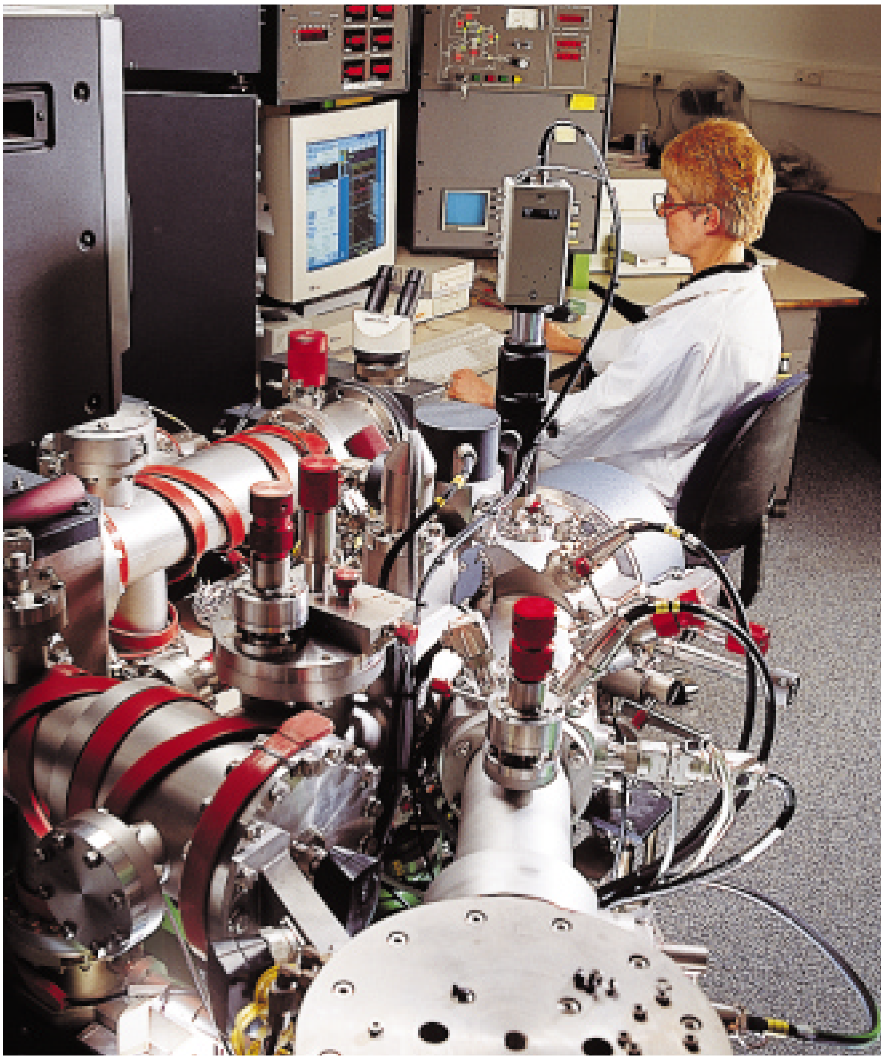
PHYSICAL TOOLS FOR STUDYING RADIO-INDUCED LESIONS

When ionizing radiation penetrates a biological medium it collides with the atoms in its path, and in the course of successive interactions it loses energy, producing observable effects such as DNA lesions in cells. Relating initial physical events to biological effects is the constant objective of researchers in radiobiology, radiotherapy and radioprotection. Two approaches are currently pursued. One is based on the correlation between absorbed dose or distributions of energy transfer and the biological effect observed. The other uses track calculations designed to follow the interactions of the incident particles throughout their path to model the initial effects of the radiation. These approaches are especially interesting because the classical views of the radiation targets could be called into question.



Antoine Gonin/CEA

The localization of radionuclides emitting short range radiation can give rise to very uneven exposures of cells and intracellular components, which demands a microdosimetric analysis of the distribution of energy deposits at the «target» scale. To study the microdistribution of radionuclides the Nuclear Protection and Safety Institute uses microanalysis techniques such as analytical microscopy by Secondary Ion Mass Spectrometry (SIMS). Operating as an electronic scanning microscope, ionic microscopy replaces the primary electron beam by a beam of ions, which makes it possible to perform elemental analysis of solid surfaces.

The limits of the classical tools

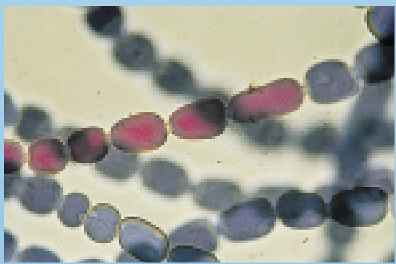
When **ionizing radiation** travels through a biological medium it loses energy by colliding with atoms in its path. These interactions result in ionization (ejection of an electron) and excitation (energy gain) of atoms in the medium along the track of the ionizing particles. At the **cell** level these initial events trigger a cascade of effects that may lead in observable **biological effects**. In simple biological systems such outcomes appear even at low incident **doses**. This well-known fact is at first sight surprising. The immediate impact of radiation on the cell is to create, directly or indirectly, lesions (see *Radiation-induced damage to nucleic acids*) similar in number to those that occur spontaneously. However, the spontaneous lesions are almost all extremely well repaired (see *The caretakers of the genome*). The physical interactions between the radiation and the cell medium thus display specific characteristics linked to how readily the radiation induces particular biological effects according to the dose delivered. This ability is termed its **biological effectiveness** (box 1).

The classical physical investigation tools, which are indispensable for the modeling of radio-induced biological lesions, make use of two parameters: *fluence*, which represents the quantity of ionizing particles entering the medium, and *effective interaction cross-section*, which measures the probability of these particles colliding with the atoms in the medium. However, these tools prove inadequate to characterize the biological effectiveness of radiation, because the extent of the primary lesions

The factors of biological effectiveness

1

The induction of **mutations** by various types of radiation, including at low doses, is analyzed in *Tradescantia*, a fairly common flowering plant that is especially sensitive to **mutagenic** agents such as ionizing radiation. *Tradescantia* bears a large number of stamens, the male organs that hold the blue-colored pollen. In the variety used, radiation creates mutations that produce a change in the color of the cells that make up the hairs of the stamens (picture). The mutation process is thus directly observable.



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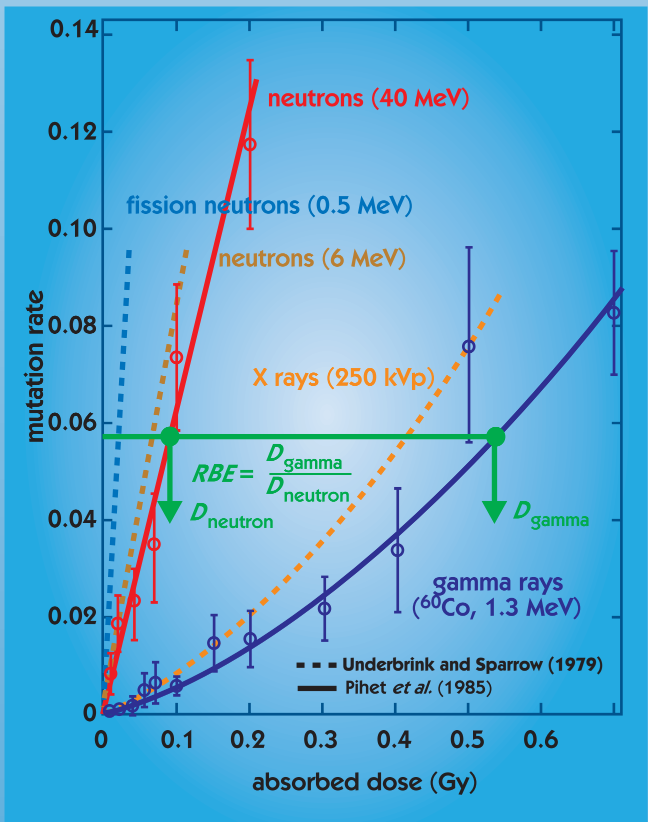
The frequency, or probability of occurrence of mutations observed according to the **absorbed dose** in *Tradescantia* is represented on the diagram opposite for ionizing radiations of different energies and types. The absorbed dose is a measure of the energy absorbed locally in the medium. It is defined by the average value of the amount of energy absorbed per unit mass of the medium at the point considered.



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Dose-effect curves show very appreciable differences right down to very low doses. When the radiation interacts with the biological medium the amplitude of the effect produced indeed depends on the energy transferred. Ionizing particles have different **lineal energy transfer** (*LET*) values, which correspond to the amount of energy absorbed by the medium per unit particle track length. Neutrons and photons are indirectly ionizing radiation, thus characterized by the average *LET* values of the charged particles they generate in the medium. The density of ionization along the particle track is on average much greater (up to 100 times) for neutrons than for photons.

In the diagram the «slope» of the dose-effect curves, which measures the extent of the damage caused to the DNA strands of *Tradescantia* illustrates the conventional classification into low-*LET* radiation, here X- and gamma rays, and high-*LET* radiation, here neutrons. The **relative biological effectiveness** (*RBE*) of one type of radiation relative to another is defined as the ratio of the absorbed doses required to produce the same biological effect on a given system.

produced depends not so much on the impact of radiation on a biological «target» as on the transfer of energy that occurs. The concept of **absorbed dose** (box 1), which is commonly used in radiobiology, is limited as a measure of bio-effect because at the microscopic level the energy is not delivered continuously but in discrete amounts of variable number and size. The interaction between the radiation and the medium is thus essentially a statistical phenomenon. An average figure does not therefore allow for the sometimes very wide statistical fluctuations in the energy deposits that occur randomly in small volumes comparable in size to cells. The absorbed dose becomes fully meaningful only under uniform conditions of exposure relative to the size of the target studied (tissue, cell, **DNA**). Accordingly it was necessary to develop tools that would provide a finer physical representation of the energy transferred at the scale of the biological targets.

Microdosimetry of ionizing radiation

2

In a biological medium the energy deposition in a volume defined by a mass m and a size d is determined by the energy transferred, ε, along a track length ℓ in the presumed target traveled by the incident and associated particles. It is expressed by the **stochastic microdosimetric quantities**, *specific energy* z and *lineal energy* y, which are the energy per unit mass of the target, and the energy per unit random track length in the target respectively.

The **statistical fluctuations** of the energy deposits are represented by:

- The frequency distribution of energy deposits or «events» taken individually, $f_1(z)$ or $f_1(y)$, and the derived dose distribution [$\sim y \cdot f_1(y)$] or the microdosimetric spectrum,

or

- The distribution of the specific energy over all the targets due to 0, 1, 2, ..., n events according to the absorbed dose D, $f(z;D)$.

The **relationship with the absorbed dose** is given by:

$$D = \lim_{m \to 0} \bar{z}$$

with

$$\bar{z} = \int z\, f(z;D)\, dz.$$

whence

$$\bar{n} = \frac{\bar{z}}{z_1},$$

which corresponds to the average number of events taking place in the presumed target for an absorbed dose D.

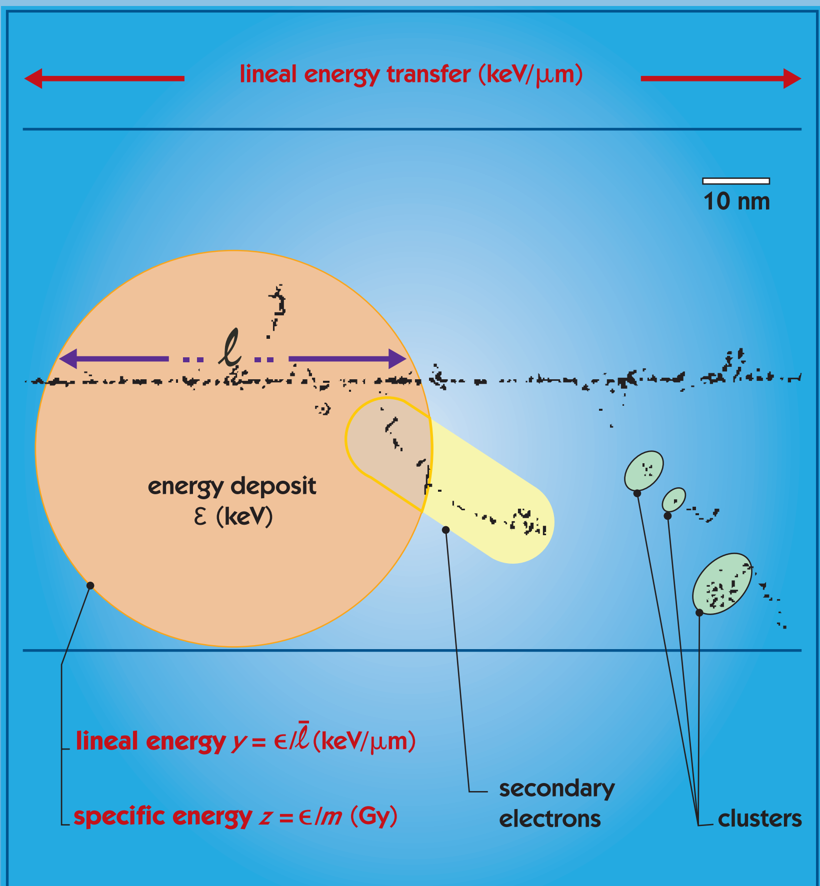
Provided we know the distributions $f(z;D)$ and $f_1(z)$, or $f_1(y)$, if the biological data are sufficient, phenomenological correlations can be sought in order to set up empirical biological response functions $e(z)$ and $e(y)$. These correlations enable us to predict the **biological effect**, $E(D)$, through a relation of the type:

$$E(D) \sim \int e(z)\, f(z;D)\, dz.$$

At low dose the **biological effectiveness** of the radiation $E(D)/D$ is obtained from a relation of the type:

$$E(D) \sim D \int e(y)\, f_1(y)\, dy.$$

The diagram below depicts an ionizing particle track and the parameters characteristic of the energy deposition in a target simulating a cell **nucleus**, generally a sphere of a few tens of nanometers to a few micrometers (1 µm = 10^{-6} m) in diameter. However, high-energy secondary electrons derived from the ionization of target atoms, which split off from the main track, finish their path in high local concentrations of ionizations. These energy depositions can have a marked impact at the subcellular level.



(From Goodhead, D. T., The Dosimetry of Ionizing Radiation, Vol II, edited by K. R. Kase, B. E. Bjängard and F. H. Attix, Academic Press Inc., London, 1987, p. 1-78)

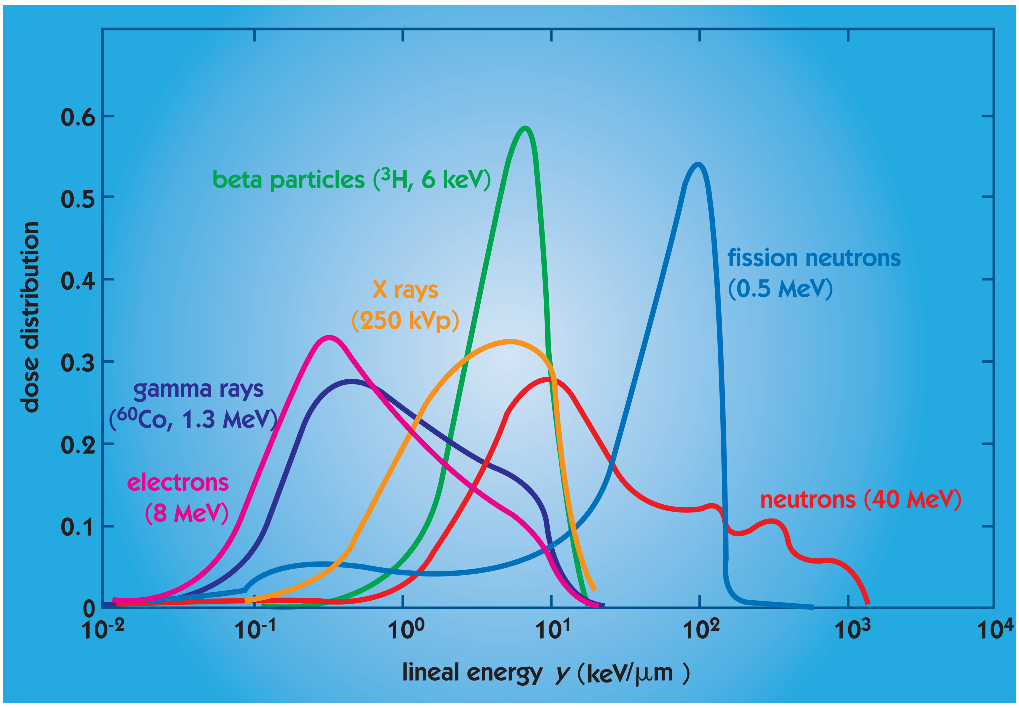


Figure 1. Microdosimetric spectra for a biological target of diameter 1 µm for different types of directly (electrons) or indirectly (gamma, X, neutrons) ionizing radiation. This representation helps us to study how well simplified relations for relative biological effectiveness can predict its variations from one type of radiation to another in different applications in radiotherapy and radioprotection.

Analysis of energy deposition

The physical impact of an ionizing particle on a biological medium is finely described by the «track» of the particle, studied interaction point by interaction point over a large number of paths. This information is made possible by techniques of random computation using the high capacities for data processing now available. Even so, such an approach remains too detailed to meet the needs of radiobiology directly unless it is simplified by reducing it to a few characteristic parameters and a statistical fluctuation about their average values.

Although the energy transferred by particles to the biological medium is on average well represented by the ionization density along the track, or by the *LET*, microdosimetry affords a different approach, focusing on the energy deposition, defined as the energy imparted in a given volume, designed to simulate a biological target, crossed by the particle track. Microdosimetry describes the fluctuations in the energy deposit (or in energy deposition) from one track to another, and the ballistics of these tracks in targets of selected dimensions exposed to a given type of radiation (box 2). This is useful because it means we can line up a broad range of radiation types, both directly and indirectly ionizing radiation, on the same representational model in order to compare the biological effectiveness of the types of radiation (Figure 1), even though their mechanisms are not fully known. Microdosimetry allows various types of ionizing radiation to be compared numerically in terms of frequency and average quantity of energy deposition, or «events», in the presumed target (Table).

Energy deposition: a powerful tool for dosimetry

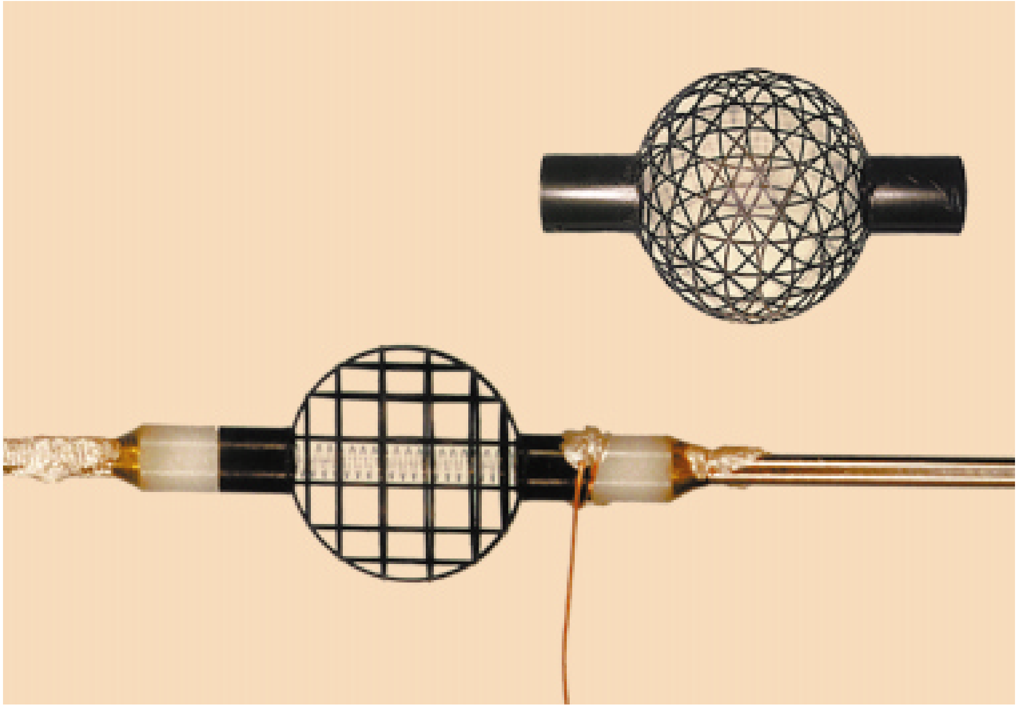
The relation between absorbed dose and the distribution of energy deposition affords a powerful tool for radiobiology and its applications in radiotherapy and radioprotection.

A comparison of the diagram in the box 1 with Figure 1 explains the overall approach, which consists in linking the observable radio-induced lesions to the

target / radiation	cell nucleus (~ 1 µm)	DNA fiber (~ 10 nm)	DNA molecule (~ 2 nm)	parameter
gamma rays (^{60}Co, 1.3 MeV)	1.2	$6 \cdot 10^{-6}$	$7 \cdot 10^{-8}$	$\bar{n}$
	0.2	60	440	σ_z
	1.6	16.8	37.4	$\overline{y_D}$
X rays (250 kVp)	0.5	$5 \cdot 10^{-6}$	$7 \cdot 10^{-8}$	$\bar{n}$
	0.3	60	440	σ_z
	3.35	17.5	38.3	$\overline{y_D}$
beta particles (^{3}H, 6 keV)	0.2	$6 \cdot 10^{-6}$	$6 \cdot 10^{-8}$	$\bar{n}$
	0.3	60	460	σ_z
	4.8	18.0	41.2	$\overline{y_D}$
fission neutrons (0.5 MeV)	9.10^{-3}	10^{-6}	$5 \cdot 10^{-8}$	$\bar{n}$
	1.3	135	730	σ_z
	85.5	90.2	104.4	$\overline{y_D}$

***Table.** Microdosimetric parameters evaluated for different types of ionizing radiation and simulated biological targets. $\bar{n}$ represents the average number of events or the frequency of energy deposits in the target for an absorbed dose D = 0.1 Gy ; σ_z, of which the unit is the Gy, corresponds to the standard deviation of the specific energy in the target for the same exposure dose, and $\overline{y_D}$, expressed in keV/µm, stands for the average value of the lineal energy, or «size» of the event, over the dose distribution.*

The proportional counter using a wall and a gas with compositions as close as possible to those of biological tissue has greatly contributed to the development of experimental microdosimetry. The photo shows detectors in which the continuous wall has been replaced by a grid to simulate a biological target as homogeneously as possible. The dimensions of the counters (a few mm to a few cm) and the gas pressure are set according to the size of the simulated target, classically about a few micrometers. However, such «miniature» counters (submillimetric) now make it possible to study targets in the nanometer range.



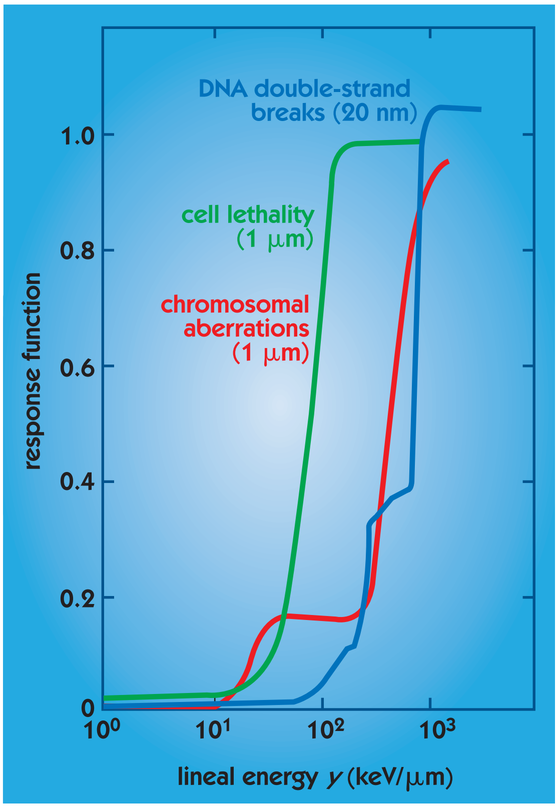
Gary Johnson/COLUMBIA UNIVERSITY/Center For Radiological Research

product of the absorbed dose and a weighting factor estimating the **relative biological effectiveness** (*RBE*) of the radiation as a function of its microdosimetric spectrum. This formulation is above all practically useful, because the microdosimetric spectra can be evaluated for almost all types of radiation and can even be measured in conditions difficult to simulate by calculation, e.g., in complex radiation fields. However, these applications remain purely phenomenological.

An essentially empirical approach

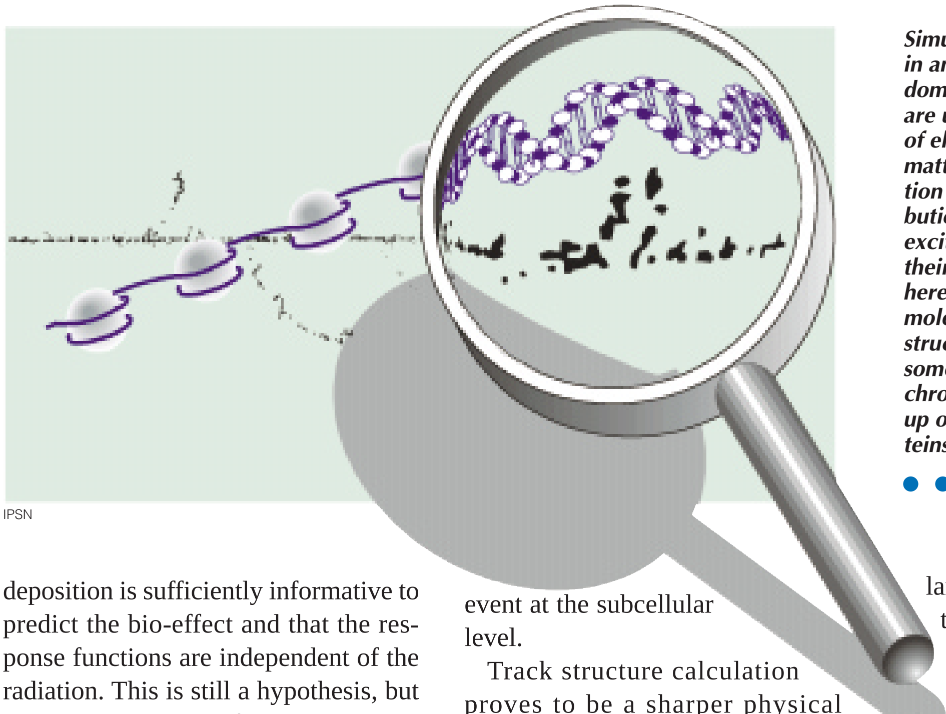
Energy deposition is an expression of primary events, i.e., ionization and excitation, triggered by the traversal of a biological target by radiation. Owing to the multitude of mechanisms that are subsequently brought into play, simple knowledge of these events does not enable us to account fully for the observed bio-effect, because of the difficulty met linking this effect to the initial molecular damage. In a first analysis only empirical correlations can thus be attempted, by bringing together energy deposition parameters and bio-effects observed under the same conditions. The dose-effect relation is a prime example. In microdosimetry this phenomenological approach comes down to looking for a function of biological effectiveness or «response function» (box 2), sometimes established purely numerically by successive adjustments of the biological data to the recorded microdosimetric spectra.

Once obtained these response functions can be combined with the microdosimetric spectra of a particular type of radiation to estimate the relevant biological parameter in accordance with the experimental data used initially to establish them. For a biological target these functions determine the probability that a given energy deposition produces a particular specific effect (Figure 2). This interpretation is fairly immediate for a well-defined type of radiation. When several types of radiation are involved it is implicitly assumed that the energy



(From Morstin, K., et al., Radiation Research 120 (1989) 383-402)

Figure 2. Examples of response functions in the form of e(y) established by comparing the biological data observed for different types of effect and the microdosimetric spectra corresponding to the same types of radiation and presumed target sizes. The curve for induced damage to DNA may be explained by the expression of two mechanisms; double-strand breaks due to single events above 600 keV/µm, and double-strand breaks resulting from two single-strand breaks for lower energy depositions. The similarity of this curve to those for chromosomal aberrations and cell lethality *are consistent with the interpretation that these outcomes ultimately result from double-strand breaks.*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Simulation of a particle track in an irradiated medium. Random computation techniques are used to describe the path of electrons and ions in the matter on the basis of interaction cross-sections. The distribution of the ionization and excitation events induced in their paths is represented here at the scale of the DNA molecule and more compact structures such as nucleosomes, elementary units of chromatin, a complex made up of cellular DNA and proteins.

deposition is sufficiently informative to predict the bio-effect and that the response functions are independent of the radiation. This is still a hypothesis, but is most likely to hold for low doses and infrequent events.

These response functions are therefore above all empirical relations, in which the size of the target (Table and Figure 1) is not a fixed parameter, but depends on the correlation obtained. This does not prevent us from interpreting the shape of these response functions a posteriori in terms of the biological criterion and presumed target size, and then relating this to certain mechanisms of lesion formation such as the interactions of DNA single-**strand** breaks or the simultaneous occurrence of double-strand breaks (Figure 2).

Track structure calculation: an indispensable research tool

Energy deposition is not sufficient to afford an understanding of the mechanisms of lesion formation. This justifies the interest of a phenomenological approach, albeit more reductive and narrower in scope. The marked bio-effects induced by radiation with an average track length in the nanometer range (1 nm = 10^{-9} m) illustrate the limits of this approach. For very low-energy electrons (less than 1 keV) in particular the dose-effect curve can, under certain conditions, resemble that obtained with high *LET* particles, which raises questions about the fine structure of the initial event at the subcellular level.

Track structure calculation proves to be a sharper physical research tool than energy deposition. It enables us to describe all the interactions of the secondary electrons that «terminate» the fate of a primary particle. Track modeling makes use of interaction cross-sections. It offers, for example, the possibility of following the distribution of intercollision distances. To access this statistical information and compare it with the observed DNA damage, a simulation is performed on a large number of tracks. It is especially interesting to study the local concentrations of ionization events or clusters of secondary electrons (box 2), and the spatial distribution of such occurrences at the scale of the DNA.

It is certainly tempting to make use of one of these parameters, which *a priori* come closer to the initial molecular damage. In comparison with the calculation of energy deposition or absorbed dose this may help us reach a more fundamental «unified concept». However, this approach is limited by the reliability of the calculations owing to the uncertainties in the basic physical data and the complexities of the biological medium. Nevertheless, track structure calculation helps gain a better understanding of the mechanisms by which the radiation acts. The ever-increasing capacity of the model to take into account initial physical processes and the transport of the chemical species created is a valuable feature. However, intercellular communication greatly complicates the model. For example, it is possible to observe effects in cells remote from those hit by the radiation.

Where do we go from here?

The choice between energy deposition and track structure calculation depends above all on the nature of the biological data that are to be interpreted. Track computation is especially suited to a mechanistic approach, because it supplies model input data that can serve to test hypotheses concerning the nature of radio-induced biological lesions. Energy deposition and the quantities derived from it are more meaningful in a physical representation of biological effectiveness, as they are supported by the correlation between the data observed for the biological system and the relevant application. One of the appeals of research in microdosimetry is without a doubt that it makes use of both approaches. However, the challenge of the coming years lies in the need to revisit the concept of radiation target. This may well prompt thorough revision of both the models of representation and the way the physical data are interpreted.

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