

The Suitability of FISH Chromosome Painting and ESR-spectroscopy of Tooth Enamel Assays for Retrospective Dose Reconstruction

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Biodosimetry/Dicentrics/Translocations/FISH/ESR (EPR) – dosimetry.

A comparative analysis of two groups of highly irradiated victims was carried out in order to evaluate the suitability of two assays for retrospective dose assessment: late translocations and electron spin resonance (ESR) dosimetry. The first group comprised 24 subjects who exhibited acute radiation syndrome (ARS) due to overexposure as a result of nuclear submarine accidents during the period 1961–1985. Their grades of ARS and individual doses were ascertained by Navy physicians who carried out primary examinations and treatment of the exposed seamen. Cytogenetic analyses were made 16–40 y after their accidents. During medical treatment seven tooth samples were collected for ESR analysis from this group. The second group consisted of ten highly irradiated men from the Chernobyl accident. Comparison was made between estimates of their average whole-body penetrating radiation doses derived from several biological parameters. In three cases ESR measurements on tooth enamel from this group were also made. Retrospective dosimetry using FISH translocations was attempted 10–13 y later. Yields of late translocations were in good agreement with initially estimated doses and with doses obtained by ESR spectroscopy analysis of tooth enamel long after exposure. It was concluded that both persisting stable translocations and ESR spectroscopy signals are suitable with similar efficiencies for retrospective biodosimetry after acute whole-body exposure.

INTRODUCTION

There is a need for doses to be determined for persons who have been exposed to above normal levels of radiation. These may be victims of acute accidental exposures or persons exposed long-term to a radiologically polluted environment. Where physical methods of dosimetry are, for various reasons, unable to provide the data, biological dosimetry can sometimes fill the information gap. This paper considers two techniques that are particularly valuable as retrospective biological dosimeters in situations where considerable periods (years) have elapsed between exposure and assessment.

These are the fluorescence in situ hybridisation (FISH) chromosome-specific painting for quantifying translocations in human lymphocytes and electron spin resonance (ESR) spectroscopy of tooth enamel. Whereas many biological changes caused by radiation are transitory, the inherent stability of some translocations over cell generations and the extremely long life span of radiation induced radicals trapped in a tooth crystalline structure have enabled them to be developed into retrospective biodosimeters.

The ESR assay has been successfully applied to assess radiation doses absorbed by victims of accidents,^{1–3)} by survivors of the A-bombs at Hiroshima and Nagasaki,^{4,5)} by clean-up workers after the Chernobyl accident,⁶⁾ by staff of nuclear chemical factories,^{7,8)} and by residents of radioactively contaminated areas.^{9–11)} Sometimes the ESR studies were accompanied by simultaneous evaluation of chromosomal aberration frequencies in lymphocytes of the study subjects, and the inter-comparison of the results proved very useful in establishing the suitability and accuracy of both assays.

The applicability of ESR and cytogenetic assays is illustrated by data on lymphocyte translocations and tooth ESR in two studies: of persons involved in the Chernobyl accident

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and of victims of radiation accidents in Russian naval submarines. All persons were seriously over exposed and exhibited acute radiation syndrome (ARS).

MATERIALS AND METHODS

The Chernobyl subjects comprised eight reactor crew members who were present at the time of the accident, one of the first fire-fighters on the scene and one of the early clean-up workers, the so-called liquidators. These ten men were among the patients evacuated to Moscow for medical treatment of acute radiation syndrome (ARS). They were chosen for this follow-up because, despite their having absorbed high radiation doses, their treatments did not involve procedures such as blood transfusion or marrow engrafting, that might have prejudiced on-going cytogenetic analyses. For each person there were data for early (soon after exposure) and late (long after exposure) dicentric and late translocations. During the follow up period three of the men provided tooth samples for ESR analysis which was done at the MRRC, Obninsk.¹²⁾

The Navy group comprised 24 ex-submariners who had exhibited ARS due to overexposure as a result of Russian nuclear powered submarine accidents during the period 1961-1985. For five men data for early dicentrics were available and 21 men were sampled both early and late. The later samples were analysed for dicentrics and translocations. The study group was complemented by ten control ex-submariners with similar ages and military service but not exposed to ionizing radiation. The grade of ARS and individual doses of these 24 subjects were ascertained by naval physicians who carried out primary examinations and treatment of the seamen. The cytogenetic analyses were made at the MRRC

and the IRSN 16-40 y after the accidents. Seven men also provided teeth for ESR analysis.¹³⁾

Initially the conventional dicentric assay¹⁴⁾ was used to evaluate the irradiated subjects and in some cases this was repeated over many years. Later FISH^{12,13)} was added to the follow-up studies. Blood samples were taken and cultured to produce first division metaphases by a standard method recommended by the International Atomic Energy Agency (IAEA).¹⁴⁾ Slides were stained using the fluorescence plus Giemsa (FPG) technique and scored for dicentrics, excess acentrics and centric rings using standard criteria. FISH 'painting' was done by highlighting with fluorescein-isothiocyanate chromosomes 2, 3, 8 for the Chernobyl and 2, 4, 12 for the Navy groups. Remaining chromosomes were counterstained with 4,6-diamidino-2-phenylindole and all centromeres were highlighted with a pan-centromere probe. A complete (two-way) translocation was scored when two bi-coloured monocentric chromosomes (with exchanged counterparts) were present in the cell. An incomplete (one-way) translocation was scored when only one bi-coloured monocentric chromosome was present. The Lucas equation for scaling to genome equivalent cell number was used.¹⁵⁾

RESULTS

Figure 1 shows the yields of dicentrics detected in highly irradiated Chernobyl survivors and naval victims sampled at various times after exposure. The solid line is the spontaneous level of dicentrics obtained from the control group which comprised 10 unexposed ex-submariners. The dotted line is the control's upper 95% confidence limit. The data points show the highly significant reduction in the dicentric yield over the follow-up time. This is to be expected because

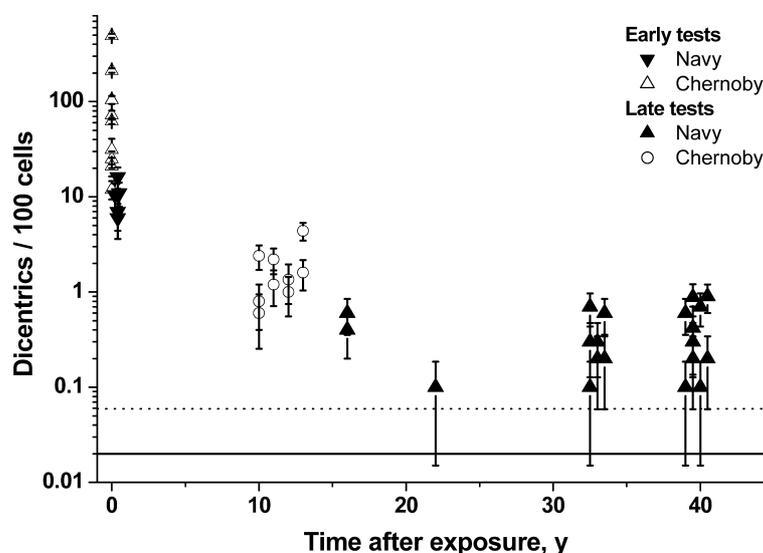


Fig. 1. The individual dicentric yields as a function of time after exposure for two groups of Russian accident cases. The solid line with its dotted upper 95% confidence limit is the background level measured in a control group of ex-naval personnel.

the dicentric is acknowledged to be an unstable type of aberration that is only suitable as a biological dosimeter soon after irradiation. Even so, many years later the residual dicentric frequency is in many instances still above control levels. However in the case of one Chernobyl and one Naval person the dicentrics had reduced to zero in 500 and 1000 scored cells respectively.

Possible statistical correlations were analysed for the individuals between their initial contemporary dose estimates based on clinical criteria (radiation field measurements, degree of ARS, blood cell counts, early dicentrics if available etc.), and late dicentrics, late translocations and ESR of tooth enamel obtained in the course of normal dental care. When combining all subjects from both data sets there are 31 persons for whom there were complete sets of initial dose estimates and late yields of dicentrics and translocations. But in only ten cases it was possible also to include dental ESR values.

Table 1 shows calculated Pearson (r) correlation coefficients for the pairs of factors within the two groups and for both groups combined. In particular, there is a close and highly significant ($p < 0.01$, where p is confidence probability) correlation between late translocation yields and ESR individual doses and this makes it possible to estimate an expected dose response from this dependence.

Figure 2 (panel A, solid circles) shows this dose response, adjusted to genome equivalence, for translocations detected in lymphocytes by FISH a long time after exposure as a function of individual dose estimated by ESR spectroscopy analysis of tooth enamel. The solid line shows the dose-response fitted by least squares methods to the linear quadratic model. The dashed line is an *in vitro* curve for genome equivalent reciprocal translocations induced by ^{60}Co irradiation and detected by FISH.¹⁵⁾

Figure 2, panel B, shows the translocation frequency detected in Hiroshima A-bomb survivors,⁴⁾ (grouped data),

Table 1. The correlation coefficients calculated between various measured parameters in the two accident groups and combined.

The examined pairs	Navy group	Chernobyl group	Combined
Contemporary absorbed doses – late dicentrics	$r = -0.14$ N=21, $p > 0.05$	$r = 0.07$ N=10, $p > 0.05$	$r = 0.41$ N=31, $p > 0.05$
Contemporary absorbed doses – late translocations	$r = 0.88$ N=21, $p < 0.01$	$r = 0.95$ N=10, $p < 0.01$	$r = 0.93$ N=31, $p < 0.01$
ESR doses – late translocations	$r = 0.97$ N=7, $p < 0.01$	$r = 0.88$ N=3, $p < 0.01$	$r = 0.98$ N=10, $p < 0.01$
ESR doses – Contemporary absorbed doses	$r = 0.97$ N=7, $p < 0.01$	$r = 0.99$ N=3, $p < 0.09$	$r = 0.94$ N=10, $p < 0.01$

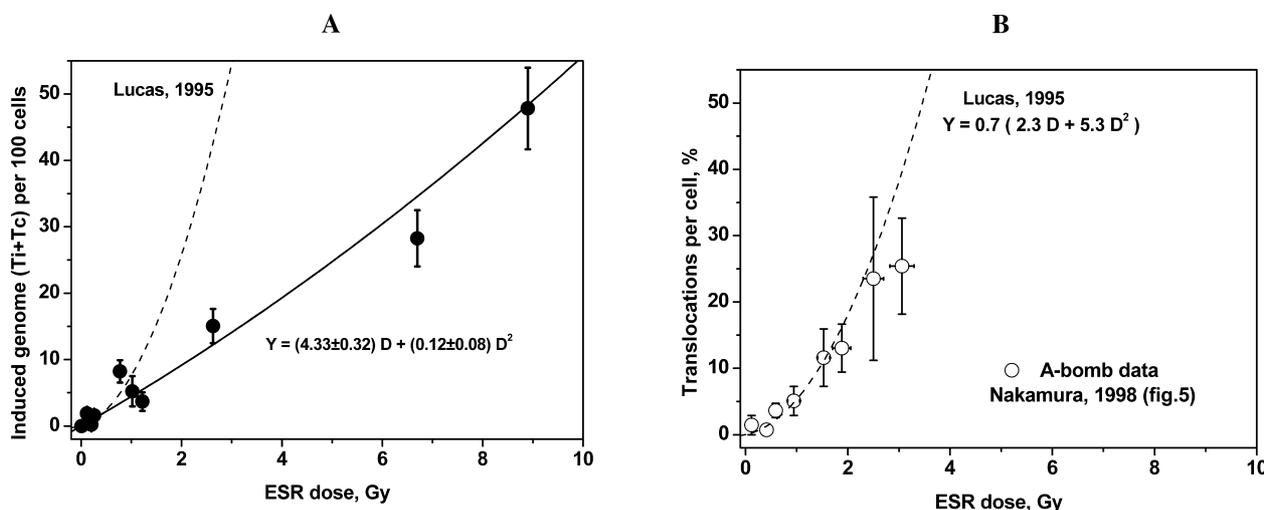


Fig. 2. Panel A. Full genome FISH translocation frequency in lymphocytes of 10 of the accident victims measured a long time after exposure as a function of their individual tooth ESR doses. The solid line is a least squares fit to the data. The dashed line is an *in vitro* FISH translocation dose response for ^{60}Co irradiation¹⁵⁾.

Panel B. Translocation frequency detected in Hiroshima A-bomb survivors⁴⁾ (grouped data) plotted against their tooth ESR doses. The dotted line is the expected translocation dose-response derived from the Lucas *et al.*¹⁵⁾ data adjusted to the 70% detection efficiency of the survivors' translocation study.

plotted against ESR-estimated doses measured from lingual sides of molars. Translocations were scored in this study by conventional Giemsa block-staining¹⁴⁾ with a detection efficiency about 70% compared with G-banding⁴⁾ and FISH methods. The numbers were taken from the original paper but plotted using the same axis scales as panel A in order to compare the data sets. The dotted line in panel B shows the same¹⁵⁾ *in vitro* curve adjusted to 70% efficiency.

DISCUSSION

It is well known that the yield of dicentrics in human lymphocytes can be used as a reliable biological dosimeter. However the big problem with dicentrics is that they are unstable and disappear from blood lymphocytes with time. So, only prompt dicentric data are the most useful and as time passes this assay tends to underestimate dose. It has been suggested (IAEA, 2001)¹⁴⁾ that for persons with normal hematology the damage disappears with a half time of ~3 y, whereas after high-acute doses the frequency reduces much faster, possibly reaching spontaneous levels over about 3 y after exposure. But such ideas are based on only a few well studied cases of accidental irradiation where dicentrics were followed over a long time. For 29 of 31 study victims reported here from both groups the yields of dicentrics had not declined to the control level during the periods of follow-up, which in some cases were over 40 y.

Despite this residue of dicentrics no correlation ($p > 0.05$) could be found between this parameter and individual initial doses (see Table 1). Nevertheless for practical purposes late dicentrics could be used qualitatively as retrospective indicators of suspected overexposure.

The present results provide a unique possibility to analyze the dose response of translocations detected long after exposure in victims with well-documented contemporarily estimated doses accompanied by extra ESR-estimated individual doses. The calculated Pearson (r) correlation coefficients shown in Table 1 point to a close correlation between contemporary doses, late translocation yields and ESR-estimated doses.

Likewise, a close correlation between ESR dosimetry from tooth enamel and cytogenetic dosimetry from lymphocytes of Hiroshima A-bomb survivors was also found.⁴⁾ In particular they studied translocation frequencies from 41 tooth donors by conventional Giemsa block-staining as a function of ESR-estimated dose from lingual surfaces of molars. The grouped data (five individuals per point) are shown in Fig. 2, panel B. It may be seen that this dose response for translocations induced by doses up to 3 Gy agrees very well with the *in vitro* curve.¹⁵⁾ This agreement was reached after converting the Lucas *et al* data to full genome equivalence¹⁴⁾ and then further reduction by 0.7 to match the 70% detection efficiency for translocations in the A-bomb survivors study.

The ESR-estimated absorbed doses for the Russian accidents presented here ranged from 0.1 to 8.9 Gy.^{12,13)} The corresponding genome equivalent translocations detected by FISH as a function of those ESR-doses are shown in Fig. 2, panel A using the same format as for panel B. The genome translocation frequency (Y) per 100 cells was fitted to the linear quadratic function by a routine least-squares method with the following result $Y = (4.33 \pm 0.32) D + (0.12 \pm 0.08) D^2$, where D is the absorbed dose in Gy.

Bearing in mind that the above curve is based on rather few individual points it is difficult to achieve a statistically high level of accuracy especially for estimation of the linear coefficient. Indeed, the value shown is somewhat higher than those reported from similar studies to derive *in vivo* linear coefficients of $(1.11 \pm 0.19)^{16)}$ and $(2.8 \pm 1.5)^{17)}$. However those studies were of workers exposed protractedly within the current dose limits over their working life, whereas the present data cover a much broader dose range up to 9 Gy received much more acutely. Therefore comparison is also made with the *in vitro* acute dose response curve¹⁵⁾ shown in Fig. 2, panel A. The quadratic coefficient derived from the Russian accident victims carries a large percentage uncertainty, as it is mainly influenced by the three highest dose cases. Even so, its absolute value is low suggesting that the pooled data are tending much more to linearity than might have been expected by comparison with traditional *in vitro* curves over a similar dose range and also the *in vivo* data from the A-bomb survivors shown here.

In addition the following brief remark concerning cytogenetic studies of Semipalatinsk people is relevant to the topic of this meeting. One of the first cytogenetic studies, of 98 residents from different places near the Semipalatinsk nuclear test site, was carried out in 1989 by the MRRC, Obninsk.¹⁸⁾ The study group included inhabitants of Semipalatinsk city and the following settlements: Shulbinsk, Chagan, Sarzhal and Kainar. Slightly enhanced levels of unstable chromosomal aberrations were found and this was confirmed in a later conventional cytogenetic study.¹⁹⁾ With regard to investigation of stable aberrations by the FISH method a re-examination of Semipalatinsk populations²⁰⁾ showed on average that the yields of translocations were consistent with expected control levels. Therefore whilst the dicentrics are suggesting, qualitatively, that these persons have been irradiated, the FISH method was unable to confirm that they had been protractedly exposed to doses in excess of 1.0 Gy.²¹⁾ A similar conclusion was reached, based on a separate FISH study of persons from Dolon village,²²⁾ where effective doses of ~ 3 Sv had been claimed but their mean translocation frequency was consistent with background.

CONCLUSIONS

- A residue of dicentric aberrations may be seen decades

after high dose irradiation. They may provide a qualitative indication of exposure but can not be used reliably as a quantitative retrospective biodosimeter.

- Both persisting stable translocations and ESR spectroscopy signals are suitable for retrospective biodosimetry after acute whole-body exposure with similar efficiency.
- The *in vivo* dose response for stable translocations appears to be more linear compared with the *in vitro* dose dependence for doses up to 3 Gy.

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