Mutagen-induced Chromosome Damage in Man

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Edinburgh
UNIVERSITY
PRESS

ISBN 0 85224 328 6

Printed in Great Britain by
R. & R. Clark Ltd
Edinburgh

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Preface

Eleven years have elapsed since the first international radiation cytogenetics symposium in Edinburgh, which proved to be a landmark in the study of the effects of ionising radiation on chromosomes in human lymphocytes. In the intervening years induced chromosome damage in lymphocytes has, under certain conditions, become established as a reliable indicator of exposure to ionising radiation, and has also been used to detect exposure to other mutagens. Considerable improvements have also been made in cytological techniques, and it therefore seemed an appropriate time to hold a second symposium, broadened to include the effects of both non-ionising radiation and chemical mutagens.

The meeting was jointly organised by the Medical Research Council and the National Radiological Protection Board and took place on 7 and 8 July 1977 at the MRC Clinical and Population Cytogenetics Unit in Edinburgh. Financial support was received from the Commission of the European Communities, Imperial Chemical Industries Ltd and the World Health Organisation. The meeting involved 60 invited participants from eighteen countries, and the 39 papers presented included both reviews and current research. Together they illustrate the vigorous development of this branch of cytogenetics in the past decade and demonstrate the considerable diversity of current activities in the field.

The first day of the meeting was mainly devoted to the effects of radiation, with a short session on the development of methods to automate the scoring of chromosome damage. The basic dose-response relationships of radiation-induced chromosome damage in human lymphocytes are now sufficiently established and cytogenetic dosimetry has taken its place in radiological protection. Outstanding problems discussed at the meeting included: the interpretation of the dose-response relationship after partial-body exposures; the need for a common denominator that would enable extrapolation between species used in experimental studies and man; and the continuing development of the technique as a fundamental radiobiological tool. It has long been evident that the circulating lymphocytes in the body form a heterogeneous cell population and the advent of methods enabling the separate study of T and B lymphocytes has led to a remarkable increase in research on the kinetics of these cells, and also on their radiation responses, both *in vivo* and *in vitro*.

The effects of chemical mutagens on human chromosomes, which occupied the second day of the meeting, is a rapidly increasing field of research. It has attracted a number of former radiation cytogeneticists as well as a new generation of researchers from the realms of cancer studies, environmental mutagenesis and toxicology. In some respects the research is at the stage reached in human radiation cytogenetics at the time of the first symposium, but many of the problems are different.

With a uniform whole-body exposure to ionising radiation approximately equivalent amounts of energy are absorbed in all parts of the body, so that the damaging events at the molecular level are, generally speaking, randomly distributed. Chemical mutagens, by contrast, comprise a wide variety of different substances, which vary in their degree of absorption, metabolism, location in the body and mode of action at the molecular level. Indeed, many chemicals may not be mutagenic *per se*, but when taken up by the body become transformed to yield active mutagenic/carcinogenic metabolites.

Ionising radiation may induce chromosome damage at all stages of the cell cycle and it is quickly transformed into aberrations, so that when cells are stimulated to enter into a mitosis, perhaps many years after irradiation, they reveal a history of their previous resting-stage exposure in the form of chromosome aberrations. This is not the case with most chemical mutagens, where DNA damage is only transformed into aberrations when the cell proceeds through a DNA replication phase. *In vivo* exposure of human peripheral blood lymphocytes to such mutagens will result in DNA damage, which may undergo repair before the cells are stimulated to enter mitosis and hence never give rise to aberrations; nevertheless, aberrations would have been developed in other cells in the body that were proliferating at the time of exposure. Chromosome aberrations in lymphocytes may therefore provide at best an inadequate, and often a misleading, measure of acute exposure of an individual to certain chemical mutagens. On the other hand, and as discussed in a number of papers at the symposium, chromosome damage in lymphocytes may provide a useful indicator of chronic exposure to chemical mutagens and a powerful method for *in vitro* studies on the actions of such substances. Studies of this kind have received a considerable impetus with the introduction of simple cytological methods to detect sister chromatid exchanges, and these techniques are being applied in efforts to monitor mutagen exposure and for *in vitro* testing of possible mutagens/carcinogens. Here, cytogenetics is clearly making a major contribution in the general upsurge of interest in the development of rapid methods for examining the mutagenic/carcinogenic potential of drugs and environmental pollutants.

An important consideration for cytogenetic monitoring is the cost, in terms of time and skill, of scoring for damaged chromosomes. Significant progress was reported at the meeting in the application of automated techniques to aberration scoring and karyotyping, although the advances have not been as rapid as many people would have wished. However, there have been dramatic developments in the use of flow systems for chromosome sorting

and detecting aberrations, and the future here is most encouraging.

Finally, although the contributions to the meeting raised some new problems and answered some old ones, a number of basic questions that have concerned researchers over many years still remain unanswered. For example, despite our considerable knowledge of the effects of radiation on human lymphocytes we still do not know how an exposure distorts the normal pooling and recycling of lymphocytes in the body, and just how representative are the few hundred cells analysed of the million or more that may be present in a small blood sample? We are naturally interested in the use of chromosome damage as a measure of levels of exposure, but, as biologists, we are really more interested in the biological effects of exposure and its consequences to the individual and to exposed populations and their descendants. A proportion of the chromosome damage that we observe is cell lethal, but a great deal is not, so that clones of karyotypically abnormal cells are detectable in individuals many years after exposure to a mutagen. This prompts the question whether the level of chromosome damage in peripheral blood lymphocytes can be equated with an individual's risk of developing a malignancy within his life expectancy. More specifically, the question has been asked whether any particular mutagen-induced chromosome changes increase the chance of occurrence of a malignant transformation in the cells that carry them. The beginnings of answers to these and other questions emerged during the meeting, and it is to be hoped that they will form the basis of an equally successful symposium to be held in the not-too-distant feature.

H. J. Evans
D. C. Lloyd

Contents

A Review of in vitro Dose-Effect Relationships

There are two principal reasons for investigating the relationship between absorbed dose and the number of chromosome aberrations per cell in lymphocytes taken from samples of human peripheral blood. The first is to obtain information about the interaction of tracks of ionising particles and biological molecules, which will incidentally throw light on chromosome structure. The other is to obtain a calibration curve for biological dosimetry, and this paper will concentrate on those aspects of the dose-effect relationship that are of importance in a dosimetry system.

As all the work described involves the stimulation of lymphocytes in culture by means of phytohaemagglutinin (PHA) it is presumed that T-type lymphocytes are the cells under consideration. Factors affecting the radiation-induced aberration yield *in vitro* of T lymphocytes are discussed briefly below.

Temperature

From the early work carried out using plant material, comprehensively reviewed by Giles (1954), it was known that temperature during irradiation affected aberration yield. Recently Bajerska and Liniecki (1969) demonstrated an effect of temperature on aberration yield in lymphocytes. Work in progress at the cytogenetic laboratory of the National Radiological Protection Board (NRPB) shows that the dicentric aberration yield increases with dose, and at 300 rad of 250 kV X-rays delivered at the rate of 100 rad min^{-1} it changes from 0.63 to 0.80 per cell as the temperature is raised from 16° to 40.5°C. Results from this type of work will be of interest to radiotherapists using a combination of local heating and radiation, which has some advantage in the treatment of cancer.

Oxygen effect

The variation of the survival of reproductive capacity in cells after irradiation at different levels of oxygen tension has been studied for many years (Okada 1970). It is now recognised that loss of reproductive capacity, at least at doses below 1000 rad, is strongly correlated with the chromosome damage that can be observed at first post-irradiation metaphase. Hence an oxygen enhancement ratio (OER) of between 2 and 3 might be expected for

chromosome aberrations. Freshly-taken venous blood for *in vitro* irradiation normally has sufficient oxygen for the aberration yield to be unaffected. Preliminary results from experiments at NRPB suggest OER values for 250 kV X-rays delivered at 100 rad min^{-1} vary from 4.7 to 2.0 as the aberration yield changes from 0.01 to 1.0 dicentrics per cell. This preliminary finding suggests that the oxygen effect is greater at low doses of low LET radiation where the dicentrics are mainly produced by single electron tracks, but more work must be done to firmly establish this observation.

Inter-Mitotic Death

It is important to know whether selective death of lymphocytes occurs in culture before they reach the first mitosis, because if this selective death occurred in cells with the higher amounts of chromosome damage it could affect the shape of the dose-response curve. However, it has always been observed that the chromosome aberrations are randomly distributed among the cells and that they follow a Poisson distribution. This observation is reassuring because it indicates that heavily damaged cells are not selectively dying in interphase. The amount of interphase death was estimated in experiments by Lloyd, Purrott and Dolphin (1973) who used a mixed culture technique. In this technique the number of irradiated cells is compared with the number of unirradiated cells reaching first metaphase in the same culture. It was found that inter-mitotic death varied exponentially with dose and could be represented by the equation $S/N = e^{-0.693D/270}$, where N is the fraction of unirradiated cells, and S is the fraction of irradiated cells reaching first metaphase, after a dose of D rad. From this equation it can be seen that, for doses of about 40 rad, 90% of the cells are capable of reaching first metaphase relative to unirradiated cells.

The cause of inter-mitotic death is not known, but it could be due to damage to the membrane or some other vital cell structure. Mixed culture experiments have not been carried out with high LET radiation and it should be interesting to observe the amount of interphase death with these radiations.

Mitotic Delay

Mitotic delay has been observed in irradiated cell cultures, and if there were selective delay of the cells containing the highest amount of chromosome damage this could affect the observed aberration yields at various culture times. As the chromosome aberrations are distributed at random among the irradiated cells cultured for 48 h this suggests that the heavily damaged cells are not at a selective disadvantage in passing through the cell cycle.

Lloyd *et al.* (1977) have investigated the effects of mitotic delay in some detail for two doses of X-rays, 150 and 400 rad, and for culture times varying from 36 to 120 h. At both the doses used the number of complete dicentrics per cell, i.e. dicentrics with the appropriate fragment visible in the metaphase, remained constant from 36 to 52 h in culture but fell off at longer times. The number of incomplete dicentrics, i.e. dicentrics without the appropriate frag-

ment present at metaphase, began to rise after 44 h in culture, indicating that some cells had already passed through into second mitosis by this time. It was also observed that complete dicentrics occurred even after 120 h at a level of about 20% of the yield at 48 h for both doses. This observation suggests that some cells take over four days to pass through to the first metaphase.

By analysing the change of the distribution of dicentrics among the cells with increasing time in culture, Lloyd *et al.* (1977) showed that cells containing dicentrics come through to first division more slowly than cells without aberrations. However the delay in reaching mitosis of cells with more dicentrics does not affect the measured aberrations per cell between 36 and 52 h in culture.

Dose Rate

A decrease of aberration yield is expected with a decrease of dose rate from the early results obtained with *Tradescantia*. Purrott and Reeder (1976a) have investigated the effects of dose rate by means of split dose experiments. In these experiments two equal doses were given separated by a few hours. When two doses of 100 rad were separated by more than 6 h there was no interaction between them. However, with two doses of 250 rad each there appeared to be interaction between the doses, as measured by dicentric damage, even when they were separated by 48 h. A possible interpretation of this latter finding is that some breaks remain open for a long time, as discussed by Lea (1946), and that the effects of these long-term breaks are more readily seen at higher total doses.

The effect of changes in dose rate on the yield of dicentrics has been investigated over a range from 1.9 to 2.8×10^{12} rad h^{-1}. Purrott and Reeder (1976b) found that the dicentric aberration yield at 100 rad of low LET radiation remains constant at rates above 25 rad h^{-1} but decreases below this rate. At 500 rad the yield was constant at rates above 200 rad h^{-1} but fell off at lower dose rates. In experiments in which the aberration yield in *Tradescantia* microspores was measured, Kirby-Smith and Dolphin (1958) found that the yield fell off at very high dose rates. They attributed this finding to the instantaneous depletion of oxygen near the chromosomes, which reduced the amount of dicentric damage. However Purrott, Reeder and Lovell (1977) did not observe this effect in chromosomes of human lymphocytes with similarly high dose rates.

Background of Aberrations in Normal Humans

When investigating the aberration yield at low doses of radiation it is essential to know the naturally occurring aberration frequency in unirradiated blood; this is particularly important in biological dosimetry where low exposure doses are most frequently encountered. The normally accepted background of dicentrics is about 1 in 3000 cells but, in a continuing study at NRPB, 11 dicentrics and 40 acentrics have been found in 6600 cells from 173 blood samples taken from new recruits. A similar study with new recruits to

the Windscale site has yielded 8 dicentrics in 8900 cells from 89 men. Hence values for the background aberrations may vary from country to country depending on the frequency of diagnostic radiological procedures, prevalence of virus disease and other agents producing aberrations in chromosomes. However the levels are low and would not significantly affect biological dosimetry except at the lowest doses.

Mathematical Representation

In the late 1960s it was customary to express the relationship between yield and dose as $Y=aD^n$, where n varied between 1 and 2. For fission neutrons $n=1$ but for low LET radiation many workers found a value of n close to 2, the exact value depending on the range of dose used in the experiment. More recently the quadratic equation $Y=\alpha D+\beta D^2$, with α and β as constants, has been used in the analysis of data on aberration yields. This equation is preferred because it has some biological significance, in that it represents aberrations formed by single tracks and by two separate tracks.

An important feature of this equation is represented by the quotient α/β; this is the dose at which equal numbers of aberrations are produced by one and two tracks. Below this dose the majority of the aberrations are produced by single tracks. These low doses are of considerable importance in biological dosimetry, and if this equation truly represents the aberration data then, at these doses, a dose-rate effect would not be expected; the aberration yield would be proportional to the total energy deposited in the body if the lymphocytes are in a reasonably uniform distribution.

Another aspect of representing aberration yields by the quadratic equation is that the RBE for high LET radiation, particularly fission neutrons, may be obtained at low doses. The aberration yield for fission neutrons is given by the equation $Y=\alpha' D$, so that at low doses the RBE for these neutrons is α'/α, which has values of 47 and 23 for fission neutrons and cyclotron-produced neutrons with a mean energy of 7.6 MeV, respectively. It also follows that there will be some high doses at which the aberration yield from neutrons will be the same as that from γ-rays. The dose at which this equality occurs is given by the quotient $(\alpha+\alpha')/\beta$, which equals 1700 rad for fission neutrons.

As the chromosome aberrations are distributed at random, Poisson statistics apply and the fraction of cells without aberrations is given by e^{-Y}, where Y is the mean number of aberrations per cell. The curve obtained when this expression is plotted against doses up to 500 rad lies close to those determined in survival experiments for human cell types. The value of the initial slope of the curve represented by e^{-Y} is given by $1/\alpha$, which has the value of about 1000 rad for 250 kV X-rays and about twice this value for ^{60}Co γ-rays (Lloyd *et al.* 1975). The slopes of the curves for X-rays and ^{60}Co γ-rays at 400 rad are 110 and 130 rad from the work of Lloyd *et al.* (1975). These values of slope are close to the D_0 values obtained in survival experiments with human cells, and lie in the range 50 to 200 rad (Whitmore and Till 1964).

Review of the Published Data on Dose-Effect Relationships

Values for α and β obtained by various authors are summarised in tables 1 and 2. The data in both tables are for dicentric or dicentric plus ring yields obtained from whole blood irradiated at 37°C. In table 1 the values of α for low LET radiation tend to be larger for the lower voltage X-rays, and most of the values of β are between 5 and 8×10^{-6} rad^{-2} for all radiations. The values for α and β depend on the dose range used in the experiments, and several dose points between 5 and 50 rad are required to establish the value of α with certainty from the measured yields. From table 1 it can be seen that there is an RBE of 3 for ^{60}Co γ-rays relative to 250 kV X-rays in the work of Lloyd *et al.* (1975), but Sasaki (1971) found a higher value for 200 kV X-rays.

The relationship between yield and dose for fission neutrons is linear and, apart from the data of Todorov *et al.* (1973), there is considerable agreement for all laboratories on the value of α, about 80×10^{-4} rad^{-1}. At higher neutron energies the value of β becomes more important and there is less agreement among the workers.

Summary

In the ten years since the previous conference at Edinburgh there has been considerable progress in harmonising dose-effect relationships, but still more is needed before this biological dosimetry system can be accepted with-

Table 1. Data on aberration yields of dicentrics, or dicentrics plus centric rings, published by various workers in which unstimulated whole blood was irradiated at 37°C with X or γ-rays at high dose rates. The equivalent doses are those corresponding to a yield of 0.1 aberrations per cell.

Author	$\alpha \times 10^{-4}$	$\beta \times 10^{-6}$	Energy/ source	Dose range (rad)	Including centric rings	Equivalent dose (rad)
X-Rays						
Liniecki *et al.* (1973)	4.03	2.62	180 kV	49–449	No	133
Sasaki (1971)	7.51	7.11	200 kV	20–400	Yes	77
Schmidt *et al.* (1972)	7.8	4.15	220 kV	25–400	No	87
Schmidt *et al.* (1976)	7.9	5.36	220 kV	25–400	No	82
Bender & Brewen (1969)	5.64	5.52	250 kV	100–300	Yes	93
Evans (1967)	29.0	0.66	250 kV	121–400	No	34
Lloyd *et al.* (1975)	4.76	6.19	250 kV	5–800	No	94
Vulpis *et al.* (1976)	4.83	—	250 kV	5–60	No	—
Leonard *et al.* (1976)	—	6.97	270 kV	100–400	No	120
Norman & Sasaki (1966)	0.85	4.28	1.9 MeV	15–800	No	143
γ-Rays						
Sasaki (1971)	0.91	6.82	^{60}Co	20–400	Yes	115
Brewen *et al.* (1972)	3.93	8.16	^{60}Co	50–400	Yes	89
Lloyd *et al.* (1975)	1.57	5.0	^{60}Co	25–800	No	127

Table 2. Values of the coefficients α and β for the equation $Y=\alpha D+\beta D^2$ given by various authors for dicentric aberrations in whole blood irradiated at 37°C with neutrons.

Author	Radiation type	$\alpha \times 10^{-4}$	$\beta \times 10^{-6}$	Dose range (rad)	Dose for $Y=0.1$ (rad)
Fission Spectra					
Biola *et al.* (1974)	Crac	90.1	—	68–317	11
	Nereide	87.4	—	100–300	11
	Harmonie 1.5 MeV max.	64.8	—	22–142	15
Scott *et al.* (1969)	BEPO $\bar{E}=0.7$ MeV	84.9	—	25–150	11
Carrano (1975)	Janus $\bar{E}=0.85$ MeV	78.4	—	25–150	13
Todorov *et al.* (1973)	IRT-2000	26.6	—	25–200	38
Lloyd *et al.* (1976)	BEPO $\bar{E}=0.7$ MeV	83.5	—	50–300	11
	AWRE $\bar{E}=0.9$ MeV	72.8	—	6–265	14
Accelerated Deuterons on Beryllium					
Sasaki (1971)	$\bar{E}=2.03$ MeV	74.5	—	14–250	13
Biola *et al.* (1974)	Louvaine $\bar{E}=6.2$ MeV	33.8	—	22–172	30
Lloyd *et al.* (1976)	Hammersmith $\bar{E}=7.6$ MeV	47.8	6.4	27–324	20
d-t Reaction					
Sasaki (1971)	$\bar{E}=14.1$ MeV	25.0	3.71	12–450	37
Bauchinger *et al.* (1975)	$\bar{E}=15.0$ Mev	14.1	3.77	31–375	60
Lloyd *et al.* (1976)	$\bar{E}=14.7$ MeV	26.2	8.8	5–303	32

out question. In particular, more work is needed to establish the factors that affect the stimulation of lymphocytes into the cell cycle and the time taken for them to reach first metaphase. These and other problems concerning lymphocytes in culture could be the subject of papers at a symposium in ten years' time.

Acknowledgements

The author wishes to thank his colleagues David Lloyd, Roy Purrott and Stuart Prosser for access to their unpublished data, and Mrs Elaine Henry for help with the manuscript.

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