid
Bq	Bequerel
BrdU	Bromodeoxyuridine
CA	Chromosomal aberration
dsb	double-strand break
ESR	Electron spin resonance
FISH	Fluorescence *in situ* hybridization
FITC	Fluorescein isothiocyanate
FPG	Fluorescence plus Giemsa
GPA	Glycophorin A
Gy	Gray
LET	Linear energy transfer
PAINT	Protocol for Aberration Identification and Nomenclature Terminology
PCC	Premature chromosome condensation
PEG	Polyethylene glycol
RBE	Relative biological effectiveness
Sv	Sievert

1 INTRODUCTION

All human beings are exposed to various natural and artificial sources of ionizing radiation. On average, a large part of the naturally occurring radiation is received from indoor radon, while that remaining is derived from natural radioactivity in the body, cosmic radiation and radioisotopes found in the environment. Among the artificial sources, radiation exposure results almost exclusively from the application of radiation and radioactive materials for diagnostic and therapeutic purposes in medicine, with x-ray examinations being the most important. Nuclear technology used in energy production and in weapon tests also increases the average radiation dose received, but in general, its contribution is very small. Large differences in radiation exposure may be encountered between individuals. Increased doses can be received by patients treated with radiotherapy, by personnel working in radiology, nuclear power and air traffic, or by inhabitants or workers in areas with high levels of naturally occurring radiation. Moreover, high individual doses are sometimes encountered in radiation accidents.

The detrimental health effects of radiation can be divided into deterministic effects emerging usually a short time after a serious exposure and stochastic effects arising several years after exposure to radiation. Accurate assessment of radiation dose is important for a better understanding of both these effects. Decisions regarding medical treatment of deterministic effects in accidentally exposed subjects are dependent on rapid assessment of dose. Radiation-induced cancer is a stochastic effect and the epidemiological studies rely on dose estimates for cohorts of people. Risk calculations for low doses and dose rates are extrapolated from studies of highly exposed populations, implying the need for a more exact approach for determination of radiation dose.

Several markers of exposure that are based on the biological effects of radiation have been developed in recent years. Measurement of biomarkers is superior to physical methods of dosimetry, since the most important cellular target of radiation damage, the deoxyribonucleic acid (DNA) molecule, is always present during exposure, unlike badge dosimeters. To function as an effective indicator of dose, the assay should be performed on easily available cells and the quantity of biomarkers must change as a

function of dose with sufficient accuracy over a large dose range. These requirements are met by chromosomal analysis of peripheral blood lymphocytes which is the most widely used technique within biological dosimetry. The standard cytogenetic method based on the detection of dicentric chromosomes has been successfully applied for dose assessment in a number of cases of accidental exposure when the accident was identified within a reasonably short period of time. However, dose estimation becomes increasingly uncertain when several months have elapsed from the exposure, because of the elimination of dicentric chromosomes from blood cells over time. Furthermore, the unstable nature of dicentrics also renders them unsuitable for quantification of cumulative doses. Assistance in overcoming this drawback in biological dosimetry has been forthcoming as a result of recent developments in molecular cytogenetics. Whole-chromosome-specific DNA probes allow the precise analysis of stable chromosome aberrations that are not selected against during cell division and are thus thought to persist over time. The new technique, termed FISH (fluorescence *in situ* hybridization) chromosome painting, is currently being tested in many laboratories, to evaluate its suitabilities and limitations in retrospective dose reconstruction. The objective of the present study was to participate in this evaluation by producing and examining data of the various aspects involved in the applicability of the FISH painting technique, above all to identify stable chromosome aberrations and to test the sensitivity of the method.

2 REVIEW OF THE LITERATURE

2.1 Radiation quantities

Radiation can be described as movement of energy. In electromagnetic radiation, such as X- and γ-rays, the energy travels in a wavelike manner or as photons. Particle radiation consists of charged components, such as alpha (α)-particles, protons and electrons, or uncharged particles, such as neutrons, all produced during decay of radioactive material. Radiation can be quantified as the absorbed dose, D, which defines the mean energy imparted to the absorbing organ or tissue. The unit of absorbed dose is the gray (Gy). The amount of energy deposited on a track distance traversed by radiation of a defined energy is given as the linear energy transfer (LET), and this quantity can differ considerably between different radiation types. To compare the influence of radiation quality on different biological endpoints, such as mutations and chromosomal aberrations (CA), the relative biological effectiveness (RBE) can be calculated. The RBE provides a ratio of the effectiveness per unit dose of a given radiation in relation to a reference radiation, usually X- or γ-rays. The RBE increases with LET up to an energy deposition of 100 keV/μm and decreases at higher LET values.

The distribution of absorbed energy influences the biological detriments for a given radiation type. For radiation protection purposes, the definition of equivalent dose has thus been introduced (ICRP 60, 1991). This can be obtained by multiplying the absorbed dose with the weighting factor for a particular radiation type. For X- or γ-rays and electrons, the factor has been given a value of 1, for neutrons of different energies the value lies between 5 and 20, and for α-particles a weighting factor of 20 is used. The concept of effective dose allows the comparison of detriment of absorbed dose to various organs using the tissue-weighting factors developed from differences in sensitivity to tumour induction (ICRP 60, 1991). The total effective dose can be calculated by summing the effective doses from all tissues derived as a product of the equivalent dose and the corresponding weighting factor from each tissue. The unit for both equivalent and effective dose is the sievert (Sv).

2.2 Principles of radiation biology

When ionizing radiation moves through a cell, the transfer of energy induces a track of primary events, ionizations, as the electrons are ejected from the atoms in the medium. This kind of direct ionizations are typical for particle radiation. For electromagnetic radiation, liberated electrons produce ionizations also during secondary collision processes. The most relevant target of radiation in a cell nucleus is the DNA molecule which can be damaged through ionization of its sugar-phosphate backbone. About two-thirds of the damage is caused by free radicals produced by ionization of water molecules in the vicinity of DNA (Ward 1985). Hydroxyl radicals are the most important products in this type of reaction and can cause severe damage through further reactions with bases and sugars in the DNA molecule.

A wide spectrum of damage to DNA and other macromolecules is known to arise in irradiated cells. For example, γ-ray irradiation with a dose of 1 Gy produces about 1000 single-strand breaks (ssbs), 40 double-strand breaks (dsbs), 150 protein-DNA crosslinks and thousands of damaged bases (Goodhead 1994). Simulated track structure analysis has revealed that at least part of the radiation-induced ionizations appear to cluster, leading to sites with complex damage consisting of multiple breaks or damaged bases (Edwards *et al.* 1996a,b). Predictions also show that the frequency of multiple damaged sites increases with increasing LET. Clustered damage is indistinguishable from simple dsbs with conventional experimental techniques. However, new methods have shown that high-LET particles induce an excess of nonrandomly occurring dsbs (Löbrich *et al.* 1996), a finding anticipated from theoretical calculations.

A particle track from low-LET radiation, such as x-rays and γ-rays, induces sparsely distributed ionizations within a cell. In contrast, thousands of ionizations are produced in a cell by a track of high-LET radiation, such as α-particles and neutrons. Therefore, a single cell will receive a relatively small dose when hit by a track of X- or γ-rays. A track of high-LET radiation, however, will result in a high dose to a single cell. When tissues or cell populations are considered at low doses, only a few cells are being hit by a track of high-LET particles, whereas low-LET radiation is distributed more uniformly among cells.

Once damage to the DNA has occurred, cellular mechanisms are activated to process the damage. This is achieved by elimination of the damaged cell through apoptosis (programmed cell death) or by repairing the DNA lesions with a wide range of enzymes. Damage to single strands is repaired efficiently, due to the complementing function of the undamaged strand that can act as a template during repair, and high fidelity is achieved. The repair of dsbs is more complicated and is more prone to sequence loss and mutations. As a consequence of enzymes rejoining the break ends, the majority of dsbs are restituted, i.e. the original configuration is achieved but a loss of bases may occur. In a minority of cases, a dsb can be misrepaired with another break close to it in space and time, producing a change in DNA configuration. Experimental data have shown that γ-ray-induced damage was repaired more efficiently than damage caused by α-particles, indicating the difficulties in repair of complex, multiple damaged sites (Hodgkins *et al.* 1996). It can be envisioned that lesions positioned close together may cause interference in the repair function of the specific enzymes involved.

The DNA dsb is considered to be the most important lesion in the formation of CAs (Obe *et al.* 1992, Natarajan *et al.* 1993, Bryant 1997). This argument is supported by data indicating a clear relationship between dsbs induced by restriction enzymes and CAs (Bryant 1984, Natarajan and Obe 1984) Further evidence has been provided by work on dsb repair-deficient cell lines that show a high frequency of CAs (Kemp and Jeggo 1986, Biedermann *et al.* 1991).

2.3 Origin of radiation-induced chromosomal aberrations

Despite several decades of studies in radiation cytogenetics, the exact mechanisms underlying production of CAs are far from understood, although a number of theories have been proposed. Two of the most discussed theories, the Breakage-and-Reunion and the Exchange Theories, were based on much sparser knowledge about the configuration of chromosomes than what is known today, and the dsb in its present meaning cannot be considered to equal the definition of a break as described in these models. It was thought that a chromosome was built up from a backbone of proteins (Lea 1946) and that DNA was swimming loosely in the nucleus during interphase and became attached to the backbone just before cell division (Darlington and LaCour 1945). The molecular structure and function

of DNA, or the repair mechanisms, were not disentangled at that time. Thus, it was not possible to account for their influence in developing the models.

Work on pollen grains of plants of the genus *Tradescantia* conducted by Sax (1938, 1940) and Lea (Lea and Catcheside 1942) was fundamental for the Breakage-and-Reunion Theory (Classic Theory). The mathematical model of this theory was developed by Lea (1946). The basic idea of the Breakage-and-Reunion Theory was that radiation induces a break in the 'backbone' of the chromosome. A break could then have three alternative fates. Firstly, the break can be restituted so that the initial configuration of the chromosome is re-established. More than 90% of all breaks are restituted. Secondly, provided that a second break is close in space and time, the loose ends can be illegitimately rejoined to form exchange-type aberrations. Thirdly, the break can remain open and appear as a terminal deletion. A linear dose effect was observed for terminal deletions, enforcing the understanding of breaks being induced in proportion to dose. Exchanges were formed, at least for low-LET radiation types, in a linear-quadratic manner, indicating that interactions between breaks had occurred. At low doses, the two breaks needed for an exchange were induced at low frequency from one track of ionizing radiation. At higher doses, the probability that the two breaks were produced from two tracks increased. For high-LET radiation types, formation of all aberration types showed a linear dose response. Based on this finding, it was postulated that exchanges were formed from breaks that were close in space and induced by the same track. This finding led to the concept that exchanges between breaks are not formed in a random manner within a nucleus, but over limited distances. The rejoining distance of breaks is dependent on the dose and radiation quality and is limited by the complicated network of chromatin threads. It has been postulated that exchanges cannot be formed by random movement of breaks within a calculated rejoining distance. Savage (1996) listed several elements that may affect the model of distance-dependent misrepair of breaks. These include the finding that breaks are not produced randomly but appear to cluster, the idea of attraction between breaks to enhance movement, the existence of repair complexes in which damaged chromatin is accumulated and the pre-existing proximity between chromosome regions for functional purposes that are vulnerable to exchange formation. The Breakage and Reunion model has received the widest support in the description of formation of CAs (Savage 1998).

The Exchange Theory introduced by Revell (1955) was originally developed for chromatid-type aberrations. This model proposed that ionizing radiation does not induce breaks in the chromosome 'backbone' but instead produces an unstable lesion. When two such lesions come together in time and space they can initiate exchange. The actual exchange will occur later or it may be reversed. This theory implies that all aberrations are formed as a result of an exchange process. Failure in one of the junctions of the process will result in incomplete exchanges producing terminal deletions. An adequate application of this theory for the origin of chromosome-type aberrations has been difficult due to problems in testing the model (Brewen and Brock 1968).

The Molecular Theory, presented by Chadwick and Leenhouts (1978), suggested that breaks and exchanges originate from a single DNA dsb. In the case of exchange formation, this model argues that a second, enzymatically induced dsb is formed in undamaged chromatin through recombinational repair mechanisms, provided there is homology between the two DNA stretches. Exchanges produced in this manner would overcome the problem with break distances as modelled in the Classic Theory (Savage 1996).

Recent advances in understanding the three-dimensional nuclear structure using FISH and confocal microscopy have led to new insights into the complex system of mechanisms in the formation of CAs. Interphase chromosomes have been found to occupy distinct territories with minimum intermingling of chromatin between chromosomes (Cremer *et al.* 1996). The three-dimensional organization has also been proposed to contain a channel structure expanding from the nuclear matrix between the surfaces of chromosome territories. The channel system continues to branch in the interior of the chromosome territories. The channel acts as the site for replication and repair for DNA strands. Exchanges between two or more chromosomes have been proposed to result from illegitimate repair of dsbs located within the same interchromosome boundary. Intrachanges within a chromosome are produced by similar repair of dsbs in the channels within a territory. Originally, this model was proposed by Savage and Papworth (1973), who claimed that interactions between chromosome territories and arm domains occur through an area shared by adjacent chromosomes. Many experiments have indicated that interactions of breaks occur over a limited distance instead of spanning the space of the entire nucleus (Savage 1996 and references therein). The model of exchange formation in the interchromosome boundary fits these findings well.

A large amount of experimental data on the formation of CAs comes from studies using the method of premature chromosome condensation (PCC), in which the chromatin in interphase cells is condensed with the help of mitosis-inducing factors from dividing cells of another species in the presence of a fusogen, a method introduced by Pantelias and Maillie (1983). PCC can also be induced chemically (Durante *et al.* 1998, Kanda *et al.* 1999). With the PCC technique, CAs can be observed directly after irradiation in the unreplicated chromatid of the G0 phase. This technique has been used to study the formation and kinetics of CAs shortly after irradiation (Hittelman & Rao 1974, Cornforth & Bedford 1983, Loucas & Geard 1994, Greinert *et al.* 1995). Recently, the PCC method has been combined with FISH using whole chromosome probes to examine exchange-type aberrations in interphase chromosomes (Evans *et al.* 1991, Brown *et al.* 1992, Kovacs *et al.* 1994, Durante *et al.* 1996a,b, Darroudi *et al.* 1998a). The inclusion of a pancentromeric probe in whole-chromosome painting has enabled reliable recognition of translocations and dicentrics (Durante *et al.* 1996b, Darroudi *et al.* 1998a).

Several investigations with the PCC technique have shown uniformly that most of the chromatin breaks induced by low-LET radiation rejoin within a few hours after exposure (Cornforth and Bedford 1983, Brown *et al.* 1992, Greinert *et al.* 1995, Durante *et al.* 1996a,b). Information concerning the formation of exchanges varies. Dicentrics were reported to form quickly and their frequency remained the same, despite the decline in number of chromosome breaks at later recovery times (Vyas *et al.* 1991). In contrast, other studies have found an increase in exchanges during a prolonged incubation time after low-LET irradiation of human G_0 lymphocytes (Greinert *et al.* 1995, Durante *et al.* 1996b). The kinetics of this increase was suggested to be composed of an early rapidly-appearing linear component and a more slowly formed quadratic term (Greinert *et al.* 1999). There are indications that the temporal behaviour of dicentrics and translocations is dependent on dose: at higher doses a clear increase in exchanges with increased incubation time was observed, whereas at low doses the initial frequency persisted (Darroudi *et al.* 1998a). It has also been shown that a vast majority of the exchange-type aberrations were incomplete in the immediate fusion, and that their frequency decreased during postirradiation time (Darroudi *et al.* 1998a, Sipi *et al.* 2000). It has been suggested that the early fast-repair mechanism is responsible for the formation of incomplete

exchanges and that complete exchanges are produced by slow repair (Darroudi *et al.* 1998a).

2.4 Classification of chromosomal aberrations

CAs induced by ionizing radiation, as viewed through a microscope on a metaphase preparation, are end products of initial lesions processed by complex cellular systems. Metaphase chromosomes can be produced by colchicin (or Colcemid) arrest during cell division of cycling cells, or mitogen-stimulated resting cells. Radiation-induced damage can be observed as chromosome or chromatid type aberrations, depending on whether the irradiated cell has resided in the G0/G1 or the G2 phase of the cell cycle, respectively. Noncycling peripheral T lymphocytes are studied for purposes of biological dose reconstruction, and radiation-induced aberrations in these cells are exclusively of the chromosome type, which is focused on in the following.

According to the most cited theory (Breakage-and-Reunion), misrepair or illegitimate rejoining of break ends produces structural aberrations between two different chromosomes (interchanges) or within one chromosome (intrachanges). In the case of an asymmetrical interchange, i.e. a dicentric chromosome, the centromeres of both chromosomes involved are located in the same piece, and an accompanying acentric fragment is present. The dicentric chromosome is easily recognizable after conventional staining of metaphase preparations. The reciprocal translocation (symmetrical interchange) involves mutual exchange of chromatin pieces, and only large rearrangements may be distinguished in solid-stained preparations. If the two breaks are located within one chromosome on each side of the centromere, the misrepair can lead to an asymmetrical intrachange producing an easily observable ring chromosome. If the two breaks on each side of the centromere are rejoined symmetrically, a pericentric inversion is produced, which in most cases remains indistinguishable in conventional analysis. A symmetrical paracentric inversion is formed when two breaks located on the same side of the centromere are misrepaired. This type of inversion leads to no variation in the chromosome arm lengths, and is therefore never observed with solid staining. In cases where breaks remain unrejoined, terminal or interstitial deletions are formed. The terminal deletions can be detected as acentric fragments, whereas interstitial deletions appear as either small double minutes or acentric rings.

In addition to simple aberrations, chromosome lesions can appear as complex configurations between two or more chromosomes. These rearrangements become more frequent at higher doses. With conventional Giemsa staining, the only complex aberrations observed in practice are polycentric chromosomes. The vast majority of complexes, therefore, remain indistinguishable; e.g. a piece of chromatin limited by two breaks in one chromosome is inserted into a break in another chromosome, i.e. an insertion is discerned if the aberrant chromosomes differ radically from their normal appearance. A very special category of abnormality is the so-called Rogue cell, a general term for cells with a high number of CAs (Awa and Neel 1986). Typically, these cells contain a large number of small deletions (double minutes) and several exchange-type aberrations, such as polycentric chromosomes. The occurrence of Rogue cells is rare and their origin is still unresolved.

2.5 The human lymphocyte

One of the most important cell types in studies involving CAs is the circulating lymphocyte. In biological dosimetry, dose assessment is predominantly based on data obtained from lymphocytes. One reason for their popularity is that they are easily obtained in large quantities from the peripheral blood. Most importantly, the vast majority of peripheral lymphocytes reside in the G0 phase of the cell cycle. Mitogen stimulation convert these resting lymphocytes into cycling cells that allows possible DNA lesions to be visualized in the metaphase chromosomes. This approach was enabled by the discovery of the mitogen phytohaemagglutinin (PHA) extracted from the plant *Phaseolus vulgaris* for stimulating human T lymphocytes to divide *in vitro* (Moorhead *et al.* 1960, Nowell 1960).

All lymphoid cells are derived from multipotent haemopoietic stem cells located in the early fetal liver and later in the bone marrow. B cells continue to differentiate in the bone marrow. In contrast, T lymphocytes develop from their precursors in the thymus, that has an active role in T-cell production early in life but this ability gradually decreases towards adulthood. Lymphocytes are produced at a rate of 10^9 cells per day in these primary lymphoid organs, from which some migrate into the secondary lymphoid tissues, such as lymph nodes, spleen and gut-associated lymphoid tissue. It has been suggested that more than 80% of lymphocytes belong to a

population of cells that are capable of recirculating through the immune system and that 1-2% of this pool recirculates each hour. Thus, it is possible to observe chromosome damage induced by irradiation not only in peripheral T lymphocytes but also in T cells that were located in primary or secondary lymphoid organs at the time of induction.

Morphologically, the majority of T cells are relatively small with a high nuclear-to-cytoplasm ratio, whereas the rest are larger cells with intracytoplasmic granules. T lymphocytes acquire the ability to recognize antigens in the thymus through recombination of the T-cell receptor (TCR) genes and finally through expression of the receptor complex on the cell surface. T cells can be further divided into subsets of T-helper and T-cytotoxic cells through specific surface markers. When an antigen is recognized by a lymphocyte, it induces the cell to proliferate. A clone of the specific antigen-binding cell is rapidly produced to achieve an immune response. Mitogen stimulation of lymphocytes closely resembles antigen stimulation. Activation of lymphocytes occurs by crosslinking the antigen receptors, which stimulates the production of cytokines and their receptors, enabling progression through the cell cycle. During activation, cells are transformed into a population of more undifferentiated blast cells with increased cell volume. The dramatic expansion of T lymphocytes caused by antigen stimulation is balanced out by apoptosis, or programmed death of cells. A portion of the activated cells escape cell death and form memory cells that possess an enhanced sensitivity for recognition of antigen. Memory T cells can be identified by a specific isoform of the leukocyte common antigen (CD45R0) separated from the more naïve or unprimed T cells that are not responsive to antigen stimulation *in vitro* and express the CD45RA isoform (Beverley 1992). Memory T cells may comprise more than half of the circulating T-cell pool.

One important issue when using lymphocytes in biological dosimetry is the lifespan of peripheral T cells. Reports of the mean lifespan of mature T lymphocytes have varied considerably, ranging from a few months to many years, based on calculations of loss of unstable CAs over time. The first attempt to define the lifespan of lymphocytes involved a long-term investigation of patients with x-ray treatment for ankylosing spondylitis (Buckton 1967a,b 1983). A mean lifetime of approximately 4.5 years, corresponding to a half-life of 3 years, was estimated from cytogenetic data encompassing up to 30 posttreatment years. However, it was recognized that the lack of analysis data from the first four years after treatment may have distorted the calculations since possible short-lived lymphocyte

populations were not included. A three-year half-life of dicentrics was also reported by Dolphin *et al.* (1973) and Lucas *et al.* (1992a). Using a model with differential half-lives for dicentrics, Bauchinger *et al.* (1989) reported values of 150 days at the end of therapy and 3.6 years at 1720 days following radiotherapy treatment of seminoma patients. In a report on the cytogenetic follow-up of the Goiania accident victims, Ramalho *et al.* (1995) proposed that unstable aberrations disappear by an exponential function formed by two subpopulations of T lymphocytes, one with cells dividing very rapidly and the other containing cells with a long lifespan. Depending on the exposure, different half-lives were obtained for the short-term subpopulation. Up to 470 days after exposure, half-lives of 110 days and 160 days were obtained for whole-body exposures of more than or less than 1 Gy, respectively. Another observation of a short half-life directly after exposure was reported by Littlefield *et al.* (1991), who found that cells containing asymmetrical aberrations declined to about 50% in 143 days in a highly exposed victim (8.3 Gy) of the 1989 San Salvador accident. In accordance with the findings of subpopulation-dependent lifespans, differences in intermitotic times have been observed in subsets of T lymphocytes bearing different isoforms of the cell- surface antigen CD45 (Michie *et al.* 1992, McClean and Mitchie 1995). Cells expressing the CD45R0 marker divide once every 22 weeks, whereas the naïve CD45RA subset of lymphocytes divides only once every 3.5 years.

2.6 Biological dose assessment using standard dicentric analysis

2.6.1 Background

Biological dosimetry using cytogenetic analysis of peripheral blood lymphocytes has been employed for almost four decades. The method was first applied in practice in dose assessment of individuals exposed during a criticality accident in 1962 where three subjects were exposed to both γ-rays and fission neutrons (Bender and Gooch 1966). Although ionizing radiation induces a variety of CAs, dicentric chromosomes became the choice for cytogenetic biodosimetry due to the easily identifiable structure and their low frequency in nonirradiated controls. Furthermore, dicentric chromosomes were found to be the most frequently produced aberration type, and similar linear-quadratic dose responses for low-LET radiation were obtained after homogeneous irradiation of resting lymphocytes both *in*

vitro and *in vivo* (Buckton *et al.* 1971, Clemenger and Scott 1971). The establishment of a dose-response curve for dicentrics was therefore a prerequisite for dose assessment. During the early years of chromosome biodosimetry, CAs were analysed from cells cultured for three days. This resulted in an increased frequency of second-division cells with an apparent decrease in the frequency of unstable aberrations. To assure analysis of first-division cells only, a two-day culture of lymphocytes soon became practice. In addition, analysis of first-division cells was clearly facilitated by the use of bromodeoxyuridine (BrdU) in the culture media, which allowed the specific recognition of different cell divisions (Perry and Wolf 1974, Crossen and Morgan 1977).

Application of the dicentric yields for dose estimation requires knowledge of the radiation type during the exposure. Dose estimation is then performed based on the specific dose-effect relationship of the particular radiation source. For acute low-LET radiation received at high dose rates, a linear-quadratic response to absorbed dose (D) is obtained that conform to the following equation:

$$Y = C + aD + bD^2$$

where Y is the yield of dicentric chromosomes, C the control level of dicentrics, and α and β are the linear and quadratic coefficients, respectively. As the LET increases, the relationship between dicentrics and dose becomes linear. Dose-response curves for 250-kVp x-rays and either ^{60}Co or ^{137}Cs γ-rays, are required due to differences in the curves at lower doses. In practice, these two curves as well as one for fission neutrons are considered to be adequate to cover a majority of cases within biodosimetry (IAEA 1986).

The statistical accuracy of the dose estimate and the lowest detection limit are dependent on the number of metaphases analysed. A dose of about 100 mGy of γ-rays can be detected by scoring 500 cells, but increasing the number of cells analysed decreases the statistical errors of the estimate (IAEA 1986). It is possible to observe doses of less than 100-mGy x-rays and 10-20-mGy fission neutrons with this method. After very high doses of several Gy, lymphocyte growth may be slowed down and there may not be many metaphases to score. However, in such cases the highly elevated frequency of dicentrics enables a reliable estimate to be obtained from less than 100 cells. The reliability of the dose estimate is usually expressed as the 95% confidence interval (CI), which limits the true dose in 95% of cases

with the same dicentric frequency. At high doses, e.g. above 6 - 8 Gy for x-rays, the dose effect becomes saturated and results in a lack of fit of the linear-quadratic curve (Lloyd and Edwards 1983).

Dicentrics analysis is generally performed to support the dosimetric data obtained from personal thermoluminescence dosimeters and other means of physical dosimetry. It is also possible that partial-body exposure, not detected by a locally placed dosimeter, can be observed by this method. More importantly, the dicentrics assay is the most accurate method for dose estimation in cases where a dosimeter has not been used, such as in radiation accidents. A reliable dose estimate is of major value when deciding on the medical treatment of individuals exposed to ionizing radiation.

2.6.2 Dose estimation of acute versus chronic exposure

Dose estimation based on the linear-quadratic relationship between dicentrics and dose is most reliable in cases where the exposure resembles the irradiation conditions of the *in vitro*-produced dose-effect curve. Most biological dosimetry laboratories have established acute dose-response curves using high-dose-rate irradiation. In many cases of overexposure, however, the dose has been received over a longer period of time. A protracted dose of low-LET radiation gives a smaller yield of dicentrics than if the same dose has been obtained within a short time. This is caused by modifications in the dose-squared coefficient of the linear-quadratic equation. During dose protraction, a smaller fraction of dicentrics from two ionization tracks is produced in a time-dependent manner as proposed by Lea and Catcheside (1942). The dose-squared coefficient is affected by the so-called G-function that considers the time period of exposure as well as the mean lifetime of breaks. As the irradiation time increases, the G-function and thus also the dose-squared coefficient approach zero. In cases where the exposure has continued for several days, the dose effect is essentially linear, thus depending solely on the α-coefficient.

2.6.3 Dose estimation of uniform versus nonuniform exposure

Another situation in which relatively reliable dose estimation of low-LET radiation can be obtained using dicentrics is in cases where the exposure is acute and distributed evenly over the whole body. In most cases of acute overexposures, however, the irradiation has occurred in a nonuniform manner covering only a part of the body. Information concerning the

uniformity of exposure is important with regard to the medical treatment of the patient. It may give essential information in cases where bone marrow transplantation is considered. Nonuniform exposure results in a fraction of lymphocytes with more CAs than expected by Poisson distribution, showing an overdistribution of aberrations. This can be tested by calculating the dispersion index, defined as the relation between the variation of dicentrics and the mean, σ^2/Y, and its test quantity U, which in case of overdistribution has a value of > 1.96 (Edwards *et al.* 1979).

In partial-body exposures a direct comparison of the dicentrics yield with the acute dose-response curve would give an average estimate of dose to the whole body. There are two methods for estimation of nonuniform exposures. The Qdr, originally developed for dose estimation of atomic bomb survivors examined more than 20 years after exposure (Sasaki and Miyata, 1968), considers only damaged cells with unstable aberrations that are assumed to have been present at the time of irradiation. Undamaged cells from the unirradiated part of the body or cells that have been produced from the precursor cells after irradiation can be ignored in this method. Qdr is the frequency of dicentrics and ring chromosomes among unstable cells, and follows the equation:

$$Qdr = Y_{dr} / [(1 - exp(-Y_{dr} - Y_{ace})]$$

where Y_{dr} and Y_{ace} are calibration curves established for dicentrics plus ring chromosomes and acentrics, respectively. The dose can be obtained by iteration of the equation. The size of the irradiated part of the body can also be estimated (Sasaki 1983).

Another method proposed by Dolphin (1969), namely the contaminated Poisson method, is based on overdistribution of dicentrics among all analysed cells. The observed distribution is composed of the Poisson distribution of cells that have been irradiated and of nonirradiated cells. The normal cells can originate from the unexposed part of the body or represent the first term (e^{-Y}) of the Poisson series. The mean yield of dicentrics (Y) in the irradiated fraction of cells can be obtained by iteration from the equation:

$$Y / (1 - e^{-Y}) = X / (N - n_0)$$

where X is the number of dicentrics observed, N the number of cells analysed and n_0 the observed number of cells without dicentrics. The mean

dose to the irradiated fraction is then determined by inserting Y in a standard dose-effect curve. The fraction (f) of cells scored that were irradiated can be calculated ($f = X / YN$), which allows the size of the irradiated fraction of the body to be estimated by considering the effects of interphase death and mitotic delay among irradiated cells.

The ability of the two approaches to estimate the proportion of irradiated cells and the absorbed dose to this fraction has been tested using both simulated partial irradiation, in which fractions of irradiated blood cells are mixed with nonirradiated cells, and by comparing doses received during partial-body radiotherapy. In *in vitro* studies, dose estimates showed good correlation with the given doses with both methods, whereas the proportion of irradiated blood cells appeared to be overestimated at higher doses (Lloyd *et al.* 1987, Barquinero *et al.* 1997). In partial-body irradiation, it was shown that the fraction of the body irradiated may not be accurately estimated and in high-dose exposure, an underestimation of dose to the fraction can be observed (Liniecki *et al.* 1983, Fong *et al.* 1995).

2.6.4 Dose estimation in radiation accidents

As mentioned above, dose assessment using dicentric analysis is most accurate in cases of accidental exposure involving whole-body acute exposure. There are only a few notable accidents, mainly within research and medicine, in which short-term, virtually homogeneous exposure has been evaluated using biological dosimetry. In a uniform exposure to ^{60}Co γ-rays, an exposed worker was analysed for the presence dicentrics and the resulting dose estimate was found to be in close agreement with the physical dose reconstruction performed with a phantom model (Brewen *et al.* 1972). In an accident at a sterilization plant, a worker received a whole-body dose of more than 20 Gy, as determined by the electron spin resonance (ESR) technique, whereas it was only possible to suggest a minimum dose of 10 Gy using dicentric analysis (Stavem *et al.* 1985).

During the Chernobyl nuclear reactor accident in 1986, several hundred people were exposed while working or participating in rescue operations at the power plant. A relatively small number of studies have been conducted in which the standard CA analysis was applied immediately after the accident. The efficiency of the standard dicentrics analysis in dose assessment decreases heavily after exposure; thus studies conducted several years after the Chernobyl accident will supply only suggestive

results of the magnitude of the dose. In a study performed by Salassidis *et al.* (1994), dose estimations for 15 subjects were calculated retrospectively 5-6 years after the Chernobyl accident using the Qdr approach. This method deals only with damaged cells that are considered to originate from the time of exposure. Similar dose estimates were obtained both from the Qdr approach and from translocation analysis. Elevated yields in chromosome-type aberrations among populations living in Chernobyl-contaminated areas were reported by Stephan and Oestreicher (1989), Salomaa *et al.* (1997) and Padovani *et al.* (1993, 1997). A prominent increase in dicentric chromosomes was observed in 35 persons living in the heavily contaminated area of Gomel (Verschaeve *et al.* 1993). In a study conducted five years after the Chernobyl reactor accident, involving more than 1700 persons from 12 cohorts, Sevan'kaev *et al.* (1995) observed a significant increase in CAs only in cohorts from the most contaminated area and in a group of children who had been evacuated from a highly contaminated area one week after the accident.

More commonly, accidentally received exposures are nonhomogeneous, and if the sources remain unrecognized they can continue to irradiate over an extended period of time. Dose estimation in this type of heterogeneous exposure pattern may thus be difficult. One such example is given by one of the most serious accidents to have occurred, namely the radiological accident in Goiania, Brazil in 1987 (IAEA 1988). It involved a strongly radioactive source, with an activity of approximately 50 TBq, containing soluble ^{137}Cs that had been removed from its housing. Fragments of the source were distributed among many persons, resulting in both external and internal exposure of nonhomogeneous, protracted as well as fractionated nature. After recognition of the accident, which was delayed for more than two weeks, dose assessment using dicentric analysis was performed for a total of 97 persons (Ramalho *et al.* 1988). The majority of the subjects showed a Poisson distribution of dicentrics. A nonuniform exposure would most probably have been concealed by the complicated pattern of irradiation. Comparison with the acute dose-response curve resulted in dose estimates of more than 0.5 Gy in 29 cases, the highest dose observed being 7.0 Gy. Since exposure in most subjects had been protracted or fractionated over a time period of hours or days, including both external and internal exposure to ^{137}Cs, application of the acute dose-response curve in these cases gave an apparent underestimation of dose. A more appropriate approach might have been to apply the G-function or the linear coefficient only, depending on the length of exposure.

Another example of nonuniform exposure to γ-rays is offered by an industrial accident that occurred in El Salvador in 1989. During malfunction of a sterilizing device containing a 666-TBq ^{60}Co source, three men became irradiated within a time period of a few minutes while trying to replace the source into its correct position (IAEA 1990). CA analysis revealed non-Poisson distribution of dicentric chromosomes in all three men, indicating that all had been exposed to nonuniform irradiation, which was in agreement with the severe skin burns observed (Littlefield *et al.* 1991). It was calculated that over 90% of the body had been exposed in each case and that this portion of the body received average doses from more than 3 Gy up to more than 8 Gy. However, as pointed out by the authors, these values included enormous differences in the distribution of dose across various parts of the body.

An accidental partial-body exposure to ^{192}Ir, resulting in serious local skin burns, was confirmed after dicentric analysis in a case where a subject had kept a radiography source in his pocket for more than two hours (Sreedevi *et al.* 1993). A clear overdispersion of dicentric chromosomes was observed. Typically for radiation accidents of this kind, the dose received was a mixture of nonuniform and more protracted than acute exposure, generating problems in dose calculations. The dose estimate could be made separately with respect to both exposure models but wide uncertainties had to be accepted. Dose protraction using the G-function resulted in a dose of 1.3 Gy, which exceeded the 0.6 Gy obtained with thermoluminescence dosimetry. The dose estimates to the irradiated fraction of the body were 4.5 Gy and 1.9 Gy, calculated using the contaminated Poisson method (Dolphin 1969) and the Qdr method (Sasaki and Miyata 1968), respectively.

Radionuclides incorporated into certain organs or tissues in the body represent another type of partial-body irradiation in which the exposure is protracted with decreasing dose-rate. A non-Poisson distribution of dicentric chromosomes was obtained in cases of internal contamination with ^{241}Am (Littlefield *et al.* 1980) and ^{131}I (Lloyd *et al.* 1976). Since the internal emitters were irradiating a pool of circulating lymphocytes, the yield of dicentrics would not have been very informative in determining the dose to the particular tissue. In contrast, irradiation caused by nuclides distributed evenly throughout the body, such as tritium absorbed as tritiated water, can be reliably quantified using chromosome analysis (Lloyd *et al.* 1986).

2.6.5 Biomonitoring of chronic exposure to radiation

Apart from direct dose assessment of radiation exposure, analysis of CAs using the standard method has been widely used in monitoring the cytogenetic effects of long-term occupational or natural radiation exposure. Since dicentric chromosomes are unstable and disappear from the circulating lymphocytes at an undefined rate, they have limited use as dosimeters for chronic exposures. An increase in the frequency of dicentrics can instead be considered to be an indication of ongoing damage to DNA.

Individuals living or working in areas with elevated natural radiation levels, derived mainly from ^{222}Rn, have been investigated using standard CA analysis. Elevated frequencies of CAs were found in uranium miners compared with controls (Brandom *et al.* 1978). Residents living adjacent to a uranium-mining location were found to have a higher frequency of cells containing chromosome-type aberrations in comparison to matched controls, although the yield of dicentrics was lower (Au *et al.* 1995). No correlation between CAs and domestic radon levels was found by Albering *et al.* (1992), whereas Bauchinger *et al.* (1994) reported a significantly increased frequency of dicentric and ring chromosomes in subjects exposed to high radon concentrations in their homes. Furthermore, elevated yields of chromosome-type aberrations were reported in individuals residing in a 'radon spa' (Pohl-Rühling and Fischer 1979, 1983).

A number of reports on cytogenetic analyses have shown an increase in CA frequencies among aircrew members who were exposed to cosmic radiation (Scheid *et al.* 1993, Heimers *et al.* 1995, Romano *et al.* 1997). However, these studies suffer either from the lack of a comparable control group or from an insufficient number of analysed cells. In a large study comprising 1000 metaphases per individual, no difference between CA yields between cabin attendants and appropriately chosen controls was displayed (Wolf *et al.* 1999). Exposure to cosmic radiation received during long-term space flights was found to result in a significant elevation of dicentrics as compared to preflight frequencies (Testard *et al.* 1996, Obe *et al.* 1997).

2.7 Retrospective biodosimetry using stable translocations

2.7.1 Background

Proliferative death of cells containing dicentric chromosomes reduces the number of such cells during division of lymphatic precursors. The replenishment of lymphocytes in the peripheral blood will consequently result in a decline in dicentric frequency. The exact elimination rate of dicentric chromosomes is, however, poorly defined. Dicentrics are therefore most suitable for dose calculations during a reasonably short period after exposure to radiation. In contrast to dicentrics, however, translocations are considered stable in cell division and so the yield should not fall with time. Analysis of translocations would therefore allow more accurate dose estimates in cases where the exposures occurred a long time previously, and it would also be informative in cases of chronic exposures. The first studies for visualization of translocations several years after exposure were performed using chromosome-banding techniques or grouping of solid-stained chromosomes according to their length and revealed the applicability of stable CAs in detection of old exposures (Littlefield and Joiner 1978, Ohtaki *et al.* 1982, Kleinerman *et al.* 1989, Kleinerman *et al.* 1990). However, these methods are very laborious, requiring skilful scoring, and were thus not extensively used within retrospective biodosimetry.

The introduction of chromosome painting with *in situ* hybridization (Pinkel *et al.* 1988) has radically changed the efficiency of discerning stable CAs, such as translocations and insertions. The method is based on the application of composite whole-chromosome probes, either directly labelled with a fluorochrome or indirectly visualized with antibodies. In general, the probes applied for hybridization cover only a few chromosome pairs equal to about 20% of the genome. Counterstaining with a different fluorochrome enables the recognition of translocations between a target and a counterstained chromosome. Labelling of centromeres further allows precise identification between translocations and dicentrics.

Due to the rapid and elegant recognition of translocations, the new technique was soon applied to serve as a tool for dose assessment in retrospective dosimetry. However, during the assessment of translocation analysis for biodosimetric purposes, it soon became clear that several factors affecting the methodology needed to be studied more carefully. New approaches to dealing with these novel types of data merged, as it was realized that the procedures routinely used in standard biodosimetry were

not applicable to translocation data. Factors influencing the applicability of the FISH technique are discussed in the following.

2.7.2 Choice of chromosomes to be painted

The use of FISH chromosome painting for biological dosimetry is founded on the assumption that chromosomes are uniformly sensitive to breakage and reunion and that the breakpoints occur at random locations. These conditions form the basis of the formula for converting the yield of exchange-type aberrations in the painted chromosomes into full genome values (Lucas *et al.* 1992b). The genomic translocation frequency, F_G, can be calculated as follows:

$$F_G = F_P / 2.05\ f_P\ (1-f_P)$$

where F_P is the frequency of translocations detected by painting and f_P is the fraction of genome painted.

A number of studies using chromosome painting have shown that the involvement of specific chromosomes in aberrations is not correlated with the predicted value. Thus, the selection of chromosomes to be painted has been and continues to be a matter for discussion. Chromosome 1 has been observed to be more sensitive to translocations than expected (Wojcik and Streffer 1998); however, opposite findings for chromosome 1 have also been reported (Fernández *et al.* 1995). In a study by Knehr *et al.* (1994), in which lymphocytes from one donor were irradiated with 3-Gy x-rays and painting was conducted with probe combinations of three chromosomes, a significantly elevated frequency of translocations was found in one combination. This indicates that the Lucas formula may not be valid. In another set of similar experiments using separate painting of single chromosomes (Knehr *et al.* 1996), chromosomes with a higher DNA content were in general less frequently involved in the formation of symmetrical translocations and dicentrics than could be expected from their DNA content. In contrast, smaller chromosomes were more frequently involved, the only exception being chromosome 4, which had the highest translocation frequency of all chromosomes analysed. Chromosome 2 was reported to be involved in fewer symmetrical translocations than predicted (Knehr *et al.* 1996). Chromosome 4 has also been represented more often than expected in other studies. Boei *et al.* (1997) observed overrepresentation of chromosome 4 in reciprocal translocations after X-irradiation of lymphocytes, whereas Stephan and Pressl (1997) found chromosome 4 more

often in both translocations and dicentrics. However, in material consisting of cells irradiated both *in vivo* and *in vitro*, Luomahaara *et al.* (1999) observed close agreement between the observed and expected yields of dicentrics and translocations among chromosomes 1, 2 and 4.

In a recent study covering all chromosomes of the female karyotype (Barquinero *et al.* 1998), the larger chromosomes were generally less involved in aberrations than expected in relation to their DNA content, whereas most of the smaller chromosomes were found in more aberrations than expected. In the same study, chromosome 4 showed no overrepresentation. Similar results were reported by Cigarrán *et al.* (1998), who however found a very good relationship between the yield of exchange-type aberrations and the surface area of interphase chromosomes.

2.7.3 Painting nomenclatures

The FISH technique introduced the need to develop a new system for description of aberrations. The existing standard classification was found to be inadequate for characterizing the highly rearranged aberrations visualized by chromosome painting. This resulted in a variety of terminology types, most of which were based on conventional scoring (ISCN 1985), containing categories of both stable and unstable aberrations and dividing exchanges into complete and incomplete events. Translocations were further recorded as two-way, in which both parts of the reciprocal exchange are observed, and one-way translocations containing only one colour-switch. Moreover, it was also discovered that a portion of the one-way translocations may in fact have been two-way translocations in which the other half has not distinguished due to the limited resolution power of the technique. Adequate comparisons between results were not possible because exact definitions of the terminology were lacking. Two nomenclature systems have been developed to serve the specific requirements imposed by the analysis of FISH-painted chromosomes. In the PAINT (Protocol for Aberration Identification and Nomenclature Terminology, Tucker *et al.* 1995), each rearranged chromosome made visible by painting is described separately, so that a reciprocal translocation is described as two chromosomes with material from the other chromosome. Distinct identification of centromeres is necessary for a reliable description with this terminology. It can be used for aberration analysis of chromosome-probe cocktails with either single or multiple colours. PAINT does not consider the mechanisms by which the aberrations are formed.

The high yield of complex exchanges visualized with the FISH technique led to the development of the S&S nomenclature system (Savage and Simpson 1994a,b, Savage and Tucker 1996), which in contrast to PAINT was specifically developed for determination of aberration origins. Complex exchanges are defined as rearrangements involving three, or more breaks in two, or more chromosomes. Savage and Simpson (1994a) classified complexes into families that are based on the number of chromosomes, chromosome arms and breaks involved. Assuming that the classic Breakage-and-Reunion Theory holds here, free interaction of breaks will produce a number of configurations that are dependent on the original number of breaks. When painted, these configurations are visualized as specific patterns. Evaluation of these patterns has shown that the frequency of visible complex exchanges is an underestimate of the true value due to the existence of apparently simple exchanges, called pseudosimples, that are in fact derived from three or more breaks (Simpson and Savage 1995a). Moreover, many observed complexes are classified as a form with fewer breaks than have in fact participated (Simpson and Savage 1995b). The S&S system as described by Simpson and Savage (1994 a,b) can only be applied for painting with single chromosome probes or multicolour cocktail probes. Cocktails of the same colour distort the pattern distributions.

The feasibility of the different nomenclature systems (PAINT, S&S and conventional scoring) for description and quantification of CAs was evaluated in both x-ray- and fission neutron-irradiated lymphocytes, analysed after single colour or multi-colour chromosome painting (Knehr *et al.* 1998, 1999). The results of the comparison indicated that conventional scoring is insufficient for an accurate description of complex exchanges, whereas PAINT provided a rapid system for this purpose. S&S is recommended when the mechanisms of aberration formation are studied. Scoring of a reciprocal translocation as two events according to the PAINT system leads to overdispersion (Finnon *et al.* 1995) and, if regarded separately, to two dose-response curves (Knehr *et al.* 1999), both of which are inconvenient for biological dosimetry purposes. A new terminology has been proposed that combines the descriptive character of PAINT and consideration of the entire rearrangement according to S&S (Knehr *et al.* 1998, 1999). Similar nomenclature was also applied in aberration scoring of a large cohort of Chernobyl cleanup workers (Littlefield *et al.* 1998).

2.7.4 Calibration curve

To achieve reliable dose estimations using retrospective translocation analysis, a calibration curve using a tested protocol for FISH chromosome painting is necessary. The same protocol should then be used in dose assessment of exposure cases. The use of a pancentromeric probe is particularly important in establishing the calibration curve for reliable sorting of dicentrics and translocations. Whether only two-way or total (two-way and one-way) translocations should be applied in retrospective dosimetry is not yet fully clarified. The decision is highly dependent on the persistence of the two types of translocation. The other potentially stable aberration type readily distinguished with chromosome painting is the insertion. In general, insertions are not considered relevant in the calibration curve for use in retrospective biodosimetry due to their rarity and because of the possible uncertainties related to the fact that they are complex rearrangements.

During the early years of translocation analysis, several reports indicated that translocations were induced far more often than dicentrics (Cremer *et al.* 1990, Natarajan *et al.* 1992, Schmid *et al.* 1992). The introduction of centromere labelling has evidently diminished this discrepancy, since this has reduced the translocations-dicentrics ratio closer to 1 (Bauchinger *et al.* 1993, Straume and Lucas 1993, Fernández *et al.* 1995, Finnon *et al.* 1995).

In the acute linear-quadratic dose-response curve for low-LET radiation, the time-dependent factor, G, modifies the quadratic term so that when the irradiation time increases the quadratic term approaches zero. Therefore, the calculated dose is very dependent on the value and reliability of the α-coefficient. The same accounts for the linear coefficient established for translocations when applied to retrospective dosimetry in cases where dose protraction is known or in chronic low-dose exposures. For retrospective biological dosimetry, the majority of cases to be studied by translocation analysis is likely to have received relatively low doses at low dose rates. The linear component of the dose-response curve therefore becomes very important in dose assessments of this kind. It is thus necessary to stress the establishment of the calibration curve for translocations, and especially of the linear part of the curve. This means that an adequate number of low-dose points and large numbers of cells at these dose points need to be analysed. Rather than using the linear term from the acute curve, the ideal situation would be to measure the α-coefficient from lymphocytes exposed to chronic radiation. However, there are indications that the α-coefficient

obtained using chronic exposure at body temperature is not different from that reported for acute dose response (Hsieh *et al.* 1999.

2.7.5 Control level of translocations

Accumulating data have shown that the background frequency of translocations in unirradiated control populations is much higher than that of dicentrics. The mean yield of dicentrics in control subjects has been established at about one in 1000 cells (IAEA 1986), whereas translocations have been found to exceed this severalfold. The mean control values of translocations have ranged between 0.002 and 0.01 in a number of studies (Bauchinger *et al.* 1993, Tucker and Senft 1994, Fernández *et al.*1995, Finnon *et al.* 1995, Lucas *et al.* 1995, Salomaa *et al.* 1997, Stephan and Pressl 1997, Snigiryova *et al.* 1997, Littlefield *et al.* 1998, Pressl *et al.* 1999). In some studies, smoking has been observed to elevate the translocation frequency (Ramsey *et al.* 1995, Moore *et al.* 1997, Tucker *et al.* 1997a, Littlefield *et al.* 1998), whereas no or only marginal relationship between smoking status and translocation yield has been reported by others (Salomaa *et al.* 1997, Pressl *et al.* 1999). However, age-dependent frequency of translocations has now become an unquestionable fact, as revealed by the significant correlation reported in a number of studies (Tucker *et al.* 1994, Ramsey *et al.* 1995, Moore *et al.* 1997, Salomaa *et al.* 1997, Tucker *et al.* 1997a, Littlefield *et al.* 1998, Pressl *et al.* 1999). In fact, this increase with age can be expected since once induced, translocations are accumulated due to their stable nature. The relationship between translocation frequency and age in unexposed control populations has been found to be nonlinear, indicating that factors other than exposure to background radiation are involved (Lucas *et al.* 1999a). It has been suggested that the increase in translocation frequency with age is caused by age-dependent biological processes (Tucker *et al.* 1999). A decrease in the repair of DNA damage with age has been observed (Wei *et al.* 1993), whereas no evidence for diminished repair capacity with age was reported by Lucas *et al.* (1999b) when comparing the linear coefficient of the dose-response curve after chronic exposure of lymphocytes from donors 24 - 79 years of age. The best fit of data obtained from subjects from 0 up to almost 100 years has shown in different studies that translocations increase with either a quadratic (Tucker *et al.* 1994), cubic (Ramsey *et al.* 1995) or linear-quadratic function of age (Lucas *et al.* 1999a), respectively. In addition, wide variability in the translocation yield within similar age-groups has been reported (Tucker and Moore 1996, Pressl *et al.* 1999). However, it has been claimed that more uniform data among individuals of the same age can be obtained with an

extensive analysis of translocations, i.e. counting up to five or six thousand genomic-equivalent cells per individual (Lucas *et al.* 1999a).

2.7.6 Persistence of translocations

Retrospective biodosimetry is based on the long-term persistence of translocations. Before the FISH technique can be reliably applied in assessment of past exposures, the persistence needs to be verified. This is best performed by follow-up studies of accidentally irradiated subjects. Since information on translocation frequencies immediately after exposure has usually not been available, comparisons with the initial dicentrics yield has been conducted. Lucas *et al.* (1992a) reported that the yield of FISH translocations six years after an accident involving intake of tritiated water was comparable to initial dicentrics yield. In a follow-up of the same person, a similar translocation frequency was still found 11 years postaccident (Lloyd *et al.* 1998). Stephan and Pressl (1997) reported that in radiation workers accidentally exposed 11 years earlier, the translocation frequencies were similar to the initial dicentric frequencies. Salassidis *et al.* (1995) found constant translocation frequencies in 11 Chernobyl victims studied between 1991 and 1994. Furthermore, persistence of translocations has been shown in rhesus monkeys exposed to whole-body radiation 28 years earlier (Lucas *et al.* 1996). However, with regard to the Goiania accident victims exposed in 1987, translocation frequencies studied during 1992 and 1995 were reported to be much lower than the initial dicentric frequencies, and a dose-dependent ratio between dicentrics and translocations was implied (Natarajan and Darroudi 1994, Natarajan *et al.* 1998, Sakamoto-Hojo *et al.* 1999).

A decline in translocation frequency with time has been reported in human (Matsumoto *et al.* 1998) and rat (Tucker *et al.* 1997b) lymphocytes cultured for several days after *in vitro* irradiation. In another follow-up experiment performed *in vitro*, in which the cell cycle number of each analysed metaphase was assessed with differential staining, no decrease in translocation yield was observed after 3-4 divisions (Guerrero-Carbajal *et al.* 1998). The same report also indicated a reduction in the yield of translocations in simulated partial-body exposure. It was postulated that the distributions of translocations and dicentrics were linked and the decline of cells containing unstable aberrations subsequently resulted in loss of translocations. The role of the nonuniform irradiation has also been offered as an explanation for the low translocation yields obtained in the Goiania victims (Lloyd 1998). These findings suggest that the elimination of

translocations after partial-body exposure clearly poses a problem to reliable dose assessment of past exposures using the FISH technique. Huber *et al.* (1999) have shown a temporal decline of translocations in lymphocytes of breast cancer patients after fractionated photon therapy of small areas of the bone marrow. The decrease was observed during 14 months in two out of five patients, implying the existence of individual differences in lymphocyte turnover.

The persistence of translocations has also been investigated in a number of *in vivo* studies on animals. A follow-up encompassing 30 days postirradiation of mouse bone marrow and peripheral blood have shown that reciprocal translocations persist at doses of 3 Gy and below (Spruill *et al.* 1996). In the same dose range, however, a decrease in nonreciprocal translocations was observed in the bone marrow. This finding indicated that irradiation of the dividing precursor cells produces chromatid exchanges which, after cell division, leads to lack of material in the nonreciprocal translocations and consequently to instability of the cell (Spruill *et al.* 1996). Xiao *et al.* (1999) similarly reported a temporal decrease in nonreciprocal translocations in x-ray-irradiated mouse bone marrow cells. This drop was explained as having been possibly caused by a decline in number of cells containing nonreciprocal translocations as part of complex rearrangements. Complexes consisting of unstable aberrations are unable to persist. In mouse splenocytes, a decline in translocations was already observed at a dose of 2 Gy, the decrease being faster for the nonreciprocal translocations than for reciprocal translocations (Hande and Natarajan 1998).

As a consequence of persistence of translocations in cell division, cells with clonal aberrations are possible due to immunologically induced expansion of T cells *in vivo*. Accurate recognition of cells containing clonal aberrations is important, since they will affect the overall aberration frequency and may therefore have an influence on biodosimetry. Clonal translocations determined using chromosome painting have been reported in both exposed individuals and unexposed control persons (Natarajan *et al.* 1991, Tucker *et al.* 1994, Salassidis *et al.* 1995, Johnson *et al.* 1999a,b) and in general the frequency of clonal translocations appears to be rather small. Individuals possessing clones were identified by applying a statistical method for indication of clonality (Johnson *et al.* 1999a). In the same study, cells with clonal aberrations appeared to be correlated with age in controls, whereas no age effect was shown in exposed persons.

2.7.7 Retrospective dosimetry after radiation accidents

Dose assessment of victims in radiation accidents that are recognized within a month or two is most reliably achieved using routine dicentric analysis. If a considerable time has passed since the accident, dose estimation can be achieved using translocation analysis by means of FISH chromosome painting. Translocation studies dealing with both high- and low-dose exposures of persons directly involved in radiation accidents or taking part in cleanup activities are discussed in the following.

In one of the first applications of FISH painting for dose assessment after a considerable time of exposure, the translocation frequencies of 20 Hiroshima atomic bomb survivors were analysed (Lucas *et al.* 1992b). The dose estimates based on the dose response for translocations were in line with the calculated DS86 dose assignments to the bone (RERF, 1987). However, both types of estimates contained large uncertainties. The workers on duty during the Chernobyl nuclear reactor accident have been extensively studied by this technique several years after exposure. Retrospective biodosimetry using translocation frequencies of 12 subjects who were working at the reactor at the time of the accident revealed whole-body equivalent doses between 1.6 and 4.4 Gy (Salassidis *et al.* 1994). Slightly higher dose estimates were derived using the Qdr method applied to data from dicentrics analysis conducted in parallel samples. The difference was suggested to be due to the inclusion of partial-body exposure in the Qdr dose, since this method considers only first-division cells with unstable aberrations. Nevertheless, comparable doses with overlapping 90% confidence intervals was obtained in most subjects (Salassidis *et al.* 1994). Sixty cleanup workers with recorded doses between 0 and 300 mGy were analysed using the FISH technique (Lloyd *et al.* 1996). Large uncertainties were obtained in the individual dose estimates derived using translocation frequencies, but when subjects were divided into groups according to the recorded dose, a reasonable correlation between translocation yields and dose was observed. In another study, 34 men with documented doses and variable working times at or near the reactor were examined by translocation analysis 6-9 years after the last assignment (Snigiryova *et al.* 1997). The mean estimate of dose based on translocations was well in line with the mean documented dose. A higher frequency of translocations was observed in subjects with repeated working periods during 1986 - 1995 than in men working a short period in 1986. However, no correlation between the individual estimates and recorded doses was obtained. FISH chromosome-

painting analysis performed on 126 Russian cleanup workers 5-10 years after the accident showed significantly elevated frequency of translocations compared with a control group (Moore *et al.* 1997). The mean recorded dose was 250 mGy with a range of 20 mGy to 2.7 Gy, whereas the mean estimate of dose based on translocations was much smaller: 90 mGy with a range of 0-510 mGy. However, the calculation was obtained from an acute dose-effect curve for translocations, and therefore an underestimation of dose may have resulted. In contrast to these findings, no increase in translocation yields among 118 Estonian cleanup workers compared with 50 control individuals was observed (Littlefield *et al.* 1998). In addition, no correlation between the stable CAs and physical dose estimates or variables corresponding to high dose exposure was seen. These results implied that the mean bone marrow doses were much less than the recorded doses. The influence of individual background information and the statistical model used on dose estimation using FISH translocations was recently described (Moore and Tucker 1999). If data on age and smoking were not accounted for, doses were found to be highly overestimated in a group of cleanup workers.

The use of translocation analysis was evaluated by repeated examinations in a case of serious overexposure of a lorry driver, who was irradiated by a ^{137}Cs source located in the door pocket of a lorry (Sevan'kaev *et al.* 1999). The exposure was both protracted, encompassing a period of about five months and nonuniform in nature. The exposure time was calculated on the basis of the dose estimate of 7.7 Gy to an extracted tooth studied with the ESR technique and the dose rate at a distance of the tooth from the source. The translocation frequencies from several samples taken over a period of 1.4 years appeared to persist. Dose reconstruction based on the mean translocation yield resulted in a dose estimate of 10 Gy, whereas a dose of 7.9 Gy was derived using the mean dicentric yields and a lymphocyte half-life of 3 years. Thus, a reasonable agreement of dose estimates between the translocation method and the dicentric as well as the physical approaches was achieved. However, the dose derived from translocation yields contained a larger uncertainty, due to the lack of an appropriate dose-response curve and the fact that a pancentromeric probe was not used.

2.7.8 Chronic exposure to low doses

The FISH technique has been applied in a number of investigations to quantify protracted or chronic radiation exposure to low doses and low dose rates among different populations. In many studies, the calculation of dose estimates has been possible only on a group level, giving the collective dose

for the entire study group. Due to the relatively low number of cells analysed per subject, determination of individual dose estimates has not been meaningful.

Fifty persons living in the area contaminated by radionuclides from the Chernobyl fallout were examined seven years after the reactor accident using translocation analysis. They were compared with a similar group of subjects from a noncontaminated control area (Salomaa *et al.* 1997). The frequency of translocations was low in both groups, but a nonsignificant increase in the mean translocation yields was observed among the residents of the highly contaminated area in comparison to the control cohort. A tentative mean dose of less than 100 mGy was estimated from the excess translocation yield in the exposure group.

During the production of nuclear weapon plutonium at the Mayak facilities in the province of Chelyabinsk, a large number of workers were exposed to protracted external γ-radiation and internal ^{239}Pu. To investigate the validity of the FISH technique in retrospective dosimetry in cases where the main part of exposure had occurred 35-40 previously, chromosome painting was applied to determine translocation frequencies in 75 Mayak workers (Salassidis *et al.* 1998). Doses of up to 9.9 Sv had been recorded with individual film dosimetry. A significantly elevated frequency of translocations was observed among the workers compared with controls from an uncontaminated area. The plutonium intake was not found to affect the yield. The estimated doses, however, were much lower than the recorded film doses. In addition, workers with similar documented doses showed notable differences in the translocation frequencies. The authors concluded that almost 40 years after low-dose-rate exposure, replacement of the natural decay of peripheral T cells by division of progenitors or precursors containing translocations is not sufficient to maintain a stable yield of translocations in circulating lymphocytes. Thus, the hypothesis of the long-term persistence of translocations is not supported by these findings.

During the release of radioactive waste from the Mayak facilities into the Techa River from 1949 to 1956, the population living on the riverbanks received both internal and external exposure to mainly ^{90}Sr and ^{137}Cs, respectively. FISH analysis conducted for retrospective biodosimetry purposes revealed that the mean translocation frequency was increased in a study group of 73 subjects residing 7-148 km downstream from the release

site (Bauchinger *et al.* 1998). A group mean dose estimate of 0.24 Gy was calculated using the linear term only, based on the assumption that mainly low-dose-rate exposure had occurred. Division into subgroups on various criteria resulted in differences in dose estimates. For example, persons living at 7-60 km distance from the release site during the period 1950-1951 exhibited a mean dose of 0.34 Gy, whereas later entering residents showed no increase of translocations compared with controls. It was also shown that within the former subgroup, the group mean dose of 0.34 Gy was composed mainly of exposure received by children and teenagers. Individual dose estimates were determined only from a few cases with an adequate number of translocations. A close correlation was observed between these and dose estimates based on whole-body count data of ^{90}Sr and tooth enamel ESR studies.

Occupational exposure to radiation comprises mainly chronic low-dose radiation of low LET. For FISH biodosimetric purposes, the occupationally exposed group can serve as a good standard of comparison due to the careful monitoring of their doses by individual dosimeters. In a study comprising 55 workers at the Sellafield nuclear facility, a significant correlation between translocation frequency and documented cumulative dose became evident (Tucker *et al.* 1997a). The exposures were obtained during an averaged working period of over 30 years and ranged from 173 to 1108 mSv, the vast majority being over 500 mSv. This finding clearly indicates that chronic exposure can be detected by accumulation of translocations. However, substantial variation among individuals was observed. The authors stated that in this type of investigation, large uncertainties in individual dose estimates are to be expected, and thus meaningful biodosimetry can only be achieved in the specific population under study. The same observation was also performed by Bauchinger *et al.* (1997), who obtained an estimated mean group dose comparable to the documented doses for a group of seven radiation workers with long-term exposures. In the same study subjects, the α-particle exposure from internally deposited ^{241}Am at effective doses of less than 60 mSv was found to be too small to induce any detectable increase in aberration yields.

Chronic exposure to naturally occurring radiation has been monitored using chromosome-painting analysis. One such source is radon, which is a major contributor to radiation exposure throughout the world. In a study of 25 residents of 9 houses with radon concentrations between 210 and 3000 Bq/m^3, a nonsignificant increase in translocation frequencies was observed (Bauchinger *et al.* 1996). It was concluded that even for subjects residing in

dwellings with the highest radon concentrations, the effective dose to the bone marrow was too low to show an effect on the translocation yields in lymphocytes. Furthermore, the majority of CAs induced by α-particles may be lethal and are thus not transmitted to circulating T cells.

2.8 Other methods of biodosimetry using blood cells

2.8.1 Blood cell count analysis

The haemopoietic system is one of the most radiosensitive tissues in the body. Therefore, radiation-induced depletion of haematopoietic cells in the bone marrow and peripheral blood can be easily observed and this character used in dose approximation. Doses of 0.5 - 1 Gy obtained as single exposures induce observable changes in blood cell frequencies. The decrease rate for different cell types in the blood is dependent on their particular cell cycle kinetics. Neutrophils and platelets have a relatively short lifespan and thus have a tendency to be depleted over days after irradiation of the haemopoietic system. A follow-up of neutrophil values for several days postirradiation was found to be well correlated with dose in victims of the Chernobyl accident (Guskova *et al.* 1988). However, in many cases of overexposure an early approximation of dose is required for efficient medical intervention. This can be achieved by counting the decrease in frequency of small lymphocytes, as these are very radiosensitive. Nadir values for lymphocytes are attained much earlier than for other cell types, and the decrease may be recognized within hours of exposure, rendering lymphocyte counts as the most sensitive of haematological dosimeters (UNSCEAR 1988). This approach was originally developed to give a rough categorization of the magnitude of exposure (Andrews *et al.* 1965). More recently, a mathematical model for lymphocyte depletion, intended only for first approximation of dose, was developed based on accidental cases with recorded haematological data and physically reconstructed doses (Goans *et al.* 1997). During the first 8 h after exposure, the decrease in lymphocytes followed a single-term exponential curve and the rate constant for this decrease correlated well with dose estimates obtained from other sources of dosimetry.

2.8.2 Premature chromosome condensation assay

In the PCC assay, the normal procedure is to fuse interphase lymphocytes with mitotic Chinese hamster ovary cells using polyethylene glycol (PEG), which results in mitotic factors inducing the nucleus to condense into chromosomes. In its original form, the method was based on the counting of excess chromosome fragments from Giemsa-stained preparations. Later, the detection of dicentrics was enabled by using C banding (Pantelias *et al.* 1993). The technique was proposed for biological dosimetry purposes more than 15 years ago (Pantelias and Maillie 1984), but was not considered reliable due to a number of technical drawbacks (Cornforth and Bedford 1993). First, the sensitivity of the PCC assay decreases rapidly over time due to rejoining of radiation-induced breaks. Second, the preparation and analysis of PCC cells are more demanding than in chromosome analysis. Although the analysis of prematurely condensed chromosomes cannot replace the conventional dicentrics assay in biological dosimetry, it may have some important potential applications. The PCC assay has the advantage of avoiding selective analysis of cells, which occurs in the conventional dicentrics assay. In partial-body exposures, the aberrant metaphases found may not represent the true fraction of damaged cells, due to prolonged mitotic delay. In high-dose exposures, lymphopaenia reduces the number of cells available for chromosome analysis and the cells that are available have a low mitotic index. High-LET radiation poses still another problematic situation for CA analysis by causing severe cell cycle delay. In PCC, these difficulties are overcome by scoring radiation-induced damage in resting cells.

The applicability of the PCC assay in biological dosimetry has been shown in studies performed *in vitro*. The technique was suggested to be useful in high-dose partial-body exposure to low-LET radiation (Blakely *et al.* 1995) and in high-LET exposures (Prasanna *et al.* 1997). Since radiation-induced chromosome breaks are rejoined rapidly, it is important to realize that separate dose-response curves must be established for different postirradiation time points (Chambrette *et al.* 1999). In a recent development of the assay, okadaic acid-induced chromosome condensation of cultured cells allowed easy scoring of ring chromosomes on Giemsa-stained slides (Kanda *et al.* 1999). After *in vitro* irradiation, PCC rings were found to increase up to a dose of 20 Gy, enabling biological dosimetry for very high exposures. Calyculin A is another chemical inducer of PCC that produces a high fraction of chromosome condensation (Durante *et al.* 1998). However, like okadaic acid it acts only on stimulated, proliferating cells, probably due to the higher level of maturation-promoting factor in these cells in comparison to G0. In an *in vivo* study of rhesus monkeys irradiated

with both total and partial-body exposure of 5 Gy, analysis of excess fragments in PCC preparations was able to detect a 6% shielding of exposure to the bone marrow, in contrast to both dicentric and micronuclei assays performed in parallel (Darroudi *et al.* 1998b).

The combination of FISH chromosome painting and PCC assay has recently been introduced, allowing reliable recognition of chromosome exchanges. Distinct identification of translocations and dicentric chromosomes was found to improve the sensitivity of the PCC assay used within biological dosimetry by extending the postirradiation time for the assay to be used (Durante *et al.* 1996a). It was shown that FISH painting of prematurely condensed chromosomes is more efficient in detecting high-LET-induced chromosome damage in comparison to metaphase analysis (Durante *et al.* 1997).

2.8.3 Micronucleus assay

A micronucleus appears in an interphase cell as a small mass of chromatin outside the main nucleus. It originates from whole chromosomes or chromosome fragments that lag behind during anaphase and are not included in the main nucleus during telophase. Therefore, expression of micronuclei requires the cells to divide. The yield of micronuclei is dependent on the cell division kinetics of the cells under study. To control the expression of micronuclei, cells are allowed to divide only once after radiation-induced damage. This criterion can be achieved using the cytokinesis-block method. A chemical called cytochalasin-B (Cyt-B) inhibits cytokinesis without interfering with division of the nucleus, thus producing a binucleated cell (Fenech and Morley 1985). The cytokinesis-block technique was proved to be superior in sensitivity of micronuclei scoring, compared with the conventional micronucleus method, in which it is impossible to detect whether a cell has completed division or not (Fenech 1991, 1997).

For biological dosimetry purposes, the cytokinesis-block micronucleus method is generally applied on peripheral blood lymphocytes. A large number of cells can be scored quickly and easily. For low-LET radiation, micronuclei display a linear-quadratic relationship with dose (Kormos and Köteles 1988, Prosser *et al.* 1988, Littlefield *et al.* 1989, Thierens *et al.* 1991). As found in CAs, micronuclei are induced more efficiently with high-LET radiation, showing a linear increase (Vral *et al.* 1994, Mill *et al.* 1996). There is no clear consensus concerning the distribution of micronuclei

between cells after radiation exposure. Some indications that micronuclei are overdispersed have been reported (Prosser *et al.* 1988), whereas yields following Poisson distribution have been observed by others (Sreedevi and Rao 1994, Verhaegen and Vral 1994, Paillole and Voisin 1998).

Although the micronucleus method is potentially able to measure exposure to ionizing radiation, it appears to possess some limiting characteristics that prevent it from becoming a widely used biodosimeter. The micronuclei frequency in nonirradiated cells has been found to show wide variability between control subjects, a fact that has direct influence on the sensitivity of the method. In a study of 47 individuals, the background yield varied between 0 and 3 micronuclei in 100 binucleated cells. This indicates that doses above 0.32 Gy can be reliably detected with this method (Paillole and Voisin 1998). Micronuclei are correlated with age, but it appears that interindividual differences also increase at higher age (Peace and Succop 1999) and that this increase has been found to be more pronounced in women (Ganguly *et al.* 1993, Fenech *et al.* 1994). However, it was recently shown that the capability of the lymphocyte micronucleus assay for detecting low doses can be increased by studying the origin of the micronucleus. (Vral *et al.* 1997, Thierens *et al.* 1999). This was achieved by performing *in situ* hybridization with a pancentromeric probe, a method applied for lymphocytes for the first time by Norppa *et al.* (1993). The majority of the spontaneous micronuclei contained a centromere, indicating that they originated from chromosome loss. Detection of chromosome-specific centromeres has indicated that the age- and gender-dependent increase of micronuclei is derived mainly from sex chromosomes (Richard *et al.* 1994, Catalán *et al.* 1995, Catalán *et al.* 1998). The radiation-induced micronuclei, on the other hand, mainly arose from fragments as revealed by their centromere-negative appearance (Vral *et al.* 1997). The low number of centromere-negative micronuclei in control samples and their predominance after radiation exposure increases the sensitivity in comparison to the classical micronuclei technique.

2.8.4 Glycophorin A assay

Detection and quantification of somatic cell mutations is another feasible approach for biological dosimetry after exposure to ionizing radiation. The most widely used mutation assay in radiation studies is the glycophorin A (GPA) method. GPA is a glycoprotein present on the surface of the red blood cell. It determines the antigenic composition of the MN blood group. The two allelic forms of glycophorin A, GPA^{M} and GPA^{N}, differ in only two amino

acid residues. About 50% of the human population are heterozygous with respect to the MN blood group. Alterations at the GPA locus in the erythrocyte progenitor cell produces variant red cells that have lost the expression of one of the alleles. This allele loss can be detected using fluorescent-labelled antibodies against the M and N forms of GPA in individuals heterozygous for the MN blood type. The method can thus not be used for M or N homozygous individuals. The GPA assay determines the frequency of erythrocytes lacking the M or N allelic forms, thus measuring the hemizygous (M/0 or N/0) and the homozygous (M/M or N/N) phenotype cells with the help of a flow cytometer. In control populations, the frequency of each variant phenotype is about 10^{-5}, although considerable variability occurs between individuals (Grant and Bigbee 1993). The frequency of GPA variants has been investigated in several studies comprising individuals exposed to ionizing radiation. Significant dose-dependent increase in mutation frequency was observed in both atomic bomb survivors, Chernobyl cleanup workers and victims of the Goiania accident exposed to high doses (Kyoizumi *et al.* 1989, Straume *et al.* 1991, Langlois *et al.* 1993, Jensen *et al.* 1995), also indicating that the assay is able to detect persistent damage. However, no prominent dose effect was evident for cleanup workers with low doses (Bigbee *et al.* 1997, Moore *et al.* 1997).

Other somatic mutation assays include mutation analysis of gene loci encoding for hypoxanthine phosphoribosyl transferase (HPRT), TCR and human leukocyte antigen A (HLA). They show potential in measuring recent exposures, but because the mutant frequency in all these genes decreases rapidly with time, they are unsuitable for retrospective dosimetry.

A broad overview of the biodosimetry methods described in the text is given in Table 1.

Table 1. *Rough characterisation of methods in biological dosimetry for low-LET radiation.*

Method	Cells studied	Optimal test period after exposure	Exposure pattern reliably detected	Applicable dose range[a], approximately
Dicentrics	Lymphocytes	Days - weeks	Acute - whole / partial body	0.1 - ≥ 5 Gy
Translocations	Lymphocytes	Retrospective	Acute / chronic - whole body	~0.3-0.5[b] - ≥ 5 Gy
PCC	Lymphocytes	Hours - days	Acute - whole / partial body	~0.1 - >10 Gy
Micronuclei	Lymphocytes	Days - weeks	Acute - whole body	~0.3 - ≥ 5 Gy ~0.1[c] - ≥ 5 Gy
GPA	Erythrocytes	Retrospective	Acute / chronic - whole body	~ 0.1 - ≥ 5 Gy
Blood cell count	Lymphocytes, Neutrophils, Platelets	Days - weeks	Acute - whole body	~0.5 - ≥ 10 Gy

a, whole body exposure; b, highly dependent on age of subject and number of cells analysed; c, with centromere detection

3 AIMS OF THE PRESENT STUDY

The general purpose of the present work was to evaluate the applicability of FISH chromosome painting for retrospective biodosimetry.

The specific aims of the study were:

1. To establish a dose-effect curve for stable CAs by means of FISH painting and to compare the response to a standard curve for unstable aberrations;

2. To test the FISH technique *in vivo* by applying it in cases of high accidental exposure;

3. To follow the time-dependent changes in CA frequencies in the accident victims for assessment of the postulated persistence of translocations;

4. To study the ability of the FISH technique to discern high-level chronic exposure to residential radon;

5. To investigate chronic exposure to low-LET radiation using FISH painting.

4 MATERIALS AND METHODS

4.1 Subjects

In the dose-response study, whole blood from two healthy donors was examined (I).

In the dose-assessment study of the accident victims in Kiisa, Estonia, 18 subjects with known or suspected overexposures were analysed for the presence of CAs both with FISH chromosome painting and solid Giemsa staining (II). The detailed exposure patterns and clinical symptoms of the subjects are described in II. A full description of the accident is given elsewhere (IAEA 1998).

Five of the Kiisa accident victims with the highest doses were followed up for the presence of time-dependent changes in CA frequencies (III).

In work investigating the correlation between CAs and radon exposure (IV), the study cohort was comprised 84 individuals divided into three matched groups with low, medium and high exposure. The procedure of selecting the cohort from the existing radon database and matching of the exposure groups is described in detail in IV.

To study the relationship between chronic exposure to low-LET radiation and translocations, 20 nuclear power plant workers, and 20 matched controls selected among STUK employees, were studied (V). The worker group consisted of 10 permanent employees from the Loviisa and Olkiluoto nuclear power plants, respectively, selected on the basis of their relatively high recorded cumulative doses.

4.2 Samples and cell culture

In all cases, blood was collected into Li-heparin tubes and processed within 24 h of blood sampling. During transportation of blood samples (II-V), care was taken to avoid extreme temperatures. In the dose-response study (I), irradiation of blood was performed at the Radiation Metrology Laboratory,

STUK, with ^{60}Co γ-rays at a dose rate of 0.24 Gy / min. The absorbed dose in blood was calculated from dose in air kerma by multiplying with a conversion factor of 1.11. The irradiated and control blood was stored at 37°C before setup of culture. In four of the studies, CA analysis using both FISH painting and standard Giemsa staining was conducted on preparations from whole blood cultures performed as described (I-IV). In one of the studies, FISH painting was performed on slides prepared from isolated lymphocyte cultures (V). The presence of second-division metaphases was investigated selectively by adding BrdU at the start of the culture (IAEA 1986).

4.3 Giemsa staining and FISH chromosome painting

For all purposes, metaphase preparations were made by placing 25-30 μl of cell suspension on a moistened slide. For standard CA analysis, air-dried preparations were stained with 4% Giemsa solution in Sørensen buffer, pH 6.8. For determination of second-cell cycle metaphases, fluorescence plus Giemsa (FPG) staining was performed (IAEA 1986).

In situ hybridization was performed using a biotin-labelled whole composite probe cocktail for chromosomes 1, 2 and 4 according to the manufacturer's protocol (Cambio, Cambridge, UK, I-V). Some modifications were made as described in the publications and are explained here only in brief. A 60-min pre-treatment with RNase (100 μg/ml) was added to improve the painting result. Hybridization of the digoxigenin-labelled centromere probe was conducted after washing off the chromosome probes, except that hybridization and analysis of centromeres were performed after scoring the slides for chromosome painting (II). Simultaneous detection and amplification of chromosome and pancentromeric probes were performed using primary antibodies against biotin and digoxigenin followed by secondary antibodies labelled with fluorescent dyes. The result was a yellow FITC label on chromosomes 1, 2 and 4 and a blue label on centromere regions (AMCA), both readily distinguishable from the red counterstain (propidium iodide). Centromere detection was achieved by a degenerate α-satellite probe produced using the polymerase chain reaction (I,II), whereas in III-V, a commercially available pancentromere probe was used.

4.4 Scoring of aberrations

For standard dicentric analysis (I, II and V), metaphases from Giemsa-stained slides were sought by a fully automated metaphase finder, and a karyotyping system was utilized for aberration analysis (Metafer and Ikaros, MetaSystems GmbH). CAs in metaphases with 46 centromeres were included in the analysis. Only dicentrics with an accompanying acentric fragment were considered to be true dicentric chromosomes.

The presence of second-cell division metaphases, i.e. displaying the harlequin effect, was determined from FPG-stained slides from cultures at all dose points and both subjects in the dose-response study (I). The percentage of such cells was less than 5% in all cases. Less than 10% of second-division cells were observed in selected samples from the other studies.

For all FISH studies, the following procedure was applied to scoring. Metaphases were located using an automated fluorescence metaphase finder (Metafer, MetaSystems GmbH, Germany). Only appropriately painted, apparently complete metaphases were scored if all parts of the 6 painted chromosomes appeared somewhere in the cell. Three colour (red, green and blue) images of aberrant metaphases were captured, digitized and stored using the ISIS system (MetaSystems). An example of an aberrant cell is shown in Figure 1.

Scoring of aberrations on FISH-painted slides was performed according to the following criteria (I-III). A bicoloured chromosome with one centromere was classified as a translocation. If both reciprocal counterparts of the translocation were present, the translocation was termed complete (two-way), and incomplete (one-way) if only one bicoloured monocentric was present. Bicoloured dicentrics with an accompanying bicoloured acentric fragment were termed complete. In cases where the accompanied fragments were painted or no bicoloured or painted fragment was visible, the dicentrics were classified as incomplete. Acentrics were scored if they were painted or bicoloured. Painted ring chromosomes, centric or acentric, were also scored. Complex exchanges, ie. originating from ≥ 3 breaks in ≥ 2 chromosomes (Savage and Simpson 1994a) were classified as single aberrations that were included in the aberration categories. For example, aberrations in the complex classified as 2G in the S&S nomenclature, i.e. a bicoloured dicentric accompanied by one bicoloured monocentric

(dic(AB)+t(Ab)), were added to the categories of incomplete dicentrics and incomplete translocations.

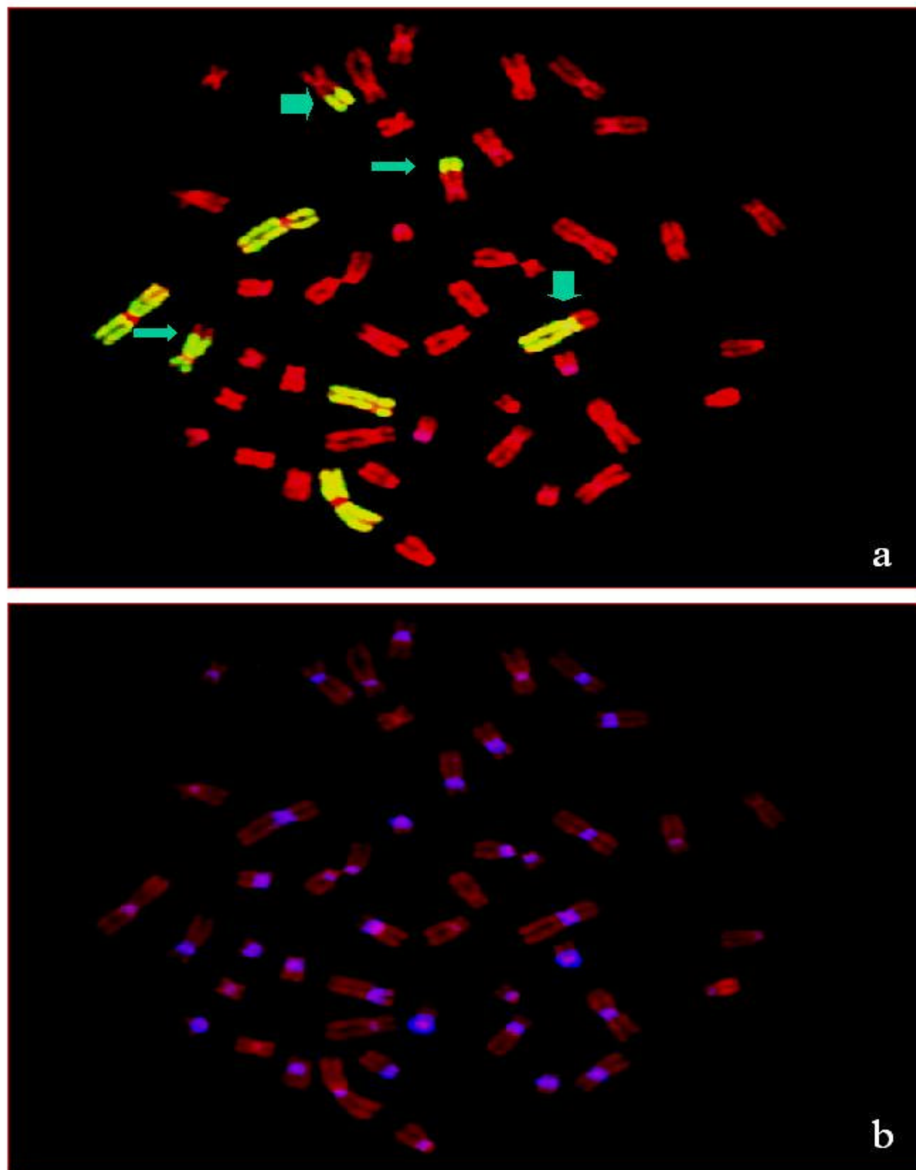

Figure 1. *An aberrant metaphase observed after FISH painting of cells obtained several years after accidental exposure to radiation. a) Green fluorescent stain of chromosomes 1, 2 and 4 and red counterstaining of all chromosomes highlights a two-way trans-location involving chromosome 2 and a red, unidentified chromosome (thick arrows) and another two-way translocation between chromosome 4 and a red chromosome (thin arrows).*

b) The same metaphase as in a) with centromeres appearing as light blue dots.

In IV and V, aberration scoring of FISH slides was based on the PAINT system of nomenclature (Tucker *et al.* 1995) and a modification thereof (Knehr *et al.* 1998, Littlefield *et al.* 1998). Bicoloured dicentrics observed in cells containing bicoloured acentrics were termed dic(BA) + ace(ba) and apparently reciprocal bicoloured translocations, i.e. two-way translocations, were called t(Ab) + t(Ba). Apparently nonreciprocal symmetrical interchanges, i.e. one-way translocations, occurring singly in cells without their reciprocal counterparts were referred to as t(Ab) or t(Ba) and the corresponding asymmetrical interchanges as dic(AB) or ace(ab). Painted acentric fragments were termed ace(b) and centric ring chromosomes r(B). Complex exchanges were handled similarly to that described above. For simplicity, the terms two-way and one-way, as defined above, will be used from hereon for describing translocations. A schematic illustration of CAs found in painting analysis is shown in Figure 2.

The genomic frequencies (F_G) of translocations and dicentrics were calculated according to Lucas *et al.* (1992b), using the equation $F_G = F_P / 2.05 f_p (1 - f_p)$, where F_P is the translocation or dicentric frequency detected by painting and f_p is the fraction of the genome painted by FISH (I-V). For chromosomes 1,2 and 4, f_p is approximately 0.22 (Morton 1991), and thus F_P / F_G is approximately 0.35.

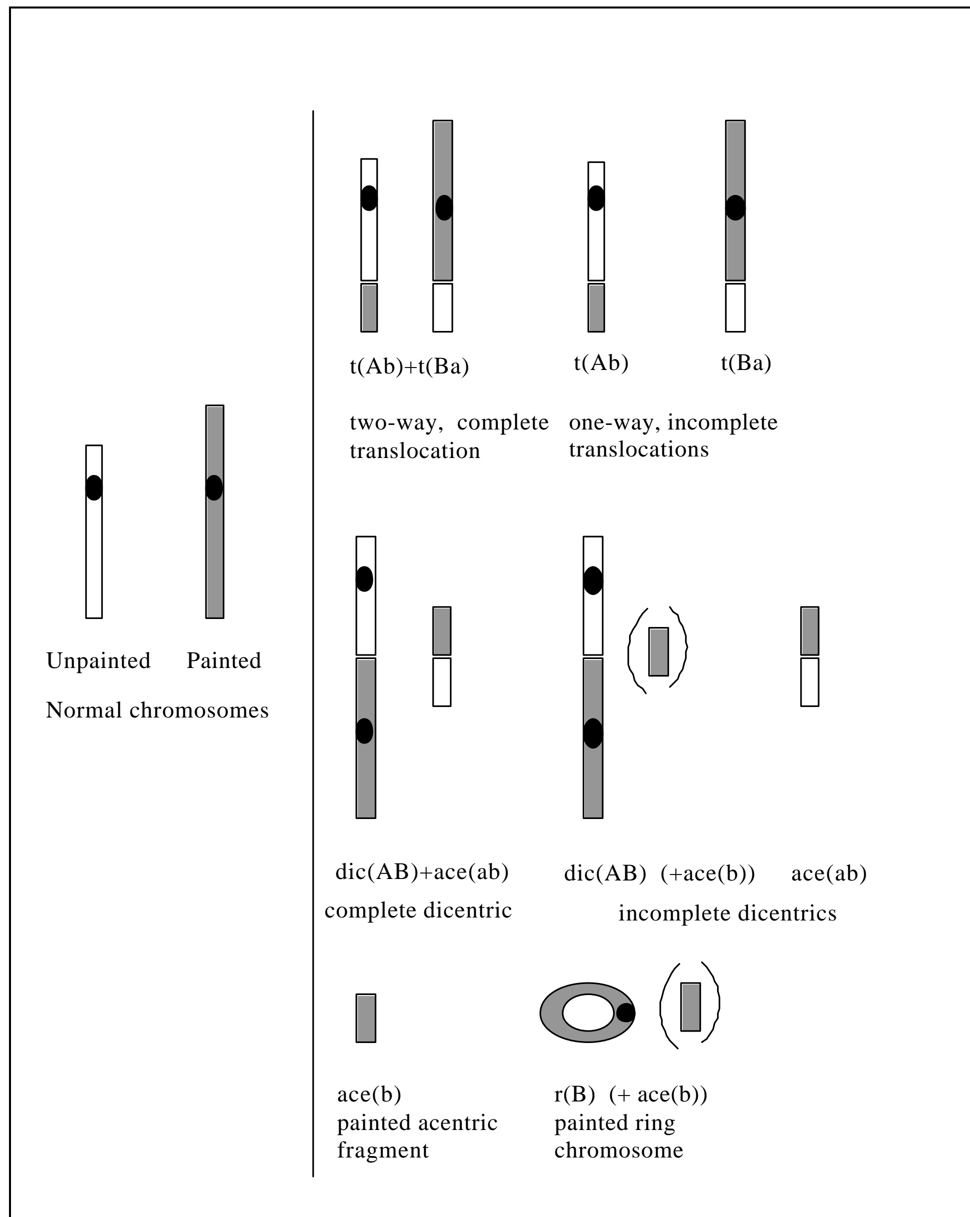

***Figure 2**. Comparison of nomenclature for CAs observed in FISH painting analysis*

4.5 Statistical analyses and dose estimation

For the dose-response study (I), curve fitting was based on the method of iteratively reweighted least squares using Poisson-based weights (Papworth 1975). The curve coefficients and their errors for painted dicentrics and translocations were converted into genomic values using the Lucas formula.

Standard dose estimations of the victims in the Kiisa accident were based on the yield of dicentric chromosomes (II). The new dose-response data were not available at the time of the dose assessment, so that a previously established linear-quadratic dose-effect curve for ^{60}Co γ-rays using a dose rate of 0.25 Gy/min was applied. The curve coefficients were $\alpha = (3.26 \pm 0.82) \times 10^{-2}$ Gy^{-1}, $\beta = (4.92 \pm 0.32) \times 10^{-2}$ Gy^{-2}, and a background frequency of 1 dicentric per 1000 cells was used. The course of the accident revealed that several subjects had been exposed to radiation over a minimum of 7 h, and in many cases the dose was delivered over several days or weeks. In cases like these, dose protraction should be accounted for by applying a time-dependent factor known as the G-function that enables modification of the dose-squared coefficient (Lea and Catcheside 1942, Schmid *et al.* 1976, Bauchinger *et al.* 1979, IAEA 1986). The G-function influences the quadratic component of the equation: as the time of irradiation increases, the quadratic term approaches zero and the curve approaches a linear response. The G-function was used to assess two cases in which the doses were protracted for 7 h. The linear function was used in cases where the dose was protracted over several weeks. The equations for the G-function are given in II. The 95% CIs were determined from standard errors of both the yields of dicentrics and the calibration curve.

To clarify whether exposure to the subjects had been nonuniform, the Poisson distribution of the dicentrics was evaluated by calculating the dispersion index σ^2/Y and its test quantity U, which in case of overdispersion show values of >1 and >1.96, respectively, as described by Edwards *et al.* (1979; II). This approach gave evidence for partial-body exposure in some of the subjects. Therefore, the Qdr method was applied, based only on the presence of damaged cells, whereas undamaged cells can be ignored (Sasaki and Miyata 1968). It is thus not influenced by normal cells from the unirradiated part of the body or by those produced after irradiation. Qdr is the frequency of dicentrics and ring chromosomes among unstable cells. The equations applied for the Qdr are given in II. The 95%

CIs were determined from the Qdr value ± 1.96 x S.E. by comparing with the Qdr curve.

Dose estimates based on the frequencies of translocations in the FISH-painted chromosomes were obtained through comparison with *in vitro* calibration curves on the yield of two-way translocations after ^{60}Co gamma irradiation (Lucas *et al.* 1995) and the yield of total (two- and one-way) translocations after ^{137}Cs gamma irradiation (Bauchinger *et al.* 1993; II).

In the follow-up study of the Kiisa accident subjects (III), acute-dose estimates at eight postirradiation sampling times were calculated from translocation data using the dose-response curve established in I. The variations in translocation frequencies between samples were tested, calculating a heterogeneity χ^2 value. The decline in dicentrics was evaluated using a model in which the decrease complied with a two component exponential function, as given in III, where the components characterize different subpopulations of lymphocytes possessing distinct lifespans.

A Poisson regression model (GENMODE procedure of SAS® software, SAS Institute, Inc. 1993) was performed to test the relationship between explanatory variables and aberration yields (IV, V). The correlation between age and translocation frequency was studied by fitting the data into several models where the yield of translocations was a function of age as described by an intercept and a linear-, second-, third- or fourth-power term (IV). A linear regression analysis was performed to study the association of cumulative dose and translocation (V). The Wilcoxon rank test was performed to clarify differences in aberration yields between groups (V).

5 RESULTS AND DISCUSSION

5.1 Dose response of stable and unstable chromosomal aberrations detected by FISH painting in comparison to standard dicentrics

FISH chromosome painting of *in vitro* ^{60}Co γ-irradiated human blood lymphocytes revealed that both translocations and dicentrics were induced at the same frequency but at different doses, resulting in similar dose-effect curves. This finding is in accordance with other reports (Straume and Lucas 1993, Fernandéz *et al.* 1995, Finnon *et al.* 1995, Lucas *et al.* 1995). The two donors displayed large differences in translocation frequencies at the control level, reflecting the age-dependent increase in translocation yields but also the interindividual variability in accumulating stable CAs. Donor-specific induction of translocations at low doses was observed. Considering total (two-way and one-way) translocations, the almost nonexistent increase in one-way translocations resulted in lower linear term than for two-way exchanges. The inclusion of complex-derived one-way translocations in the total translocation data increased their yield at higher doses. Consequently, in comparison with two-way translocations, a larger quadratic term was obtained for totals. The dose response of dicentrics scored from standard Giemsa-stained preparations fitted better with the curve for total than two-way exchanges.

In the fitted curves for translocations, particularly total translocations, large standard errors for the α-coefficient were obtained. This implies that the number of cells scored at low doses was not sufficient for a precise determination of the linear term of total translocations. The large uncertainties of the α-coefficients were to a certain degree also caused by the relatively high frequency of translocations at the control level. Using the linear component of total translocation would in this case result in less reliable dose estimates of past exposures than that obtained by using the α-term for two-way translocations. Therefore, two-way translocations are preferred in retrospective dose reconstruction with the FISH technique. To illustrate the established curve, the calculated dose estimates for both acute and protracted exposure for a given number of translocations observed are shown in Table 2. Increasing the number of cells scored from 1500 cells

(Table 2a) to 3000 (Table 2b) had only a slight effect on the sensitivity; the dose with a confidence interval that does not include zero, decreased from about 0.5 to 0.4 Gy. Moreover, reliable detection of acute dose was achieved at lower dose levels than for protracted exposure. In fact, nearly 10 000 cells (more than 3000 cell equivalents) should be scored to be able to determine a protracted dose of about 1 Gy with sufficient confidence.

Table 2. *Dose estimates and 95% confidence intervals (CI) calculated for different numbers of two-way translocations observed. The linear-quadratic dose-response curve for acute exposure (a) and linear dose response for protracted exposure (b) established in I were used. The control frequency of the two donors was used as the baseline*

Table 2a

1500 cells (525 cell equivalents) analysed		Acute dose (Gy)	3000 cells (1050 cell equivalents) analysed	
No of translocations	95% CI (Gy)		95 % CI (Gy)	No of translocations
4	0; 0.33	0.06	0; 0.27	8
6	0; 0.47	0.21	0; 0.42	12
8	0; 0.58	0.33	0; 0.53	16
10	0; 0.68	0.42	0.07; 0.63	20
15	0.17; 0.88	0.61	0.27; 1.06	30
20	0.34; 1.26	0.76	0.42; 1.22	40
30	0.59; 1.52	1.01	0.89; 1.49	60

Table 2b

1500 cells (525 cell equivalents) analysed		Protracted dose (Gy)	3000 cells (1050 cell equivalents) analysed	
No of translocations	95% CI (Gy)		95 % CI (Gy)	No of translocations
4	0; 0.57	0.06	0; 0.43	8
6	0; 0.94	0.32	0; 0.77	12
8	0; 1.45	0.57	0; 1.3	16
10	0; 1.91	0.82	0; 1.76	20
15	0; 3.1	1.45	0; 2.91	30
20	0; 4.23	2.08	0.09; 4.09	40
30	0.11; 6.58	3.35	0.25; 6.44	60

5.2 FISH chromosome painting and standard scoring of dicentrics after accidental exposure to ionizing radiation

Standard dicentric analysis of 18 subjects involved in the Kiisa accident revealed five subjects with dicentric yields significantly higher than control values. In these five individuals, dose estimations from 0.3 to 1.1 Gy were obtained from calculations based on the acute dose-response curve. Using dose protraction, either with G-function modification of the β-term or the linear term only, dose estimates increased to values between 0.5 and 2.7 Gy. It should be pointed out here that the applied dose-response curve for dicentrics was based on an earlier dataset where the α-coefficient was insufficiently determined. Using the linear term from the recently established dose-effect curve (I), the protracted dose estimates for the accident victims are up to 2.5 times higher than the ones calculated with the earlier α-coefficient. However, the protracted estimates have large errors and in all cases, partial overlap of the 95% CIs with both values of α are obtained. This example emphasises the importance of adequately determined linear coefficients in estimating cases of dose protraction. It also addresses the weakness in extrapolating protracted doses from coefficients defining acute dose response.

Non-Poisson distribution of dicentrics among cells, denoting nonuniform exposure, was clearly shown in two of the cases and indicative in one. The medical information on these subjects, such as skin burns, supported the findings of partial-body exposure. Dose estimates to the irradiated part of the body, as calculated with the Qdr approach, were higher than the acute or protracted whole-body estimates in two of the nonuniform cases, indicating that a great deal of the exposure had been received during a brief proximity to the radiation source. In one case, the partial-body estimate did not exceed the value of the protracted whole-body dose, suggesting that apart from the nonuniform exposure, a considerable part of the exposure came from protracted irradiation distributed uniformly in the body.

The feasibility of obtaining blood samples from victims soon after the accident was recognized, allowing determination of the frequency of stable CAs directly after irradiation *in vivo*, and thus providing an ideal reference point for future follow-up of aberration frequencies. In the FISH chromosome-painting analysis, translocations were found at an approximately equal frequency as dicentrics in almost all cases, thus

showing good agreement with *in vitro* studies (Straume and Lucas 1993, Fernandéz *et al.* 1995, Finnon *et al.* 1995). The equality was not confirmed in one elderly person, who showed a whole-genome translocation yield of about 5 per 100 cells in comparison to about 6 dicentrics in 1000 cells. In all subjects who had received a considerable dose, one-way (incomplete) translocations were observed at a much higher frequency than incomplete dicentrics. Painting analysis also revealed that dicentrics were obtained at comparable frequencies in both painting and standard analysis. The feature of the chromosome-painting technique that enabled distinguishing of persistent aberrations also enabled the distinguishing of a distinct translocation of clonal origin involving chromosome 1 in one of the accident victims. For all cases with exposures to moderate or high doses, dose estimates based on the translocation yields were in agreement with those obtained from standard dicentric analysis.

5.3 Follow-up of chromosomal aberrations over time

A follow-up of five highly exposed victims comprising eight samples taken within two years of the Kiisa accident was conducted to study the short-term development of CA frequencies. In general, both two-way and one-way translocation frequencies remained at similar levels during the two-year period. Although some variations between frequencies existed, the differences were in general nonsignificant. In one of the subjects, however, the yields were significantly decreased for one-way translocations and showed a notable decline for two-way translocations. The exposure received by this person had contained both protracted whole-body and short partial-body exposure. Based on *in vitro* findings it was suggested that in partial-body exposure the distributions of dicentrics and translocations are linked, whereby the temporal decline of dicentrics will directly decrease the frequency of translocations (Guerrero-Carbajal *et al.* 1998). In all subjects with high exposures, dicentrics decreased very rapidly during the study period. This finding is in line with other reports on the rapid disappearance of unstable aberrations from peripheral blood lymphocytes after exposure (Bauchinger *et al.* 1989, Littlefield *et al.* 1991, Ramalho *et al.* 1995). In one of the subjects, the clonally occurring one-way translocation encountered in the first sample was still observed in the second and third samples, but was absent in subsequent samples. The influence of the clone on the overall translocation frequency was relatively small and would not have a major

effect on dose estimates. However, the clone was considered to be one cell in all calculations.

Retrospective dose reconstruction using two-way translocation data showed that acute dose estimates remained constant during the two-year follow-up, as anticipated from the general persistence of translocations. Even though the frequencies at different sampling times showed large differences in some cases, only rather small deviations in acute dose estimates were obtained. This was because most of the values fell on the quadratic part of the curve. For the protracted dose calculations, however, the linear relationship between dose estimates and aberration frequency would result in much larger differences in estimates at various sampling occasions.

In a separate study investigating the distribution of radiation-induced breakpoints in chromosomes 1, 2 and 4, data from all eight samples of the Kiisa accident victims (II, III) as well as the data in the dose-effect study (I) were included (Luomahaara *et al.* 1999). The number of exchanges involving these chromosomes was in proportion to their DNA content in both the *in vitro* dataset and in the majority of the sampling points and subjects from the *in vivo* data.

5.4 Chronic exposure to radon and chromosomal aberrations detected with FISH painting

The effect of chronic exposure to residential radon on CAs was investigated using FISH chromosome painting (IV). Despite a large number of scored metaphases, no increase in the frequency of translocations was observed with increasing radon level. The same was true for all unstable CAs, complex exchanges and rogue cells. The results imply that the bone marrow and blood dose from radon-derived α-particles at the concentrations studied may have been too low to induce a detectable yield of aberrations. These findings are in agreement with results obtained by Bauchinger *et al.* (1996), who found no significant increase in translocations in relation to elevated residential radon. However, in a previous study unstable aberrations were found to be significantly correlated with the presence of radon (Bauchinger *et al.* 1994).

The translocation frequency among the radon cohort showed a strong association with age. The relationship could be described equally well with

models comprising an intercept and linear, second, third or fourth-power terms. The use of two-way instead of total translocations gave similar results but slightly smaller correlations. In addition, the range of translocation yields became larger with age. At about 50 years of age, the translocation frequency varied from 0.002 to 0.021, implying that large uncertainties in dose reconstruction using translocations should be considered, due to the large interindividual variability in control level. However, a notable increase in the number of scored cells was suggested to decrease the differences in translocation frequencies between individuals of the same age (Lucas et al. 1999a).

5.5 Stable and unstable chromosomal aberrations in nuclear power plant workers

CA analysis of 20 nuclear power plant workers from two Finnish plants using the standard Giemsa method revealed no differences in unstable aberration frequencies in comparison to a group of 20 age-, gender- and smoking-matched controls. No correlation of the unstable aberrations with any confounding factor was displayed. In FISH chromosome-painting analysis of the same individuals, a nonsignificant increase in translocation frequencies among the workers was observed. When the datasets from the two power plants were considered separately, workers at Plant 1 showed a significant increase in translocation yields compared with their controls, whereas at Plant 2, the workers and controls displayed equal frequencies. This can be partially explained by the higher cumulative doses among workers at Plant 1 and partially by the higher translocation frequency among the controls at Plant 2. When the translocation yield was plotted against individual cumulative dose determined from thermoluminescence dosimeters, the slope of two-way translocations was almost three aberrations per 1000 cells for a 100-mSv increase in accumulated dose. The Poisson regression model for the pooled data showed a significant association between cumulative exposure and translocations. In accordance with several other reports, the translocation yield was significantly correlated with age, whereas no clear relationship with smoking was obtained. In addition, high individual variability in translocation frequencies was shown in this study. This demonstrates the fact that the conditions in the present study were not adequate for reasonable dose estimation on an individual level. A collective dose estimate calculated using the linear term from the γ-ray curve resulted in a higher value than the documented mean

dose. However, the workers were not exposed solely to direct γ-rays, but also to scattered radiation with lower energy and thus the calculation based on the γ-ray curve may be overestimated. The results in this study indicate that the chromosome painting assay is able to detect chronic exposure to low-LET radiation on a group level.

6 CONCLUSIONS AND FUTURE PROSPECTS

Translocation analysis using FISH chromosome painting has proven its superior applicability in retrospective biological dosimetry in comparison to standard dicentric analysis. In delayed sampling, translocation analysis allows direct measurement of radiation-induced CAs and circumvents the need for rough approximations of lymphocyte half-life in extrapolation of unstable aberrations. The application of FISH chromosome-painting analysis for retrospective dosimetry has required new considerations concerning the dose-effect relationship of CAs. Translocations found several years after exposure are expected to be of the transmissible types that are not lost during cell division. It has been recommended that the dose-response curve to be applied for purposes of retrospective dose estimation should be established from two-way translocations in stable cells, i.e. karyotypically normal cells and cells containing monocentric abnormalities (Finnon *et al.* 1999). One-way translocations are excluded since a portion of them are incomplete or involved in complex rearrangements and are thus considered unstable. This is supported by data from both animal studies and partially also from follow-up of accident victims in the present study, which indicate that the yield of one-way translocations declines over time. Furthermore, it has become evident that it is exceedingly important to determine the linear part of the dose-response curve with sufficient precision, as the majority of cases to be studied by translocation analysis are likely to have received chronic exposure.

During the more than 10 years of FISH-painting analyses, several investigations have addressed the question of translocation persistence over time. The reports are based on comparisons with initial dicentric data, obtained from *in vitro* experiments or coming from animal studies. Conflicting results concerning persistence have been emerging, and thus the value of the FISH technique as a retrospective dosimeter has not yet been fully established. Several factors may influence the frequency of translocations observed over long periods of postexposure. CAs seen in peripheral lymphocytes several years after induction have been derived from cells that belonged to progenitor or precursor population of T cells at the time of irradiation. Radiation sensitivity may not, however, be similar for these proliferating cells and for the resting T lymphocytes. Moreover,

although translocations do not interfere with cell division mechanisms, undetected deletions may occur during illegitimate repair of dsbs that lead to chromosomal exchanges. These lesions can affect cell survival. An indisputable approach to evaluate the stability of translocations in the peripheral blood of humans can be achieved with a prospective study using repeated sampling of individuals exposed to radiation. The two-year follow-up of the Kiisa accident victims has provided evidence for the general persistence of translocations. However, the follow-up was too short for conclusions of persistence during a longer time interval. Sampling is thus continued to determine the long-term stability of translocations. Furthermore, follow-up of these subjects will give information on how efficiently the translocation yields are able to reflect the initial damage induced by radiation exposure of heterogeneous patterns, i.e. mixtures of whole- and partial-body as well as acute and protracted exposures.

The age-dependent control level of translocation frequency reduces the sensitivity of FISH biodosimetry and thus the applicability of the method. It is obvious that age is the major factor when assessing the lower detection limit for retrospective biodosimetry with the FISH technique. The problem with high control level becomes more pronounced in retrospective dosimetry at the individual level where no previous information on the translocation frequency is available. The situation is complicated further by the observed person-to-person variation in background yields and perhaps more importantly by the individual susceptibility in formation of radiation-induced CAs. The sensitivity of the FISH assay can be improved by increasing the number of cells scored, e.g. by automatic metaphase finding. It is clear, however, that the existing data are not sufficient to provide a definite answer regarding the lowest detectable dose. In the light of present knowledge, it can be stated that the use of translocations as an individual retrospective dosimeter is supported at least in cases of considerable whole-body exposures. In partial-body exposures, however, there are indications that the elimination of correlated unstable aberrations reduces the frequency of translocations over time, which would thus result in an underestimation of dose.

Reliable dose reconstruction is a very important factor in epidemiological studies for deriving risk estimates for radiation-induced cancer. In this respect, FISH chromosome painting is far more efficient than standard dicentric analysis. In many investigations, translocation measurement has already shown its potential in examining old or chronic exposures to low-

LET radiation in cohorts, although some conflicting data do exist. The results available on for chronic exposure to radon in indoor environments do not, however, support the use of translocations in detecting exposure to α-particles. Although the lymphatic precursor cells may be exposed to small amounts of α-radiation, the majority of induced CAs would most probably be unstable, resulting in cell death. Recently, based on *in vitro* experiments, transmissible-type complex exchanges have been suggested to serve as biomarkers for exposure to α-particles (Anderson *et al.* 2000).

The principal application of translocation analysis performed using the FISH technique may not be in demonstrating accidental exposures to high doses, as these are usually recognized without longer delay and are thus reliably monitored with the dicentrics assay. Rather, the technique shows potential in assessing chronic exposure to low-LET radiation, particularly if large cohorts are investigated. Although a large amount of data has been, and is, produced within the field of FISH chromosome painting, extensive studies concerning the persistence and control values of translocations are still required to generate general guidelines for the use of translocations as a retrospective dosimeter.

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