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COMMENTARY ON THE PANEL DISCUSSION

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Auger emitters present at one and the same time a potentially valuable tool in biological and radiobiological research, a prospect of therapeutic application and a possibly serious radiological hazard. The reason for the qualifications on each of these statements is the uncertainty in our knowledge of the mechanisms by which Auger emitters achieve these effects. The process of Auger decay is a complex one involving the release of many electrons, mostly of low energy; a charging up of the residual atom, as a consequence; and, in the case of spontaneous Auger decay, the transmutation of the decaying radionuclide. Most attention has been focussed on the role of the electrons and, to date, little is understood of the consequences of charging. Theoretical calculations taking into account the stochastic nature of the decay predict intense deposition of energy close to (a few nanometers) the source of the event, and pioneering work in the field confirms that, under laboratory conditions, damage in DNA is limited to the region close to the site of decay. But the cell is altogether a more complex entity, and it is certainly a challenge to confirm this finding in DNA in a cellular environment

Nevertheless, the unique properties of Auger emitters are generally regarded as being due to this very highly localized irradiation albeit that some findings are not entirely supportive of this position. However, there is considerable potential for experimental work in this area as the following discussion indicates. Although the subject is still in the early stages of development, for example, fundamental dosimetric issues can still cause

disputes (see below), it has developed considerably since the last meeting in this series four years ago.

There has been a long running debate in radiobiology in general over whether radiation effects on DNA are due to direct absorption of ionizing energy or mediated indirectly by radiation induced free radicals. Humm (Boston) sought to clarify the concepts, direct and indirect, in the context of Auger effects. Martin (Melbourne) had spoken of direct excitation followed by energy migration, Pomplun (Jülich) had spoken of ionizations within the bound water of DNA. Hofer (Talahassee) had suggested that the extent of indirect effect was measured by oxygen enhancement ratio. Adelstein (Boston) agreed that careful definitions were important. Watery radicals generated close to the DNA probably had no 'choice' but to react with it and therefore could not be classified as 'indirect' on the basis that scavengers can intervene to prevent indirect effects. However, some molecules, such as cysteamine, were able to 'repair' damage by hydrogen donation in addition to scavenging the radicals. This restitution effect need have nothing to do with 'indirect' effect. In simple molecular systems it was possible to distinguish these different effects but in cells this was not possible. Experiments with ^{125}I incorporated at specific sites in the DNA held some promise of resolving these issues especially with a strong model building input.

Sastry (Amherst) urged the retention of the conventional definitions rather than trying to redefine the terms in relation to the effects of Auger emitters. Phenomena such as oxygen effect were a product of the subsequent chemistry and as such not relevant. Halpern (Jülich) pointed out that microdosimetric models of DNA damage based on events initiated over time scales of 10^{-16} s by Auger electron tracks had to relate to measurements made on vastly greater time scales. He wondered about the relevance of such extrapolations, as the microdosimetry is essentially concerned with the physics of energy deposition, but the chemistry eventually responsible for the biological damage that occurs much later.

Clearly this debate is far from resolved and recent work with Auger emitters has highlighted new aspects. For example, the confirmation by Martin of his earlier experiments so successfully explained by 'direct' effect, the fascinating results of Yasui (DeKalb) who finds no evidence for DNA protein cross-linking in cells with Auger emitters located in the nucleus in contrast to X irradiation, the results of Rao and colleagues (Newark) who showed a protective effect of cysteamine *in vivo* and the results of Hofer

(Talahassee) indicating a low-LET like survival curve in cells pulse labeled and harvested for freezing and ^{125}I decay accumulation shortly after labeling as opposed to a high-LET like response at later times. These results seem to highlight the essential complexity of the cell in relation to *in vitro* systems. In my view we have to build models of predictive power based on these mechanisms and design experiments to test the predictions.

A second contentious issue is that of the induced Auger cascade in cold elements and the related issue of the sensitization of radiation effects in DNA containing cold bromine or iodine. This first effect we shall refer to as enhancement and it results from stimulating a cold atom with radiation at or above the absorption energies of inner shell electrons, so called edges. The second effect we shall refer to as sensitization and is due to the extra radiosensitivity that is observed when halides are incorporated into DNA. One question is, do 'edge' effects give radiobiological enhancement? Some experimenters, notably Hieda (Rikkyo), Kobayashi (Tsukuba) and Maezawa (Tokai), claim they do, producing experimental evidence from relatively simple molecules while some theoreticians, notably Humm (Boston), predict only very tiny enhancements. Earlier in the meeting Hieda (Rikkyo) and Halpern (Jülich) disputed different methods of converting exposure to dose in the experiments in which solutions of DNA concentrated in a buffer are irradiated with low energy X rays. Hieda used the combined f-factors for DNA and buffer, while Halpern (Jülich) used only the f-factors for the DNA with no account taken of the buffer. It seems that these two approaches lead, respectively, to somewhat over- or underestimated values of the electron dose to the DNA. The issue of which approach gave the better approximation was unresolved at the meeting. Goodhead (Chilton) maintained that if the dosimetry, including geometric considerations, was clearly defined there should be no room for dispute. It seems strange that there should be dispute over the fundamentals of dosimetry in spite of the obvious complexity of the physics involved.

A somewhat curious result was that reported by Laster (Upton) in which iodine-(cold) gave an enhancement in cell killing with radiations above the appropriate edge but bromine did not. Laster (Upton) was confident this was not a problem of dosimetry and suggested that the effect might be due to saturation since the bromine replacement was three times higher than that for iodine. Humm (Boston) pointed out that because the energy of the K edge for bromine is much lower than that for iodine and the photoelectric cross section is sharply dependent upon energy, the much more numerous

'background atoms', *e.g.* oxygen, would tend to mask the effects from bromine more than those from iodine. Halpern (Jülich) drew attention to the greater extent of charge build up in the case of iodine - a problem that remains open. Laster (Upton) added, in support of the suggestion that a saturation effect was being observed, that the radiosensitization effect of iodine at 10% replacement was about the same (a factor 2.2) as that for bromine at about 60% replacement. Schneiderman (Omaha) noted that no account was taken of the differences in growth of cells with bromodeoxyuridine. This modified their response even without exposure to radiation.

Goodhead (Chilton) questioned the validity of an explanation based upon a saturation effect. This could only arise if allowance had not been made for attenuation of the primary beam as it passed through the sample *i.e.* it would be the consequence of incomplete dosimetry.

Clearly experiments of this kind have many complexities and variables which need careful control and there is a need to design experiments to distinguish between the 'edge' effects and those of radiosensitization. It is important to understand the mechanisms involved because of the potential therapeutic benefit, yet experiments are difficult to compare because of differences in percent replacement, sample geometry *etc.* Such problems might be overcome if theoreticians were to try to predict the most advantageous conditions under which to resolve the contributions of edge effect and radiosensitization. Halpern (Jülich) suggested utilizing L5178Y s/s murine lymphoma cells since the sensitization by BrUdR is very small.

Martin (Melbourne) asked how the double strand break yields from Auger enhancement for iodine and bromine compared. Humm (Boston) said that for iodine it was 0.8 DSB/photoelectric interaction and for bromine 0.4. He warned of the importance, in this instance, of distinguishing between dose and fluence. Below the edge the photoelectric cross-section is 20 times smaller than above it. So to get the same number of interactions 20 times greater fluence is required.

Kobayashi (Tsukuba) pointed out that to convert exposure to dose one is required to know the relevant volume. Considering the cell nucleus as a target, the conversion factor above the edge would be only a few percent larger than below the edge of phosphorous.

Rao (Newark) drew attention to the results of experiments of Kassis (Boston) with ^{125}I, ^{123}I, and ^{77}Br in which the effects of these widely different sized Auger cascades produced about the same effect in terms of dose to the nucleus. Each of these emitters should cause different levels of local strand break damage; yet this does not seem to influence the biological effect. Sastry (Amherst) agreed that dose to the nucleus did seem, in these circumstances, to have meaning. Hofer (Talahassee) had described results from experiments in which synchronized cells at the beginning of S phase were pulse labeled with ^{125}I and then allowed different times of development into S phase before being frozen to allow ^{125}I decay to occur. Samples frozen shortly after pulse labeling produced low-LET like survival curves whereas cells frozen five hours after labeling show characteristic high-LET curves. He proposed that the high-LET effect resulted from the irradiation of something more than the DNA - perhaps some superstructural element in chromatin. Clearly, much remains to be explained. The highly innovative nature of Hofer's (Talahassee) experiments demonstrates as clearly as anything that to believe the current dogma can be stifling. It would at least seem that any assumption that the effects of Auger emitters were entirely explained in terms of damage within two DNA diameters of the decay site was a gross over simplification.

Halpern (Jülich) questioned the accuracy of radiobiological data to permit the kinds of model construction involving complex numerical analysis. He drew particular attention to the implied accuracy in the figure of 17.5 eV for the formation of a single strand break in DNA. Pomplun (Jülich) agreed insofar as there are a lot of phenomena which could not have yet been considered in the models due to the lack of appropriate, and what is most important, coherent data. Adelstein (Boston) noted two results which suggested that there was a finer relationship between strand breaks and survival. One of these was the experiments of Hofer (Talahassee) referred to above, the other work by Makrigiorgos (Boston) that showed that the ratios of D_o to double strand break yields for incorporated ^{123}I and ^{125}I were not the same. Clearly here was a disparity that current modelling devoted to explaining effects entirely in terms of locally induced strand break yields could not resolve, particularly since the results from these experiments were robust. This was an area of experimental biology where the theoretical basis was relatively quantitative.

What emerges is a clear need for interaction between experimentalists and theoreticians but one should not assume that the theory will not need substantial revision in the future. There seem to be relatively few examples of

theory leading the subject. As suggested above, it would be nice to see some purely theoretically based predictions that can be meaningfully tested. There are still fundamental differences of theoretical approach which was made evident in a short exchange between Sastry (Amherst) and Pomplun (Jülich) concerning the validity of the 'frozen orbital' approach for calculating electron energy spectra. This ended with a plea from both Sastry (Amherst) and Pomplun (Jülich) for a clear identification of the limitations of any calculation and a careful definition of terms.

Auger emitters are increasingly used diagnostically in nuclear medicine and as such present a radiological hazard. How should they be catered for in radiological protection? Johanson (Uppsala) noted that the ICRP had first proposed to use a value of $Q = 5$ for Auger emitters but in the final recommendations had dropped the idea. When iodine-125 was brought close to the DNA, as in the case of transfer by thyroid hormone, the effect was definitely akin to high-LET. Perhaps the assumption made by ICRP was that only a small fraction of the iodine became bound in this way.

Howell (Newark) warned that using Q alone would not be enough - something similar to a distribution factor (like N previously employed by the ICRP but now dropped) would be required to take account of subcellular distribution of the nuclides. The important factor in determining the effectiveness of an Auger emitter was chemical form because it seemed that many Auger emitters resulted in the same biological effectiveness if they were identically distributed within the cell. Kassis (Boston) agreed that was largely a matter of the identity of the pharmaceutical and not the isotope.

Adelstein (Boston) drew attention to two observations, one published in 1982 by Commerford *et al.* , suggesting that if the Auger emitter is in the nucleus it did not matter where. This result might suggest that the highly localized nature of the Auger decay did not necessarily indicate that damage was similarly localized. Clearly, in the light of some of the results discussed at this meeting, this question should be re-addressed. The second, a more recent result by the Harvard group seems to indicate dramatic transformation frequencies at doses which barely affect cell survival. These two results have important implications both for radiological protection and fundamental understanding of radiobiology.

From this short discussion it is clear that questions continue to arise in this field which, although being explored by relatively few workers, is no

doubt one of the more vigorous and innovative areas of radiobiology. The geographical separation of workers makes collaboration somewhat difficult but there does seem to be a need for more coherence in both theoretical and experimental approaches. The radiological protection implications deserve much more attention by the granting bodies given the importance of Auger emitters in nuclear medicine, but there can be no doubt that Auger emitters are an increasingly important tool in exploring biology in general and may well find important applications in therapy.