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J. Booz and H. G. Ebert

Volume II

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THE MOLECULAR ION EXPERIMENT**

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Abstract

In early simplifications of the Theory of Dual Radiation Action it was assumed that ionizing radiations produce lesions which arise from the combination of pairs of sublesions that are produced in "sites", i.e. limited regions in the cell nucleus in which this combination can take place. In a more realistic treatment, the probability of combination depends on the separations at which the sublesions can be produced and the probability that combination occurs if such initial separations intervene. The molecular ion experiment is designed to determine this dependence. Pairs of ions with varying mean spacing are generated by the break-up of molecular ions and subsequent divergence by multiple coulomb scattering in a foil. The emergent ions traverse mammalian cells attached to the exit side of the foil.

The principles of the experimental techniques are discussed and some initial results are presented.

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Background and objective:

The concepts of microdosimetry were developed because the cells of higher organisms usually respond to ionizing radiation in a non-linear manner, in that different distributions of absorbed energy result in different frequencies of effects. The identification of pertinent microdosimetric quantities must however be governed by the nature of the dependence of effect frequency on energy concentration and by the magnitude of the domains in which energy concentration is significant.

The Theory of Dual Radiation Action provides answers to both of these questions by its postulate that radiation injury is due to lesions which in turn result from the pairing of sublesions that have been produced at separations that are typically 1 μm. In an early formulation (1) it was stated that $\varepsilon(z)$, the yield of lesions, is proportional to the square of the specific energy, z, or

$$\varepsilon(z) = K\, z^2 \tag{1}$$

where K is a constant and z is the value of the specific energy in sites (i.e. subnuclear volumes) having a diameter of about 1 μm.

Eq. (1) is equivalent to

$$\varepsilon(D) = K(\zeta D + D^2) \tag{2}$$

where D is the absorbed dose and ζ the dose mean of the specific energy produced by individual events in the sites.

It was recognized that this formulation can only be an approximation for a number of reasons. These include the necessity of corrections for saturation and for variation of K with radiation quality. An additional simplification consists in the assumed existence of sites in which there is constant probability of interaction between sublesions. There is no cytological evidence for the existence of such volumes and it seems much more likely that sublesions are produced throughout the nucleus and that they combine with a probability that depends on their separation. The initial separation of combining sublesions is then on the average about equal to the radius of the hypothetical sites.

In a recent generalization (2) of the Theory of Dual Radiation Action the site concept has been eliminated and the relation given in Eq. 2 has been replaced by

$$\varepsilon(D) = K(\xi D + D^2) \tag{3}$$

ξ is proportional to the integrated product of three functions:

$s(x)dx$, the expected volume of matrix at a distance x to x + dx from a sublesion. The matrix is that portion of nuclear material in which sublesions can be produced.

$g(x)$, the average probability that a pair of sublesions separated by x combines to form a lesion.

and $t(x)dx$, the mean energy deposited by the same event at a distance x to x + dx from an energy deposit.

The normalized product

$$\Phi(x) = g(x)\ s(x) / \int_0^\infty g(x)\ s(x)\ dx \tag{4}$$

may be termed the availability of adjacent loci. If sublesions are produced randomly in the matrix, i.e. if energy is uniformly imparted to the medium, $\Phi(x)dx$ is the fraction of lesions resulting from two sublesions that were produced at a separation x to x + dx. The site radius applicable to the site model should be comparable to $\int_0^\infty x\,\Phi(x)\,dx$.

Since $\Phi(x)$ characterizes the dependence of sublesion combination on separation, it is a basic function of radiobiology. The determination of its values for inactivation of tissue culture cells is the objective of the molecular ion experiment.

Basic plan of experiment:

The most direct determination of $\Phi(x)$ would be one in which radiation is administered in pairs of energy deposits of varying separation. However this does not seem feasible because of the inherent track pattern of energy deposition.

A less direct but achievable method consists in the employment of short tracks of varying length. Monoenergetic x rays having energies of the order of one keV or monoenergetic neutrons of somewhat higher energy could be employed to determine the value of the integral given above over various finite intervals. Other studies with such radiations have in fact been carried out (3,4,5). There is however a major difficulty in that, as already mentioned, K which depends on the effectiveness with which sublesions are produced is very likely to depend on LET. The nature of this dependence is largely unknown but it may be expected to be especially pronounced near the ends of tracks.

This difficulty can be avoided if the irradiation is by pairs of parallel tracks with variable spacing and this is the modality that has been adopted. Because of problems of scattering and the minute separations required, it was deemed impractical to utilize collimated particles, and uncollimated molecular ions are employed instead. Although protons as well as deuterons are used and the molecules can be triatomic as well as diatomic, the remainder of this discussion will deal with diatomic deuterium molecules.

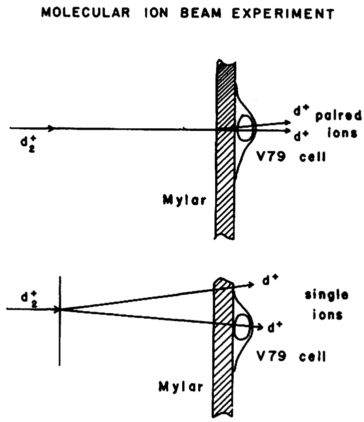
The ion source of the RARAF Van-de-Graaff accelerator provides an ample supply of D_2^+ ions which are readily segregated by a magnetic field. When these molecules strike a foil, the electron joining the deuterons is stripped off and the two nuclei proceed independently in tracks that are not strictly rectilinear because of multiple coulomb scattering. It can be shown that for any foil thickness, a, the separation, s, of the emerging particles is very nearly distributed as

$$f(s) = s/\beta^2 \cdot \exp(-s^2/\beta^2) \qquad (5)$$

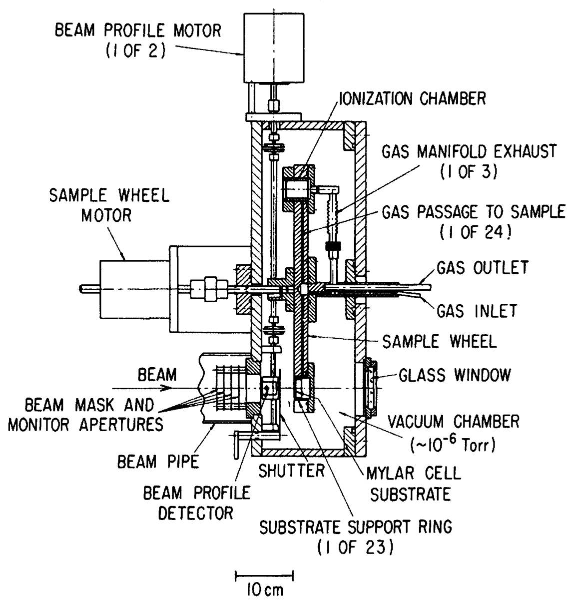
where β is a constant that is inversely proportional to particle energy, proportional to the square root of a, and dependent on the material of the foil. Scattering of single particles was studied to determine β and to obtain slight corrections to Eq. 5.

In this irradiation modality there is no fixed separation of tracks but a distribution having a width that depends on β. However this poses no substantial problem since t(x) can be calculated for either condition following approaches previously outlined (6).

The basic experiment consists in irradiating cells attached to thin sheets of mylar with deuteron molecules that are incident on the opposite surface. The survival of cells is compared with that obtained with equal numbers of single deuterons of the same energy. This condition is attained by the simple expedient of introducing a very thin scattering foil into the beam pipe at some distance upstream from the cells. There is negligible probability that following break-up by this foil,both deuterons pass through the same cell. The ratio of survivals for uncorrelated and correlated deuterons is studied as a function of foil thickness, allowance being made for



1. Schematic representation of irradiation by molecular ions. The molecular ions dissociate either in a foil contiguous to the cells or they are dissociated at some distance upstream from the cells. The lower sketch is not to scale since the thickness of the cells and the supporting mylar are of the order of a micrometer while the separation of the breakup foil from the foil-cell combination is on the order of meters.



2. A schematic cross-sectional drawing of the irradiation facility of the molecular ion experiment. The major components are identified.

an additional mean separation due to scattering in the cells which are about as thick as the thinnest foil employed ($\sim$ 4 μm). The mean separation at median cell depth has,in experiments carried out thus far,ranged from about 90 to about 250 nm.

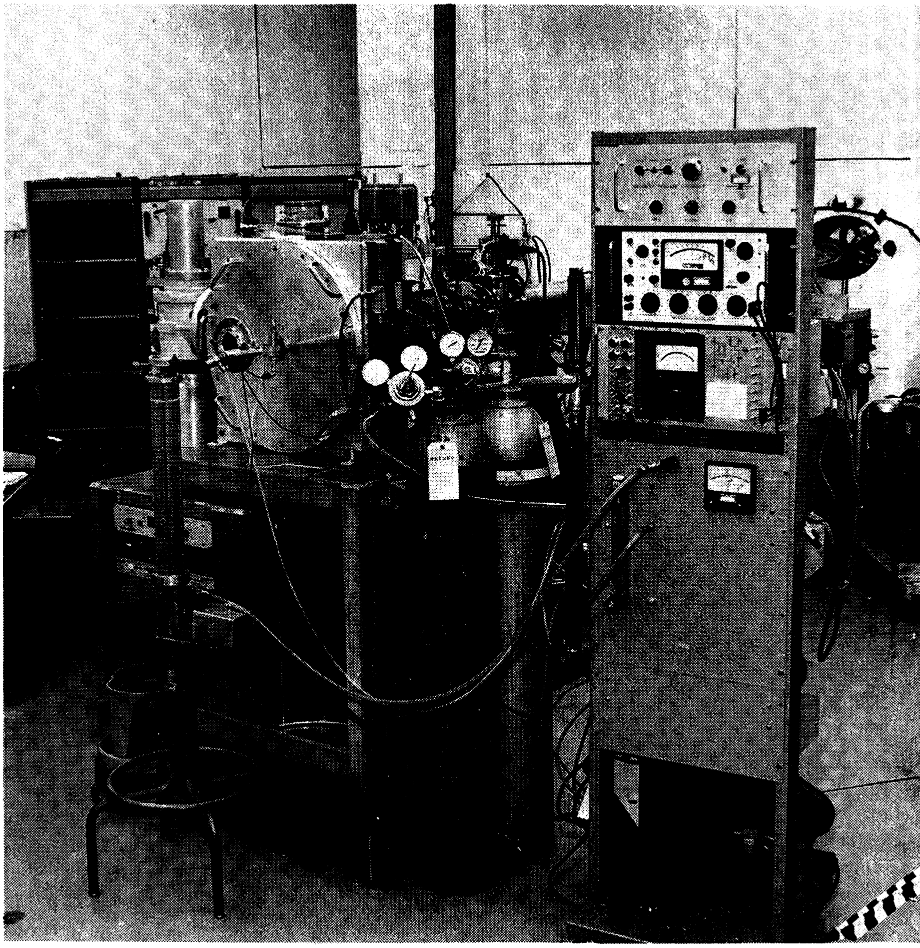
In these experiments V79 hamster cells are utilized and they are synchronized because it was anticipated that $\Phi(x)$ might depend on cell age.

Technical features:

A series of papers will describe the various details of the molecular ion research. Although the principle of the experiments is relatively simple, substantial practical difficulties had to be overcome. Here only a few salient features will be mentioned.

The need to irradiate the cells in dishes having a diameter of several cm results in a mechanical problem since the thin foils are liable to rupture when subjected to a pressure differential of one atmosphere between the vacuum chamber and the cell environment. It has thus been found necessary to expose the cells when they are in gas at 1/10 atmosphere pressure. This in turn requires rapid evacuation and careful control of humidity. However even under the optimum conditions available, it is not possible to avoid the trauma that is caused by the irradiation environment together with that introduced by synchronization with hydroxyurea. In order to reduce the limits of error, a considerable number of dishes are irradiated to equal numbers of associated or random particles with other dishes serving as controls.(Fig. 1)

The dishes are placed into a large wheel that rotates at a rate that is proportional to the dose rate as measured by a current monitor. The beam is delimited by a slit and the wheel rotates in steps of 5 min of arc



3. Photograph of the irradiation facility for the molecular ion experiment. Most of the apparatus surrounding the irradiation chamber (large aluminum box) serves to control the cell environment.

when ever a predetermined fluence has passed through the slit. This ensures uniformity of irradiation. Two of the positions on the wheel are occupied by dosimeters. All of the operations including wheel motion, insertion of the break-up foil, dosimetry, etc. are controlled by an on-line computer. Fig. 2 is a schematic drawing of the irradiation chamber and Fig. 3 is a photograph showing some of the peripheral equipment as well.

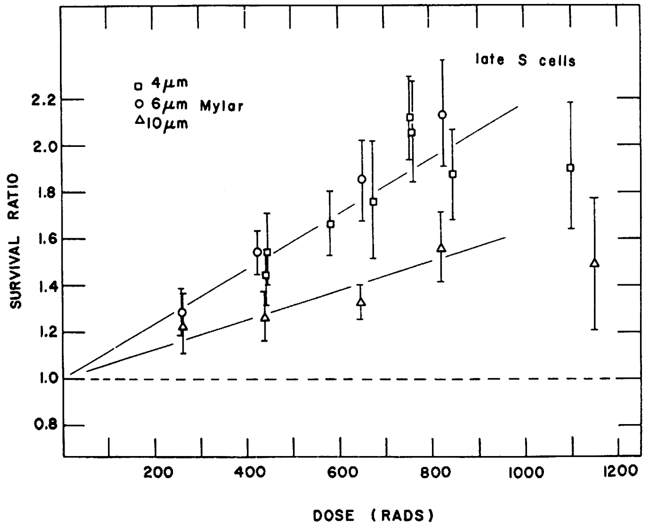
The assay of cell survival is carried out by standard techniques.

Initial results:

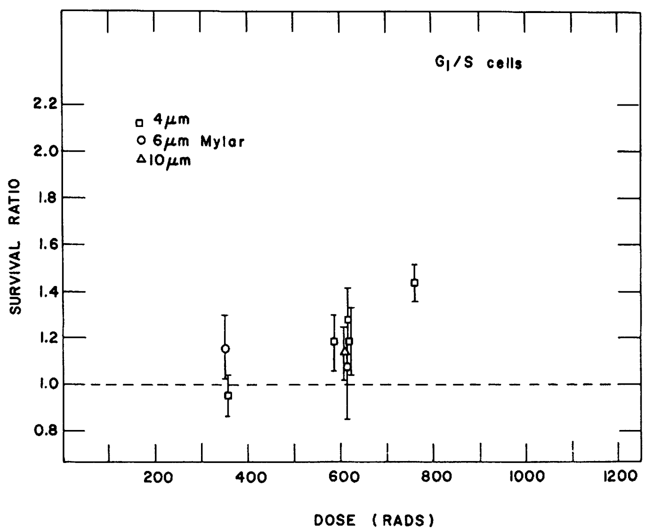
Although a considerable number of irradiations have been carried out, the results obtained to date are for only 3 foil thicknesses. They are given primarily to show that the expected effect does indeed exist. As also anticipated, it is more pronounced in late S than at the G_1/S interface presumably because of differences in the aggregation of DNA.

Fig. 4 shows the survival ratio as a function of absorbed dose for cells in late S for three different foil thicknesses and Fig. 5 shows the corresponding data for G_1/S. The survival ratio is defined for equal doses as the ratio of survival with random particles and that with associated particles. It is evident that in general, with increasing correlation (i.e. with decreasing foil thickness), the effectiveness of deuterons increases, particularly in late S.

No definitive attempt has been made to determine $\Phi(x)$ although the methods for such an analysis are at hand, including adequate formulation of $t(x)$. However, it would appear on the basis of initial considerations that $\Phi(x)$ may turn out to have a complex shape in which the function decreases rather rapidly at low x but much more slowly at large x. Verification of this impression must await the results of further experiments.



4. Preliminary data on the relation between the survival ratio (ratio of survivals with uncorrelated and correlated ions) as a function of absorbed dose. The data are for cells in late S phase attached to foil of the indicated thicknesses.



5. Preliminary data on the relation between the survival ratio (ratio of survivals with uncorrelated and correlated ions) as a function of absorbed dose. The data are for cells in G_1/S interface attached to foil of the indicated thicknesses.

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William Gross, Leon Goodman, and E.J. Hall made major technical contributions to this work and we are grateful for their help.

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