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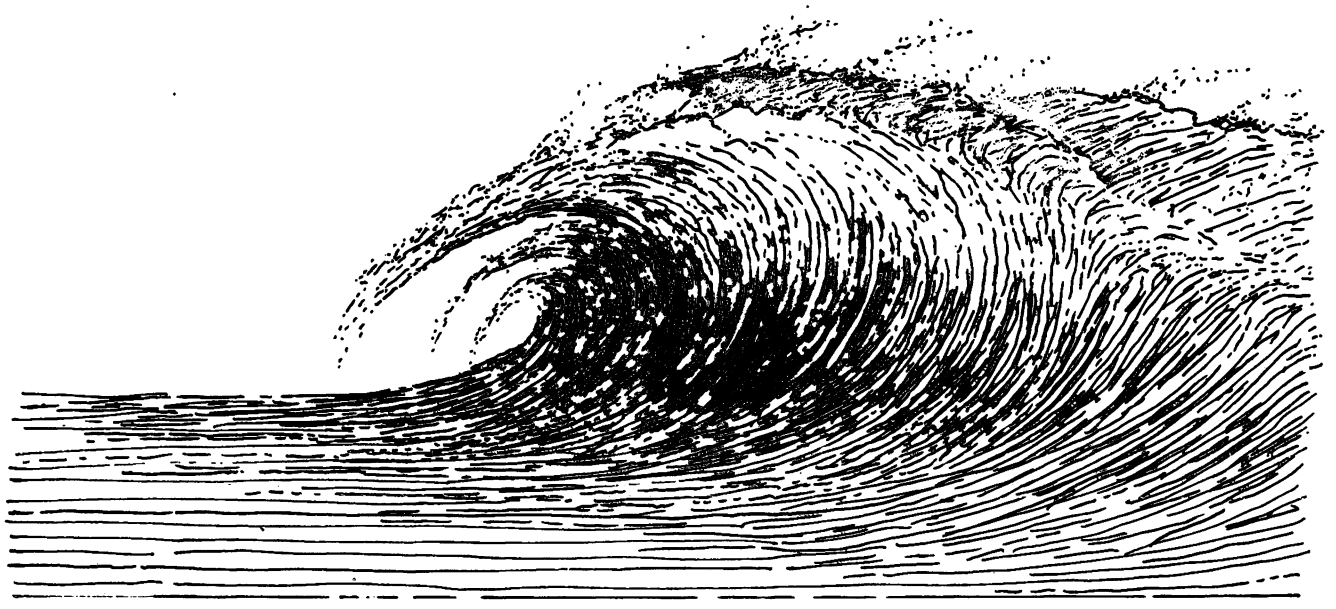
Wasser – natürliche Kraftquelle



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Towards a cumulative biological dosimeter based on chromosome painting and digital image analysis

An approach for a long-term (cumulative) biological dosimeter is described, based on the idea that stem cells with irradiation-induced reciprocal translocations and their progeny would neither lose nor gain genetic material and thus should retain the same proliferative potential as non-irradiated cells. Rapid detection of chromosome translocations has become possible in irradiated human lymphocytes by a newly developed fluorescent in situ hybridization method called "chromosome painting". We have used this approach to score chromosome aberrations, including translocation events, in over 8000 chromosomes painted in lymphocytes from two patients exposed to an X-ray contrast medium containing ^{232}Th and from two age-matched control persons. The percentage of both the total fraction of aberrant painted chromosomes and of translocations was found significantly higher in exposed patients. A program was developed which can automatically determine the number of normal and aberrant painted chromosomes and classify evaluated cells as "normal" or "aberrant" within 1 to 2 seconds.

Zur Entwicklung eines biologischen Langzeitdosimeters mittels chromosomaler In-situ-Suppressions-Hybridisierung und automatischer Bildanalyse. *Es wird ein Ansatz für ein biologisches Langzeitdosimeter zur kumulativen Erfassung von Strahlenschäden vorgestellt. Er beruht auf der Annahme, daß Stammzellen und daraus abgeleitete Zellpopulationen mit strahleninduzierten reziproken Translokationen genetisches Material weder verlieren noch gewinnen und damit in der Regel die gleiche Proliferationskapazität wie unbestrahlte Zellen behalten. Mittels einer neuen Fluoreszenz-in-situ-Hybridisierungsmethode wurden Chromosomenaberrationen in über 8000 spezifisch gefärbten Metaphasechromosomen zweier Thorotrast-Patienten und zweier Kontrollpersonen gleichen Alters quantitativ bestimmt. Der Prozentsatz der aberranten Chromosomen insgesamt sowie der Translokationen war in den Lymphozyten der Thorotrast-Patienten signifikant höher. Es wurde ein Programm entwickelt, mit dem die Zahl normaler und aberranter Chromosomen innerhalb von 1 bis 2 Sekunden automatisch bestimmt werden kann.*

1 Introduction

Biological dosimetry of radiation-induced chromosomal damage in human cells may lead to an estimate of the radiation exposure even in cases where no reliable physical data are available. A variety of approaches may be used to monitor chromosomal damage by light microscopic observations (for a review, see [1]). These include the scoring of dicentric metaphase chromosomes, i.e., chromosomes with two centromeres, in cultured human lymphocytes isolated from blood samples of exposed patients [2], and the evaluation of interphase cells with micronuclei derived from acentric chromosome fragments [3], or of prematurely condensed chromosomes [4]. To monitor large chromosome numbers flow cytometry may also be applied [5-7].

The induction of dicentric chromosomes or acentric chromosome fragments may likely result in genetically imbalanced cells during subsequent cell cycles. Dicentric chromosomes may become involved in breakage-fusion cycles, while acentric fragments cannot be distributed properly during mitosis and may be lost. As a consequence the affected cells may die. For this reason biological dosimeters based on the scoring of dicentrics or micronuclei may be particularly useful to evaluate irradiation damage during the first cell cycles after an acute irradiation event. In contrast, irradiation-induced *reciprocal* translocations do not result in gross genetic imbalances and the affected cells may therefore be expected to retain the same proliferative potential as normal cells. Accordingly, a biological dosimeter based on the scoring of reciprocal translocations should be particularly useful to monitor the effects of a single exposure even many years after such an event has taken place, as well as cumulative effects of multiple or chronic exposures.

The development of such a long-term (cumulative) biological dosimeter has been impractical so far for various reasons. A reliable scoring of translocation events could only be obtained by the analysis of banded chromosomes. Such analyses depend on skilled personnel and are time consuming. While a skilled technician may analyse some 30 homogeneously stained metaphase spreads per hour for dicentric chromosomes, the rate drops to a few cells per hour at best for a thorough analysis of structural aberrations in banded metaphase spreads. Considerable efforts have therefore been made to automate such analyses [8-10]. Automation becomes particularly important in the case of low-dose irradiation, where thousands of cells have to be evaluated to obtain statistically significant results [11]. Automatic preselection of supposedly dicentric chromosomes by digital image analysis has been successfully applied to *homogeneously* stained samples. The minimum evaluation time obtained with a multiprocessor system has been about 10 seconds per metaphase spread [8]. A reliable automated image evaluation of banding patterns of aberrant chromosomes following irradiation has not been possible so far [12].

To overcome these limitations, we have developed a new approach which is particularly useful for the unequivocal

and easy identification of irradiation-induced chromosome translocations in human cells [13]. This approach makes use of recent developments in molecular genetics. Individual human chromosomes were isolated by fluorescence activated sorting [14-16]. DNA fragments isolated from large numbers of a given chromosome type were integrated in appropriate vectors, e.g., lambda-phages, and amplified in bacterial strains to form a chromosome specific DNA library. A technique called chromosomal in situ suppression(CISS)-hybridization [17-19] or "chromosome painting" [20] allows the specific binding (by DNA-DNA hybridization) of chemically modified library DNA fragments (probes) to their respective chromosomal target sites. These binding sites can be visualized in various ways, including indirect immunofluorescence and colorimetric procedures. In the present experiments, probes were labeled with biotin and detected with fluoresceine-conjugated avidin, a protein which binds specifically to biotin [21].

In previous experiments [13] we used CISS-hybridization of chromosome no. 1 and no. 7 to establish dose-response curves for aberrations of these chromosomes in ^{60}Co-gamma-irradiated human lymphocytes. In agreement with other studies [2, 22], a linear increase of aberrations (including translocations, inserts, deletions, fragments and dicentrics) was obtained with the square of the irradiation dose (range 0 to 8 Gy).

In this report we describe the first application of chromosome painting to detect chromosome aberrations induced by ionizing radiation in lymphocytes of patients exposed to the X-ray contrast medium Thorotrast [23]. Thorotrast consists of a 25% stabilized colloidal solution of thorium dioxide and contains the radioactive nuclide ^{232}Th (half-life 1.4×10^{10} years), which decays under emission of alpha-particles (more than 90% of the total radioactivity) and sparsely ionizing beta- and gamma-rays which may be neglected [24]. Thorium dioxide proved to be an excellent X-ray contrast medium, in particular for the visualization of the vascular system [23], and was used as a contrast medium for radiography in man from the late 1920s to the early 1950s despite warnings about possible risks of late effects of its radioactivity. After intravascular injection the compound is permanently stored in the organs of the reticuloendothelial system, mainly in the liver (59%), spleen (29%), red bone marrow (9%), the lymph nodes and other organs (3%) [23]. Continuous irradiation of tissues led to the development of malignant primary hepatic neoplasms and myeloid leukemias. A few epidemiological studies were performed with patients who had received injections of Thorotrast [25-28], including studies of chromosome aberrations in these patients [29-33]. Some reports described a correlation between the volume of intravascularly injected Thorotrast (which is proportional to the dose rate delivered by the decay of ^{232}Th in red bone marrow and spleen) and the number of chromosome aberrations in patients [29, 34] and in animal studies [35, 36], while such a clear-cut response was not found in other studies [37, 38].

In our present experiments metaphase spreads were prepared from phytohemagglutine-stimulated lymphocyte cultures of two patients and two age-matched control persons. In about 4000 spreads chromosomes no. 1 to no. 5 were individually painted by CISS-hybridization and scored for aberrations. In addition, a program METSTAT was developed for automated evaluation of metaphase spreads with painted chromosomes. Preliminary results are reported for the rapid automated classification of metaphase spreads as "normal", i.e., spreads containing two painted chromosomes as expected in normal, diploid cells, or "aberrant", i.e., spreads containing more than two painted chromosomes, mostly cells with a translocation.

2 Materials and Methods

2.1 Cell material

10 ml of blood was obtained from two control persons and two patients suffering from the intravascular injection of approx. 10 ml of Thorotrast (no. 5597: 60 year old female, 42 years of exposure, 2.6×10^3 Bq of ^{208}Tl measured with whole-body counter; no. 5870: 67 year old female, 47 years of exposure, 2.6×10^3 Bq of ^{208}Tl; this activity corresponds to approximate dose rates of 13 cGy/a in the liver, 40 cGy/a in the spleen and 4 cGy/a in the red bone marrow). The control persons were treated in the same hospital and at the same time as the patients but were not exposed to Thorotrast (no. 7506: 63 year old male; no. 7524: 56 year old male). Lymphocytes were isolated, stimulated with phytohemagglutine (PHA) to divide, and cultured for 72 h using standard techniques [39]. Colcemid arrested metaphase spreads were obtained after hypotonic treatment (0.075 M KCl) and fixation with methanol/acetic acid (3:1 per volume). In other experiments, human lymphocytes from a healthy male donor (46, XY) were cultivated, irradiated at room temperature with 8 Gy of ^{60}Co-gamma-rays (1.17 and 1.33 MeV) and fixed as described above (for further details, see [13]).

2.2 DNA libraries and CISS-hybridization

Phage DNA libraries from sorted human chromosomes no. 1 to no. 5 were obtained from the American Type Culture Collection (no. 1: LA01NS01; no. 2: LL02NS01, no. 3: LA03NS02, no. 4: LA04NS02, no. 5: LA05NS01). Amplification of these libraries, isolation of the DNA, chemical modification by nicktranslation with biotin-11-dUTP [21] and CISS-hybridization were carried out as described in detail elsewhere [17]. In most experiments, biotinylated library DNA from a single chromosome was used; in one experiment the biotinylated library DNA from chromosomes 1 and 2 was combined. Hybridized chromosomes were detected by using fluoresceine-isothiocyanate(FITC)-conjugated avidin, which binds specifically to the biotinylated DNA (green fluorescence). For signal amplification the protocol of Ref. [40] was used. Metaphase chromosomes were counterstained with propidium iodide (PI, red fluorescence) and 4,6-diamidino-2-phenylindol-dihydrochloride (DAPI, blue fluorescence).

2.3 Microscopy

The microscopic evaluation was performed with a Zeiss photomicroscope III equipped with epifluorescence. Pictures were taken at high numerical aperture with Agfachrome 1000 ASA diapositive films.

2.4 Digital image analysis

Microphotographs on diapositive films were digitized using a drum scanning densitometer (Color Scandig 2605; Joyce Loebl) and a VAX PDP 11/3600 computer; 256 gray levels were distinguished. Hybridized areas (FITC plus PI fluorescence) and non-hybridized areas (PI plus background FITC fluorescence) could be distinguished due to their different gray levels without the need of additional filters. The digitized images were transferred to an IBM compatible personal computer with an INTEL 80386 microprocessor and a 25

MHz clock. For evaluation, a program called METSTAT was written in Turbo C 2.0. This program can be divided into four parts, viz., contrast enhancing, segmentation, counting and surveying, and classification.

2.4.1 Contrast enhancing

The contrast of painted and unpainted chromosomes in the digitized image of metaphase spreads may vary within a wide range in different experiments, depending both on the efficiency with which chromosome specific sequences hybridize to their respective target chromosome and the efficiency with which nonspecific signals can be suppressed [17]. A low contrast can severely disturb the segmentation routine, i.e., the setting of the threshold in a given metaphase spread which separates painted chromosome regions from non-painted ones. Therefore the algorithm analyses the gray value histogram (channels 0 to 255) and determines the positions of T_1 and T_2, where T_1 is the beginning of the gray value histogram > 0, while T_2 is the first maximum of the gray value histogram $\leqq 255$. The difference $T_2 - T_1$ is used to set a relation between a quadratic look-up table operation and the actual contrast. The gray value pic(x,y) of every pixel of the digitized image is then replaced by

$$\text{pic}(x,y)_{\text{new}} = (\text{pic}(x,y)_{\text{original}})^2 / (T_2 - T_1 + C_1),$$

where C_1 is an empirically determined constant.

2.4.2 Segmentation

The contrast enhancement enlarges the histogram over the complete gray value scale. For this new gray value histogram the values T_{1*}, T_{2*} are determined as described above for T_1, T_2. The following rather simple algorithm was developed for the segmentation of painted chromosome material, non-painted chromosomes and image background and proved to be less time consuming than entropy methods:

$$TH = T_{1*} + (T_{2*} - T_{1*})\, C_2 .$$

C_2 is an empirically determined constant which depends on the system used for digitization of the image.

All image pixels with gray values below TH are considered to belong to painted chromosome regions.

2.4.3 Counting and surveying

A "signal" is defined as a contiguous area of pixels with a gray value smaller than TH. The image matrix is scanned column by column until the first pixel fulfilling this condition appears. Starting from this position, all directly neighbouring pixels having also gray values less than TH are evaluated. Summing up, these pixel positions allow to calculate the "center of gravity" for each signal. For further evaluation of the image, the gray values of the recognized areas are set to 254 and the program starts again. If no more new areas are found, the counting routine lists up the number of signals, their area in number of pixels and their position.

2.4.4 Classification

From the number of recognized signals and their size, the metaphases are classified as "normal" or "aberrant". The program first determines the size of the largest signal of the image. All signals smaller than 1/10 of the largest one are considered to be artifacts due to the staining procedure or to segmentation errors and are consequently neglected. A metaphase spread classified as "normal" shows two signals, whereas an "aberrant" metaphase spread contains more than two signals. If the number of signals below the 1/10 threshold is above a certain limit (empirically chosen as 3 in the present series of images), the whole metaphase spread is discarded from the evaluation (classification: "excluded").

3 Results

3.1 CISS-hybridization of chromosomes no. 1 to no. 5 in metaphase spreads from PHA-stimulated lymphocytes of Thorotrast patients and control persons

The CISS-hybridization experiments were performed with the biotinylated libraries from chromosomes no. 1 to no. 5 as probes. As examples, Fig. 1 (a, b) shows two metaphase spreads from the control person (no. 7524) after CISS-hybridization with the combined chromosomes no. 1 and no. 2 libraries. While the selectively stained chromosomes appear normal in one metaphase spread (a), a deleted chromosome no. 1 and two translocations were detected in the other metaphase spread (b). For comparison, Fig. 1 (c) shows the chromosomes of this metaphase spread after DAPI staining. Fig. 1 (d) presents an example of a metaphase spread from the Thorotrast patient (no. 5597) after CISS-hybridization of chromosome no. 5. Besides a normal chromosome no. 5, a deleted chromosome no. 5 and a translocation of chromosome no. 5 material can clearly be seen (compare the DAPI stained chromosome in Fig. 1 (e)).

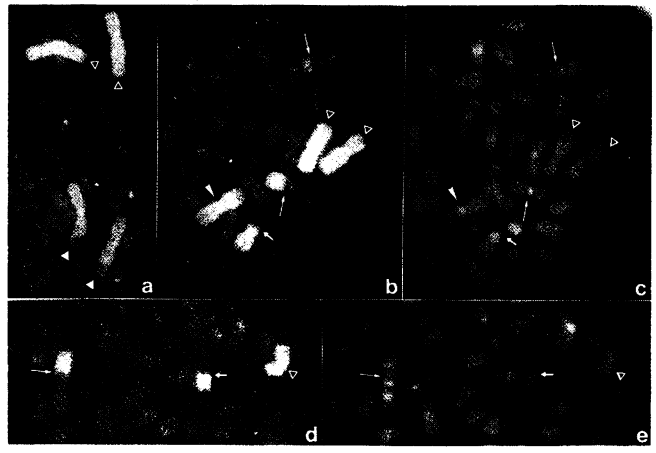

Fig. 1. Metaphase spreads from PHA stimulated lymphocytes from control persons (a-c) and Thorotrast patients (d, e) after CISS-hybridization with biotinylated libraries from sorted human chromosomes; detection with avidin-FITC and counterstaining with PI (a, b, d) and DAPI (c, e). In panels (a, b, d) painted chromosome material appears white due to a bright, green FITC fluorescence, non-painted chromosomes or chromosome regions are slightly visible due to their red PI fluorescence.
(a, b) Metaphase spreads from a control person (no. 7524) after simultaneous CISS-hybridization with library DNA from both chromosomes no. 1 and no. 2. In (a) the two homologous chromosomes no. 1 (closed triangles) and no. 2 (open triangles) are painted along their entire length. In (b) another metaphase spread from the same person shows two normal painted chromosomes no. 2 (open triangles), one normal painted chromosome no. 1 (arrowhead), one deleted chromosome no. 1 (short arrow) and two translocation chromosomes containing painted chromosome material presumably derived from the deleted chromosome no. 1 (large arrows point to the breakpoints of two translocation chromosomes). (c) The same metaphase is shown after counterstaining with DAPI. (d) Metaphase spread from a Thorotrast patient (no. 5597) after CISS-hybridization with chromosome no. 5 library DNA. A normal chromosome no. 5 is indicated by an open triangle. In addition, a painted fragment (short arrow) and a translocation chromosome containing chromosome no. 5 material can be seen (large arrow points to the breakpoint). (e) The same metaphase after DAPI counterstaining

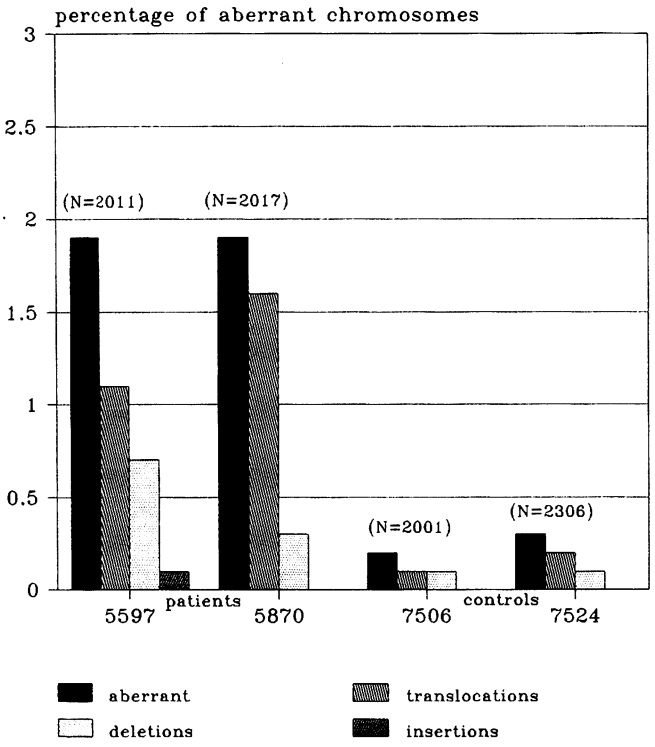

Fig. 2. Percentages of painted aberrant chromosomes after CISS-hybridization with chromosome libraries no. 1 to no. 5 in human lymphocytes from two Thorotrast patients (no. 5597 and no. 5870) and two control persons (no. 7506 and no. 7524). Percentages were calculated from the Table (number of painted aberrant chromosomes)/(total number of painted chromosomes evaluated in all experiments from each person). For statistical evaluation a Z-test was performed. The difference in the aberration scores between patients and control persons was significant with a probability ≧ 99.8% both for the total percentage of aberrant chromosomes and the percentage of translocation chromosomes

For aberration scoring, the CISS-hybridized chromosome preparations from the two Thorotrast patients and the two age-matched control persons were coded to avoid any possibility of a biased evaluation. The Table shows the results obtained by direct microscopic observation. Five experiments (one for each of the chromosomes no. 1 to no. 5) were performed with lymphocytes from each Thorotrast patient and from the control person no. 7506. For the control person no. 7524, an additional double CISS-hybridization experiment using the combined libraries from chromosomes 1 and 2 is also presented (compare Fig. 1 (a-c)). Some 400 (range 305..408) painted chromosomes were evaluated in each CISS-hybridization experiment. These results are summarized graphically in Fig. 2. Translocations, deletions, and in rare cases also insertions were identified both in Thorotrast patients and in age-matched control persons. The percentage of both the total of aberrant chromosomes and translocations was significantly higher ($p \leqq 0.002$) in the Thorotrast patients than in the control persons (see legend to Fig. 2).

3.2 Digital image analysis of CISS-hybridized metaphase spreads

The CISS-hybridization technique is particularly suited for application of a rapid digital image analysis approach, since digitized images of painted normal and aberrant chromosomes can be detected and discriminated as specifically fluorescent regions of various size by an automated threshold procedure. For this purpose a program METSTAT was developed (see Section 2.4). Every step in the image analysis performed by this program can be displayed on the monitor. To reduce the execution time, only the final result is routinely displayed for each cell (number of signals and signal areas; classification of the metaphase spread as "normal", "aberrant", "excluded"). On an AT 80386 computer, a total of 1 to 2 seconds was required per cell to run the METSTAT program (including the reading of the image file).

Fig. 3 presents an example of the application of this program to a metaphase spread from a control person (no. 7506) classified as "aberrant". To test the reliability of classifications achieved by METSTAT with the classifications performed by a cytogeneticist, we used digitized images produced from color slides of 140 human lymphocyte metaphase spreads obtained from non-irradiated cultures and from cultures irradiated with 8 Gy of ^{60}Co-gamma rays after CISS-hybridization with the chromosome no. 1 library [13]. These images were taken under various conditions, their mean brightness differing up to almost 50%. Metaphase spreads containing two normal painted chromosomes were classified by the cytogeneticist as "normal", while spreads containing three and more signals from normal and/or aberrant painted chromosomes were classified as "aberrant". In 131 out of 140 cells the classification obtained by METSTAT was in agreement with the cytogeneticist's classification: 118 cells were correctly classified as normal and 13 cells as aberrant. Seven cells were automatically excluded from scoring according to criteria described above (Section 2.4). From these cells, six were classified as normal and one as aberrant by the cytogeneticist. Only two false classifications were made by the program. One was "false positive", i.e., the metaphase spread was classified "aberrant" by METSTAT, but "normal" after direct microscopic inspection. The other one was "false negative", i.e., classified "normal" by the system, but judged "aberrant" by the investigator.

4 Discussion

This study has been intended as a first step towards the development of a long-term (cumulative) biological dosimeter (see Introduction). Using cultured lymphocytes from Thoro-

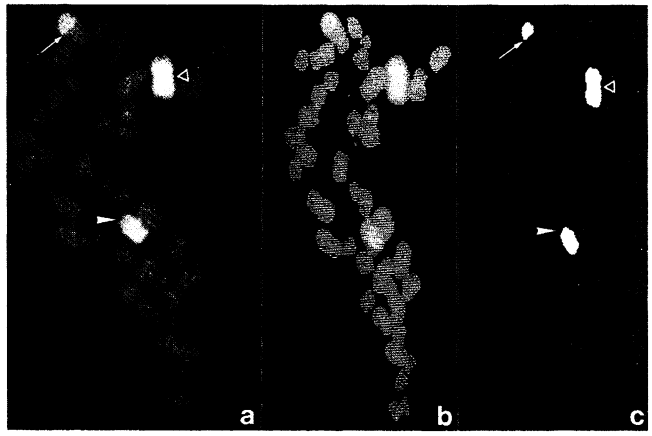

Fig. 3. Automatic segmentation of painted chromosomes in an aberrant lymphocyte metaphase spread obtained from the control person no. 7506 after CISS-hybridization with a chromosome no. 1 library. (a) Microphotograph of the metaphase spread before segmentation. (b) The same metaphase spread after digitization and background subtraction. (c) The same metaphase spread after automated segmentation of painted chromosomes by the program METSTAT. Three signals were obtained, one signal from a normal chromosome no. 1 (compare open triangles in (a) and (c)), one deleted chromosome no. 1 (compare arrow heads in (a) and (c)) and one chromosome with a translocation of chromosome no. 1 material (compare arrows in (a) and (c)). Photographs in (b) and (c) were taken from the monitor

Table. Evaluation of lymphocyte chromosomes from Thorotrast patients and control persons after CISS-hybridization with chromosome DNA libraries no. 1 to no. 5
In one experiment (control person no. 7524) the biotinylated DNA libraries for chromosomes 1 and 2 were combined, in all other experiments a single library was used

	Chromosome no.	No. of evaluated painted chromosomes	No. of translocations	No. of deletions	No. of insertions	No. of aberrant chromosomes	
						total	Fraction %
Patient 5597	1	405	8	5	–	13	3.2
	2	402	1	3	1	5	1.2
	3	400	5	4	1	10	2.5
	4	401	2	–	–	2	0.5
	5	403	6	2	–	8	2.0
Patient 5870	1	408	11	4	1	16	3.9
	2	400	5	–	–	5	1.2
	3	405	9	1	–	10	2.5
	4	402	3	2	–	5	1.2
	5	402	4	–	–	4	1.0
Control person 7506	1	401	1	2	–	3	0.7
	2	400	–	–	–	–	–
	3	400	–	–	1	1	0.2
	4	400	–	–	–	–	–
	5	400	1	–	–	1	0.2
Control person 7524	1	400	–	–	–	–	–
	2	401	2	–	–	2	0.5
	1+2	305	5	1	–	6	2.0
	3	400	–	–	–	–	–
	4	400	–	–	–	–	–
	5	400	–	–	–	–	–

trast patients and control persons, as well as cultured human lymphocytes irradiated with a ^{60}Co-source as model cases, our results indicate that two basic requirements of such a dosimeter may be fulfilled.

Firstly, our study demonstrates the usefulness of chromosome painting by CISS-hybridization for the unequivocal and rapid detection of chromosome translocations in patients chronically exposed to ionizing irradiation [13].

Secondly, it can be shown that a relatively simple algorithm can be used for rapid automated classification of metaphase spreads with normal and aberrant painted chromosomes. In the following we will discuss the present limitations and the potential of such an approach in biological dosimetry.

In contrast to other types of aberrations used in biological dosimetry, such as dicentrics or acentric fragments (micronuclei), we would predict (see Introduction) that reciprocal translocations should (a) be largely retained in an irradiated stem cell population of the red bone marrow even many cell cycles after an acute single irradiation event, and (b) accumulate in a chronically irradiated stem cell population. If so, we would expect that the percentage of reciprocal translocation events detectable in differentiated cells, e.g., lymphocytes, should also reflect the level of such translocations present in the stem cell population from which these differentiated cells are derived. While chromosome painting will clearly facilitate further tests of these predictions, the long-term reliability of a biological dosimeter based on translocation scoring may be limited by the fact that only a fraction of the detected translocations would be strictly reciprocal, while other translocations would not. Non-reciprocal translocations would also result in genetically imbalanced cells, which may be lost from a stem cell population. Notably, cell death is not the only important biological consequence of irradiation exposure. One should also consider the possibility that the chance induction of tumor specific chromosome aberrations, in particular tumor specific reciprocal translocations, may be causally related with the frequent development of malignant neoplasms in Thorotrast patients. While the number of translocation events scored in two Thorotrast patients was significantly higher than in two age-matched control persons (range between 56 and 67 years), more patients and control persons have to be studied to confirm this result.

Counting of the number and size of automatically segmented, painted chromosomes was sufficient for a reliable classification of metaphase spreads by the program METSTAT. While this classification was in agreement with a cytogeneticist's classification with few exceptions, it should be noted that color slides from well analyzable spreads were used as material for this comparison. The time needed for digitization of these slides by a drum scanning densitometer was excessively long (about 10 minutes per metaphase spread) as compared to the image analysis time. To accelerate the analysis, the scoring routine should be combined with an automatic metaphase finder and digitization should be made directly from the microscopic specimen. This may be achieved, for example, by a highly sensitive CCD-camera suitable for fluorescent imaging. Such a system could considerably shorten the evaluation time necessary for the fully automated analysis per cell with an envisaged goal of several hundred metaphase spreads per hour. While an improved version of METSTAT necessary for such an automated classification of unselected metaphase spreads will require considerably more sophistication, it should be noted that the segmentation of painted chromosome material can be achieved even in spreads with clumped chromosomes which could not be used for normal cytogenetic evaluation.

In contrast, image analysis procedures used for the automated detection of chromosome aberrations after conventional staining [10] require the separate segmentation and analysis of *all* individual chromosomes of a metaphase spread. In the latter case it is much more difficult to define algorithms which can rapidly discriminate between normal and aberrant chromosomes with high reliability.

The sensitivity of our present experiments to detect metaphase spreads with aberrant chromosomes was limited by the fact that (with one exception, see the Table, control person no. 7524) only one chromosome type was "painted" per cell. The yield of detectable aberrations per cell could be largely increased if several chromosome types were *simultaneously* hybridized. For theoretical reasons (*J. W. Gray*, personal communication) the simultaneous painting of human chromosomes no. 1 to no. 4 (including some 20% of total human genomic DNA) is expected to give a maximum yield of detectable aberrations if one labeling method and one color (e.g., fluoresceine) is used for all hybridized chromosomes. Note that exchanges between chromosomes can be obscured if the participating chromosomes are both painted with the same color. This limitation, however, may be overcome in the future by application of multi-probe hybridization and multicolor detection protocols [41], which make use of probes labeled by different chemical procedures and detected by different fluorochromes. Double in situ hybridization protocols allow the painting of two chromosomes participating in reciprocal translocations in different colors. As a long-term goal, these procedures should make it possible to stain several subsets of chromosomes simultaneously in different colors and thus include most or even all chromosomes of a given metaphase spread in translocation scoring.

Finally, biological dosimetry based on chromosome aberration scoring would be very much facilitated if a rapid automated detection of aberrations could be implemented directly in interphase nuclei. In this respect, it is an intriguing thought to apply the possibilities of the rapidly evolving field of interphase cytogenetics (e.g., [13, 19, 40, 42-48]) also to the problem of biological dosimetry.

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