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The Correlation between Mutation Frequency and Cell Survival following Different Mutagenic Treatments

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Summary. A direct mathematical relationship between mutation frequency per survivor and cell survival is derived from theoretical considerations of the molecular effects of radiation in a cell. It is shown that the relationship is satisfied by analysis of the various data on radiation induced mutations available in the literature. The analysis implies that a common type of lesion may lead to mutation and cell death and is derived on the assumption that radiation-induced double strand breaks in DNA are the critical lesions. The mathematical relationship is independent of the way in which the lesion which leads to mutations and cell death is induced, so the analysis has consequently been applied to other mutagenic treatments such as UV light and chemicals. It is concluded that, although the lesions induced by chemicals may not be the same as those induced by radiation, it is probable that for the chemicals considered common basic damage to the DNA molecule is implicated as the critical lesion.

Introduction

A theoretical model has been developed to explain the radiation-induced killing of cells (Chadwick and Leenhouts, 1973). The basic assumption of this model is that radiation induced DNA double strand breaks are the most critical lesions which lead to cell death. In an extension of the model, radiation-induced DNA double strand breaks have been related to chromosome aberrations (Leenhouts and Chadwick, 1974a) and it has been shown that a relationship exists between cell survival and chromosome aberrations following radiation (Chadwick and Leenhouts, 1974). It is possible that DNA double strand breaks also lead to mutations, either through loss of chromosome material in a deletion or through the mis-repair of the break giving base pair changes in the DNA, so it was proposed that the same theoretical considerations could be applied to radiation-induced mutations (Leenhouts and Chadwick, 1974b).

In this paper we derive the general relationship between cell survival and mutation induction from the radiation model, demonstrate that there is experimental evidence which supports the relationship, and consider the possible application of the relationship to other mutagenic agents.

Radiation Induced Mutations and Cell Survival

We have assumed that the DNA double strand break is the critical lesion which leads to cell death and that following a radiation dose D the mean number of double strand breaks per cell is given by

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

where α represents the number of double strand breaks induced in one radiation event (proportional to dose) and β represents the number of unrepaired single strand breaks which combine to produce a double strand break (proportional to the square of the dose). Then in a uniform population of cells, survival is given by the equation

$$S = e^{-pN} = e^{-p(\alpha D+\beta D^2)}, \quad (1)$$

where p is the average probability that a double strand break leads to cell death.

If q is the average probability that a double strand break leads to a specific mutation, then the equation for the mutation frequency per survivor is given by

$$M = 1 - e^{-qN} = 1 - e^{-q(\alpha D+\beta D^2)} \quad (2)$$

When $M \leqslant 1$ equation (2) may be approximated by

$$M = qN = q(\alpha D + \beta D^2) \quad (3)$$

and equations (1) and (3) can be combined to give the general relationship between mutation frequency per survivor and survival as

$$\ln S = -(p/q)M \quad (4)$$

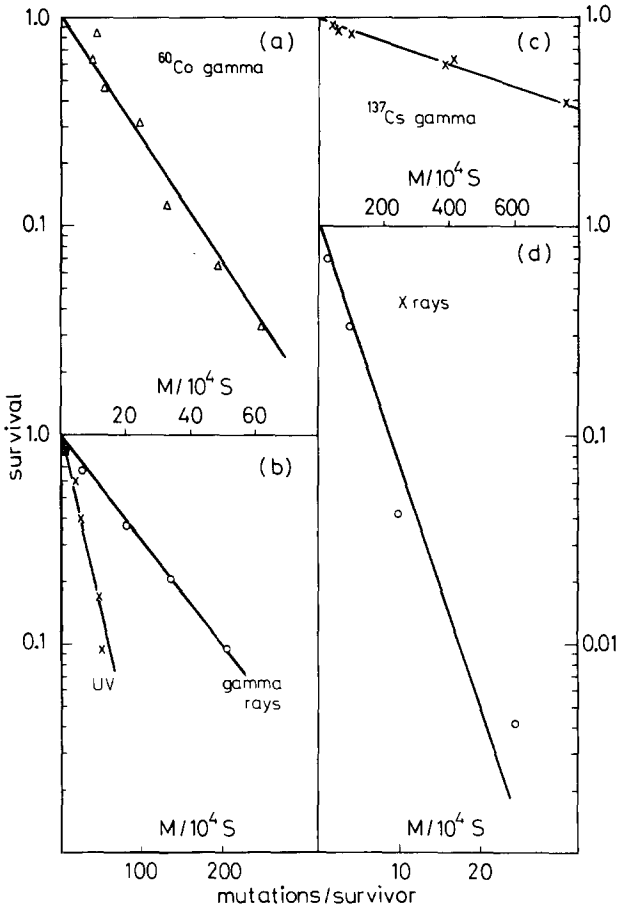

Fig.1. Analysis of mutation and cell survival according to lnS = (p/q)M for ionizing radiation. a) γ-ray induced red to white mutations in *Schizosaccharomyces pombe* ad-7 (Hannan 1975); b) γ and UV induced 8-azg resistance in Chinese hamster cells (Bridges and Huckle, 1970); c) γ-ray induced waxy mutation in maize (Eriksson, 1963); d) γ-ray 8-azg resistance in Chinese hamster cells (Chu, 1971)

According to equation (4), a plot of survival on a logarithmic scale, versus mutation frequency per survivor on a linear scale, should give a straight line curve with a slope (-p/q) passing through the origin (S = 1, M = 0). Figure 1 illustrates four examples of this analysis applied to data, mainly taken from the literature, which are in good agreement with the theoretical proposals, even though the original survival and mutation dose relationships are non-linear (when the logarithm of survival and mutation dose relationships are linear, equation 4 holds automatically without necessarily implying any causal relationship between the two phenomena).

Application to other mutagenic agents

The equation lnS = -(p/q)M is independent of the way, or kinetics, by which the lesion is formed and depends only on the assumption that the critical lesion leading to either cell killing or mutation is the same. The analysis has therefore been applied to other mutagenic agents, such as ultra-violet light and chemicals, in addition to ionizing radiation. The results of this analysis are illustrated in Figs.2 and 3. The straight line curves found in accordance with equation (4) can be explained by assuming that there is a similar causal relationship between cell killing and mutation induction, following treatment with a variety of chemicals and ultra-violet light, to that existing for ionizing radiation.

Discussion

The critical lesion

The straight line curves shown in Figs.1-3 analysed according to equation (4) can be interpreted by assuming that in each case the type of lesion which leads to mutation induction is the same as the type of lesion which leads to cell death. The similarity between the analyses for the different mutagenic treatments does not imply that the lesion induced by chemicals is exactly the same as the lesion induced by ionizing radiation, although, when a similar value for p/q is found for a chemical treatment (Roberts, Sturrock and Ward, 1974) and a radiation treatment (Richold and Holt, 1974) for the same mutation in the same cells (see Fig.2a), this does suggest that the same, or at least a similar, type of lesion may be involved following the different mutagenic treatments. It is known that many of the chemical mutagens react with the DNA molecule either to form complexes or to cause geometric disruptions of the double helix and some cause single strand breakage (Setlow and Setlow, 1972). It is generally accepted that DNA is the most important target molecule for the action of radiation and ultra-violet light on the cell. We have developed mathematical relationships which can be used to describe the action of radiation on cell killing and mutation induction by assuming that the DNA double strand break is the most important radiation-induced lesion leading to both biological end points. We can not conclude from this analysis alone that the DNA double strand break is the critical lesion

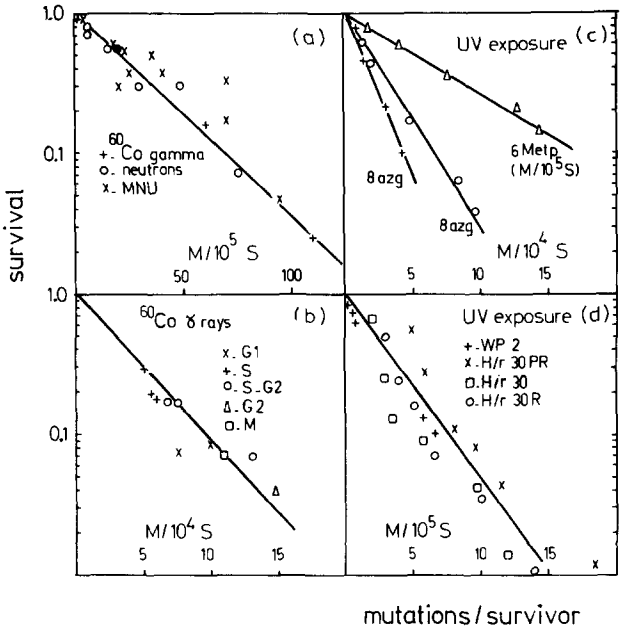

Fig.2. Analysis of mutation induction and cell survival according to lnS = (-p/q)M for different radiation types. a) γ-ray, neutron and N-methyl-N-nitrosourea (NMU) induced 8-azg resistance in Chinese hamster cells (Richold and Holt, 1974, Roberts et al., 1974); b) γ-ray induced 8-azg resistance in Chinese hamster cells at different stages of the cell cycle (Arlett and Potter, 1971); c) UV induced 8-azg resistance and 6 methyl purine resistance in *Aspergillus nidulans* (Arlett, 1966); d) UV induced mutation in *E.coli* H/r30 and H/r 30R with and without photoreversion and *E.coli* W.P2 with photoreversion (Doudney, 1966)

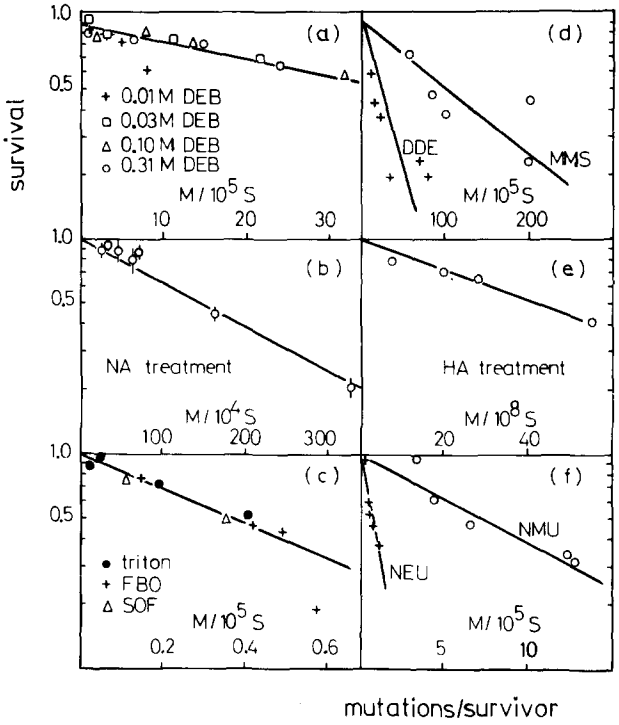

Fig.3. Analysis of mutation induction and cell survival according to lnS = (-p/q)M for different chemical treatments. a) reversion of ad-3A 38701 mutant in *Neurospora* by different concentrations of diepoxybutane (DEB) (Kilbey, 1974); b) induction of red ad-6 and ad-7 mutants in *Schizosaccharomyces pombe* 972 h⁻ by nitrous acid (Abbondandolo and Bonatti, 1970); c) induction of gene conversion in yeast strain D4 in the presence of different optical brighteners, Uvitex SOF, 2,5-dibenzoxazolylthiophene and Blanko-phor FBO in triton (Kilbey and Zetterberg, 1973); d) induction of 8-azg resistance in Chinese hamster cells following treatment with methyl methanesulfonate (MMS) and 1,1-dichloro-2,2 bis (p-chlorophenyl) ethylene (DDE) (Kelly-Garvert and Legator, 1973); e) induction of reversion mutations in *Neurospora crassa* by hydroxyaimine (HA) (Malling, 1966); f) induction of methionine reversion in D19h⁻ *Schizosaccharomyces pombe* by N-nitroso-N-methylmethane (NMU) and N-nitroso-N-ethylmethane (NEU) (Guglielminetti et al., 1966)

for all mutagenic treatments but suggest that common basic damage to the DNA molecule is definitely implicated as the critical lesion leading both to mutation induction and cell reproductive death by different mutagens.

The value of p/q

Although variations in methodology from one experimenter to another may lead to differences in the efficiency of mutation expression and thus to differences in the value of p/q, for the same treatment in the same cell it should be possible to find some consistency under controlled experimental conditions. The relationship in equation (4) is independent of how the critical lesion is formed; thus, in the case of a random-non-specific agent such as ionizing radiation the value of p/q should be independent of the type or dose-rate of the ionizing radiation. This is illustrated in Fig.2(a) by the data of Richold and Holt (1974) for neutrons and gamma rays. Chemicals, which may be more specific in their interaction with the cell, will probably give different values of p/q varying from chemical to

chemical. However, one chemical should give the same value of p/q independently of concentration-time effects, at least at the acute level; this is illustrated in Fig. 3a using the data of Kilbey (1974) for different "dose-rates" of the chemical diepoxybutane (DEB). One important question is whether the value of p/q is an absolute constant for a specific treatment or whether the value can be influenced by altering the circumstances in the cell during and after the treatment. We suspect that factors which affect the repair of lesions in the DNA will influence the value of p/q: for instance, a misrepaired DNA double strand break might lead to a mutation but be less critical for cell survival than an unrepaired double strand break.

In view of the increasing concern for the mutagenic and possibly carcinogenic hazards from many environmental chemicals and ionizing radiation, we believe that this form of analysis will be valuable for comparing various hazards and may eventually provide an initial basis for the rationalisation of the hazards from chemicals to those of radiation, which has been suggested by Bridges (1974), and for which there exist internationally accepted norms.

It is also possible that this analysis could be used with specifically selected mutagens to learn more about the basic mechanisms involved in the formation of the critical lesion.

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