# Radiobiological Evaluatory Report

## Dose-effect relationships:
## Components of the survival curve - adaptive response

M.C. JOINER

Gray Laboratory Cancer Research Trust
P.O. Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, UK

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Tel: Int + 44 1923 828611
Fax: Int + 44 1923 835210
Email: joiner@graylab.ac.uk

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## Summary

Radioprotective mechanisms, or stress responses, exist that are upregulated in response to exposure to small doses of ionizing radiation and other DNA-damaging agents. Phenomenologically, there are two ways in which these *induced* mechanisms operate. First, a small conditioning dose (generally below 30 cGy) may protect against a subsequent, separate, exposure to radiation that may be substantially larger than the initial dose. This has been termed the adaptive response. Second, the response to *single* doses may itself be dose-dependent so that small acute radiation exposures, or exposures at very low dose rates, are more effective per unit dose than larger exposures above the threshold where the induced radioprotection is triggered. This combination has been termed low-dose hypersensitivity (HRS) and induced radioresistance (IRR) as the dose increases.

In studies on cell lethality, both the adaptive response and HRS/IRR have been well documented with yeast, bacteria, protozoa, algae, higher plant cells, insect cells, mammalian and human cells *in vitro*, and in studies on animal models *in vivo*. There is indirect evidence that the HRS/IRR phenomenon in response to single doses is a manifestation of the same underlying mechanism that determines the adaptive response in the two-dose case and that it can be triggered by high and low LET radiations as well as a variety of other stress-inducing agents such as hydrogen peroxide and chemotherapeutic agents although exact homology remains to be tested. Little is currently known about the precise nature of this underlying mechanism, but there is evidence that it may operate by increasing the amount and rate of DNA repair, rather than by indirect mechanisms such as modulation of cell-cycle progression or apoptosis. Changed expression of some genes, only in response to low and not high doses, may occur within a few hours of irradiation and this would be rapid enough to explain the phenomenon of induced radioresistance although its specific molecular components have yet to be identified.

There is some evidence of apparent inverse dose or dose-rate effects for mutational or transformational endpoints following radiation exposure, but it has so far not been possible to unequivocally ascribe these to the effects of dose reduction or protraction rather than to modulation of the response by cell-cycle progression of cell proliferation which is generally an accompanying feature of dose-protraction protocols. In contrast, the inverse dose effect observed in the cell-killing response has been convincingly linked to a direct effect of dose or dose intensity. If cells exhibit low-dose HRS in the killing and in the transformation endpoints to the same extent, then cancer risk estimates would be unaffected. However, if cells exhibit low-dose HRS for cell killing but less or not at all for transformation, then acute doses up to 15 cGy might produce no significant risk of malignancy in those tissues and in some cases could even be protective.

## Introduction

The response to radiation exposure can be significantly determined by active or programmed biological intervention as well as by passive physical responses. The process of apoptosis is an example, where cells actively promote their own death in response to injury. In contrast, mammalian cells can also upregulate or induce the more efficient repair and control of DNA damage when faced by excessive injury which demands more aggressive repair to retain cell viability than can be provided by the constitutive processes that are available to cope with background damage to DNA, replication and housekeeping. The adaptive response to small radiation doses is a manifestation of this process of induced resistance and has been the subject of studies over many years particularly in human lymphocytes (see for example Wolff, 1992; Wolff, et al., 1988; Wolff, et al., 1989). This document focuses on another manifestation of induced resistance which may also be of concern to radioprotection, specifically the phenomenon by which cells die with excessive sensitivity to small, single, doses of ionizing radiation but are more resistant (per unit dose) to larger single doses. This may be because the greater amount of injury produced by larger doses is above some threshold for triggering an "adaptive"-type response, which then upregulates radioresistance on a similar timescale (or less) to the rate of repair of DNA damage. Typically, cells exhibit hyper radiosensitivity (HRS) to very low radiation doses (<0·3 Gy) which is not predicted by back extrapolating the cell-survival response from higher doses. As the dose is increased above about 0·3 Gy, there is increased radioresistance (IRR) until at doses beyond about 1 Gy, radioresistance is maximal and the cell survival follows the usual downward-bending curve with increasing dose.

## HRS/IRR in non-mammalian systems

As early as 1963, Eriksson (1963) described HRS/IRR experiments on irradiated maize plants detailing both *mutation induction* and *lethality* in pollen grains after acute low-dose gamma-ray exposures. Dose-response relationships from this work, presented by Chadwick and Leenhouts (1975), seemed to indicate hypersensitivity to doses less than 0·5 Gy compared with higher doses, for both these endpoints. Calkins (1967) raised the possibility of a threshold dose above which cells would acquire radioresistance; this idea was proposed to explain the shapes of such dose-survival relationships and, in experiments on the protozoan *Tetrahymena pyriformis*, a real *increase* in cell survival as the radiation dose was raised above this hypothetical level. The shapes of the survival curves of budding yeast (Beam, et al., 1954) and algae (Horsley and Pujara, 1969) also demonstrate a similar pattern in the low-dose region.

The *adaptive response* has also been detected in lower cell systems. An early example is the work of Hillova and Drasil (1967) with the green unicellular alga *Chlamydomonas*. In sporelings of the fern *Osmunda*, Hendry (1986) showed that prior irradiation would increase the radioresistance by a factor of 3–4 to subsequent exposures given 5 hours later but that this radioprotective effect had apparently decayed by 24 hours. Based on this and evidence from

many other studies, Hendry concluded that this type of response was probably a feature of all photosynthesising cell types. Yeasts also demonstrate similar adaptive responses to ionizing radiation, as summarised by Boreham and Mitchel (1991), as well as the HRS/IRR pattern (Beam, et al., 1954).

The well-known variation of radiosensitivity of cells during progression in the cell cycle has been proposed to explain induced-resistance phenomena but it is not the explanation. For HRS/IRR, the argument was that low doses of radiation would eliminate cells predominantly in sensitive phases of the cycle and that higher doses would then need to kill cells in resistant phases of the cycle (this is a "two-population" explanation similar to the situation of oxic and hypoxic cell populations in tumours which respond with differing sensitivity). For the adaptive response, the argument was that a first dose of radiation would produce partial synchrony by eliminating cells predominantly in sensitive phases of the cycle. The surviving cohort(s) of cells would then re-present in resistant phases of the cycle some time later, for the second, larger, radiation dose. Several pieces of evidence rule these arguments out in these non-mammalian cell systems. For example, Howard and Cowie (1976) have shown that in the desmid *Closterium*, initial doses, which were only 10% of those needed to produce any measurable response "off the shoulder", induced a factor of almost two increase in both incremental (increased $D_0$) and absolute radioresistance to a subsequent larger dose. This increased radioresistance was significant within 1 hour following the small conditioning dose but required 6 hours to reach maximum. The data could not be explained by the known variation in radiosensitivity of these cells within the cell cycle and subsequently (Howard and Cowie, 1978) the same authors showed that cells kept in darkness, hence in cycle arrest, demonstrated the same adaptive response and that this could be inhibited by the presence of cycloheximide during the period between the conditioning dose and the subsequent challenge dose. Other studies on lower organisms have reached similar conclusions. For example, Horsley and Laszlo (1971; 1973) have examined *synchronous* cultures of the green alga *Oedogonium* and found that a first dose of radiation induced resistance to a subsequent dose, that was hugely in excess of any change in sensitivity that could be explained on the basis of cell-cycle progression between the two doses. Bryant (1972) has also tested the alga *Chlamydomonas* and Santier *et al.* (1985) have tested the green alga *Chlorella*, and the same picture emerges.

In single-dose studies, Koval (1984) has found multiphasic cell-survival curves demonstrating HRS/IRR for the lepidopteran insect cell line TN-368 irradiated in either air or nitrogen. A similar oxygen enhancement ratio was found for both the low-dose (sensitive) component and the high-dose (more resistant) components of survival. In this and subsequent papers (Koval, 1986; Koval, 1988) it was shown that this low-dose substructure in the single-dose survival curve for these cells did result from induction of radioresistance with increasing dose. The survival curve for nitrogen demonstrated a transition region between the low and high resistance portions where the slope (change in survival with dose) was actually zero. Following

on from the ideas of Beam *et al.* (1954), if the substructure in this survival curve was explained as the sum of the individual responses of two or more cell populations with differing radiosensitivity, as in the cell-cycle phase explanation outlined above, then a zero-slope could only arise if the sensitivity of one of the populations was negative in this range of dose, therefore indicating that survival would be greater than 100% for that cell population alone in response to radiation. This is clearly not sensible, for cell-cycle phase or any other variations in radiosensitivity. It is more reasonable, based on the evidence for adaptive responses in photosynthesising cells, to explain these survival curve shapes as a result of *dose-dependent* radiosensitivity, i.e. induced radioresistance.

## HRS/IRR in mammalian systems

The existence of HRS/IRR and the adaptive response in mammalian systems is of considerable relevance to radioprotection. Improvements in the methodology of clonogenic assays within the last decade (Palcic, et al., 1983; Palcic and Jaggi, 1986; Spadinger and Palcic, 1992; Spadinger and Palcic, 1993; Spadinger, et al., 1989; Spadinger, et al., 1990; Wouters and Skarsgard, 1994) have made it possible to examine the response of *mammalian* cellular systems to radiation with sufficient accuracy to resolve changes in radiosensitivity at doses less than 1 Gy where cell survival approaches 100%. Conventional colony assays cannot reliably measure radiation-produced mammalian cell death in this low-dose region. Prior to these developments, similar studies were generally possible only in non-mammalian systems which respond at higher doses, as reviewed above. The two main protocols for improving the accuracy of cell-survival measurement both determine the number of cells *precisely* that are "at risk" in a colony-forming assay. This is achieved using either a fluorescence-activated cell sorter (Wouters and Skarsgard, 1994) to *plate* an exact number of cells or microscopic scanning to *identify* an exact number of cells after plating (Spadinger and Palcic, 1993). Using the latter technique, Marples & Joiner (Marples and Joiner, 1993; Marples, et al., 1992) were first to define in mammalian cells (V79 hamster fibroblasts) HRS and IRR in the dose range less than 1 Gy. As illustrated in Figure 1, cells exhibit extreme sensitivity to very low radiation doses which is not predicted by back extrapolating the response from higher doses. As the dose is increased above about 0·3 Gy, the radioresistance increases until at doses beyond about 1 Gy, radioresistance is maximal and cell-survival follows the usual downward-bending curve with increasing dose. With V79 cells, HRS/IRR features can be demonstrated following X-irradiation but not following *single-dose* irradiation with high-LET neutrons, but this does not mean that high-LET radiation doesn't induce radioresistance in adaptive-response protocols (see later). As with the non-mammalian systems reviewed above, this low-dose substructure cannot be explained by any differential sensitivity of cells in different phases of the cell cycle (Marples and Joiner, 1993). As with the lepidopteran-cell studies (Koval, 1984), V79 cells demonstrate the HRS/IRR phenomenon under both oxic and hypoxic conditions (Figure 2) and this leads to a generally decreasing Oxygen Enhancement Ratio with decreasing dose, but with a dose range between 2 down to 0·6 Gy over

which a small *increase* is seen (Marples, et al., 1994a). Therefore, there is significant homology in the HRS/IRR phenomenon between mammalian and non-mammalian systems, leading one to suspect that the effects result from a conserved stress-response mechanism.

## HRS/IRR in human cells

There is now definitive data on the very low-dose response of 15 different human cell lines, twelve from the Gray Laboratory group using the microscopic cell-location assay (Lambin, et al., 1994a; Lambin, et al., 1994b; Lambin, et al., 1994c; Lambin, et al., 1996; Lambin, et al., 1993, Short & Joiner unpublished) and a further four from the group in Vancouver (Wouters and Skarsgard, 1994; Wouters, et al., 1996) who have measured the extent of low-dose hypersensitivity using the FACS assay. The Vancouver group has also tested the HT29 line (Wouters and Skarsgard, 1994; Wouters, et al., 1996), and produced closely similar data using their techniques, to the data published by the Gray Laboratory group on HT29 cells demonstrating HRS (Lambin, et al., 1993). Therefore, low-dose hypersensitivity in mammalian cells has now been well documented by two laboratories using different assay techniques and different conditions of cell growth, handling and irradiation. The current library of 15 human cell lines tested by the two laboratories consists of three colorectal carcinoma lines, one bladder carcinoma line, three melanoma lines, one prostate carcinoma line, one cervical squamous carcinoma line, one lung adenocarcinoma line, one neuroblastoma line, three glioma lines and one non-malignant lung epithelial line. It is those cell lines most radioresistant to 2-Gy doses that demonstrate the most marked HRS: these are the HT29 (colorectal carcinoma), U1 (melanoma), Be11 (melanoma), RT112 (bladder carcinoma) T98G and A7 (glioma). Four cell lines show no evidence of HRS: HX142 (neuroblastoma), SiHa (cervical carcinoma) and SW48 (colorectal carcinoma). Three of these are very radiosensitive at 2 Gy, but in U373, with $SF_2$ equal to 0.54, HRS has so far been undetectable in our laboratory. At present it is not clear whether this results from lack of resolution in the assay coupled with very efficient IRR or whether this is a truly HRS/IRR-deficient, resistant cell line. Figure 3 shows example data from three relatively resistant cell lines and three more sensitive lines. Figure 4 summarises all the data from the 15 cell lines tested so far and shows the general pattern of increased extent of HRS/IRR in more radioresistant cell lines (with U373 and SiHa being exceptions). An implication of these data is that the property of intrinsic radiosensitivity may be dose dependent at least for certain cell lines. Further, the radiosensitivity in the *initial* low-dose region of the survival curve is similar for all the cell lines regardless of the extent of their high-dose radiation response (Lambin, et al., 1996).

There are broadly two mechanistic hypotheses to explain differences in radiosensitivity between cell types. On one hand, greater radiosensitivity would result from the incidence of a larger number of lesions per gray than the average for most cell lines (Peacock, et al., 1992; Radford, 1985), or alternatively, greater radiosensitivity would be due to a deficiency in the repair of lesions, i.e. quality or quantity of repair (Giaccia, et al., 1992; Malaise, et al., 1989;

Powell and McMillan, 1994). The very rapid change in radiosensitivity over the first gray in V79 and HT29 cells supports the latter hypothesis and if this phenomenon is a manifestation of "induced" radioprotection (paralleling the adaptive response) then a consequence is that resistance to radiation doses greater than 1 Gy would be determined, at least in part, by the amount of such "induced radioprotection".

## HRS/IRR in vivo

There is evidence that hypersensitivity *in vitro* can translate into additional effectiveness of fractionated radiotherapy given in very small doses per fraction. Thus when the dose per fraction is reduced below 1 Gy, the total dose needed to produce damage *decreases* in skin (Joiner, et al., 1986), kidney (Joiner and Johns, 1988), lung (Parkins and Fowler, 1986) and in the only example tested so far of an experimental tumour system exposed to as many as 126 fractions (Beck-Bornholdt, et al., 1989). This "reverse" fractionation effect is precisely that expected from the HRS/IRR pattern of cell survival following low doses in cell lines, but would imply a decay of adaptive resistance in these mammalian systems over the period between fractions. In these studies, this interval was 7–8 hours.

## Are HRS/IRR and the adaptive response homologous phenomena?

If the adaptive response and HRS/IRR are consequences of the same underlying mechanisms, then following a small conditioning dose, there should be no evidence of HRS in response to a second challenge dose. This hypothesis has been tested in the V79 hamster cell system, which is one of the most reproducible models for studying HRS/IRR (Marples and Joiner, 1995). Specifically, it has been confirmed that either small "priming" doses of X rays or hydrogen peroxide induce resistance to a "challenge" dose of radiation given a few hours later. This adaptation is dose dependent (Figure 5, upper panels) with priming doses of 20 cGy being more effective in abolishing challenge-dose HRS than higher doses. The adaptive response takes several hours to reach maximum and then decays away; by at most 24 hours (Figure 5, lower panels; intervals between 6 and 24 hours were not tested in this study) cells have returned to their hypersensitive baseline (Marples and Joiner, 1995). Figure 6 (upper) shows that the induction of increased radioresistance after single doses of X rays in V79 cells is inhibited by cycloheximide which demonstrates the need for protein synthesis in the development of IRR (Marples and Joiner, 1995). Cycloheximide also inhibits the *adaptive response* in the same cell line (Figure 6, lower), illustrating further homology between the two induced-resistance phenomena.

In the HT29, RT112 and T98G human cell lines, the decay of radioresistance and return to a hypersensitive state occurs by 6-8 hours following an initial small dose (Aleman & Joiner; Short & Joiner, unpublished). This decay of induced radioresistance is essential to understanding the effect of very low-dose fractionation, as in the normal tissue studies described above, in which each successive radiation treatment must produce the hypersensitive response in order to

derive an increased effect of the overall schedule. Little is yet known about this decay time (hence optimum interval between fractions) in mammalian cells except for the limited examples cited above, but it might be expected that it would depend on the size of the priming or conditioning dose, with smaller priming doses associated with a faster return of radioresponse to the baseline hypersensitive state following induced resistance. An implication for radioprotection is that successive small radiation exposures would each illicit a sensitive cell-killing effect. These data also imply that continuous, very protracted exposure at dose rates less than about 10 cGy per hour would also result in a sensitive lethal response. This hypothesis remains to be tested although there may be some evidence that low *dose-rate* HRS can be detected using mutational assays (see below).

## Mechanisms underlying induced radioresistance

At low single doses, where HRS dominates, cells can be up to 20 times more sensitive than at doses greater than 1 Gy (which trigger IRR) at which measurements of the cell-killing effects of radiation exposure are usually made. For the cell lines tested so far, the amount of this *change* in radiosensitivity over the first gray is correlated with intrinsic radioresistance (at higher doses) and hypersensitivity to low single doses (compared with doses >1 Gy) is not even seen with very "sensitive" cell lines, for example SW48 and HX142. If this change reflects induced repair mechanisms as proposed above, then intrinsic radiosensitivity (to doses of 1 Gy and above) could be linked to the repair ability of cells. There is accumulating evidence that the induced radioresistance, inferred from Figures 1 and 3, and adaptive responses to small conditioning doses of radiation, may indeed be due to increased repair or repair fidelity. For example, the adaptive response in lymphocytes is blocked by 3-aminobenzamide (Wolff, 1992), suggesting that repair pathways involving poly(ADP-ribose) polymerase may be involved. 3-aminobenzamide also blocks the development of IRR in single-dose HRS/IRR in V79 cells (Marples and Joiner, 1997), so that radiation response continues to follow the low-dose hypersensitive pattern out to higher doses. Some of the strongest evidence for inducible repair comes from studies measuring the reactivation of radiation-damaged virus (e.g. adenovirus 5) by the DNA-repair mechanisms of a subsequently-infected host mammalian cell. The host cells are usually not irradiated so the endpoint is a functional test only of host-cell repair and repair fidelity (Eady, et al., 1992). However, in some cases, if the host cells are pretreated with small doses of DNA-damaging agents, increased ability to reactivate damaged virus can be measured compared with untreated host cells. This apparent induced repair has been seen with cells given conditioning doses of UV (Jeeves and Rainbow, 1983), gamma rays (Jeeves and Rainbow, 1979) and chemicals (Sarasin and Hanawalt, 1978). In further support of repair involvement, the *rad52* mutant of *Saccharomyces cerevisiae*, which is *deficient* in recombinational repair, does *not* seem to show an adaptive response (Mitchel and Morrison, 1987). Although there is no evidence for specific mutations responsible for the radiosensitivity of either SW48 or HX142, repair involvement is also supported by Skov et al (1994) who measured the low-dose response of 3

hamster cell lines specifically defective in DNA repair, compared with their parental lines. The V79-derived double-strand break repair-deficient line (XR-V15B) showed a purely exponential survival response with no increased radioresistance in the zero to half-gray range compared with the V79B wild type. The UV-20 line, which is defective in excision repair, also appeared to respond exponentially with no evidence of induced radioresistance. However, the *single-strand* break repair-deficient line EM9 did show some low-dose hypersensitivity and induced radioresistance. This suggests that the dual phenomena of low-dose hypersensitivity and induced radioresistance at higher doses (HRS/IRR) may be linked to double-strand break and excision repair mechanisms. Clearly, it would be useful to test this hypothesis further, specifically in SW48 and HX142 cells using reactivation of radiation-damaged adenovirus in the presence or absence of small conditioning doses. Support for a direct link between induced radiation resistance and DNA dsb repair comes recently from Wojewodzka (1995) in studies with human lymphocytes and Ikushima *et al* (1996) in studies with V79 cells, who have both indicated that small conditioning doses of X-rays or hydrogen peroxide lead to faster and more complete rejoining of DNA double-strand breaks following exposure to a higher challenge dose of radiation several hours later.

At the Gray Laboratory, we have tested two other hypotheses to explain HRS/IRR. First, HRS might reflect apoptosis (hence high sensitivity) at low doses as a means of removing genomically-unstable cells from the population; as the dose increased apoptosis would be down-regulated to allow cell-population survival as a priority. In work on a range of cell lines demonstrating HRS/IRR, we have found no evidence to support this hypothesis. However, more sensitive methods for detecting apoptosis, specifically following low-dose radiation exposures, are under development (Matthews, et al., 1995) and may shed further light on this question. Second, cell-cycle delay increases with dose in many cell lines, this would imply a longer available time for repair with larger doses and hence more sensitivity to lower doses. However, in a range of cell lines, we have found no consistent correlation between HRS/IRR and a cell-cycle delay versus dose relationship.

Mammalian cell studies with single doses (Lambin, et al., 1993; Marples, et al., 1994b) suggest that high-LET radiations are less able to induce radioresistance than X rays at similar levels of cell killing. In yeast, small neutron conditioning doses are less efficient than X rays at producing adaptive protection against subsequent large X-ray exposures and hypoxia reduces the efficiency of X-ray conditioning doses (Boreham and Mitchel, 1991). Therefore damage by free-radicals and low-LET radiations might be particularly good at inducing radioprotection, supporting the notion that at the very small doses (a few centigray) needed to produce adaptive responses in human lymphocytes (Wolff, 1992), only single-strand breaks or actual ionisations themselves would be abundant enough to be candidates for the primary inducing event. Small doses of hydrogen peroxide as well as X-rays can induce protection against subsequent doses of ionizing radiation, supporting the notion of oxidative species or single-strand breaks as inducers

(Gupta and Bhattacharjee, 1988; Marples and Joiner, 1995). This is exampled in Figure 7 where V79 cells pretreated with 100 μM (but not 10 μM) hydrogen peroxide for 20 minutes lose their HRS in response to small, single X-ray exposures delivered 90 minutes later. Priming or conditioning treatment with small doses of $^{60}Co$ γ rays also protects human fibroblasts against subsequent doses of X rays (Azzam, et al., 1992). However, Marples & Skov (1996) reported that 20-cGy doses of high-LET (d(4)-Be) neutrons could produce an adaptive resistance to subsequent 1-Gy challenge doses of X-rays in V79 cells and that this adaptation was at least as large as that induced by 20-cGy doses of X rays. This apparent contradiction with the lack of HRS/IRR noted in previous work with this cell line (Figure 1) can be resolved by considering that the overall response of these cells to neutrons is extremely steep, indeed insignificantly different from a linear relationship between log survival and dose. In single-dose exposures therefore, cells are killed by complex lesions on which repair is largely ineffective. However, cells which survive a small dose of neutrons are still effectively adapted to subsequent doses of lower LET radiation where repair is important in determining response. This explanation is supported by (Lambin, et al., 1993) who showed that the response of HT29 cells to single neutron doses was characterised by a detectable curvature on the relationship between log survival and dose. In this more resistant cell line which is more prone to X-ray HRS, there is evidence of HRS with small doses of neutrons being more effective than predicted by extrapolation from higher doses.

Skov et al (1995) have shown that HRS is diminished in cells labelled with $^{14}C$- or $^{3}H$-labelled thymidine. This implies that such cells are relatively radioresistant at low doses of X rays, presumably because of induced radioprotection resulting from continuous low-dose rate exposure from isotopic decay in DNA. Pretreatment with cisplatin also increases resistance to subsequent low-dose radiation exposures by abolishing HRS (Marples and Skov, 1995). It therefore seems likely that most DNA-damaging agents can "prematurely" trigger the putative protective mechanism so that HRS is lost when cells are exposed to subsequent doses of radiation. However, while damage to DNA is a strong candidate for the inducing trigger, this has not been proven and radiation damage to other cellular structures, particularly membranes, could also be important in initiating radioprotective signals.

Changes in gene transcription and/or protein levels following radiation have been widely reported. However, most of these studies have been in the higher radiation dose range. Robson et al (1995; 1997; Robson, Joiner & Arrand, pers. comm.) have reported no change in expression of a panel of 16 genes reported previously to be high-dose regulated, 90 minutes following exposure of L132 cells to 0·5 Gy X rays. This is a dose which is at about the trigger level for single-dose IRR in this cell line (Singh, et al., 1994). Only the *Gadd45* gene was upregulated slightly. However, following differential cDNA library screening to detect for changes in message, a novel gene was identified. The message (clone 8.6) is downregulated maximally following doses of 0·2 Gy, reaching minimum levels by 1 hour following radiation

and returning to normal levels by 8 hours. This is similar to the dose and time scale of the adaptive response. The 8.6 gene appears to have homology with four heat-shock related proteins, including peptidyl prolyl cis-trans isomerase (PPIase), p59 protein (mouse and human, also with PPIase activity), oestrogen-receptor binding immunophilin and yeast heat-shock protein. The first three of these have been reported to bind to HSP90. It is known that PPIases are present in complexes with HSP90, HSP70 and a variety of steroid hormone receptors (Lebeau, et al., 1992; Renoir, et al., 1990). Several kinases are also known to be involved in this complex, notably some tyrosine kinases encoded by oncogenes (Brugge, et al., 1981; Lindquist and Craig, 1988). It has been suggested that HSP90 may regulate these kinases by keeping them inactive during transportation to their proper location in the plasma membrane (Brugge, et al., 1983); in this regard HSP90 could be regarded as a molecular chaperone. A hypothesis is that the protein product of the 8.6 gene may stabilise these complexes and if downregulated following radiation, release of kinases would occur with a possible initiation of signal transduction which might ultimately result in increased transcription of repair factors or modulation of cell-cycle progression.

## Speculation on implications for cancer risk

The existence of HRS in the cell-survival response implies that cancer risk from small acute exposures to ionising radiation might be lower than current estimates, if the result of such HRS were to protect the cell population from mutational and initiating events in some cells by readily eliminating those cells from the population. In this sense, HRS would be a protective response at the organismal level. However for such a hypothesis to be correct, would require that a hypersensitivity to *mutation* following low-dose exposure either did not exist or if it did exist, it was present at a differential between low and high-dose sensitivity that was less than summarised in Figure 4 for the cell-killing HRS.

Several recent papers have reviewed the available data on the dependence of mutation frequency on dose rate (Amundson and Chen, 1996; Colussi and Lohman, 1997; Furuno Fukushi, et al., 1996) and the phenomenon of the *mutagenic* adaptive response whereby cells pre-exposed to very small doses of radiation exhibit less pronounced sensitivity to mutation following subsequent high-dose exposure compared with non pre-exposed cells (Rigaud and Moustacchi, 1996). The existence of an inverse dose-rate effect, with continuous exposures typically less than 10 cGy per hour inducing a higher mutation frequency than similar doses given at higher dose rates, has been documented in both rodent and human cell lines. However, this phenomenon is controversial. It has not been seen universally even in studies using the same cell lines and endpoints. Most studies have used either the *hprt* or *tk* loci and it remains to be seen whether any inverse dose-rate effects can be seen in more critical loci associated with oncogenes or tumour-supressor genes. Critically, the issue of cell-cycle progression cannot be ignored in

any studies with protracted continuous exposures and the cases where an inverse dose-rate effect for mutation has been reported have not been able to definitively rule this out.

In contrast, the evidence for a mutational adaptive response is more compelling, since cell-cycle and progression effects can be largely ruled out as radiation exposures can be given as acute, high dose-rate exposures. These experimental protocols are similar to the methods used in defining the cell-killing adaptive response, as reviewed above. As with the inverse dose-rate studies, *hprt* and *tk* endpoints have dominated the work to date. Given the apparent similarities between HRS/IRR and the adaptive response in cell killing (reviewed above), it would not be unreasonable, based on the existence of mutational adaption, to postulate the existence of mutational HRS/IRR in the response to single, acute doses. However, at the present time there is no direct evidence supporting the existence of single-dose mutational HRS/IRR in mammalian cells, which contrasts with the situation for the cell lethality endpoints where the existence of HRS/IRR is incontrovertible.

In the region of HRS, cell survival falls rapidly with increasing dose ($D$) over the first 10–20 cGy, at a rate corresponding to a value of $\alpha$ equal to approximately 1 $Gy^{-1}$ in the relationship Surviving Fraction $= e^{-\alpha D}$. Because of this exquisite sensitivity, mutation (based on current estimates in the absence of mutational HRS) would be more than compensated for by cell kill at doses less than about 10 cGy which would lead to overall transformational risk for the cell population being similar or even lower than the value for unirradiated cells. Enormous caution should be sounded in reaching this conclusion, as it depends completely on the assumption of lack of mutational HRS which remains to be proven. However, it seems reasonable to conclude that, based on our present knowledge of HRS, current risk estimates from exposure to low, acute radiation doses probably represent the upper boundary of the true cancer risk.

## Conclusions

Hypersensitivity to cell killing by low, single, acute radiation doses coupled with increasing radioresistance with dose, could result from the same mechanism which controls the adaptive response. There is considerable homology between these two phenomena, although definitive proof of this hypothesis awaits the identification of the underlying mechanisms. There is accumulating evidence that the actual rate and extent of repair of DNA damage is implicated in this mechanism, rather than indirect effects like, for example, cell-cycle control or apoptosis. In addition, a significant component of resistance to cell killing by high radiation doses appears to be this induced repair. At the present time, a definitive conclusion as to the effect of cell-killing HRS on cancer-risk estimates at low doses cannot be made, since the existence of an equivalent HRS for mutation induction cannot be ruled out. However, in those cases where cell-killing HRS was present in the absence of mutational HRS, cancer risk below acute exposures of about 10 cGy would be lower than currently estimated from high-dose exposure data.

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## Figure legends

**Figure 1** Survival of V79-379A Chinese hamster cells irradiated with single doses of 250 kVp X rays (X) or d(4)-Be neutrons (N) *in vitro*. Data points are mean ± SEM. At X-ray doses >1 Gy, the dose-survival relationship conforms to a conventional Linear-Quadratic (LQ) model. When extrapolated back below 0·6 Gy (dotted line in inset), this prediction from the high-dose data underpredicts the hypersensitive response to X rays actually seen (solid line). The response of V79 cells to neutrons is described by a simple exponential survival curve (dashed line) and there is no indication of increased sensitivity at low doses in this system. Data from Marples and Joiner, 1993.

**Figure 2** Survival of V79-379A Chinese hamster cells irradiated with single doses of 250 kVp X rays in air (Oxic) or under nitrogen (Hypoxic) *in vitro*. Data points are mean ± SEM. Hypersensitivity to low X-ray doses is evident for both conditions of oxygenation, as shown in the detailed inset as the data points and broken lines compared with predictions (solid lines) extrapolated from the fits of a Linear-Quadratic model to the data at high doses. Data from Marples, et al., 1994a.

**Figure 3** Survival of six human tumour cell lines following irradiation with 240 kVp X rays. The solid lines show the fit to the data of a model describing the induction of radioresistance with increasing dose (Lambin, et al., 1996). The dotted lines show the expectation if the response to high doses only is considered in the fit. The amount of excess response at low doses is correlated with the overall radioresistance as assessed by the survival response at 2 Gy and above. The most radioresistant cell lines show the most pronounced substructure in the response as the dose is reduced below 1 Gy; in contrast there is no evidence of low-dose hypersensitivity in the two most radiosensitive lines.

**Figure 4** The differential between radiosensitivity at very low doses and high doses, expressed as the ratio of $\alpha$ in the sensitive and resistant forms of the modified Linear-Quadratic equation developed to fit the low-dose substructure in the survival curve (for example solid lines in Figure 3, Lambin, et al., 1996). The diagram summarises data from 15 cell lines studied in two laboratories (see text). Two values are shown for the HT29 cell line, ⊙ from Lambin, et al., 1993; ⊡ from Wouters, et al., 1996. Higher values of $\alpha_S/\alpha_R$ indicate more pronounced low-dose hypersensitivity compared with the response extrapolated from high doses. Cells can potentially be a factor up to twenty times more sensitive to killing by low doses than expected on the basis of extrapolation from the high-dose response.

**Figure 5** Survival of V79-379A cells (mean ± SD) irradiated with single doses of 250 kVp X rays ( ● ) or irradiated with the indicated priming dose of X rays 6 h preceding the challenge doses shown on the dose axis (upper panels, ○ ) or irradiated with a 0·2-Gy priming dose at three intervals before the challenge doses shown in the dose axis (lower panels, ○ ). For the priming experiments, the data are normalised to account for the cell killing which results from the priming treatment alone. An adaptive response, maximum at 6 h following 0·2 Gy, abolishes hypersensitivity to low X-ray doses (HRS). Data from Marples and Joiner, 1995.

**Figure 6** Survival of V79-379A cells following single-dose exposures (upper panel) or adaptive response schedules (lower panel) of 250 kVp X rays. Unmodified response to single X-ray doses (both panels, ● ). Cycloheximide (33 μM) abolishes the development of IRR in single-dose exposures, if present between 1·5 h prior to and 6 h following irradiation (upper panel, ○ ). A priming dose of 0·2 Gy prior to challenge-dose irradiation abolishes HRS (lower panel, ■ ) but the presence of cycloheximide (33 μM) during the interval between the priming and challenge doses inhibits development of the adaptive response which again allows HRS to occur ( ○ ). Data from Marples and Joiner, 1995.

**Figure 7** Survival of V79-379A cells irradiated with single doses of 250 kVp X rays ( ● ), or pretreated with hydrogen peroxide on ice for 20 min, 1·5 h prior to a challenge dose of X rays shown on the dose axis ( ○ ). Hydrogen peroxide at a concentration of 100 μM is needed to elicit an adaptive response, which abolishes HRS in response to subsequent irradiation. Data from Marples and Joiner, 1995.

Figure 1

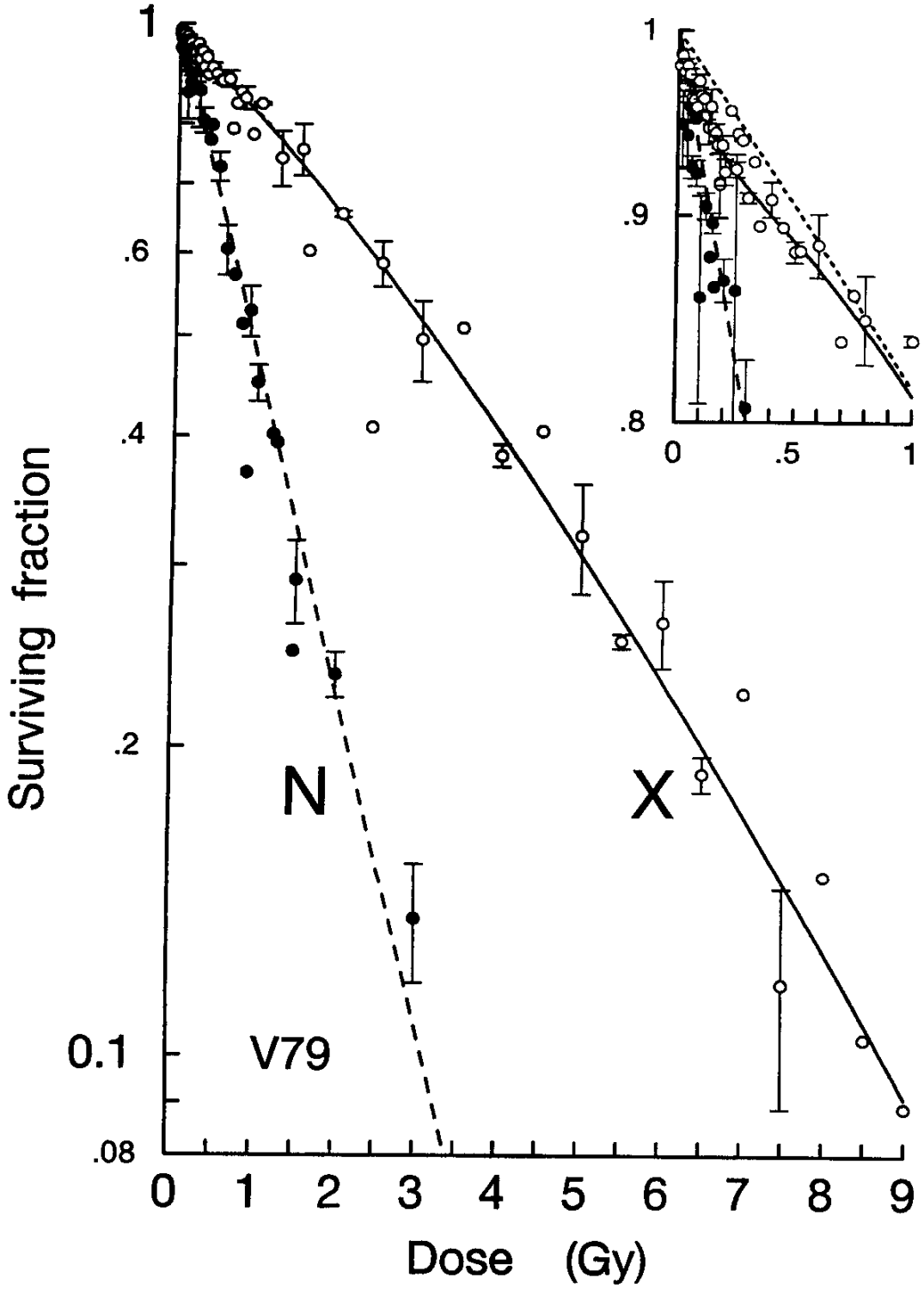
Surviving fraction
1
.6
.4
.2
0.1
.08
N
X
V79
0 1 2 3 4 5 6 7 8 9
Dose (Gy)
1
.9
.8
0
.5
1

Figure 2

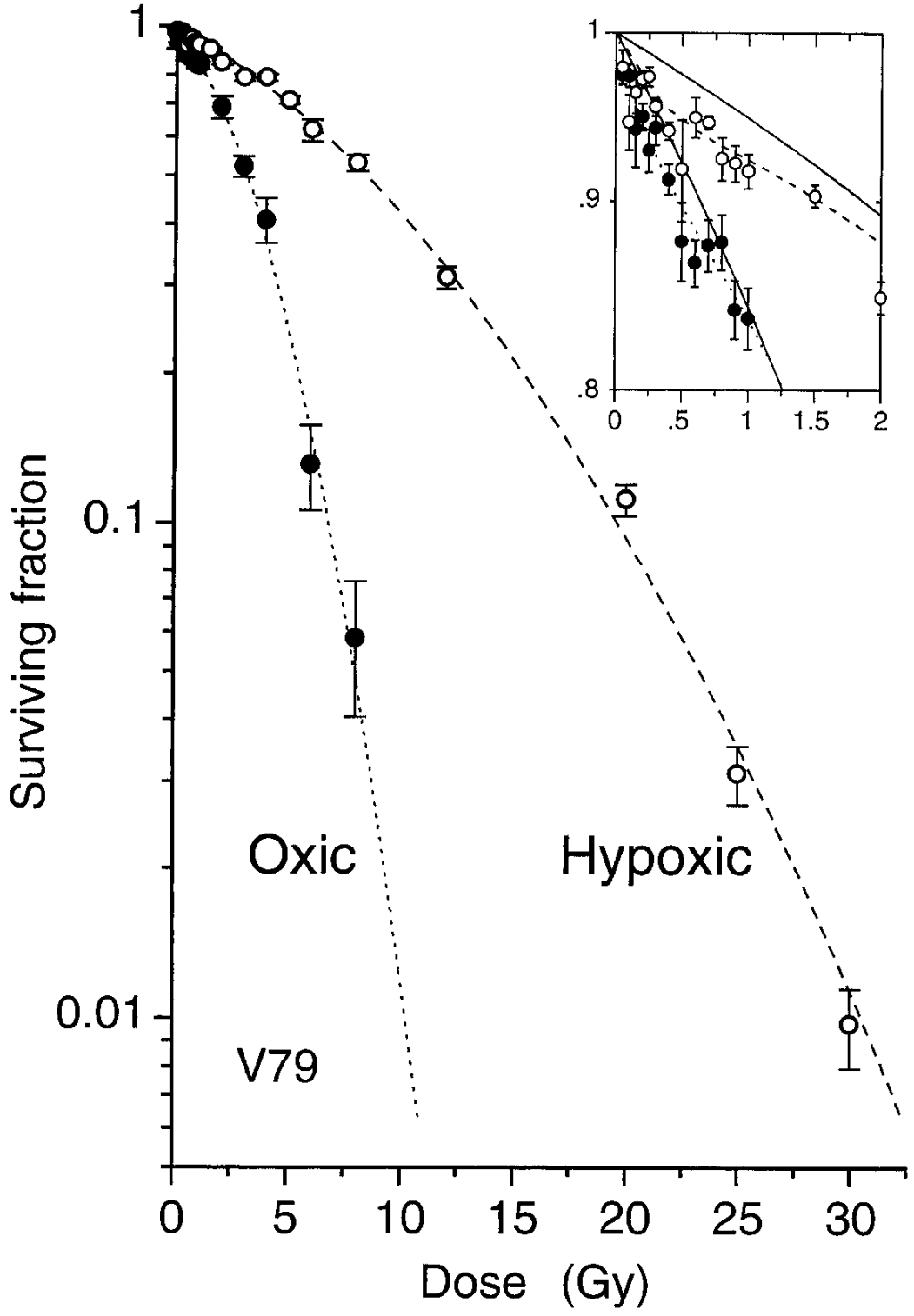
Surviving fraction
1
0.1
0.01
Oxic
Hypoxic
V79
0
5
10
15
20
25
30
Dose (Gy)
1
.9
.8
0
.5
1
1.5
2

Figure 3

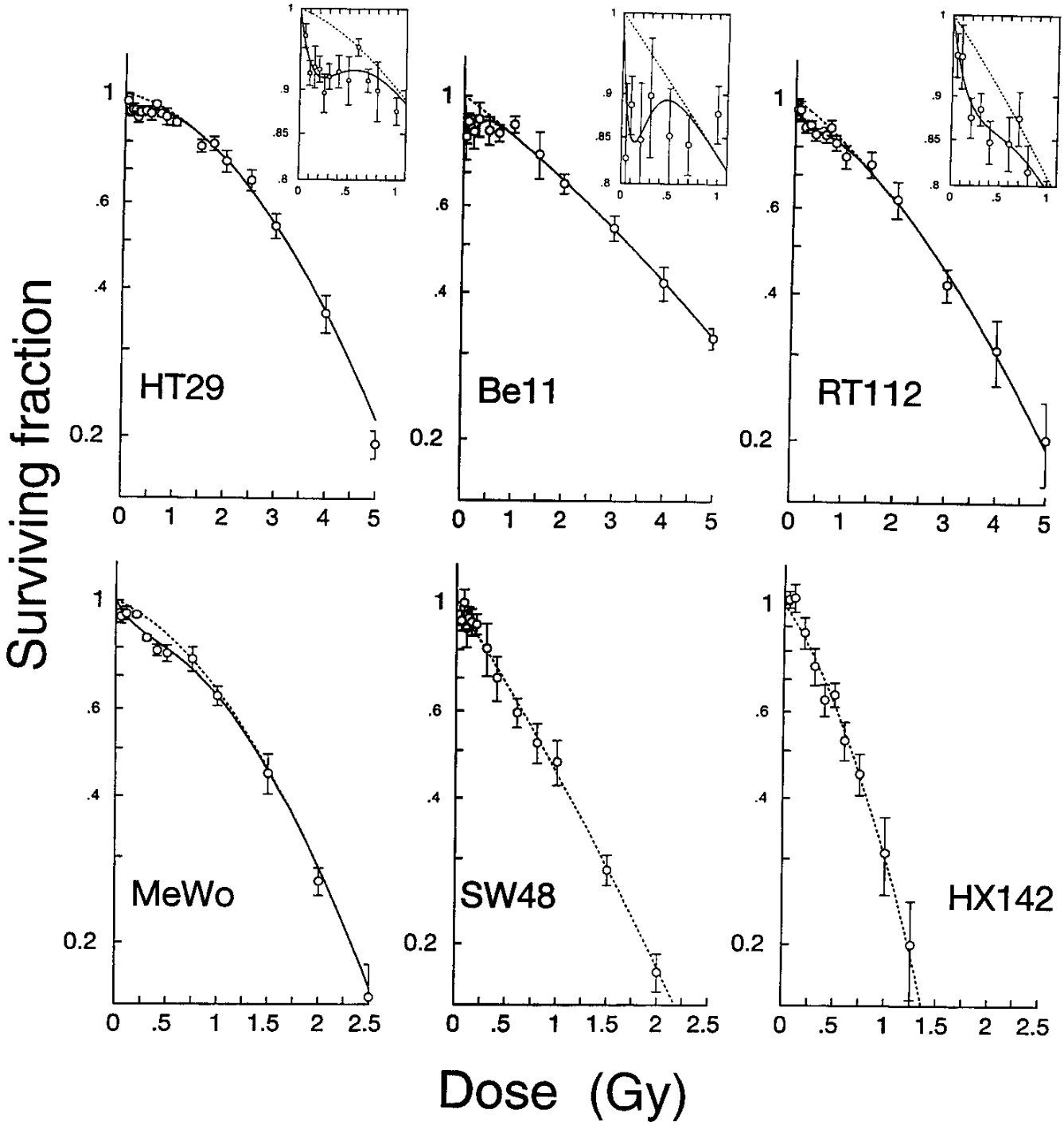

Figure 4

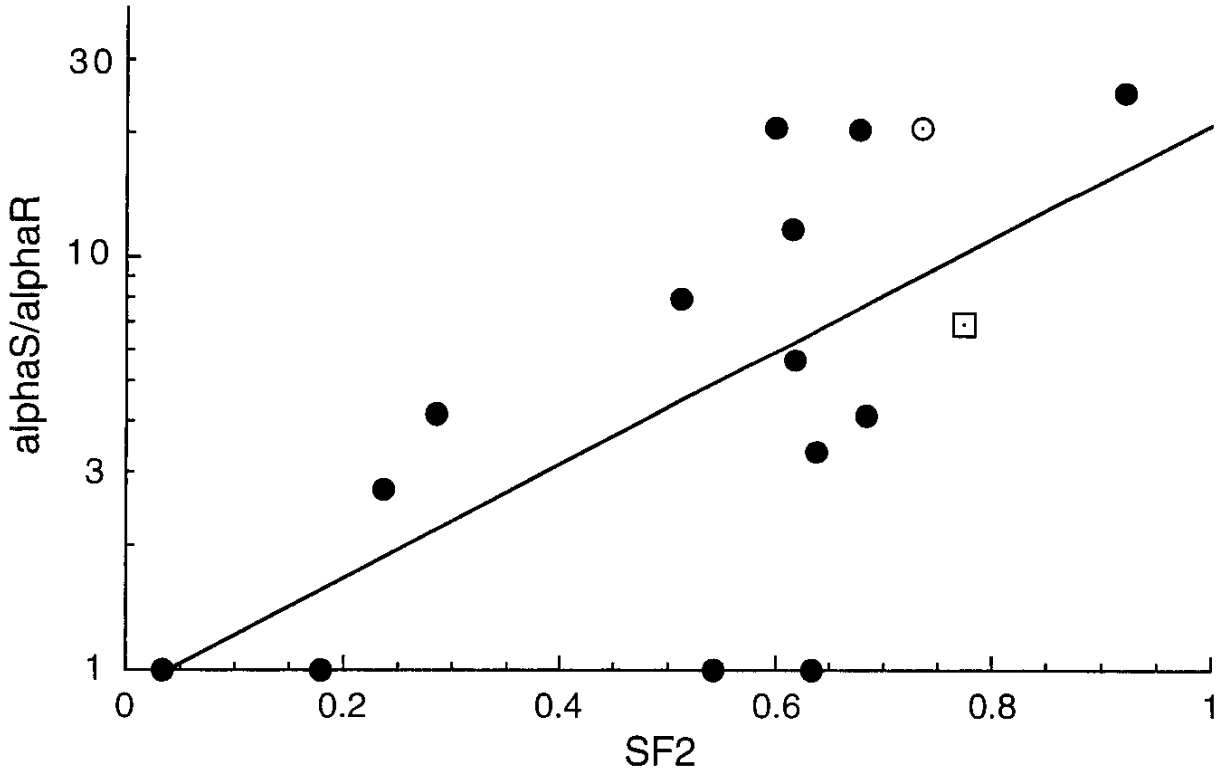

Figure 5

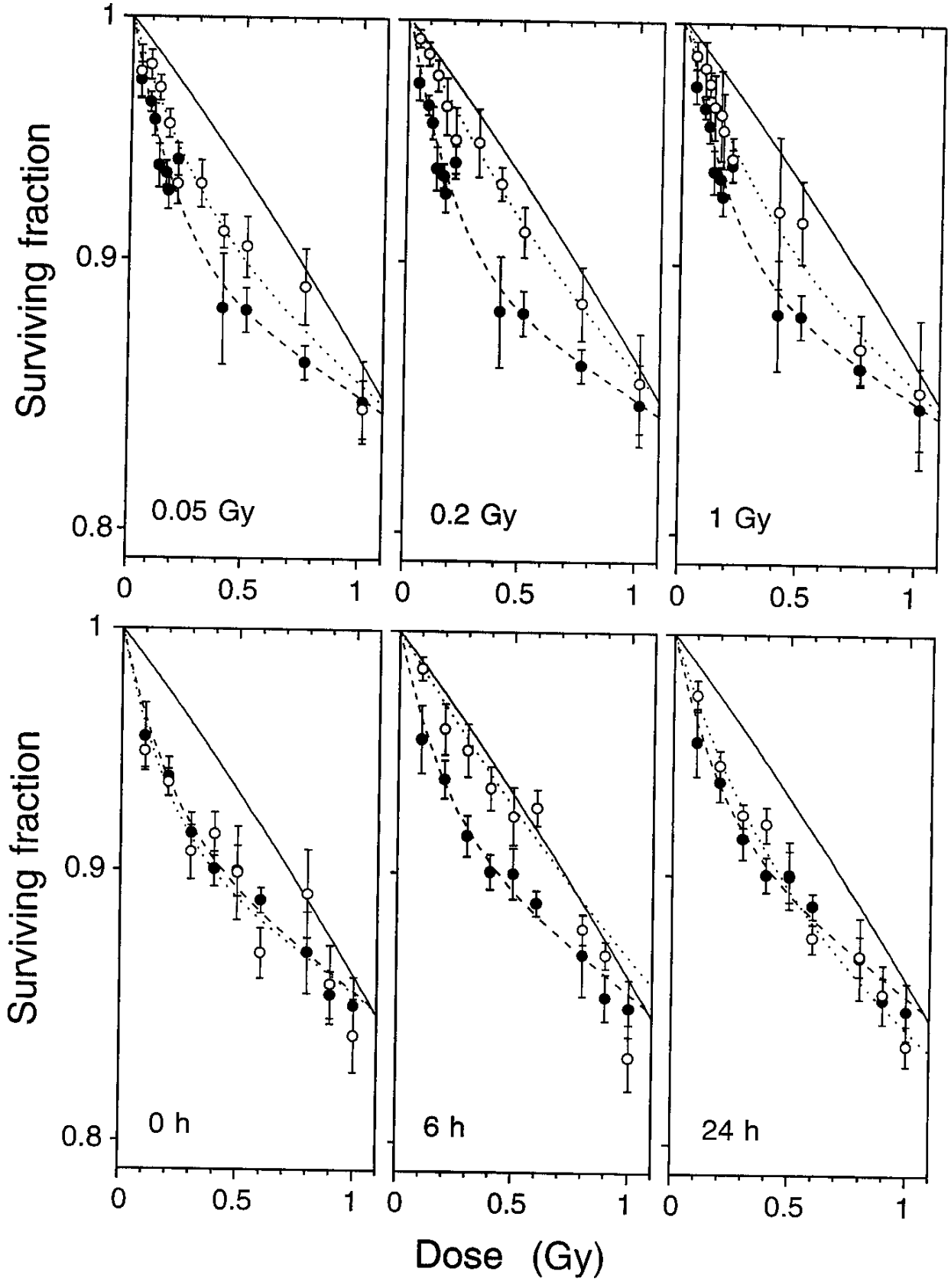
Surviving fraction
1
0.9
0.8
0.05 Gy
0.2 Gy
1 Gy
0 h
6 h
24 h
0
0.5
1
Dose (Gy)

Figure 6

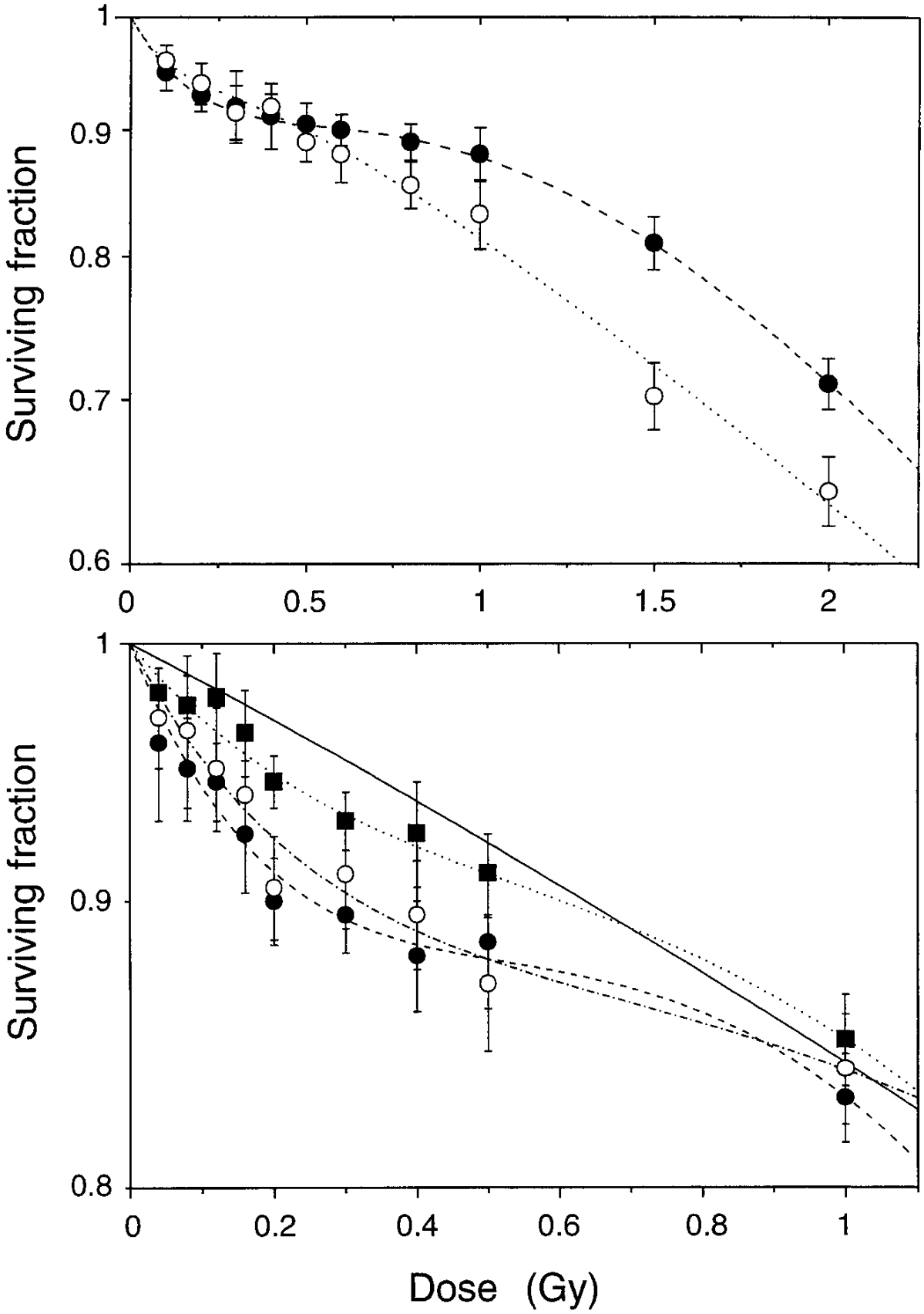
Surviving fraction
1
0.9
0.8
0.7
0.6
0
0.5
1
1.5
2
Surviving fraction
1
0.9
0.8
0
0.2
0.4
0.6
0.8
1
Dose (Gy)

Figure 7