Section 1

Introduction

(To be drafted later)

Section 2

NCRP Subcommittee 1-6 Linearity of Dose Response

2.0 DNA REPAIR AND PROCESSING AFTER LOW DOSES AND LOW DOSE RATES OF IONIZING RADIATION

2.1 Ionizing radiation-induced DNA lesions and their repair

2.1.1 Repair of base damage and base loss
(i) N-glycosylases
(ii) Apurinic and apyrimidinic site endonucleases
(review by Demple and Harrison, 1994)

2.1.2 Repair of single strand breaks (ssb). This process may not be so simple as generally assumed. Nearly all results have been based on experiments with DNA irradiated in solution. However, the the kinds of ssb formed inside irradiated cells, although not well characterized, differ from those formed in solutions of DNA.

2.1.3 Repair of double strand breaks
(a) by ligation
(b) by recombination
(Review by Jeggo, Taccioli and Jackson, 1995)

2.1.4 Repair of DNA-protein crosslinks
(Review by Oleinick, et al, 1990)

2.1.5 Repair of complex DNA damage (local multiply damaged sites [lmds]) The repair of these (hypothetical) lesions is probably very complicated and may lead to a high frequency of misrepair. The frequency of their formation probably increases with increasing LET.

The formation of all the above DNA lesions is a simple linear function of dose at all dose rates and at all doses in the radiobiological range; presumably the probability of misrepair is a constant in all cases. DNA damage is the initiator of all consequent radiobiological effects, and misrepair of this damage is the cause of chromosomal aberrations, mutation , and radiation-induced cancer. At radiobiological doses and in the absence of complicating factors (such as cell cycle delays), however, neither the rate of induction of DNA damages nor the rate of their repair will cause deviations from linearity in the dose response. Therefore the discussion of DNA damage and repair in the report should not be extensive.

2.1.6 Mismatch repair (defective in nonpolyposis colorectal cancer, in some sporadic colon cancers, and in some other cancers). This repair system corrects mismatches formed during normal semiconservative DNA synthesis. Because extra mismatches may be induced behind the growing point when cells are irradiated in S phase, the failure of this repair system to recognize them could lead to a deviation from linearity in the dose response for carcinogenesis.
(Review by Kolodner, 1995)

2.2 Cell cycle check points (role of p53, ATM, and other proteins)
[a] G1 to S
[b] S phase
[c] G2 to M
(Reviews by Enoch and Norbury, 1995; Cox and Lane, 1995)

2.3 Programmed cell death (apoptosis)
(Review by Hengartner, 1995)

2.4 Dose response relationships for DNA repair as impacted by cell cycle checkpoints and programmed cell death
(a) low dose and LET-little if any effect
(b) dose rate-possibly large effect, shifting from linearity at moderately low dose rates to higher than expected at very low dose rates (or vice versa). This result may occur when the time between successive ionizations in the DNA is longer than the delay in the cell cycle progression caused by the first ionization. The cell will then have moved into the next phase of the cell cycle, which may be either more or less radiosensitive (in terms of carcinogenesis)than the one in which it was delayed.

2.5 The adaptive response. Although this phenomenon is very probably due to an induced DNA repair system, it is unlikely that it will cause any deviation from linearity at low single doses, simply because no adapting dose has previously occurred. As dose rate changes, a shift from linearity might occur in the time frame around when the protective effect of the first (adapting) ionizing event dissipates and a second ionizing event occurs.
(extensively reviewed in UNSCEAR, 1994).

Section 3

NCRP SC 1-6, Linearity of Dose Response

Mutation in vivo

1. Human *in vivo*

a. A-bomb survivors

Hirai, Y, Y Kusunoki, S Kyoizumi, AA Awa, DJ Pawel, N Nakamura and M Akiyama 1995 Mutant frequency at the hprt locus in peripheral blood T-lymphocytes of atomic bomb survivors Mutat. Res 329: 183-96.
Among 254 survivors (171 exposed and 83 unexposed), there was a weak but significant effect. Control frequency was 10^{-5}, and ln(slope/Gy) was 0.104.

Hakoda, M, M Akiyama, Y Hirai, S Kyoizumi and AA Awa (1988) In vivo T cell frequency in atomic bomb survivors carrying outlying values of chromosome aberration frequencies. Mutat. Res 202: 203-8.
Here they have compared hprt- MF in survivors with high levels of chromosome aberrations, or in those with background levels (calculated total doses were 248 and 273 cGy, respectively). MF in the high aberration group were nearly twice as high (mean = 6.7 vs 3.7 x 10^{-6}; unexposed controls were 3.4 x 10^{-6}). As expected, lots of scatter in the data.

Langlois, RG, M Akiyama, Y Kusunoki, BR DuPont, DH Moore 2nd, WL Bigbee, SG Grant and RH Jensen (1993) Analysis of somatic cell mutations at the glycophorin A locus in atomic bomb survivors: a comparative study of assay methods. Radiat. Res 136: 111-7.
Significant dose-related increases seen in this set of 39 exposed survivors. Data were fitted to linear curves but there was lots of scatter.

b. Other exposed groups

(i) Cancer patients

Messing, K and WEC Bradley, 1985 In vivo mutant frequency rises among breast cancer patients after exposure to high doses of gamma-irradiation. Mutat. Res., 152: 107-12.
Exposures to 4 Gy, fractionated. Mutant frequency of 7 x 10^{-6} mutants/cell/Gy.

Saia-trepat, M, J Cole, MHL Green, O Rigaud, JR Vilcoq and E Moustacchi (1990) Genotoxic effects of radiotherapy and chemotherapy on the circulating lymphocytes of breast cancer patients. III. Measurement of mutant frequency to 6-thioguanine resistance. Mutagenesis 5: 593-598.
Increases in MF were attributed entirely to RT (1.8 Gy 5 times/wk to a total of 45 Gy), without effect from CT (fluorouracil, adriamycin and cyclophosphamide), regardless of the order of treatment.

Normal Controls	= 17.73±1.09(SE) x 10^{-6}.
Cancer patients before therapy	= 20.60±1.14
Chemo first	= 26.19±1.16
RT first	= 36.43±1.24
RT followed by CT	= 37.92±1.24
CT followed by RT	= 38.69+1.14

Nicklas, JA, JP O'Neill, TC Hunter, MT Falta, MJ Lippert, D Jacobson-Kram, JR Williams and RJ Albertini 1990 In vivo ionizing radiations produce deletions in the hprt gene of human T lymphocytes. Mutat. Res, 250:383-396.

Patients were studied before and after treatment with RIT with I-131. Mean MF was 11.5±5.1 x 10^{-6} pre and 27.8±16.1 post. Known doses ranged from 8.5 to 152.3 mCi, and a dose-response curve was fitted well to either a first or second order equation. Analysis of individual mutants indicated that there was an excess of deletions, which is consistent with induction by radiation. However, patients also received a 21 Gy dose with an external beam to the affected organ (liver), and chemotherapy as well. So attributing the increase to the RIT seems questionable.

(ii) Nuclear medicine patients

Seifert, AM WEC Bradley and K Messing (1987) Exposure of nuclear medicine patients to ionizing radiation is associated with rises in HPRT- mutant frequency in peripheral T-lymphocytes Mutat. Res 191: 57-63.
This is for Tc-99m. Pre-exposure blood draw served as control. Mean MF increased from 2.09±3.18 x 10^{-6} to 7.62 x 10^{-6}. According to the supplier, the dose to the blood is 1-1.5 cGy. However, based on other studies of micronuclei formation, the authors speculate that the biologically effective dose was higher, by up to a factor of 3. The authors estimate 10^{-4} to 10^{-5} induced mutants/cell/Gy.

Kelsey, K.T., K.J. Donohoe, A. Memisoglu, B. Baxter, M. Caggana and H.L. Liber (1991). In vivo exposure of human lymphocytes to technetium-99^{m} in nuclear medicine patients does not induce detectable genetic effects., Mutation Res., 264:213-218.
This study did not reproduce the Seifert et al results.

Bachand, M, AM Seifert and K Messing 1991. Nuclear medicine patients do not have higher mutant frequencies after exposure to thallium-201. Mutat. Res 262:1-6.
They speculate that the difference between Th and Tc has to do with effective dose to LCs. Th is a K analog and distributes to organs, while Tc adsorbs to RBCs and is found with LCs.

Kelsey, K.T., K.J. Donohoe, B. Baxter, A. Memisoglu, J.B. Little, M. Caggana and H.L. Liber (1991). Genotoxic and mutagenic effects of the diagnostic use of thallium 201 in nuclear medicine. Mutation Res., 260: 239-246.
Also no effect of thallium 201

(ii) Radiation technicians

Messing, K, J Ferraris, WEC Bradley, J Swartz and AM Seifert (1989) Mutant frequency of radiotherapy technicians appears to be associated with recent dose of ionizing radiation. Health Physics 57: 537-44.
This group was exposed largely to gamma, with an average dose 2.2 mSv in the previous 6 months before the assay. These technicians had an average MF of 12.8 x 10^{-6} in 1986 versus 9.5 in the controls; the same individuals were at 7.7 versus 3.1 in the controls in 1984. The differences between the time points were ascribed to laboratory procedures. The implication is that the 2.2 mSv average dose yielded a 50-100% increase in mutant frequency.

(iv) Miners

(v) Radiation accidents

Jensen, RH, RG Langlois, WL Bigbee, SG Grant, and DH Moore 2nd Elevated frequency of glycophorin A mutations in erythrocytes from Chernobyl accident victims. Radiation Research 1995, 141(2):129
Data from people exposed after the Chernobyl accident showed increased variant frequencies at the GPA locus. These mutations are the result of large-scale alterations. Data were fitted linearly, but the scatter is very large.

c. Comparison among genes - hprt vs GPA

2. Animal in vivo

Russell, WL and EM Kelly 1982
Specific locus mutation frequencies in mouse stem spermatogonia at very low radiation dose rates. Proc Natl Acad Sci 79: 539-41
Mutation frequencies in male mice and the estimation of genetic hazards of radiation in men. Proc Natl Acad Sci 79: 542-4
With the specific locus test, linear DR obtained for both acute and chronic dosing protocols. DR effect seen down to 0.8 R/min, but not below.

Lorenz, R, W Deubel, K Leuner, T Gollner, F Hochhauser and K Hempel 1994, Dose and dose-rate dependence of the frequency of hprt deficient T lymphocytes in the spleen of the 137Cs gamma-irradiated mouse. IJRB 66(3): 319-26.
In vivo assay with hprt in T-cells. Treated with Cs137. Doses 0.3-6 Gy. Dose rates = 0.5 Gy/min, 1 Gy/day, 1 Gy/wk. Mutants scored 8-10 or 30-40 weeks after treatment. Data fitted to L or to LQ equations.

- Acute irradiation, with MF at 8-10 wks, dose-response = Linear quadratic.
- Low DR, with MF at 8-10 wks, dose-response = Linear

Schiestl, R.H., Khogali, F. and Carls, N. 1994. Reversion of the mouse pink-eyed unstable mutation induced by low doses of X-rays. Science 266: 1573-6.

This in vivo reversion assay detects DNA deletions, since the mutation is a gene duplication. X-ray-induced reversion occurred linearly between 0.01 and 1 Gy. This is an example of a specific type of mutation being induced with linear kinetics.

Mutagenesis in vitro

1. Acute, low LET radiation.

a. Assays at the hprt locus

The most commonly used genetic locus for mutation study in mammalian cells is the hprt locus, for several reasons. It is easily selected for with purine analogs such as 6-thioguanine. It is X-linked and thus hemizygous in all mammalian species (this is advantageous in that there is no second compensating allele to mask phenotypic changes after a mutation, but disadvantageous for two reasons: (i) mutational mechanisms that involve the homologous chromosome do not function, and (ii) very large deletion events may include an essential gene that will result in cell death).

In some human cell systems, there is a linear dose-response with no apparent threshold. These include human fibroblasts (e.g., Cox and Masson, 1979) and human lymphoblasts (REF). Generally, there is little data below 50 cGy, so thresholds cannot be ruled out. However, Grosovsky and Little (1985) did a fractionated experiment in which lymphoblast cells were treated daily with 1-10 cGy of acute X-rays. The final observed MF was equal to that seen for a single acute exposure, suggesting that the increments were additive, and a dose as low as 1 cGy was effective at inducing mutation.

In other human cell systems, notably T-lymphocytes there is a non-linear dose-response (Vijayalaxmi and Evans, 1984; Sanderson et al, 1984).

In the majority of rodent studies, the dose-response is non-linear.

Within the same laboratory, human fibroblast versus rodent V79 have maintained this linear versus non-linear trend (Thacker et al, 1979; Cox and Masson, 1979)

b. Assays at other genetic loci

Studies at the heterozygous tk locus have been done in both human and mouse cell systems. Mutations at this locus can arise by all of the same pathways as at hprt, but in addition, can arise from mechanisms involving the homologous chromosome, and by very large intrachromosomal deletions .

In L5178Y mouse cells, the dose-response curve was reported to be non-linear (Nakamura and Okada, 1981, 1982). However, later experiments with a different subclone indicated a linear response (Moore et al, 1988).

In TK6 human lymphoblast cells, the curve is linear (REF; Konig and Kiefer, 1988). However, if the p53 tumor suppressor gene is mutated in these cells(Xia et al, 1995), the dose-response curve becomes non-linear (Amundson et al, 1993).

Studies at the dhfr locus, where one mechanism by which mutants can arise is by gene amplification, have shown that the dose-response is non-linear in EMT-9 mice (Hahn et al, 1990). However, in L5178Y mouse lymphoma cells, mutation was linear (Nakamura and Okada,

2. Acute exposure to high LET radiation

Generally these dose-response cures are linear, especially in human (e.g., Cox and Masson 1979; Nakamura et al, 1982; ETC, ETC - More refs). In rodent cells, curves sometimes are linear, and sometimes curvilinear. The non-linear curves often fit better to linear equations than they do for the low LET radiation.

3. Chronic exposure to low LET radiation

In human lymphoblast cells (where the acute dose-response is linear), chronic exposure to either gamma-radiation (Konig and Kiefer, 1988) or beta particles from tritiated water (Liber et al, Tabocchini et al) showed no evidence of a dose-rate effect.

In L5178Y mouse lymphoma cells, lowering the dose-rate from 50 to 0.8 cGy/min resulted in the dose-response going from non-linear to linear. At the dhfr locus in these cells, the lower dose-rate was less mutagenic, but the dose-response was still linear in shape (Nakamura and Okada, 1981).

In V79 cells, Crompton et al (1985) reported that lowering the dose-rate from 4 Gy/min to 50 mGy/hr decreased the mutagenic efficiency of gamma-rays; however, decreasing the dose-rate still further to 8 mGy/hr led to a dramatic increase in mutagenic

efficiency, to at least several-fold higher than the acute treatment. In these experiments all dose-response curves were non-linear.

4. Chronic exposure to high LET radiation ???

5. Differences among mutational classes

From molecular analyses of radiation-induced mutants, it appears likely that mutations that are studied in vitro and in vivo arise from several different mechanisms.

Point mutations (base pair substitutions and small insertions or deletions) likely arise from damaged bases that are mis-replicated or mis-repaired. Unless there are saturable levels of radical scavengers or of DNA repair pathways for base damage, this sort of DNA damage should form linearly with dose, and so the mutations resulting in this fashion should also arise linearly.

Large-scale deletions of thousands or millions of base pairs are thought to arise from one or more double strand breaks. If in fact two or more "hits" are required, one would imagine that deletions should follow non-linear (quadratic or higher) kinetics. At autosomal heterozygous genes, LOH by recombination (gene conversion or strand exchange) "unmasks" a recessive allele. Similarly, such events have been thought to require multiple hits and threfore are expected to follow non-linear kinetics.

In support of these ideas:

DNA amplifications arise with non-linear kinetics in EMT-9 mouse cells (Hahn et al, 1990).

In CHO cells, Nagasawa and Little (Radiation Research meeting, 199X) utilized PCR of the exons of hprt to characterize X-ray-induced mutants. First of all, they showed that overall mutation fit best to a linear quadratic equation. However, they found that point mutations and also partial deletion mutations arose with linear kinetics; only the total gene deletions arose non-linearly as a function of dose. Thus the total gene deletions dictated the non-linear nature of the dose-response curve as a whole.

On the other hand, Moore et al (1988) reported that at the tk locus in L5178Y mouse lymphoma cells, that gamma-rays induced both large colony (thought to arise from small intragenic alterations) and small colony (thought to arise from large multi-locus alterations including both deletion and recombinational events) mutants with linear kinetics. In TK6 human lymphoblast cells, X-ray-induced LOH events (again a combination of deletions and recombinations) arise with linear kinetics.

6. Differences with respect to repair capacity

Human cells with a double mutation in the Rb gene have the same linear dose-response curve after treatment with gamma-rays as do normal fibroblasts (Wang et al, 1986).

Human lymphoblast cells with mutant p53 are considerably more mutable by X-rays, and they develop a non-linear dose-response curve shape. They also are more mutable by high LET radiation. (CHECK w AK and or SA for details)

Recently, L5178Y mouse lymphoma cells were found to be mutant at the p53 locus. However, it is not certain whether all of the various strains of this line that have been used in mutagenesis research carry this alteration.

Xrs-irs lines

In vitro dose-response data for mutagenicity

Cell type	Locus	Dose-rate	Radiation type	Result	Reference
EMT-6 mouse	dhfr, arising by amplification; hprt	Acute	X-rays	Non-linear for both loci	Hahn, P, B. Nevaldine and WF Morgan 1990, X-ray induction of methotrexate resistance due to dhfr gene amplification. Somat. Cell Molec. Geent 16: 413-23
CHO	hprt	Acute	X-rays alpha	X-rays: overall DR slightly curvilinear - Large D curvilinear, but point and partial D are linear Alpha: overall L, Large Deletion: curvilinear Partial Deletion: linear Point mutation: Linear	Nagasawa, H and Little, JB, unpublished (But presented at a Radiation research meeting)
Human diploid fibroblasts	hprt	Acute	He at LETs of 20-90 keV/μm; B at LETs of 110-200; N at 470	RBE maxima at 90-200 keV/μm; All curves linear	Cox, R and WK Masson 1979, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. III. Human diploid fibroblasts, Int. J Radiat. Biol. 36: 149-60
V79 (same lab as above)	hprt	Acute	He at LETs of 20-90 keV/μm; B at LETs of 110-200; N at 470	Curves generally linear quadratic, occasionally linear	Thacker, J, A Stretch and MA Stephens 1979, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. II. Chinese hamster V79 cells, Int. J Radiat Biol 36:137-48
L5178Y	hprt mtx-r	Acute	γ-rays; fast neutrons	hprt: γ is curvilinear, N is linear mtx: both linear (unclear whether 1-step mtx selection is via amplification)	Nakamura, N, S Suzuki, A Ito and S Okada, 1982 Mutations induced by γ-rays and fast neutrons in cultured mammalian cells Differences in dose-response and RBE with methotrexatre- and 6-thioguanine-resistant systems, Mutat. Res 104: 383
CHO-AL	large-scale mutations	Acute; 0.58 - 17 cGy/min	γ-rays; neutrons	Linear; Linear, with high doses becoming less effective; lowest E (.33 Mev) most effective mutagen	Hei, TK, EJ Hall and CW Waldren 1988 Mutation induction and relative biological effectiveness of neutrons in mammalian cells. Radiat Res 115: 281-291.
					Amy K

Human TK6 LB	hprt tk	2.7 mGy/hr; 27 mGy/hr; Acute	γ-rays	No dose-rate effect; Linear curves	Konig, F and J Kiefer 1988 Lack of dpse-rate effect for mutation induction by γ-rays in human TK6 cells, Int. J. Radiat. Biol., 54:891-7
Human TK6	hprt	1-10 cGy/day (fractionated acute)	X-rays	No difference between Fract and Acute	Grosovosky, AJ and JB Little 1985 Evidence for linear response for the induction of mutations in human cells by X-ray exposures below a0 rads Proc Natl Acad Sci 82:2092-5
L5178Y mouse lymphoma	hprt mtx-r	50 cGy/min 0.8 cGy/min	γ-rays	hprt goes from non-linear to linear; mtx is linear at both rates, but less effective at lower; suggest two components; one shows dose-rate dependency, and the other does not	Nakamura, N and S Okada 1981 Dose-rate effects of gamma-ray induced mutations in cultures mammalian cells. Mutat. Res., 83: 127-35
Human LC in vitro	hprt	Acute	X-rays	non-linear	Vijayalaxmi and HJ Evans 1984 Measurement of spontaneous and x-irradiation-induced 6-thio-guanine-resistant human blood lymphocytes using a T-cell cloning technique Mutat. Res 125: 87-94
Human LC in vitro	hprt	Acute	X-rays	non-linear	Sanderson, BJS, JL Dempsey and AA Morely, 1984, Mutations in human lymphocytes: Effect of X- and UV-irradiation, Mutat. Res. 140: 223-7
V79 hamster	hprt	8 mGy/hr 50 mGy/hr 4 Gy/min	γ-rays	All non-linear; Inverse dose-rate effect - lowest dr was much more mutagenic	Crompton, NEA, F Zolzer, E Schneider and J Kiefer 1985 Increased mutant induction by very low dose-rate γ-irradiation Naturewissenschaften 72: 439-40
L5178Y	hprt	30 Gy/hr; 20cGy/hr; 6.3 mGy/hr	γ-rays	non-linear; linear and less effective than acute; linear and more effective than medium dose (only difference from acute is in the highest dose (4 Gy)	Furuno-Fukushi, I AM Ueno and H Matsudaira 1988 Mutation induction by very low dose-rate γ-rays in cultured mouse leukemia cells L5178Y Radiation Research 115:273-80.
Human	hprt	Acute	γ-rays	Rb cell lines and normal lines have similar linear DR	Wang, Y, WC Parks, JC Wigle, VM Maher and JJ McCormick 1986, Fibroblasts from patients with inherited disposition to retinoblastoma exhibit normal sensitivity to the mutagenic

					effects of ionizing radiation Mutat res 175: 107-14
L5178Y	tk - large clone - small clone	Acute	γ-rays	All curves linear	Moore, MM, A Amtower, GHS Strauss and C Doerr 1986 Genotoxicity of γ-irradiation in L5178Y cells Mutat Res 174: 149-54.
V79	hprt (8AG)	Acute 1.2 rad/sec and 105 rad/sec - both gave similar results	γ-rays	All curves non-linear, Splitting the dose reduces the overall MF, with the greatest effect for smaller # of fractions (i.e., approaches a limiting value as the # of fractions increases).	Asquith, JC 1977 The effect of dose fractionation on γ-radiation induced mutations in mammalian cells. Mutat Res 43: 91-100.

1. (MEDLINE result)
Schweizer PM.
Linear dose-response relationship and no inverse dose-rate effect observed for low X-ray dose-induced mitotic recombination in Drosophila melanogaster.
International Journal of Radiation Biology, 1995 Mar, 67(3):303-13.
(UI: 95205002)

Abstract: Mitotic recombination has emerged lately as a surprisingly common cause of recessive functional gene loss in mammalian cells and has been implicated in tumour suppressor gene loss in human neoplasms. In an assay, primarily monitoring mitotic recombination in Drosophila melanogaster, the ability of low dose acute- and chronic X-ray irradiation to induce clonal expression of recessive mutations of formally heterozygous loci was investigated. Mosaic spots of recessive wing-hair misshape mutations (mwh and flr) and of hair-into-bristles transforming mutation (w3tio) were enhanced by a factor of two over control level following irradiation of heterozygous larvae to doses as low as 0.01, 0.03 or 0.1 Gy X-rays. The frequencies of mosaic spots induced with eight doses in the interval 0.01-2.0 Gy was linearly related to the dose. The regression lines show no significant intercept at zero dose. During the entire larval developmental period exposure of the exponentially growing target cell population to conditions of chronic irradiation at dose-rate of 15.7 x 10(-5) Gy/min provided no evidence of an inverse dose-rate effect as reported in yeast. In Drosophila, the probability of mitotic recombination per induced DNA double-strand break appears to be at least one order of magnitude higher than in man.

Adaptive response:

2. (MEDLINE result)
Schappi-Buchi C.
On the genetic background of the adaptive response to X-rays in Drosophila melanogaster.
International Journal of Radiation Biology, 1994 Apr, 65(4):427-35.
(UI: 94209794)

Abstract: The effects of a low dose (0.1-20 mGy) preirradiation with X-rays followed by a higher dose (2 Gy) of the same radiation on the recovery of the genetic damage induced as dominant lethals in mature oocytes (stage 14) of different strains of Drosophila melanogaster were investigated. The response was shown to be dependent on the genotype of the flies tested, since lower frequencies of dominant lethals (DL) were only obtained in strains carrying the white mutation. Based on these observations experiments to locate the genetic factor responsible for the adaptive response (AR) were performed. This factor was found to be in a specific region of the X-chromosome. Additional experiments were carried out to give information on the minimal dose required to induce the AR. The results showed that the lowest dose needed is 0.2 mGy. Increasing the conditioning X-ray dose had no influence on the response.

Low dose-rate studies:

1. Furuno-Fukushi I; Ueno AM; Matsudaira H.
Mutation induction by very low dose rate gamma rays in cultured mouse leukemia cells L5178Y.
Radiation Research, 1988 Aug, 115(2):273-80.
(UI: 88304497)

Abstract: Induction of cell killing and mutation to 6-thioguanine resistance was studied in growing mouse leukemia cells in culture following gamma rays at dose rates of 30 Gy/h, 20 cGy/h, and 6.3 mGy/h, i.e., acute, low dose rate, and very low dose rate irradiation. A marked increase was observed in the cell survival with decreasing dose rate; no reduction in the surviving fraction was detected after irradiation at 6.3 mGy/h until a total dose of 4 Gy. Similarly, the induced mutation frequency decreased after low dose rate irradiation compared to acute irradiation. However, the frequency after irradiation at 6.3 mGy/h was unexpectedly high and remained at a level which was intermediate between acute and low dose rate irradiation. No appreciable changes were observed in the responses to acute gamma rays (in terms of cell killing and mutation induction) in the cells which had experienced very low dose rate irradiation.

Acute exposure to high LET radiation:

In human cells, the curves are linear. There is limited data to suggest that for very high LET the curves for individual types of heavy ions diverge and LET is not a good predictor (Kiefer).

some references to add and discuss:

1. Kronenberg A; Little JB.
Locus specificity for mutation induction in human cells exposed to accelerated heavy ions.
International Journal of Radiation Biology, 1989 Jun, 55(6):913-24.
(UI: 89278995)

Abstract: The relative efficiencies of two types of densely ionizing particles were compared for the induction of mutations at two distinct genetic loci in human cells. Mutations to 6-thioguanine resistance (hgprt locus) or to trifluorothymidine resistance (tk) locus were scored in TK6 human lymphoblastoid cells exposed to graded doses of 40Ar ions (470 MeV/amu, LET = 95-97 keV/microns) or 28Si ions (456 MeV/amu, 61 keV/microns). The autosomal tk locus was more efficiently mutated than the X-linked hgprt locus following heavy particle irradiations. This was predominantly due to the contribution of a class of slowly growing mutants scored at the tk locus. Silicon ions were more efficient per unit dose than argon ions for the induction of mutants at either locus. When the mutant yield for a particular ion was compared with particle fluence, similar numbers of hgprt

mutants are induced by equal numbers of 40Ar or 28Si ions. Comparison of the number of tk mutants with particle fluence demonstrates an increased efficiency for 28Si ions over 40Ar. These data suggest that the LET-RBE relationship may be different for individual genetic loci in human cells.

1. (MED90 result)
Whaley JM; Little JB.
Efficient mutation induction by 125I and 131I decays in DNA of human cells.
Radiation Research, 1990 Jul, 123(1):68-74.
(UI: 90319385)

Abstract: To examine the role of radiation energy deposition in DNA on cellular effects, we investigated the ability of 125IdUrd and 131IdUrd to kill cells and induce mutations at the hprt locus. We employed human lymphoblastoid cells proficient (TK6) or deficient (SE30) in the ability to incorporate a thymidine analog into DNA by way of the thymidine kinase (TK) scavenger pathway. Iodine-125 releases a shower of low-energy Auger electrons upon decay which deposit most of their energy within 20 nm of the decay site, whereas 131I is a high-energy beta/gamma emitter that is generally considered to emit sparsely ionizing radiation. Although 125IdUrd incorporated into cellular DNA was very effective at producing toxic and mutagenic effects in TK6 cells, virtually no effect was seen in TK-deficient cells incubated with similar levels of 125IdUrd in the extracellular medium. In response to 131IdUrd treatment, 0.45 X 10(-6) mutants were induced per centigray dose deposited within the nucleus in TK-proficient cells, whereas few mutations were induced in TK-deficient cells at doses up to 38 cGy from 131I decays occurring in the medium. The differences in biological response between TK6 and SE30 cells cannot be explained by differential radiosensitivity or IdUrd sensitization of the cell lines involved. We conclude that both 125I and 131I decays occurring while incorporated into DNA are more effective at inducing cell killing and mutations in human cells than either nonincorporated decays or low-LET radiations. These results suggest that localized energy deposition is an important factor in producing biologically important damage by both of these isotopes, and that residual lesions following the decay of DNA-incorporated radioisotopes may contribute to the toxic and mutagenic effects observed in TK-proficient cells. Furthermore, they emphasize that certain beta/gamma-emitting isotopes such as 131I may be particularly hazardous when incorporated into DNA.

2. (MED90 result)
Whaley JM; Kassis AI; Kinsey BM; Adelstein SJ; Little JB.
Mutation induction by 125iodoacetylproflavine, a DNA-intercalating agent, in human cells.
International Journal of Radiation Biology, 1990 Jun, 57(6):1087-103.
(UI: 90270767)

Abstract: Survival and the induction of mutations at the hprt and tk loci were

measured in TK6 human lymphoblastoid cells following treatment with the DNA-intercalating agent 125iodoacetylproflavine (125IAP). 125IAP was readily taken up into the cells, was localized to the nucleus, and was released rapidly following resuspension of the cells in fresh medium. Treatment with 125IAP for 24 h yielded a D0 of 110 decays/cell and an induced mutant fraction of 0.13 x 10(-6) per decay at the hprt locus and 0.4 x 10(-6) per decay at the tk locus. Molecular analyses of 125IAP-induced hprt mutants by Southern blot revealed a high proportion of large-scale changes at this locus. When these results are compared with those observed with 125IdUrd, 125IAP shows a reduced effectiveness per decay, related perhaps to the non-covalent nature of intercalator binding, resulting in reduced energy deposition in the DNA.

3. (MED90 result)
Whaley JM; Little JB.
Molecular characterization of hprt mutants induced by low- and high-LET radiations in human cells.
Mutation Research, 1990 Jan, 243(1):35-45.
(UI: 90136688)

Abstract: Southern blotting techniques were employed to examine the spectrum of molecular alterations in DNA induced by internally emitting iodine isotopes and X-rays at and around the hprt locus in a human lymphoblastoid cell line. We analyzed 165 mutant clones using a cDNA probe for the human hprt locus, and 3 anonymous sequence probes for regions of the X chromosome which are linked to hprt. The results were compared with those for 35 spontaneously arising mutant clones. The majority of ionizing radiation-induced mutants showed changes in the normal restriction patterns at the hprt locus, whereas very few alterations were seen at linked markers along the X chromosome. Total hprt coding sequence deletions comprised 30-48% of the changes observed at this locus, while partial deletions and rearrangements comprised 14-54% of the observed changes. In the case of mutants induced by [125I]dUrd, a densely ionizing radiation, the spectrum of alterations was dose-dependent; at low doses it was not significantly different from that seen after sparsely ionizing X-ray exposure, whereas a higher proportion of gene deletions and rearrangements occurred after high doses of this incorporated isotope. Changes were rarely observed in the 3 linked markers examined. Overall, these results indicate that the distribution of mutational events at the hprt locus in irradiated human cells may not only be LET-dependent but dose-dependent, and that deletions involving large regions of the X chromosome surrounding the hprt locus are rare events.

5. (MEDLINE result)
Tsuboi K; Yang TC; Chen DJ.
Charged-particle mutagenesis. 1. Cytotoxic and mutagenic effects of high-LET charged iron particles on human skin fibroblasts.
Radiation Research, 1992 Feb, 129(2):171-6.
(UI: 92132001)

Abstract: Cytotoxic and mutagenic effects of high-LET charged iron (56Fe) particles were measured quantitatively using primary cultures of human skin fibroblasts. Argon and lanthanum particles and gamma rays were used in comparative studies. The span of LETs selected was from 150 keV/microns (330 MeV/u) to 920 keV/microns (600 MeV/u). Mutations were scored at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus using 6-thio-guanine (6-TG) for selection. Exposure to these high-LET charged particles resulted in exponential survival curves. Mutation induction, however, was fitted by the linear model. The relative biological effectiveness (RBE) for cell killing ranged from 3.7 to 1.3, while that for mutation induction ranged from 5.7 to 0.5. Both the RBE for cell killing and the RBE for mutagenesis decreased with increasing LET over the range of 1.50 to 920 keV/microns. The inactivation cross section (sigma i) and the action cross section for mutation induction (sigma m) ranged from 32.9 to 92.0 microns2 and 1.45 to 5.56 X 10(-3) microns2; the maximum values were obtained by 56Fe with an LET of 200 keV/microns. The mutagenicity (sigma m/sigma i) ranged from 2.05 to 7.99 X 10(-5) with an inverse relationship to LET.

6. (MEDLINE result)
Metting NF; Palayoor ST; Macklis RM; Atcher RW; Liber HL; Little JB.
Induction of mutations by bismuth-212 alpha particles at two genetic loci in human B-lymphoblasts.
Radiation Research, 1992 Dec, 132(3):339 45.
(UI: 93117343)

Abstract: The human lymphoblast cell line TK6 was exposed to the alpha-particle-emitting radon daughter 212Bi by adding DTPA-chelated 212Bi directly to the cell suspension. Cytotoxicity and mutagenicity at two genetic loci were measured, and the molecular nature of mutant clones was studied by Southern blot analysis. Induced mutant fractions were 2.5 x 10(-5)/Gy at the hprt locus and 3.75 x 10(-5)/Gy at the tk locus. Molecular analysis of HPRT- mutant DNAs showed a high frequency (69%) of clones with partial or full deletions of the hprt gene among radiation-induced mutants compared with spontaneous mutants (31%). Chi-squared analyses of mutational spectra show a significant difference ($P < or = 0.005$) between spontaneous mutants and alpha-particle-induced mutants. Comparison with published studies of accelerator-produced heavy-ion exposures of TK6 cells indicates that the induction of mutations at the hprt locus, and perhaps a subset of mutations at the tk locus, is a simple linear function of particle fluence regardless of the ion species or its LET.

7. (MEDLINE result)
Stoll U; Schmidt A; Schneider E; Kiefer J.
Killing and mutation of Chinese hamster V79 cells exposed to accelerated oxygen and neon ions.
Radiation Research, 1995 Jun, 142(3):288-94.
(UI: 95281735)

Abstract: Mutation induction by accelerated heavy ions to 6-thioguanine resistance (HPRT system) in Chinese hamster V79 cells was investigated using oxygen and neon ions with energies between 1.9 and 400 MeV/mu, corresponding to LET values between 18 and 754 keV/microns, respectively. Because of technical limitations most experiments could be performed only once. Inactivation and mutation induction cross sections, sigma i and sigma m, were obtained from the slopes of the exponential survival and the linear mutation induction curves, respectively. Both parameters increased with LET up to about 200 keV/microns, where the curves separated for the two types of ions. Calculated RBEs were higher for mutation induction than for killing for all LET values.

8. (MED90 result)
Kranert T; Schneider E; Kiefer J.
Mutation induction in V79 Chinese hamster cells by very heavy ions.
International Journal of Radiation Biology, 1990 Dec, 58(6):975-87.
(UI: 91061017)

Abstract: Mutation induction (resistance to 6-thioguanine) in Chinese hamster fibroblasts (V79) by exposure to accelerated heavy ions (O, Ne, Ca, Ti, Ni, Xe, Pb and U with energies between 5 and 14.8 MeV/u) was investigated, covering a range of LET from 300 to about 15,700 KeV/micron. The LET-dependence of the mutation induction cross-section (sigma m) has, in a similar way to inactivation (sigma i), to be described by separate curves for each ion. Both sigma m and mutagenicity (sigma m/sigma i) decrease with increasing specific energy for any given ion. Relative biological effectiveness for mutation induction was found to be significantly smaller than unity for the ions and energies investigated.

9. (MED85 result)
Grdina DJ; Sigdestad CP; Carnes BA.
Protection by WR1065 and WR151326 against fission-neutron-induced mutations at the HGPRT locus in V79 cells.
Radiation Research, 1989 Mar, 117(3):500-10.
(UI: 89185396)

Abstract: The radioprotectors WR1065 and WR151326, each at a concentration of 4 mM, protect against cell killing and mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79 Chinese hamster fibroblast cells exposed to fission-spectrum neutrons (mean energy of 0.85 MeV) from the JANUS reactor. Significant protection against neutron-induced cell lethality occurred only when the radioprotectors were present during irradiation; e.g., D0's and n's were 82 Gy, 1.27 for control cells; 97 Gy, 1.51 for WR1065-protected cells; and 120 Gy, 1.00 for WR151326-protected cells, respectively. Mutation induction by JANUS fission-spectrum neutrons was linear over the dose range tested giving rise to a mutation frequency of 109.3 x 10(-6)/Gy. In comparison with 60Co gamma rays (mutation frequency 8.7 X 10(-6)/Gy), JANUS neutrons, at a dose rate of 24 cGy/min, were over 12 times more effective in inducing HGPRT mutations. Both WR1065 and WR151326 afforded protection against the induction of mutants by neutrons, even when they were administered up to 3

h after irradiation; i.e., mutation frequencies were 40.9, 48.8 and 68.6 X 10(-6)/Gy for WR1065 present during, present immediately after, or added 3 h after irradiation, respectively; and 61.7, 47.8, and 68.5 X 10(-6)/Gy for WR151326 present at the same times.

10. (MED85 result)
Grdina DJ; Nagy B; Hill CK; Sigdestad CP.
Protection against radiation-induced mutagenesis in V79 cells by 2-[(aminopropyl)amino] ethanethiol under conditions of acute hypoxia.
Radiation Research, 1989 Feb, 117(2):251-8.
(UI: 89161129)

Abstract: The effects of the radioprotector 2-[(aminopropyl)amino] ethanethiol (WR-1065) on radiation-induced cell killing and mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79 Chinese hamster cells under hypoxic or aerobic conditions were examined. Conditions of acute hypoxia were attained by gassing 10(6) cells in 1-ml volumes in individual glass ampoules for 2 min with nitrogen. Ampoules were then sealed and incubated at 37 degrees C for 60 min. Following this treatment, cell survival after irradiation as expected was significantly enhanced. The effect of acute hypoxia on the formation of HGPRT mutants by irradiation was also investigated. Mutation frequencies were determined with a 6-day expression time and corrected for the number of spontaneous background mutants. Although mutation induction was approximately linear as a function of radiation dose under most conditions tested, it was significantly reduced in cell populations made acutely hypoxic prior to irradiation. Protection against mutation induction was apparent and similar when cells were irradiated in the presence of the radioprotector, regardless of whether they were also hypoxic or aerated. If cells were irradiated in air and then made hypoxic, no significant protection was still observed. These results suggest that the antimutagenic effect of WR-1065 is not due solely to its ability to scavenge radiation-induced oxygen-free radicals, but rather that it may also modulate these effects through the scavenging of metabolically induced free radicals and/or the chemical repair of radiation-induced DNA lesions.

11. (MED85 result)
Hill CK; Nagy B; Perraino C; Grdina DJ.
2-[(Aminopropyl)amino]ethanethiol (WR1065) is anti-neoplastic and anti-mutagenic when given during 60Co gamma-ray irradiation.
Carcinogenesis, 1986 Apr, 7(4):665-8.
(UI: 86190342)

Abstract: We have studied the effect of 2-[(aminopropyl)amino]ethanethiol (WR1065) on the induction of neoplastic transformation using 10T1/2 cells and on mutation at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus using Chinese hamster V79 cells. Here we report the first observations that treatment of 10T1/2 cells with 1 mM WR1065 for a total of 35 min during irradiation with 60Co gamma-rays significantly reduces the incidence of neoplastic transformation while having no effect on cell viability. In a similar experiment with V79 cells in which 4 mM WR1065 was

used, we found a significant reduction in mutation frequency at the HGPRT locus and significant protection against cell killing. These results suggest that WR1065 acts to modulate both acute damage and sub-lethal processes that lead to mutation and neoplastic transformation. Beyond the purely mechanistic approach of these studies, the potential application of these agents to minimizing the long-term neoplastic effects of radiation or chemotherapeutic agents currently in use for treating potentially curable cancer patients should be further investigated.

12. (MED85 result)
Grdina DJ; Nagy B; Hill CK; Wells RL; Peraino C.
The radioprotector WR1065 reduces radiation-induced mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in V79 cells.
Carcinogenesis, 1985 Jun, 6(6):929-31.
(UI: 85228594)

Abstract: N-(2-mercaptoethyl) 1,3-diaminopropane (WR1065) protects against radiation-induced cell killing and mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in V79 Chinese hamster lung fibroblast cells. At a concentration of 4 mM, WR1065 was found to be effective in protecting against radiation-induced cell lethality only if present during irradiation, e.g., a dose modification factor (DMF) of 1.9. No protective effect was observed if the protector was added within 5 min after irradiation or 3 h later, e.g., DMFs of 1.0 and 1.1, respectively. The effect of WR1065 on radiation-induced mutation, expressed as resistance to the cytotoxic purine analogue 6 thioguanine (HGPRT), was also investigated. In contrast to the treatment-schedule dependence for protection by WR1065 against cell killing, this agent was effective in reducing radiation-induced mutations regardless of when it was administered. Following a dose of 10 Gy of 60Co gamma-rays, the mutation frequencies observed per 10(6) survivors were 77 +/- 8, 27 +/- 6, 42 +/- 7, and 42 +/- 7 for radiation only, and WR1065 present during, immediately after, or 3 h after irradiation. These data suggest that although a segment of radiation-induced damage leading to reproductive death cannot be modulated through the postirradiation action of WR1065, processes leading to the fixation of gross genetic damage and mutation induction in surviving cells can be effectively altered and interfered with leading to a marked reduction in mutation frequency.

13. (MED85 result)
Mei MT; Craise LM; Yang TC.
Induction of proline prototrophs in CHO-K1 cells by heavy ions.
International Journal of Radiation Biology and Related Studiesin Physics, Chemistry and Medicine, 1986 Aug, 50(2):213-24.
(UI: 86277140)

Abstract: Using an established mammalian cell line, Chinese hamster ovary cells (CHO-K1), we have observed the induction of prototrophs by various heavy ions. This cell line requires proline for normal growth in medium with low serum concentration. X-rays, three types of heavy particles (600 MeV/u

iron, 670 MeV/u neon, and 320 MeV/u silicon ions), ethylmethane sulphonate and 5-azacytidine were used to induce revertants which were proline independent. Log-phase cells treated with 5-azacytidine showed a very high reversion frequency. The induction frequency per viable cell appears to be dose dependent for these four types of radiation, and the dose-response curves are approximately linear. Our results also indicate that the effectiveness of high-LET particles in inducing proline prototrophs is much greater than that of low-LET radiation. The RBE value for the induction of prototrophs was calculated for neon, silicon, and iron particles and found to be about 1.3, 1.7 and 4.5, respectively. At equal survival level, the reversion frequency for X-rays and EMS was about the same.

Chronic exposure to high LET radiation (neutrons):

4. (MED90 result)
Kronenberg A.
Perspectives on fast-neutron mutagenesis of human lymphoblastoid cells.
Radiation Research, 1991 Oct, 128(1 Suppl):S87-93.
(UI: 92021460)

Abstract: The effects of low-fluence exposures to (Pu, Be) neutrons (En = 4.2 MeV) have been studied in a sensitive human B-lymphoblastoid cell line, TK6. Mutations were scored for two genetic loci, hypoxanthine phosphoribosyltransferase (hgprt) and thymidine kinase (tk), as a function of dose and dose rate. For exposures limited to less than one cell cycle, the mutation frequency for the hgprt locus was 1.92 X 10(-7)/cGy. When exposures were protracted over multiple cell generations, mutation yields were increased to 6.07 X 10(-7)/cGy. Similar yields were obtained for the induction of tk-deficient mutants with a normal cell generation time (tk-ng) when exposures were carried out at very low dose rates over multiple cell generations. In the series of data presented here, the results obtained for short-duration neutron exposures are compared with data obtained for monoenergetic heavy charged particles of defined linear energy transfer (LET) produced at the BEVALAC accelerator at Lawrence Berkeley Laboratory. TK6 cells have been exposed to beams ranging in atomic number from 20Ne to 40Ar over an energy range from 330 to 670 MeV/amu. Mutation induction was evaluated for both loci for a subset of these beams. The results obtained with 20Ne ions of 425 MeV/amu (LET = 32 keV/microns) and 28Si ions of 670 MeV/amu (LET = 50 keV/microns) closely resemble the mutation yields obtained for brief exposures to (Pu, Be) neutrons. The nature of alterations in DNA structure induced within the tk locus of tk-ng mutants is reviewed for a series of neutron-induced mutants and a series of mutants induced by exposure to 40Ar ions (470 MeV/amu, LET = 95 keV/microns). The mutational spectra for these two types of mutants were similar and were dominated by allele loss mutations. Multilocus deletions inclusive of the c-erbA1 locus were common among tk-deficient mutants induced by these densely ionizing radiations. For the mutants induced by 40Ar ions, it is likely that the mutations were produced by the traversal of the chromosome by a single particle.

Differences among mutational classes:

Chromosomal scale mutations:

15. (MEDLINE result)
McGuinness SM; Shibuya ML; Ueno AM; Vannais DB; Waldren CA.
Mutant quantity and quality in mammalian cells (AL) exposed to cesium-137 gamma radiation: effect of caffeine.
Radiation Research, 1995 Jun, 142(3):247-55.
(UI: 95281729)

Abstract: We examined the effect of caffeine (1,3,7-trimethylxanthine) on the quantity and quality of mutations in cultured mammalian AL human-hamster hybrid cells exposed to 137Cs gamma radiation. At a dose (1.5 mg/ml for 16 h) that reduced the plating efficiency (PE) by 20%, caffeine was not itself a significant mutagen, but it increased by approximately twofold the slope of the dose-response curve for induction of S1- mutants by 137Cs gamma radiation. Molecular analysis of 235 S1- mutants using a series of DNA probes mapped to the human chromosome 11 in the AL hybrid cells revealed that 73 to 85% of the mutations in unexposed cells and in cells treated with caffeine alone, 137Cs gamma rays alone or 137Cs gamma rays plus caffeine were large deletions involving millions of base pairs of DNA. Most of these deletions were contiguous with the region of the MIC1 gene at 11p13 that encodes the S1 cell surface antigen. In other mutants that had suffered multiple marker loss, the deletions were intermittent along chromosome 11. These "complex" mutations were rare for 137Cs gamma irradiation (1/63 = 1.5%) but relatively prevalent (23-50%) for other exposure conditions. Thus caffeine appears to alter both the quantity and quality of mutations induced by 137Cs gamma irradiation.

LOH for densely ionizing radiations: (more references available to confirm, extend this)

2. Kronenberg A; Little JB.
Molecular characterization of thymidine kinase mutants of human cells induced by densely ionizing radiation.
Mutation Research, 1989 Apr, 211(2):215-24.
(UI: 89181728)

Abstract: In order to characterize the nature of mutants induced by densely ionizing radiations at an autosomal locus, we have isolated a series of 99 thymidine kinase (tk) mutants of human TK6 lymphoblastoid cells irradiated with either fast neutrons or accelerated argon ions. Individual mutant clones were examined for alterations in their restriction fragment pattern after hybridization with a human cDNA probe for tk. A restriction fragment

length polymorphism (RFLP) allowed identification of the active tk allele. Among the neutron-induced mutants, 34/52 exhibited loss of the previously active allele while 6/52 exhibited intragenic rearrangements. Among the argon-induced mutants 27/46 exhibited allele loss and 10/46 showed rearrangements within the tk locus. The remaining mutants had restriction patterns indistinguishable from the TK6 parent. Each of the mutant clones was further examined for structural alterations within the c-erbA1 locus which has been localized to chromosome 17q11-q22, at some unknown distance from the human tk locus at chromosome 17q21-q22. A substantial proportion (54%) of tk mutants induced by densely ionizing radiation showed loss of the c-erb locus on the homologous chromosome, suggesting that the mutations involve large-scale genetic changes.

Hprt induction vs. chromosomal scale induction by high LET:

1. Kronenberg A; Gauny S; Criddle K; Vannais D; Ueno A; Kraemer S; Waldren CA. Heavy ion mutagenesis: linear energy transfer effects and genetic linkage. Radiation and Environmental Biophysics, 1995 Jun, 34(2):73-8. (UI: 95380618)

Abstract: We have characterized a series of 69 independent mutants at the endogenous hprt locus of human TK6 lymphoblasts and over 200 independent S1-deficient mutants of the human x hamster hybrid cell line AL arising spontaneously or following low-fluence exposures to densely ionizing Fe ions (600 MeV/amu, linear energy transfer = 190 keV/microns). We find that large deletions are common. The entire hprt gene (> 44 kb) was missing in 19/39 Fe-induced mutants, while only 2/30 spontaneous mutants lost the entire hprt coding sequence. When the gene of interest (S1 locus = M1C1 gene) is located on a nonessential human chromosome 11, multilocus deletions of several million base pairs are observed frequently. The S1 mutation frequency is more than 50-fold greater than the frequency of hprt mutants in the same cells. Taken together, these results suggest that low-fluence exposures to Fe ions are often cytotoxic due to their ability to create multilocus deletions that may often include the loss of essential genes. In addition, the tumorigenic potential of these HZE heavy ions may be due to the high potential for loss of tumor suppressor genes. The relative insensitivity of the hprt locus to mutation is likely due to tight linkage to a gene that is required for viability.

General comment: (just me rambling, Howard, so we can discuss this next week...) While it is imaginable that large scale deletions should theoretically require two double strand breaks, linear dose responses can still result it:

The deletions result if one break is put in by the radiation and the second break is enzymatically produced,

The deletions result due to clustered ionizations: this can occur both for low LET radiation and for high LET radiations.

The mutations are totally non-targeted, and occur as a delayed effect in response to the radiation exposure.

Section 4

NCRP Subcommittee 1-6 **Linearity of Dose Response**

Chromosome Aberrations - Low doses and low dose rates of ionizing radiation

[redacted]

1. Summary of types of DNA damage induced by low and high LET radiations
   (i) Single strand breaks
   (ii) Double strand breaks
   (iii) Base damages
   (iv) Multiply damaged sites

   This section will be a brief description of each of these types of DNA damage, referring back to Section 2 (lesions induced in DNA by ionizing radiations).

2. Summary of modes of repair of different types of DNA damage
   - Repair of oxidative damage

     Short description of basic enzymatic processes (review by Demple and Harrison, 1994). Link to Section 2 (if covered there).

   - Repair of strand breaks

     Short description of basic process (review by Jeggo et al., 1995; Jackson, 1996) describing similarities with V(D)J recombination, and role of Ku 70, Ku 80 and DNA-PKcs, p53 (p21 and GADD 45) in the process. Link to Section 2 (if covered there).

   - It is possible that multiply damaged sites are difficult to repair, and have a high probability of producing an aberration.

- Repair, replication and cell cycle control

  Description of importance of an association of cell cycle arrest in G_1 prior to replication, at G_2 prior to mitosis, and perhaps in S and mitosis with DNA repair, so that DNA damage will not remain at the time of replication or division. Failure to repair will lead to chromosome aberrations. Describe known checkpoints and known association with DNA repair (Lydell and Weinert, 1996; Bates and Vonsden, 1996).

- Repair and the cell cycle

  Describe variations in repair rates in different stages of the cell cycle. (if not in Section 2). Inducible genes (by DNA damage) might be cell cycle specific (Ch. 13 Friedberg, Walker and Siede).

3. Mechanisms of formation of chromosome aberrations

   (i) Low LET radiations

   - Errors of repair and replication

     Chromosome aberrations can arise by errors of DNA repair (G_1 and G_2, DNA not replicated proximate to exposure) and by errors of DNA replication (for a particular DNA region that is replicated fairly proximately to exposure). The types of aberrations (chromosome-type or chromatid-type) induced will be dependent upon whether they are produced prior to or after DNA replication. DNA dsb are converted into aberrations by misrepair; DNA base damages can be converted into aberrations by misrepair or S-phase replication errors. The process of aberration formation itself probably involves recombination repair rather than simple ligational errors (Preston, 1995). This would need a short description of the two basic models, breakage first and Revell hypothesis.

- Deletions, intrachanges and interchanges

These error processes can lead to different classes of aberration. A complete description of all types can be found in Savage (1997). For the present discussion it is only necessary to describe the basic classes. For both chromosome-type and chromatid-type aberrations, these are deletions (terminal and interstitial), intrachanges (rings) and interchanges (dicentrics and reciprocal translocations). Terminal deletions can arise from a failure of dsb to repair or a failure to complete recombinational repair of dsb or base damages. The other aberration types are a consequence of misrepair. Thus, aberrations can be used to measure repair kinetics and fidelity.

(ii) High LET radiations

- Errors of repair and replication

As for low LET radiations. Higher frequency of aberrations per unit dose. Probability of converting DNA damage into aberration is higher for high LET radiations (misrepair more likely).

- Classes of aberrations are the same as for low LET radiations. The relative frequencies of the different types is different for high vs. low LET radiations (Savage, 1996; Brenner and Sachs, 1994).

4. Shapes of dose response curves

(i) Low LET radiations

The dose response curves for all aberration types (chromosome and chromatid) fit the same general formula $Y=\alpha D+\beta D^2$, i.e. they can be formed by a one-track or a two-track process. It has been suggested that the two processes involve different types of DNA damage (one-track DNA dsb; two-track DNA base alterations). The fact that all aberration types (including chromatid deletions and chromosome-type terminal deletions)

fit a linear-quadratic curve suggests that some form of incomplete recombinational repair leads to all types, rather than simple breakage and misrepair or failure to repair. An exception might be multiply damaged sites that can lead to deletions if not repaired, but whose formation could be proportional to D^2 (i.e. nonlinear with dose).

- Effects of dose rate

In simple terms, at low dose rates chromosome aberration dose response curves will be linear, the contribution from two-track aberrations will be negligible, thus, $Y=\propto D$. The aberration frequencies at low doses (<5cGy) will be effectively identical following acute or chronic exposures.

(ii) High LET radiations

The dose response curves for all aberration types are linear with dose, indicating a one-track process of formation of the DNA damage involved. Aberration frequencies are related to LET such that RBE increases up to a maximum at about 100 KeV/μ and then decreases at higher LET's. This increase in effectiveness can be due to the higher frequencies of adjacent DNA damages from the dense ionization tracks, and/or to differences in DNA damages produced (double strand gaps vs. dsb, for example).

- Effects of dose rate

Since the dose response curve for acute exposures is linear, exclusively one-track aberrations being formed, there is no reduction in yield or change in shape of the curve for low dose rates. The RBE for aberrations induced by low, chronic exposures (<5cGy) of high LET radiations will be similar to that for low level, acute exposures.

5. Distribution of aberrations within and among cells - random vs. non-random

(i) Intercellular distribution

For LET radiations chromosome aberrations are distributed randomly among cells at high and low dose rates. For high LET radiations, as reflective of the distribution of ionization tracks, the distribution of aberrations is non random, with a higher than expected number of cells with multiple aberrations.

(ii) Interchromosomal distribution

The distribution among chromosomes might vary with cell type, for example, in lymphocytes higher frequency background aberrations than predicted at specific fragile sites, and some evidence for increase over expected in irradiated lymphocytes. Other examples of particular chromosomes being involved in aberrations more often than Poisson prediction for low LET exposures. No evidence for high LET radiations.

(iii) Intrachromosomal distribution

For low LET radiations, there is evidence showing that along a chromosome there are "hot spots" for aberration formation. These include light band regions and internal telomere-like DNA sequences. For high LET radiations, there is limited evidence to suggest similar localizations of aberrations.

- Is there evidence that specific chromosomal regions are more or less susceptible to aberration formation?

Thus, as indicated above, there is some evidence to suggest that specific chromosomal regions are more susceptible to aberration formation. There is also some evidence showing that DNA repair after ionizing radiation is non-uniform (most rapid in transcribed regions), whether this leads to more or less aberrations would be a matter for debate.

6. Uncertainties in shape of dose response curve at low doses

(i) Define non linear and threshold responses

A nonlinear dose response, such as $Y=\propto D+\beta D^2$, will show a continually changing slope at high exposures but will be essentially linear at low exposures (5cGy). The magnitude of the low dose response will be defined by $\propto$. If $\propto$ is small and β large, i.e. the curve approaches $Y=D^2$, the slope at low doses will be greatly reduced compared to higher $\propto$ values.

A threshold response is one that has no increase in aberrations until some amount of dose (or DNA damage) is reached. The difference between a threshold response and that for $Y=\beta D^2$ at low doses will be insignificant, but will be significant for $Y=\propto D+\beta D^2$.

(ii) Effect of adaptive response

In cases where an adaptive response has been demonstrated, (low LET radiations) the yield of aberrations is reduced by a factor of about 2. Thus, the shape of the curve at low doses will be reduced maximally by a factor of 2, but will still have a positive slope. It is possible (or arguable) that the adaptive response reduces the two-track component of the dose response curve, and thus will not result in any change of slope at low doses (<5cGy).

(iii) Saturability of DNA repair

Unlikely that DNA repair that correctly rejoins broken ends or completes the excision process would saturate at very low doses (<5cGy). Discuss whether or not DNA repair could be (or has been shown to be) error-free at low doses, i.e. would result in a threshold for chromosome aberrations. Less likely for high LET radiations.

(iv) Inducibility of DNA repair - dose response and relationship to aberrations

Studies of radiation-induced cell cycle check points have utilised high doses. Little is known about the operation of checkpoints at low levels of induced DNA damage. If a G_1/S checkpoint is not induced at low Xray doses this could increase the chromosome aberration frequency as a result of replication errors on a damaged temp-plate. The outcome will be a steeper dose response curve, not a threshold.

(v) Genetic susceptibility

Genetic susceptibilities that would alter radiation sensitivity are most likely to be those that involve housekeeping processes such as DNA repair, DNA replication, cell cycle control genes. It is most likely that the outcome will be an increased slope at low doses, or a non-threshold, if the arguments above on how a threshold might be obtained.

7. Association of chromosome aberrations to cancer

How do studies of chromosome aberrations at low doses impact on cancer dose response?

(i) Hematopoietic tumors

Chromosomal alterations are most frequently translocation involving a breakpoint in the T cell antigen receptor loci or immunoglobulin loci and adjacent to an oncogene. The product is frequently a fusion protein (review by Rabbitts, 1994). There appears to be a single genetic alteration for any particular tumor type.

Thus progression would be predicted to be rapid, and it is. Studies of the dose-response curve for chromosome aberrations and factors that influence those are pertinent to the dose response for tumor formation.

(ii) Solid tumors

- Mammary tumors

At the chromosomal level proposed that there are 5 morphological stages each involving specific chromosome alteration or gene mutation (Sandberg, 1993).

- Colorectal cancer

  Specific stages and associated mutations and chromosome losses described by Fearon and Vogelstein (1990). Involvment of mismatch repair processes.

- Bladder cancer

  Proposed that there are 5 stages associated with gene alterations or chromosome losses (Sandberg, 1993). Thus, progression would be lengthy and multiple changes in single cell are needed. Studies of chromosome aberration induction at low doses would be partially useful for describing the tumor dose response curve. Multiple steps could allow for positive slope for chromosome aberrations, but threshold for tumors themselves.

8. Biological dosimetry using chromosome aberrations

   Types of study

   - Acute exposures

     A-bomb survivors (Awa et al.) long range retrospective

     Accidental, occupational exposures (Lloyd et al.) recent retrospective

     Medical exposures (e.g. Buckton et al., ankylosing spondolytics; Littlefield et al., childhood thyroid exposures)

   - Chronic exposures

     Shipyard workers (Evans et al.)

     Atomic energy workers (Lloyd et al.)

     High background areas (e.g. Monozite sands)

Data in general fit $Y=\propto D + \beta D^2$ for acute, and $Y=\propto D$ for chronic. Thus, they are not suggestive of a threshold. The simplest view would be no threshold for cancer given the role of chromosome damage and mutations in tumor formation. However, this might hold for hematopoietic tumors but not necessarily for solid tumors–multiple steps, one cell.

9. Summary and conclusions

(i) New information needed

(ii) Studies to accomplish this

- Mechanisms of tumor formation better understood, especially role of specific mutations and chromosome alterations.
- Kinetics and fidelity of DNA repair at low doses.
- Inducibility of repair at low doses

Section 5

ONCOGENIC TRANSFORMATION IN VITRO

1. Dose Response Relationships

a) Rodent fibroblasts. Extensive data are available for 3T3 and C3H 10T1/2 cells for both high and low LET radiations. One of the most remarkable features of transformation in rodent cells is the high frequency, much too high to be accounted by a mutational event. This is not true for human cell lines. No-one has ever succeeded in transforming primary human cells with any dose of any type of radiation. Even immortalized human cells are transformed at only low frequency even by α-particles.

b) Human Cells. Dose response relationships are available for a hybrid cell line (HeLa normal fibroblast), missing a suppressor gene, and for a sarcoma line into which the Rb gene is transfected. Several point estimates are available of transformation frequencies for immortalized human epithelial cells exposed to α-particles, but no dose response curves are available because the frequencies are several orders of magnitude lower than for rodent cells.

2. Descriptions

- Dose response curves for rodent cells are empirical - molecular mechanisms are not understood.
- Frequency too high for the cause to be a single mutation.
- There is evidence that transformation is a multistage event and that the initial event may have a high probability.

3. Shape of curve

- Most dose response curves appear to be linear at low to intermediate doses-reaching a plateau at higher doses
- Data are available down to doses of about 10 cGy of γ-rays, or 1 cGy of neutrons.
- Marked variation of sensitivity through the cell cycle. Window of sensitivity of γ-rays in G2/M.
- There is some evidence that the dose response curve has a complex shape. While the data do not exclude the possibility of linearity at low doses, they suggest caution is needed to extrapolate from intermediate to low doses. This complex dose response curve may reflect the variation of sensitivity through the cell cycle.

4. Dose-rate

- Sparing effect for low LET radiation.
- Increased effect of high LET radiations shown for low dose-rate or fractionated exposures - the so-called inverse d/r effect.
- Biophysical models of the inverse dose-rate effect based on the variation of sensitivity through the cycle.
- The dose level at which all dose-rate effects disappear provides information in the relevant target size.

6. Modulation

The frequency of transformation following a given X-ray dose can be modified by post-irradiation manipulations.

- Increased by tumor promoting agent (TPA)
- decreased by protease inhibitors
- The age-response function can be flattened by the post irradiation addition of TPA

7. Genomic Instability

Immortalized human epithelial cells show progressive instability following irradiation, involving chromosomal aberrations, loss of anchorage dependent and eventually the ability to form a tumor in immune suppressed animals. These phenotypic changes are paralleled by changes at the molecular level, including p53 mutations and an overexpression of cyclin D1.

Section 6

(3)

[redacted]

ncrp sc 1-6 B
II. Animal Models and *In vivo-In vitro* Studies

I. Introduction and Generalizations

It seems to me that if there is general agreement about a series of premises, the data can be more readily interpreted. Perhaps we can discuss the following in more detail:

- Are the following generalizations and inferences about radiation effects and carcinogenesis generally acceptable?

1. Carcinogenesis is a multistage process, minimally involving *initiation*, *promotion* and *progression*.

2. In most instances, radiation carcinogenesis experiments deal with radiogenic initiation, infrequently with radiogenic promotion.

3. The single radiation dose-carcinogenesis response relationships of greatest interest to the Committee are those that predominantly presuppose radiation to be acting as the initiator.

4. The multiple low radiation dose-carcinogenesis response and the low radiation dose rate-carcinogenesis response relationships may involve radiation as an initiator and/or a promoter.

5. Radiation causes both point mutations and chromosomal breaks with rearrangements during repair. Such genetic events in unirradiated or otherwise treated mammalian cells occur at frequencies of 10^{-7} - 10^{-5} per cultured cell generation, and are increased by one to three orders of magnitude by radiation doses that permit significant cell survival.

6. Radiation causes non-mutational ("epigenetic") events or processes such as chromosomal instability and increased chromatid exchange rates, changes in DNA methylation patterns which alter gene expression, and induction of some specific enzymes. These effects occur at very high frequencies in cultured cell systems and some of them persist for two to several cell generations.

7. Leukemias arise from pluripotential or committed incompletely differentiated precursor cells.

8. Carcinomas also most generally arise from incompletely differentiated cells, either precursor cells or cells that have dedifferentiated. Such cells generally represent small subpopulations of the total epithelial cell population of a tissue.

9. Where measured, radiogenic initiation is a highly common event within the relatively small subpopulations of cancer susceptible cells.

- Inference #1: Hence although the possibility of initiation by a mutation at any one of a large number of genetic loci can not be excluded, radiogenic initiation is most likely to generally be an epigenetic process. The mutations that become prominent during carcinogenesis may be rare later events that occur during promotion/progression and are increased in frequency by the radiation-induced epigenetic changes or promoting conditions. Alternatively, in some cases such mutations may be the result of expansion of small populations of preexisting mutant cells.

- Inference #2: Radiogenic promotion by chronic exposure to radiation at low dose rates or to multiple small doses at high dose rates may act through the same epigenetic pathway(s) as are responsible for initiation.

- Inference #3: Those conditions which stimulate terminal differentiation would be expected to reduce the frequency of progression to cancer.

Section 7

Annotated Outline of Epidemiologic Material for NCRP SC 1-6 on Low-Dose Linearity

Interpretation of epidemiologic data

- Weight of evidence approach
  - ◊ Examine the consistencies in all good-quality sources of data

    ** Two approaches will be taken. When dose-response data and analyses are available for relevant cancer endpoints, these will be presented. In addition, the risks seen in the strongest of the low-dose worker (or other) studies will be summarized.

  - ◊ Recognize that small numbers & subgroup analysis can lead to apparent irregularities in the data

    ** Will discuss the limitations of epidemiologic data, particularly in the low-dose range. There are reciprocal issues of detecting a risk and of ruling out large risks at low doses.

Cautions in use of epidemiologic data to evaluate low-dose effects

- Weaknesses of some study designs

  ** Both ends of the spectrum of preconceptions (i.e., the hormesis camp and the catastropic-risks camp) have placed undue reliance on selected results that are generated by weak studies. This section will aim to provide some qualification and tempering of the interpretation of data from weak studies.

  - ◊ Aggregate ("ecological") studies

    ** Greenland and others have pointed out the large potential for (generally undetectable) biases in this type of study. Several of these will be summarized, and it will be mentioned that they apply to various studies in the literature (e.g., B Cohen's radon-lung cancer study).

  - ◊ Case-control studies

    ** Problems here have to do with sample selection biases, and especially with information bias in the case where people's self-reports are used to characterize past exposures.

- Limitations of epidemiologic data

** For risk assessment, epidemiologic data are usually high on validity but low in precision.

◊ Reduced statistical power and precision in the low-dose range

** Examples will be given of how power & precision diminish at lower doses. The implication will be emphasized that null results in such circumstances are not a strong basis for inferring no effect.

◊ Few data available permitting high- and low-LET comparisons

** The main high-LET data are the radium dial painters, thorotrast patients and radon-exposed workers.

◊ Heterogeneity of human populations

◊ Leads to less precision in risk estimates

◊ Genetic, age and gender variations provide insights

** Heterogeneity potentially stems from the amounts and types of other carcinogenic exposures, as well as genetic and other factors. Substantial gender variations occur for only a few cancer sites. Age variation may apply to a number of sites, although thyroid and breast are perhaps the most marked. Genetic variation will be discussed in another section, below.

◊ Heterogeneity among studies due to variations in radiation parameters

** Variations in dose rate or dose fractionation, total dose (or dose range), localized vs. total-body irradiation, mixed types of radiation (gamma, neutron, etc.)

Examination of epidemiologic data for dose-linearity and low-dose risks

- Desirable characteristics of an epidemiologic model system

** Tumor site with low background rate and high radiation sensitivity; groups with substantial and well-quantified exposures, long follow-up period.

Major sources of information:

- RERF atomic bomb study
- ~10 large medical-irradiation series that have informative data for various cancer sites.
- A few case-control studies that have objective (rather than self-report) data

- A few of the largest radiation-worker studies (where large is defined in terms of person-year Sv).

Review of dose-linearity & low-dose data for various cancer sites

- Leukemia

  ◊ Postnatal exposure

  ** Review of dose-response data and selected low-dose studies (see *Science*, p. 1821-22, 29 Mar. 1996; BEIR V; UNSCEAR 1994)

  ◊ Prenatal exposure

  ** Review of the available case-control studies (of which the Stewart-Kneale and study is the largest) and of cohort studies (mainly MacMahon-Monson study). Comparison with Japanese atomic-bomb results

- Thyroid cancer

  ** Variety of studies available with external radiation (little fractionation) and a few with radioiodine exposure. Strong age effect discussed.

- Breast cancer

  ** Summary of available studies with dose-response data. Age effect discussed. Interactions of radiation with other risk factors for breast cancer.

- Lung cancer

  ◊ Inverse dose-rate effect for high-LET radiation (radon)

  ◊ Direct dose-fractionation effect for low-LET radiation (fluoroscopic examinations)

  ** Also comparison of radon and atomic-bomb risk estimates. Evidence on dose-response relationships.

- Colon cancer

  ** Examination of shape of dose-response curves. Status of findings from low-dose groups.

Impact of host susceptibility factors on dose linearity

** Theoretical impact of this. Mention GAO report (Libossi ?) thesis. Implications of this for a dose threshold.

- Known genetic factors
  - ◊ Retinoblastoma and Rb1 gene
    - ** Summary of findings re: susceptibility to radiation-induced cancers.
  - ◊ Nevoid basal cell carcinoma syndrome
    - ** Summary of findings re: skin cancer induction by radiation.
- Possible genetic factors
  - ◊ Potential to examine genetic heterogeneity for breast cancer – BRCA1, BRCA2, ATM
    - ** Controversy regarding ATM heterozygotes (Michael Swift). What evidence is available regarding radiation-sensitivity for breast cancer and the BRCA1/2 genes?
  - ◊ Colon cancer mismatch repair genes (MSH2 and MLH1) and APC gene
    - ** Any indications of radiation-sensitivity in those with mutated genes?

Interactions of radiation with other agents

- Lung cancer – smoking
  - ** Radon & smoking; atomic-bomb & smoking.
- Skin cancer and UVR
  - ** Magnitude of ionizing radiation risk for skin cancer in darker colored populations compared with caucasian populations.

Implications of Epidemiologic Data for Dose Linearity

- Relate to existing models
  - ** Linear-quadratic formulation or Moolgavkar models both show some risk at low doses.
- Discuss how alternative models (e.g., threshold model) would have to be shown superior (in multiple/pooled studies) before non-linearity could be accepted
  - ** Not sufficient to pluck out a few studies as cases-in-point for a threshold, because of the low statistical power in such studies.

Major gaps in information

- More information on protracted or highly fractionated radiation exposures.
- Information on how genetic factors affect radiation risk.
- Information on the temporal course of risks, especially in relation to the influence of types of malignancy, age, gender and genetic factors.
- Low dose studies have limited precision and possible biases, so it is unlikely that epidemiologic data could ever provide definitive results that would conclusively demonstrate a threshold or a hormetic effect.

Section 8

Interpretation of Adaptive Responses

(To be drafted later)

Section 9

Conclusions

(To be drafted later)