A REVIEW: SOME BIOCHEMICAL EFFECTS OF HIGH LET RADIATIONS
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Abstract

The natural environment of outer space may theoretically produce a significant exposure of high LET radiation to the space traveler. The use of nuclear reactor power systems may increase this exposure.

Since biological endpoints of radiation damage are inevitably due to biochemical changes, it becomes of interest to consider the effect of high LET radiations at the biochemical level. There are qualitative and quantitative differences in the biological damage observed after exposure to high LET radiation (such as heavy ions, protons, neutrons and mesons) as compared to that caused by low LET radiations (such as electrons, x-rays, and gamma rays). This review is concerned with these differences, which are ultimately reflected at the biochemical, cellular and even whole animal levels. In general, high LET radiations seem to produce biochemical damage which is more severe and possibly less repairable. Experimental data for these effects will be presented in terms of biochemical RBE's with consideration of both early and late manifestations.

An LET independent process by which significant biochemical damage may result from protons, neutrons and mesons will be discussed.

Introduction

The natural environment of outer space may produce a significant exposure of high LET radiation to the space traveler. This exposure consists of a wide variety of particles including high energy heavy ions, and perhaps some neutrons. The interaction of these particles with the space capsule may produce secondary particles such as mesons which may have significant mean life and penetration ability to be of some hazard (ref. 1). The contemplated use of nuclear reactor power systems as propulsion systems may further increase the exposure, since it is probable that weight requirements will necessarily restrict the shielding so there may be some exposure to epithermal and thermal energy neutrons.

In a review of radiobiology literature one becomes impressed by some general aspects in which there are significant differences in the biological response to high LET radiations (such as neutrons, heavy ions, protons, and mesons) and low LET radiations (such as electrons and x-rays):

1. The incidence of mutations and chromosome abnormalities after high LET radiation is impressively higher than that observed after low LET radiation. The type of genetic change observed is quite variable ranging from subtle almost undetectable mutations to rather drastic endpoints such as carcinogenesis and reproductive death.

2. There is apparently a deficiency of cellular repair following radiation damage from high LET particles which is contrasted with significant or complete repair after low LET radiations (ref. 2).

3. There is relatively little oxygen dependence in the production of cellular damage with high LET radiations, contrary to a great oxygen dependence of low LET radiation (ref. 3).

From this general information, one may speculate that there are some rather significant differences between the effects of high and low LET radiations at the biochemical level. The purpose of this review, therefore, is to explore some of the currently available literature in this field (which is remarkably sparse). Also, since the purpose of a symposium is not only to exchange ideas, but perhaps to attempt to define further areas of needed research, I would like to offer some speculations and opinions.

One of the problems in discussing the biochemical effects of high LET radiation is that the biochemical effects of low LET radiation are not well understood. The term "high LET" radiation itself introduces complexities since this covers a variety of types of radiation and, as will be seen from some of the data, the physical and biological manifestations of high LET radiations are highly dependent on the energy and type of particle being discussed. However, there are some points that can be made by giving a brief summary of some of the types of biochemical experiments being done.

In attempting to describe the types of experiments that have been done with high LET radiation on biochemical processes, it seems useful to discuss these experiments in terms of biochemical RBE's since, although one has to be very specific in using this term, it has both fundamental and practical implications. The studies that will be

\*LET can be defined as the rate of energy loss along the track of an ionizing particle with units such as KeV/micron.

\*RBE is the absorbed dose of standard radiation (60Co or 220 KV x-rays) required to produce a biological endpoint.

\*absorbed dose of test radiation required to produce the same biological endpoint.
discussed are those that were directed toward the study of DNA and RNA metabolism, since these macromolecules are involved in the master coding processes of the cell and, therefore, ultimately responsible for the transmission of genetic information and for cellular reproductive processes. And, there is substantial evidence that low LET radiation causes a perturbation of the functional integrity of DNA, which somehow is related to reproductive death of the cell (ref. 4).

As an introduction to some known sites of radiation damage at the biochemical level, Fig. 1 demonstrates, in a simplified manner, three processes by which DNA is ultimately related to cell function and reproduction. These processes are:

1. DNA replication, the process by which DNA duplicates itself, so that at mitosis (cell division) two genetically identical cells are produced.

2. RNA transcription, the process by which several types of RNA (messenger, ribosomal, and transfer RNA's) are formed from one of the DNA strands. These RNA's have variable lifetimes and functions within the cell and are essential intermediate molecules for transferring the DNA instructions for ultimate protein synthesis.

3. Translation, the process by which proteins are made through the appropriate assembly of a sequence of different amino acids.

This diagram is indeed oversimplified since there are many other intermediate steps involving various enzymes and energy providing compounds, but it is useful for illustration of some general sites and mechanisms of radiation damage by high and low LET radiation.

There is considerable radiobiological data indicating that double and single strand breaks of DNA are produced by ionizing radiation (ref. 13). There is also some evidence that double strand breaks occur in DNA more frequently from high LET radiation than from low LET radiation and this is an attractive means of explaining the deficiency in repair noted for high LET radiation (ref. 14). One example of this repair deficiency is demonstrated in cellular "survival curves" where high LET radiations have smaller or absent shoulder regions (ref. 15). These aspects of cellular radiobiology will undoubtedly be discussed in more detail in other parts of this symposium.) However, the full significance of single and double strand breaks to ultimate cell death is at present unknown, since there is also evidence that both of these lesions may be repairable (ref. 16), and even that double strand breaks may not be as well correlated with "LET" as has been assumed (ref. 17). However, it is conceivable that either double or single strand breaks, if they are not repaired, could cause drastic perturbations of either DNA replication or transcription.

There is also evidence that the bases of DNA can be significantly damaged by either high or low LET radiation (ref. 6), and this type of damage could be expressed functionally as either inhibition of replication and transcription or by causing the production of a defective quality of DNA or RNA --- and either of these types of damage could lead to various biological endpoints.

With this background we can now discuss some specific experiments on the biochemical effects of high LET radiations. One such experiment by Yatvin et al. (ref. 18), involved the study of fission neutrons as compared to x-rays on polysomes. In Fig. 1, it is seen that polysomes are messenger RNA-ribosomal RNA complexes which are involved in the transmission of information at both the transcription and translation levels. It had been shown by Curtis that fast neutrons (which in tissue produce energetic protons by elastic scatter and protons and α's by nuclear reactions with nitrogen and oxygen) that there was a significant deficit in the ability of regenerating liver to repair induced chromosome abnormalities (ref. 19). It was therefore of interest to study in this same system the DNA-RNA transcription function by studying polysomes. The details of the experiment are a little complex for this discussion, but essentially it was found that fast neutron radiation was not significantly more damaging than x-radiation --- specifically, the polysome pattern following both types of radiation showed an initial decrease, but followed by a recovery in the number of heavy aggregates at 36 hours after irradiation (implying the possibility that if the DNA-RNA transcription apparatus is damaged, then it would seem to be temporarily damaged, and "repaired" to the same extent for both types of radiation). Whenever whole animal irradiations are done, however, there are many abscopal effects to be considered and, in general, there are usually many interpretations.
As another method of looking for transcription damage following high and low LET radiation, we utilized the regenerating liver system. The system and experiments are described elsewhere (ref. 7), but, in general, these experiments were concerned with immediate and delayed damage (following fission or 2.6 MeV cyclotron neutrons as compared to x-rays) in addition to the possible cell cycle dependence of biochemical damage. Our conclusions following 2.6 MeV cyclotron neutrons, was that there appeared to be immediate inhibition of rapidly labeled RNA synthesis rate (which probably is m-RNA synthesis due to the design of the experiment in this semisynchronous system.) It is known that cell reproductive death has variable sensitivity to low LET radiation as a function of cell cycle (ref. 20), but our studies with fast neutrons seem to indicate an inhibition of RNA synthesis rates during early G1 and early S, and G2, while no inhibition at any stage was observed following x-irradiation. In addition, comparing either fission or cyclotron neutrons versus x-rays at one month or nine months following irradiation, we observed statistically significant depressions in the rapidly labeled RNA synthesis rate at early G1 and early S phases following the neutron radiations, but no significant change was noted with x-rays. Our conclusions were that there appears to be both immediate and delayed inhibition of rapidly labeled RNA synthesis rates (probably reflecting inhibition of transcription) in a rather cell cycle independent manner and at low doses (300 rads) following fast neutron radiation, but not for x-ray irradiation. These results, along with other experiments to be described, are summarized in Fig. 2.

Another whole animal experiment was done by Tsuya and Okano (ref. 5) in which the effect of fast neutrons on DNA synthesis was studied. Their procedure was to irradiate mice with various energies of fast neutrons ranging from 0.43 MeV to 1.8 MeV and then to label with 3H-Thymidine approximately 2½ hours after irradiation. Specimens were then taken from spleen, thymus, bone marrow and intestine and the rate of DNA synthesis as compared to control was determined in each of these organs. In this system the RBE for inhibition of DNA synthesis was found to vary according to cell type, and neutron energy, i.e., for bone marrow cells the RBE value for 0.43 MeV neutrons is 3.6 (at 500 rads) and was approximately 1.2 (at 90 rads) for 14 MeV neutrons. The RBE for DNA synthesis for 14.1 MeV neutrons was also found to be 1.1 for thymocytes and 1.8 for intestine cells. The value of the experiment is that it points out that biochemical RBE's are very dependent on cell type and neutron energy.

In other experiments by Tokarskaya and Kuzin (ref. 6), a reactor (which had the usual significant gamma contamination) was used to study the effect of fission neutrons on DNA synthesis in pea sprouts. The technique of this experiment was to irradiate dry seeds and to cause them to germinate several days later. The DNA synthesis rate was then compared to control and it was found, for doses of radiation from 1000 to 10,000 rads, that the inhibition of DNA synthesis rate by fission neutrons was always greater than that of the gamma irradiated seeds. The comparison between the fission neutron and gamma irradiated DNA synthesis rates was variable, but the RBE was 7-10 with gamma dose around 10,000 rads, while it was approximately 1.3 with doses around 1,000 rads. In addition biochemical analysis of the DNA after the neutron irradiation showed that neutron irradiation of 10,000 rads led to rather selective damage to the adenine base by deamination and conversion to hypoxanthine. This resulted in an ultimate shift of the AT to GC ratio in the DNA from 1.0 to 0.67. However, for 10,000 rads gamma dose, the AT to GC ratio changed from 1.0 to 0.92, so a 'qualitative' RBE of 92/67 = 1.4 was obtained. Thus from both a 'quantitative' and 'qualitative' point of view, neutron damage to DNA was more severe than gamma. This qualitative change seen with neutrons and not by gamma rays makes the enhanced mutation rate from neutrons in many animal and plant systems more understandable, since a change in the coefficient of specificity in DNA could easily alter transcription and thus invite mutations.

Duzin and Vainson (ref. 8) irradiated Hela cells with particles or x-rays and one hour later studied the effect on DNA synthesis rate by autoradiography. They found an RBE of 1 and thus could not associate a depression in DNA synthesis rate with cell death. This correlates with the low LET results of others in that it supports the concept that the process of DNA replication may not be responsible for cell death.

Hutchinson (ref. 11) also found a "qualitative" RBE change in DNA as a function of LET when he irradiated, with various heavy ions, streptomycin-resistant pneumoccus bacteria and then extracted the DNA, which was subsequently tested for ability to transform streptomycin-sensitive pneumoccus. He found that the RBE for inhibition of transforming activity rose to 2.5 when the LET was increased.
to 100-500 MeV/cm²/gm and then decreased to 1.0 again at about 3,000 MeV/cm²/gm.

There has been only one experiment regarding the possible effects of \( \pi^- \) mesons on DNA (ref. 12). This was done by Raju et al., where they studied some genetic changes of specific loci in diploid yeast cells. The RBE obtained for inducing these changes was 1.5, so again one may postulate a change in the quality of the DNA, which is reasonable when one considers that \( \pi^- \) mesons can modify DNA by annihilation of atoms, as well as by high LET particle tracks.

Some interesting experiments were done by Huston and Pollard (ref. 9) in which they irradiated E. Coli (prelabeled with \(^3\)H-Thymidine) with several energies of protons as compared to \(^{60}\)Co. They immediately post radiation measured the degradation of the DNA and found that 1.3 MeV protons (LET = 240 MeV/cm) was degraded much more rapidly as a function of dose than for 4.75 MeV protons (LET = 110 MeV/cm) or \(^{60}\)Co rays. Biochemical RBE’s can thus be obtained (by dividing slope ratios) and for 1.3 MeV protons the RBE = 6, while for 4.75 MeV protons, the RBE = 2. Thus, this system again proved to be very energy dependent and those changes observed in DNA may be thought of as qualitative changes. Also, the authors raised the possibility that the results may be due to some damage at the transcription level.

As was mentioned initially, there is a great tendency in the literature to associate qualitative changes (such as cell death and mutation) with the concept of LET. There certainly is justification for this when one looks at data such as that of Barendsen (ref. 21) and Skarsgard (ref. 22) where these end points seem to be well correlated with LET changes. However, LET concepts do not completely explain some biological effects, so it may be useful to look for other mechanisms than LET associated electron orbital ionizations, when trying to explain particle radiation effects. There is some evidence for a type of radiation damage that need not be correlated with ionization and LET associated events. This can be described by the following experiments:

Esochard (ref. 10) used thermal neutrons to irradiate tomato seeds, studied the resulting genetic mutations, and found RBE’s from 1.5-3.0. His conclusion was that an important part of the enhanced RBE of thermal neutrons was due to the \(^{14}\)N \((n,p)\) \(^{14}\)C nuclear reaction where \(^{14}\)N is naturally present in the plant cell (and in the DNA). He found that if \(^{14}\)N was substituted in the plant nutrients that the enhanced RBE did not occur (since \(^{15}\)N does not absorb thermal neutrons). Also by controlling the \(^{10}\)B concentration in the plant, the \(^{10}\)B \((n,\alpha)\) \(^{7}\)Li reaction effect was studied and by analysis, he found that the \(^{14}\)N reaction had a larger effect per rad on RBE (since the \(^{10}\)B reaction usually occurred in the membrane or cytoplasm) and the \(^{14}\)N is to some extent in nuclear DNA. Thus, when the \(^{14}\)N \((n,p)\) \(^{14}\)C reaction occurs in DNA, two types of damaging events can occur.

1. The ejected proton has an energy of 0.59 MeV and an LET of 45 KeV/\(\mu\)m, and this could cause double strand breaks (which, however, might be reparable).

2. The \(^{14}\)C atom formed has enough energy (42 KeV) to recoil and break all chemical bonds, thus leaving an empty space in the DNA strand --- the efficiency of biological systems to repair this kind of damage is unknown (and perhaps is non-existent, since only in recent history have living things been exposed to particles such as neutrons and \( \pi^- \) mesons, so there may have been no evolutionary development to cope with this type of lesion).

Another fascinating type of experiment by Jung and Zimmer (ref. 23) and more recently by Watt and Hughes (ref. 24) is the study of the inactivation of enzymes such as ribonuclease by very low energy (less than 100 KeV) protons. They have noted that the enzyme inactivation is highly dependent on energy as is shown in Fig. 3. The curve denoted by \(^{15}\)N signifies the enzyme inactivation cross section reaches a minimum at 1 KeV, but then rises again steeply below 1 KeV. The electronic stopping cross section \(\sigma_e\) is noted to fall and become negligible at 1 KeV, while the nuclear stopping cross section, \(\sigma_n\), begins to rise at 10 KeV and thus rises in conjunction with the enzyme inactivation cross section, indicating a very strong correlation between nuclear elastic scatter (displacing a hydrogen or heavy atom from the enzyme molecule) and the biological inactivation of ribonuclease! Thus, this type of damage really had nothing to do with LET concepts (except the casual association that low energy protons do in fact provide fairly high LET’s). There is of course no reason why this type of physical lesion is not induced in DNA and RNA by irradiation with various energies of neutrons, protons, \( \pi^- \) mesons, and (even \( \alpha \) particles and other heavy particles) and such damage must be of real significance to the functional integrity of the macromolecule.

It is known that even small changes in the hydrogen atom movement perturbs hydrogen bonds in DNA and may be responsible for tautomeric shifts in base pairing and mutations (ref. 25) Thus, even a gentle elastic collision could cause major alterations in the DNA. One might argue that an LET dependent ionization type of damage is still the most significant and this is usually hard to disprove, since the system is 'saturated' with LET dependent electronic ionizations. The answer to this question will have to come from experiments like those of Jung and Zimmer and Watt and Hughes. The point is, however, (as originally implied by Platzman (ref. 26), that ionization related damage may be much more reparable than nuclear type damage is interesting and should be vigorously pursued. It is certainly conceivable that even very small perturbations (such as the removal of a single key atom) in the DNA or RNA structure could lead to drastic endpoints, since these molecules are capable of great biochemical amplification of damage.

If nuclear interactions and reactions, therefore, are important (and it seems they might be) when evaluating biochemical responses to particle
radiation, then new avenues are opened to investigators in many fields; as an example, a better knowledge of these reactions and their biochemical effects might enable and promote the synthesis of new types of drugs ('neutron sensitizers') which might then be used to supplement the possible value of fast and lower energy neutrons in the treatment of human malignancies. (Attempts to develop such drug sensitizers for thermal neutrons (ref. 27) and low LET radiations (ref. 28) have already been made, but the results thus far are disappointing. However, the development of drugs that might utilize nuclear reactions associated with higher neutron energies, such as resonance and threshold reactions, might be rewarding.)

When discussing neutrons (which due to their neutral charge have the capability for producing an especially large number and variety of nuclear reactions), it is possible to group their reaction types into several categories, (a) elastic scatter, (b) inelastic scatter, (c) resonance absorption, (d) thermal absorption -- and all these reactions are highly and (as in the case of resonance absorption) precisely energy dependent. For biochemical purposes, their reactions can be more simply categorized into reactions which cause atomic displacements or atomic transmutations.

As theoretical examples, the mechanism and possible significance of damage to an A-T base pair of DNA by some possible nuclear interactions and reactions are described in Fig. 4, 5, or 6. The actual significance of these types of interactions and reactions is at present unproved. But, in reviewing the data of Fig. 2, one becomes impressed at the rather large energy and particle dependence of the RBE's observed, (by comparing the results of the proton-DNA degradation experiments (ref. 9), the thermal neutron effect on tomato seeds genetics (ref. 10), the fission neutron base ratio experiments (ref. 6), the immediate and delayed inhibition of RNA synthesis experiments (ref. 6, 7), and the experiments of transformation of DNA) (ref. 11). Such particle and energy dependence is highly suggestive of nuclear process cross sections. Also, in each of these cases (although there is a wide range of biological systems utilized from bacteria to the whole animal), there is direct correlation between the implied and measured functional integrity of DNA. In addition, there is great variation (from immediate to 9 months later) in the time from radiation to observations, suggesting the possibility that these findings reflect the presence of a DNA or RNA lesion(s) which might not be repairable, even over long periods of time. An interesting experiment would be to try to correlate nuclear damage (σp) to DNA with lack of repair (as shown by lack of a high LET e.s.c. shoulder).

In closing, it is relevant to mention some ways in which the better knowledge of biochemical effects of ionizing radiation (and in particular "high" LET radiation) is applicable to problems such as manned space flight. As has been mentioned, one biological endpoint that is accentuated by "high" LET radiations is cell death and we should learn more about this endpoint by study at the molecular level. (The development of good, quick response, RBE dependent, biochemical dosimeters should also be stimulated by this type of research). Life threatening, massive cellular death, however, will occur only with large exposures; and for the lower doses that are currently being recorded in manned spacecraft, perhaps this endpoint is not the most realistic hazard. A more incidious hazard which might be observed with chronic low exposures to "high" LET particles is carcinogenesis. From the data presented, it seems likely that even very small perturbations (such as perhaps a single atom displacement, or annihilation) in DNA or even RNA (in view of Temin's recent work) (ref. 29) could theoretically be directly related to the development of an endpoint such as carcinogenesis. Thus, by further study at the biochemical level and by acquiring accurate, specific biochemical RBE's such diverse fields as space flight health physics (where this information would be very useful in assigning quality factors) and the radiotherapy of human malignancies might be mutually benefitted.

Fig. 3: Calculated stopping cross sections for electronic (e) and nuclear (σn) processes as a function of proton energy and experimentally determined inactivation of yeast mutants resulting from radiation (σk) as a function of proton energy. (All curves were redrawn from ref. 13.)
SOME POSSIBLE ATOMIC DISPLACEMENTS AND ATOMIC TRANSMUTATIONS FROM NEUTRON INTERACTIONS WITH THE PHOSPHATE LINKAGES IN DNA

Fig. 6: Fast neutron (Φ fast) and thermal neutron (Φ thermal) reactions with atoms in the phosphate linkage of DNA. The 31P absorbs a thermal neutron and forms 32P which is radioactive and decays to 32S, which is not able to maintain proper chemical bonds and the strand breaks. The Φ displaces the oxygen atom, leaving an atomic void and a broken DNA strand.
REFERENCES


