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Radiation Induced DNA Double Strand Breaks and Chromosome Aberrations

H. P. LEENHOUTS and K. H. CHADWICK

Association Euratom — ITAL, Wageningen (The Netherlands)

Summary. This paper presents arguments which favour an alternative approach to the interpretation of radiation induced chromosome aberrations. Starting from the modern concept that a chromosome has a DNA double stranded helix backbone and that the induction of DNA double strand breaks has a quadratic dose relationship, it is concluded that most chromosome aberrations arise from only one chromosome break. The direct correlation between chromosome aberrations and cell death derived from the model is demonstrated by the analysis of experimental results. The effect of dose rate, LET and the occurrence of chromatid aberrations after irradiation in G1 are all logically explained by the theoretical model.

Introduction

In a recent publication (Chadwick and Leenhouts, 1973) we have developed a theory, which is based on the induction of double strand breaks in the DNA helix, to explain radiation induced cell killing. The application of this theory to the dependence of cell survival on radiation dose rate and the variation of radiation sensitivity in the cell cycle gave analyses which were both logical and could be explained by the behaviour and metabolism of the DNA molecules in the cell.

If this theory is considered in the light of the modern proposals of chromosome structure (DuPraw, 1965; Callan, 1967; Whitehouse, 1967; DuPraw, 1970; Prescott, 1970; Crick, 1971; Laird, 1971) certain arguments can be developed which question the validity of the basic assumptions of the current theories on radiation induced chromosome aberrations. These arguments and their consequences are presented here in the form of an alternative theoretical approach.

DNA Double Strand Breaks, Cell Killing and Chromosome Aberrations

The DNA helix forms a structured molecular target for radiation which induces double and single strand breaks in the sugar-phosphate chains of the helix. In aqueous solution the induction of double strand breaks in DNA molecules has been found to have a quadratic relation with radiation dose (Hagen, 1967; Freifelder and Trumbo, 1969).

A double strand break can be induced in one radiation event when both strands of the helix are broken simultaneously (proportional to dose), or by the combination of two unrepaired single strand breaks which are induced in two independent radiation events (proportional to the square of the dose). Thus if allowance is made for repair processes the total number of DNA double strand breaks after a dose D can be calculated to be

$$N = \alpha D + \beta D^2 .$$

The basic tenet of the new molecular theory is that the induction of double strand breaks in DNA in the cell also has a quadratic dose relation and that this molecular damage forms the critical biological lesion.

In the molecular theory of cell survival we have assumed that each double strand break in a cell has a probability p of leading to cell death so that the probability for cell survival is given by the equation

$$S = e^{-pN} = e^{-p(\alpha D + \beta D^2)} .$$

Recent experimental work on chromosome structure (DuPraw, 1965; Laird, 1971) supports the view that the chromosome has a single long DNA double helix backbone (Callan, 1967; Whitehouse, 1967; DuPraw, 1970; Prescott, 1970; Crick, 1971). Thus, on the basis of this concept a break in the DNA double strand helix is in fact a break in the chromosome backbone. This means that the induction of chromosome backbone breaks will also have a quadratic dose relation given by

$$N = \alpha D + \beta D^2 .$$

This straight forward conclusion is of fundamental importance as it contradicts the basic assumption of the current theories of chromosome aberrations (Sax, 1939, 1940, 1941; Lea, 1955; Revell, 1955, 1959, 1963, 1966). These theories, which were developed prior to the recent considerations on chromosome structure, assume that the primary break or lesion in the chromosome is linearly related to radiation dose.

The conclusion that the induction of chromosome backbone breaks is quadratic forms the starting point of the new proposal that a chromosome back-

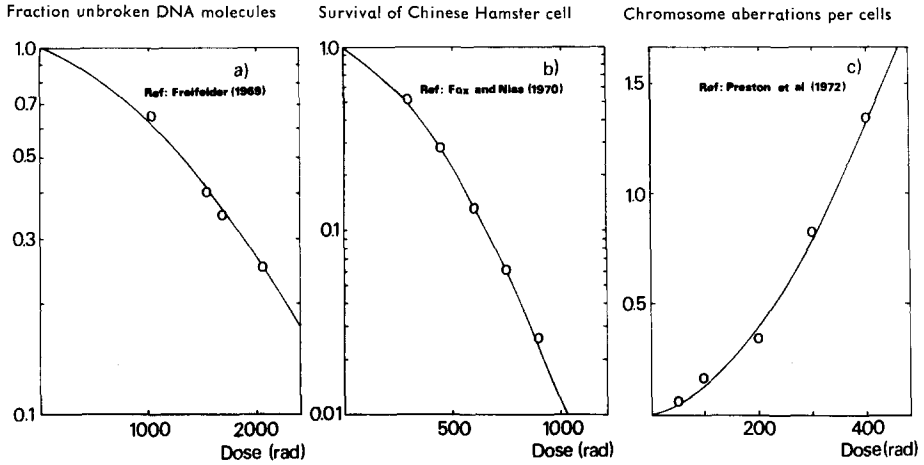

Fig. 1. Comparison of dose kinetics for DNA double strand breaks, cell survival and chromosome aberrations

bone break may lead to a chromosome aberration. Thus, the yield, Y, of a chromosome aberration can be written as

$$Y = k\,N = k\,(\alpha\,D + \beta\,D^2)$$

where k is a constant which is dependent upon the aberration type being scored and the scoring efficiency.

The interpretation of experimental results should be made on the basis of the parameters which form the coefficients α and β and which have been given special radiobiological significance in reference 4 where the complete derivation of the quadratic equation has been presented.

In Fig. 1 we present some evidence which supports the theory which has just been developed. Fig. 1a shows the dose kinetics found for the induction of DNA double strand breaks in DNA obtained from the phage B3 and irradiated in buffer solution (Freifelder and Trumbo, 1969), analysed according to the quadratic dose relationship. Fig. 1b shows the survival of chinese hamster cells following irradiation (Fox and Nias, 1970) analysed according to the molecular theory and Fig. 1c shows the induction of dicentrics plus centric rings plus acentric fragments in chinese hamster cells (Preston *et al.*, 1972) analysed according to the quadratic relationship.

In Table 1 we present the details of the coefficients which have been derived from the analyses of the curves shown in Fig. 1. In the last column of the Table the coefficients for double strand breakage per nucleotide pair have been calculated using an estimate of the number of nucleotide pairs per mammalian cell given by Swanson, Merz and Young (1967). It has been assumed that the probability p for cell death per double strand break is unity and that each double strand break causes a chromosome aberration. These assumptions form an oversimplification which does not however detract from the convincing agreement between the coefficients found in all three cases. This agreement between the coefficients is a strong indication that the same radiation induced process, i. e. DNA double strand breakage, is involved directly as the basic mechanism leading to radiation induced cell death and chromosome aberrations.

The Correlation between Cell Killing and Chromosome Aberrations

A direct extension of these arguments is that we predict that for measurements carried out on the

Table 1. *Comparison of the induction of DNA double strand breaks, cell survival and chromosome aberrations after irradiation*

Process	Analysis	Numerical values	Nucleotide pairs per molecule or cell	Coefficient for d. s. b. per nucleotide pair	Ref.
DNA double strand breaks in buffer	Fraction of unbroken DNA molecules $F = e^{-\alpha D-\beta D^2}$	$\alpha = 2.3 \times 10^{-4}$ rad^{-1} $\beta = 2.2 \times 10^{-7}$ rad^{-2}	1.4×10^8	$\alpha^1 = 1.6 \times 10^{-12}$ rad^{-1} $\beta^1 = 1.6 \times 10^{-15}$ rad^{-2}	Freifelder and Trumbo (1969) Fig. 1a
Survival of chinese hamster cells	Survival of cells $S = e^{-p\alpha D-p\beta D^2}$	$p\,\alpha = 1.6 \times 10^{-3}$ rad^{-1} $p\,\beta = 2.8 \times 10^{-6}$ rad^{-2}	$\sim 3 \times 10^9$	* $\alpha^1 = 5.3 \times 10^{-13}$ rad^{-1} $\beta^1 = 9.3 \times 10^{-16}$ rad^{-2}	Fox and Nias (1970) Fig. 1b
Chromosome aberrations in chinese hamster cells	dicentrics + centric rings + acentric fragments per cell $Y = k\,\alpha\,D + k\,\beta\,D^2$	$k\,\alpha = 6 \times 10^{-4}$ rad^{-1} $k\,\beta = 6.7 \times 10^{-6}$ rad^{-2}	$\sim 3 \times 10^9$	** $\alpha^1 = 2.0 \times 10^{-13}$ rad^{-1} $\beta^1 = 2.2 \times 10^{-15}$ rad^{-2}	Preston *et al.* (1972) Fig. 1c

* It is assumed that the probability p for cell death per double strand break = 1.
** It is assumed that each double strand break causes a chromosome aberration.

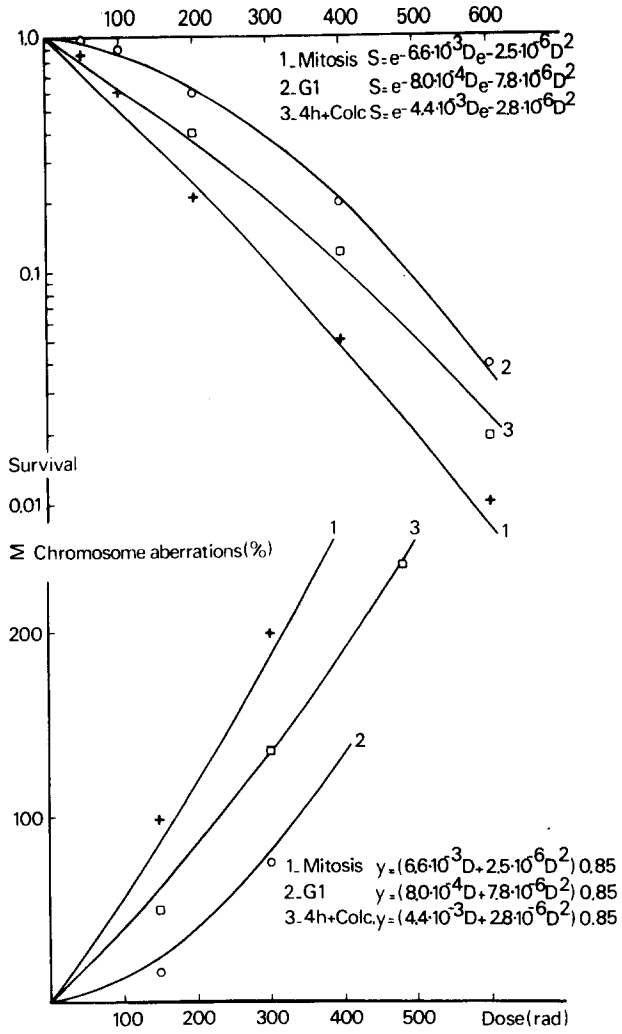

Fig. 2. Correlation between cell killing and total chromosome aberrations in synchronised chinese hamster cell (Dewey *et al.*, 1971)

same cell under the same conditions the coefficients derived for cell survival and chromosome aberrations will be related to each other by a constant factor. One such correlation is illustrated in Fig. 2 for synchronized chinese hamster cells irradiated at different stages of the cell cycle (Dewey *et al.*, 1971). The coefficients $p\,\alpha$ and $p\,\beta$ derived for the cell survival curves, multiplied by a constant 'K', give curves which fit in each case the total production of chromosome aberrations. It is interesting to note that the correlation is found in different phases of the cell cycle, this means that the changing shape of the cell survival curve through the cell cycle is mimicked by the chromosome aberration curve. This variation in radiation sensitivity through the cell cycle has been analysed to provide strong support for the association between DNA double strand breaks and cell killing (Chadwick and Leenhouts, 1973, 1973a).

Starting from the basic mechanism of a DNA double strand break leading to a chromosome aberration we can now consider how this mechanism is related to the various chromosome configurations found at mitosis and what consequences arise from this mechanism.

The Terminal Deletion

The simplest explanation for the occurrence of this chromosome aberration is that proposed in the classical theory (Sax, 1939, 1940, 1941; Lea, 1955) that the terminal deletion arises from a single break in the chromosome. The classical theory predicted that the terminal deletions would exhibit a linear dose relationship. We would expect that an aberration which arises from a single break in the chromosome backbone would have a quadratic dose relationship in general. Fig. 3 shows the curve for terminal deletions found in *Wallabia bicolor* by Brewen and Brock (1968) fitted with a quadratic equation which is in agreement with our theoretical expectations.

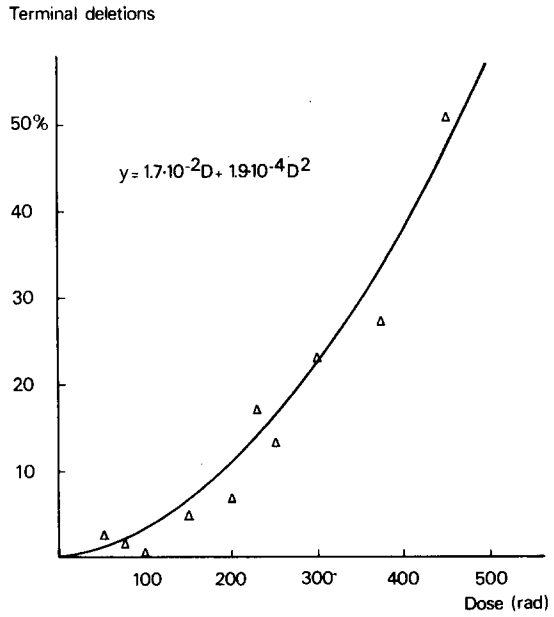

Fig. 3. Terminal deletions in *Wallabia bicolor* fitted to $Y = k\,(\alpha\,D + \beta\,D^2)$ (Brewen and Brock, 1968)

It is worth noting, at this point, that under certain experimental conditions the general quadratic relationship may be reduced to a linear dose relationship. This occurs when the coefficient β is close or equal to zero. This is important because the actual dose relationship for terminal deletions, linear or quadratic, has been a topic of some controversy between different schools of radiation cytologists.

The Exchange Aberrations

The dose relationship for exchange aberrations has often been fitted using a quadratic dose relationship and as a result these aberrations have been interpreted in the classical and exchange theories as arising from two chromosome breaks.

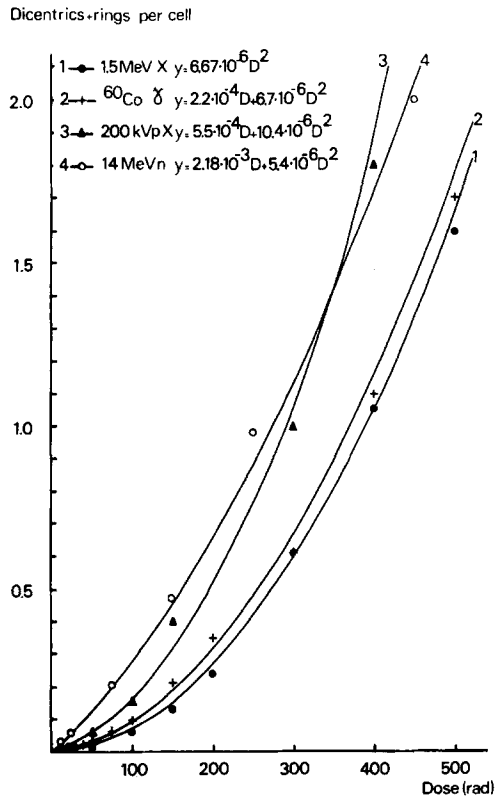

Fig. 4. The effect of different radiation qualities on the production of dicentrics and rings in whole blood (Unscear, 1969)

The consequence of the arguments presented in this paper is that an exchange aberration which arises from only one break in the chromosome backbone would have a quadratic dose relationship and would therefore fit the experimentally determined dose relationships.

Dicentric and ring aberrations form one group of easily recognisable and accurately scorable exchange aberrations. Fig. 4 presents the fitting of a quadratic equation to the production of rings and dicentrics in blood for different qualities of radiation (Unscear, 1969). The theory predicts, in analogy with the theory for cell killing (Chadwick and Leenhouts, 1973), that as the LET of the incident radiation increases the α coefficient will increase and dominate and the relation will eventually become linear with dose. The value of the α coefficient (Fig. 4) is increasing consistently with increasing radiation quality and the limiting RBE of 4.0 found for the 14 MeV neutrons against the 200 kVp X-rays is in agreement with that found for cell killing in the T1-g kidney cells (Chadwick and Leenhouts, 1973).

If we accept that the basic mechanism involved in a chromosome aberration is a radiation induced DNA double strand break and that an exchange aberration must be developed from one chromosome break it is necessary to find an explanation for the considerable variety of exchange aberration configur-

Aberration type	G1	IRR.	Joining	Metaphase
Terminal deletions + minutes				
Centric rings				
Acentric rings				
Pericentric inversion				
Dicentric and fragment				
Symmetrical interchange				

Fig. 5. Diagrammatic representation of the formation of chromosome type aberrations at mitosis on the basis of rejoining between a broken and normal chromosome end

Aberration type	G2	IRR.	Joining	Anaphase
Chromatid break				
Isochromatid break				
Symmetrical intrachange				
Asymmetrical intrachange				
Symmetrical interchange				
Symmetrical interchange				
Asymmetrical interchange				
Asymmetrical interchange				

Fig. 6. Diagrammatic representation of the formation of chromatid type aberrations at mitosis on the basis of rejoining between a broken and normal chromosome end

ations which have been found at mitosis following irradiation. These configurations have been explained previously by assuming they arose through the incorrect rejoining of two chromosome breaks.

We suggest that the various configurations found at mitosis can be explained on the basis of one chromosome break if it is assumed that a broken chromosome end can rejoin with a normal chromosome end. The formation of these configurations on this assumption is illustrated diagrammatically in Figs. 5 and 6.

The Joining of a Broken Chromosome End to a Normal Chromosome End

The process of joining between a normal end and a broken chromosome end is a process which has not been considered before, at least in the field of radiation cytology. Indeed, it has not been necessary to consider it until now, and as a consequence there appears to be little information available on the biochemical nature of the chromosome ends and no direct scientific evidence either for or against assuming that the process can occur.

Some recent indirect evidence in favour of the assumption can be found in a paper by Brewen *et al.* (1973). Brewen *et al.* find a quadratic dose relationship for dicentrics and for terminal deletions in six different species which have a similar amount of DNA per cell. The yield of dicentrics was found to be linearly related to the effective chromosome arm number of the different species, although there was no similar relationship for terminal deletions. If we assume that the joining of a break to a normal chromosome end can form a dicentric we would expect to find that the yield of dicentrics was directly dependent on the number of chromosome ends available. The terminal deletion, on the other hand, is according to us, independent of any rejoining or exchange and thus independent of chromosome arm number.

The explanation of exchange aberrations on the basis of one chromosome break plus the rejoining of a broken and normal end means that certain types of chromosome aberrations will occur only rarely at mitosis. The true reciprocal translocation, for instance, which must involve the exchange of two fragments between two chromosomes, will arise from two breaks and the dose relationship will contain terms in up to the fourth power of the dose. The theory does not exclude completely the possibility that a true reciprocal translocation type of aberration may occur but it does imply that this aberration will be rare.

The translocation type of aberration predicted by the theory involves one chromosome break plus transfer of the fragment to another chromosome. This aberration will be almost impossible to identify separately from a reciprocal translocation at mitosis using the normal cytological techniques. In this respect, it is perhaps relevant to note that some assumed reciprocal translocations have recently been shown to be non-reciprocal (Francke, 1972).

We have restricted ourselves to the description of aberration configurations at mitosis in Figs. 5 and 6 because we do not know in which way and to what extent the various processes such as 'crossing over', occurring in meiosis will affect the structure and transmission of these aberrations.

The Dose Rate Effect and its Consequences

In direct analogy with the arguments used in the molecular theory of cell survival, the dose rate effect is explained on the basis of the enzymatic repair of DNA single strand breaks which is a well known biochemical phenomenon (Painter, 1970). Briefly, this means that as the dose rate is reduced the DNA single strand breaks will have more chance for repair and fewer DNA double strand breaks will result from the combination of two independently produced single strand breaks. The result of this is that the α coefficient should remain constant and the β coefficient should decrease with decreasing dose rate. This effect is shown in Fig. 7 for the effect of dose rate on exchange aberrations in *Tradescantia* microspores (Sax, 1940, 1941).

This explanation of the dose rate effect is not unlike that used in the classical and exchange theories. There are however two important differences, firstly the repair process we propose occurs at the molecular level and secondly, the dose rate effect occurs in the production of the chromosome break and not in the formation of the configuration.

This has an important consequence as it means that we can no longer use the dose rate effect to determine the time limits for chromosome rejoining.

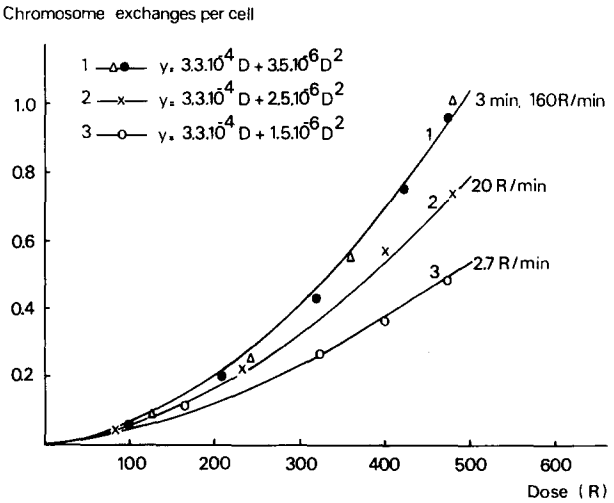

Fig. 7. The effect of dose rate on the induction of exchange aberrations in *Tradescantia* microspores (Sax, 1940, 1941 see Lea, 1955). The curves have been fitted to the experimental points by holding α constant and by varying β

Thus, a break produced by irradiation in G1 may be replicated in S before rejoining in G2 to give a typically 'isochromatid' type of aberration. We are no longer able to identify the phase in which the cell was irradiated from the type of aberration which we find at mitosis and we expect that some chromatid type of aberrations may arise from cells irradiated in G1 or early S. It is encouraging to note that this effect has been found experimentally by Dewey *et al.* (1969, 1970) and Wolff (1969).

Conclusion

We have presented an alternative theoretical approach to radiation induced chromosome aberrations which is based on the interaction of radiation with the DNA double helix molecule and on the modern concept of chromosome structure.

It has been shown that the theory explains consequently and without any further assumptions the dose relationship, the LET effect, the dose rate effect and the fact that chromatid aberrations may arise in cells irradiated in the G1 or early S phase of the cell cycle. The theoretically predicted direct quantitative relation between cell death and chromosome aberrations has been demonstrated by analysis of experimental observations.

The various chromosome configurations found at mitosis can be described if it is assumed that a broken chromosome end can join to a normal chromosome end. In view of the arguments presented here it is necessary to investigate and demonstrate experimentally the occurrence of this process of joining and to learn more about the biochemical nature of normal chromosome ends.

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H. P. Leenhouts
K. H. Chadwick
Association Euratom — ITAL
Postbus 48
Wageningen (The Netherlands)