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EFFECTS OF SPATIAL-TEMPORAL DISTRIBUTION OF PRIMARY EVENTS

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ABSTRACT

Three general methods whereby we can improve our understanding of the biophysical effect of a physical agent are: (1) Attempt to follow the series of processes taking place beginning with the interaction of the agent and the biological system. (2) Try to unravel the causal chain backward beginning with the effect. (3) Search for basic rules that govern the overall process. A few years ago Rossi, considering these approaches in an evening lecture to the Radiation Research Society, concluded that in radiobiology only the third line of attack holds any real promise, at least at this time. A modified copy of his address makes up the first section of this paper to develop this argument and to furnish a simple introduction to the subject. Microdosimetry provides some of the physical concepts required for what we term the "theory of dual radiation action." In the second section we summarize the current status of this theory.

INTRODUCTION TO MICRODOSIMETRY*

I sometimes wonder how the Radiation Research Society has been so successful despite the fact that both physicists and biologists belong to it. They have rather different scientific temperaments. The physicist, by and large an analytical fellow, prefers to find simplicity in the complicated, say, by reducing the universe to a differential equation. The biologist is more likely to find complexity in the simple; for example, when contemplating a bacterium or even a bacteriophage, he discovers enough to fill at least a monograph. The two personality types rarely interact. At a university they are likely to encounter

*Outline of an address presented by Harald H. Rossi at the 18th Annual Meeting of the Radiation Research Society, March 1–5, 1970, in Dallas.

each other only at president's teas or at faculty meetings convened to deal with student riots. Here, however, they belong to the same society, go to the same meetings, elect each other officers, and occasionally even listen to each other's papers. This is surprising since their respective fields of radiation research are still divided by a no-man's-land.

The physicist, starting with the terminals of his X-ray machine or accelerator, can work his way down to atomic phenomena in irradiated tissues and perhaps also to the level of active radicals or other simple molecular species. At the other extreme, the biologist, who is at the far end of the radiation research spectrum exploring tissue effects with the radiologist, can work his way back perhaps to mutations or chromosome breaks. Between the two points of furthest penetration there remains a very large, very black area of ignorance. The detailed phenomena involved are almost certainly terribly complex. This becomes evident if we ask a radiochemist to relate what happens when X rays interact with as innocuous a substance as pure water.

It has occurred to many that, even if we did not know the details of radiation biophysical processes, we might gain a general comprehension and an ability to predict using some basic laws in the manner of geneticists, who were quite successful before anybody knew about DNA. Physicists have been particularly active in this sort of endeavor since the founding of target theory half a century ago. Many a young physicist, believing perhaps that there was not much left to be accomplished in dosimetry, decided to contribute to the progress of radiobiology by theoretical methods. This work usually starts with a demand for "hard" data, almost always a survival curve. As a rule the analysis results in a combination of exponentials with a few adjustable parameters. At this point somebody quotes an old mathematics professor to the effect that with three parameters you can fit an elephant and with four you can make him walk.

If the physicist, in an attempt to reduce the number of free parameters, asks for some additional information, the biologist might accidentally, or perhaps by design, hand him a curve that looks different but was obtained for the same organism on a day when the horse serum came from a different supplier or perhaps when all aspects of the experiment seemed to be the same except for the results. He might well tell the upset physicist that the curve would also have looked different if he had asked another technician to run the experiment. The physicist finally realizes that despite apparent identity the survival curve is not the same thing as the decay curve of a radioisotope since cells do not always act the same way and are not all alike.

At this point the physicist either takes a job with NASA or decides to straighten out radiobiology personally. If he does the latter, he quickly finds out that the cell is at least as complicated as a walking elephant. He patiently investigates not only the survival curve but also its dependence on dose rate, on LET, and perhaps on position of the cell in its cycle. He may go on to explore the effects of oxygen tension and radioprotective agents and perhaps such related subjects as cell proliferation and microcirculation. About this time he becomes a

full-fledged radiobiologist and is quite likely so wrapped up in his work that he hardly remembers how he became involved in it. He may, in turn, smile indulgently at brash, naïve physicists trying to explain what his experiments mean.

We all know examples of this case history. Lest I be misunderstood, let me make it quite clear that such physicists have made extremely important contributions to radiobiology, without which the rest of my talk would be impossible. Indeed my question is this, In view of recent advances made by these men and other radiobiologists, have we come to the point where we should again attempt to interpret the biological action of radiation in terms of simple underlying mechanisms?

An indication that this may be so is given in Fig. 1, which shows the logarithm of relative biological effectiveness (RBE) as a function of the logarithm of dose of high-LET radiation (actually neutron radiation) for a variety of effects on plants and mammals. I could spend, and indeed I have spent, an hour discussing the implications of this rather disorderly looking set of curves, but, in the interest of time, I must restrict myself to just a few main points.

1. The figure demonstrates the by now generally accepted fact that RBE for systems such as these is a function of dose.

2. The abscissa is a dose and the ordinate is a ratio of doses. Hence a numerical scale of biological effect is obviated. Some of the effects involve such nondigital notions as "opacification" or "redness," but these can be quite well represented since all that is involved is the criterion of equal effect.

3. The lines are essentially straight and their slope is between 0 and -0.5.

4. Except for one effect, the RBE's continue to increase as the doses decrease throughout the ranges investigated. There are, of course, other systems where this is not true, e.g., bacteria and even simpler systems. There may also be instances where a constant RBE at low doses is found in mammalian cells in tissue culture. However, as will be seen, in such cases the conclusions I am about to make are not applicable rather than contradicted.

The biological effects shown here must be caused by energy deposition in one or more locations in the cell or tissue irradiated. We may consider regions (sites) in which the required energy can be deposited by a single neutron secondary. As the dose is reduced, the one-particle inactivation must become dominant since the probability of one particle per site decreases linearly with dose and the probability of, for instance, two particles decreases with the square of the dose. At higher doses, particularly when the LET is low (e.g., for 14-MeV neutrons), inactivation by two particles may be more important.

If low-LET particles (in these cases electrons) could also singly initiate site inactivation, the RBE would have some fixed value at low doses which is equal to the relative frequency with which single high- and low-LET particles inactivate at a given absorbed dose. Except for the case of *Tradescantia* (where millions of stamen hairs had to be examined to extend significant experimenta-

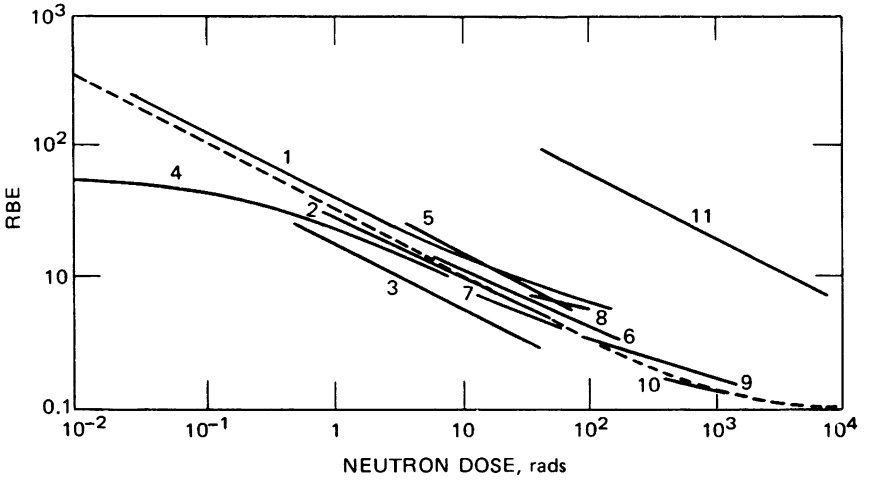

Fig. 1 Relative biological effectiveness of neutrons as a function of absorbed dose of neutrons for various biological end points. The general shape of the relation is shown by the dashed curve.

Ref.	Curve	End point	Neutron energy	Estimated ζ_n, rads	Dia. (d), μm
1	1	Opacification of the murine lens	430 keV	150	3
1	2	Opacification of the murine lens	1.8 MeV	840	2
1	3	Opacification of the murine lens	14 MeV	260	3
2	4	Mutations of *Tradescantia* stamen hairs (blue to pink)	430 keV	800	1.8
3	5	Mammary neoplasm in the Sprague–Dawley rat	Fission	2,200	1
4	6	Chromosome aberrations in human lymphocytes	Fission	1,300	1.4
5	7	Growth reduction of *Vicia Faba* root, aerated	3.7 Mev	600	2
5	8	Growth reduction of *Vicia Faba* root, anoxic	3.7 MeV	2,000	1.3
6	9	Skin damage (human, rat, mouse, and pig)	6 Mev	1,200	1.5
7	10	Inactivation of intestinal crypt cells in the mouse	14 MeV	800	2
8	11	Various effects on seeds of *Zea Mays*	Fission	400,000	0.15

tion into the range of constant RBE), this is not observed, and we must conclude that single electrons do not inactivate sites in the effects and dose ranges covered by the figure. It may be worth mentioning that this statement applies with high probability. For a given absorbed dose, the aggregate length of electron track produced by X or gamma rays is roughly a hundred times larger than that of the recoil tracks generated by neutrons. Some of the RBE values, and hence the dose ratios, approach 100. Therefore even with 10,000 times more track, and thus 10,000 times more-frequent traversal of sites, the electrons often show no signs of single-event inactivation.

However, we can show quite easily that a limiting slope of -0.5 is consistent with two-electron inactivation. In virtually all instances where the slope is between 0 and -0.5, the experiments indicate two-particle action by neutrons as well. The symptoms include recovery phenomena that did not occur at lower neutron energies (i.e., higher LET) or break frequencies of chromosomes that are not proportional to dose. Thus in these instances it seems more reasonable to assume that the two-particle inactivation by high-LET radiation is important than to assume that a single-particle inactivation by low-LET radiation is becoming evident. It is rather easy to calculate the general dependence of RBE on neutron dose under the condition that neutron secondaries can inactivate in either one or two events, but electrons can inactivate in two events only. The general shape of the relation is shown by the dashed curve, which can be shifted horizontally, depending on neutron sensitivity, and vertically, depending on the relative X-ray and neutron sensitivity of the system under investigation. For instance, Smith, Combatti, and Rossi[8] have shown that, if wet rather than dry seeds are irradiated, the neutron sensitivity changes little but the X-ray sensitivity increases by a factor of 10. This would bring curve 11 generally in line with the others.

Note that the dashed curve changes shape very slowly and that long stretches at intermediate slopes can be regarded as straight, particularly if the limited accuracy of the biological data is taken into account.

I submit that the reasoning presented is difficult to dispute. We may, of course, question the underlying assumptions by, for instance, claiming that, instead of acting on the same sites, high- and low-LET radiations act on different sites, the high-LET radiations perhaps damaging membranes and the low-LET radiations producing damage in DNA. Or we might think that differences in energy density lead to the production of entirely different chemical species that inactivate at different rates; thus high-LET radiation might make H_2O_2 and low-LET radiation HO_2. Neither approach could readily account for the single- or double-particle vs. double-particle inactivation characteristics, which seem quite attractive considering that the cell carries various components in duplicate, e.g., the two strands of the DNA, the two chromatids in the chromosomes, and the two chromosomes in a diploid cell. We are, for instance, tempted to assume that heavy particles having a high probability of inactivating a target inactivate two targets when they happen to traverse them both. On the other hand,

electrons might have a very low probability of inactivating a target they traverse and thus a negligible probability of "getting" both targets in a single pass. Although none of these features really prove the validity of my approach, they seem to provide at least a good motivation for further consideration of biological action on sites containing dual targets.

However, when we ask what size targets at what target separation in the site and what levels of energy could be involved, attempts to provide numerical information lead to the sometimes surprising discovery that, rather than not knowing enough biology, we do not know enough physics.

We are quite familiar with the interaction of almost any radiation with tissue down to the point of knowing how many particles of what energy are produced per rad of absorbed dose. We know that, depending on their charge and velocity, these particles expend energy at varying rates known as the LET. We also know that the principal mode of energy loss is a transfer of energy to electrons which may be excited or may be given enough energy that they leave their parent atom. If these electrons have sufficient energy to initiate appreciable further electronic displacements, we call them delta rays. When we reach this level, information rather rapidly becomes hazy. The rate of energy deposition and the range and track curvature of delta rays and their progeny are poorly known. All we know for certain is that most of what arose originally as kinetic energy of the primary charged particle ultimately, within a small fraction of a second, appears as heat. The heat diffuses throughout the biological specimen and, once it has degenerated to this level, it is certainly of no biological consequence. I have previously given some reasons why only energy transfers of the magnitude required to produce an ion pair in a gas should be considered. The data in Fig. 1 make me think that even larger energies are required to inactivate each target in a site. If I am wrong, the basic concepts would remain the same, but the task of microdosimetry would be very much more difficult. At any rate, I shall use the terms "energy" or "energy deposition" to mean the loss of kinetic energy which charged particles have suffered when traversing the volume under discussion, and I shall assume that, when this volume is filled with a gas, the ionization produced is proportional to the energy loss.

Having defined more or less concisely what I mean by locally deposited energy, I can proceed to a microdosimetric view of irradiated tissue. Figure 2 is a schematic representation of the energy per unit mass vs. mass for constant dose. If the medium is irradiated by gamma rays and if we consider large masses of the same magnitude at random, we always find essentially the same energy. If we reduce the mass (m) somewhat, the energy (E) will be similarly reduced, and thus the ratio of E to m will remain the same. However, if we repeat this procedure with smaller and smaller masses, we will observe increasing fluctuations caused by the fact that the energy is deposited by comparably small numbers of electrons. Both the number of these electrons and the energy deposited by them are subject to statistical fluctuations that must become so great that ratios very different from the absorbed dose will be observed; these

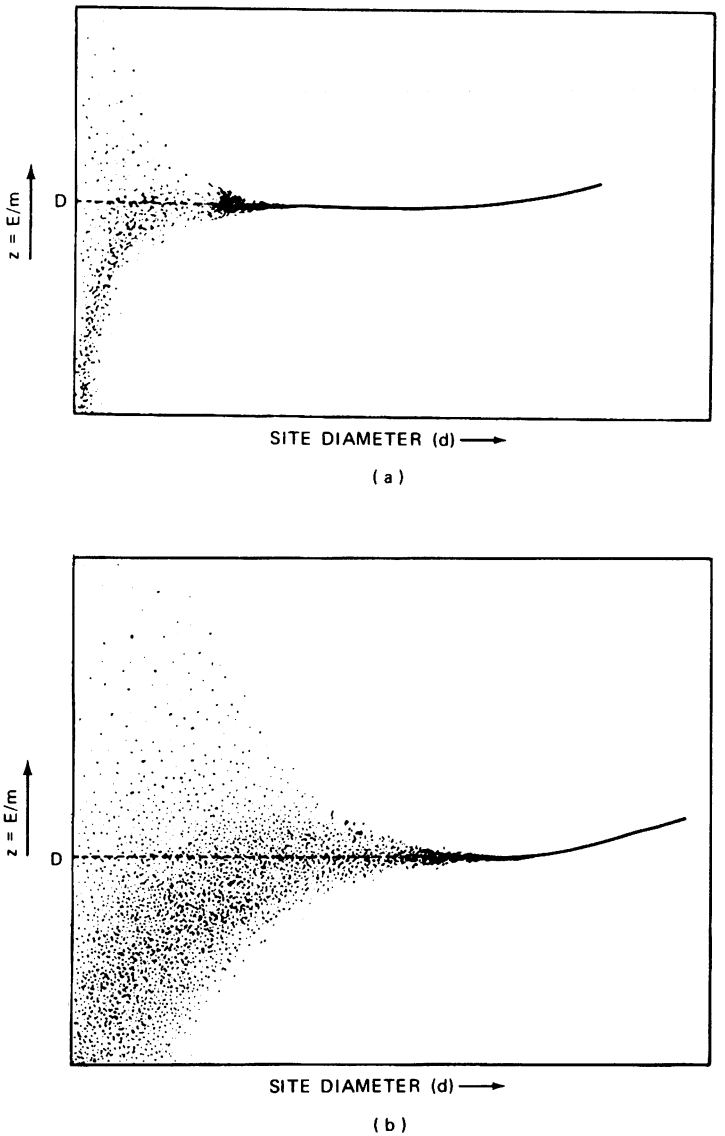

Fig. 2 Schematic representation of the fluctuations in specific energy as a function of site diameter. (a) Gamma rays. (b) Neutrons.

ultimately include cases where E becomes 0. The ratio E/m used to be called the local energy density but is now known as the specific energy (z). In small regions z can differ greatly from the absorbed dose, ranging from 0 to values that may be a 100 or many thousand times larger than the dose.

The situation for neutrons is essentially similar except that the much higher LET of neutron secondaries results in a much greater energy deposition per particle. This, in turn, results in more pronounced fluctuations. If, for instance, we look for a z value that is 10 times larger than the absorbed dose, we are far more likely to find it for neutrons than for gamma rays at most values of m.

Figure 3 gives a schematic representation of two volumes and of a particle that traverses them. If the energy deposited in a volume is E_v, it will be approximately $2E_v$ when the linear dimensions of the volume are twice as large. Consequently the lineal energy, y, defined as the ratio of the energy and the average diameter ($\bar{d}$) of the volume in which it is deposited, is to the first approximation independent of size; y is defined for single events only but z may refer to one or to several events. It is evident that each value of y represents an increment of z, but, when several events occur, the exact relation between the possible values of y and the resultant value of z, although unique, is rather complex. A number of investigators, particularly A. M. Kellerer, have furnished the mathematical apparatus that permits us to calculate the probability of any value of z for any dose if the relative probabilities of all values of y are known.

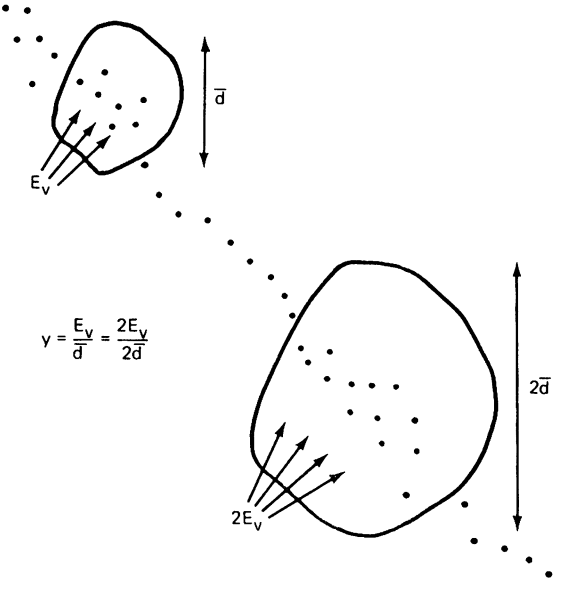

Fig. 3 Schematic diagram illustrating the approximate invariance of lineal energy with site diameter.

We can, of course, consider y or z for volumes of any size or shape, but it appears that shape is usually comparatively unimportant.

Evidently the $\bar{d}$ values in which we might be interested cannot be larger than those of cells, and very likely they are of the order of a micrometer or less. It would be extremely difficult to determine accurately the pattern of energy deposition by single particles in volumes of this size. However, a simple solution of the problem is to magnify greatly the dimensions of the volume by replacing the tissue within it by tissue-equivalent gas that may have a density 100,000 times smaller. The tissue surrounding the volume of interest is replaced by tissue-equivalent plastic. This leads to designs of the type shown in Fig. 4, where

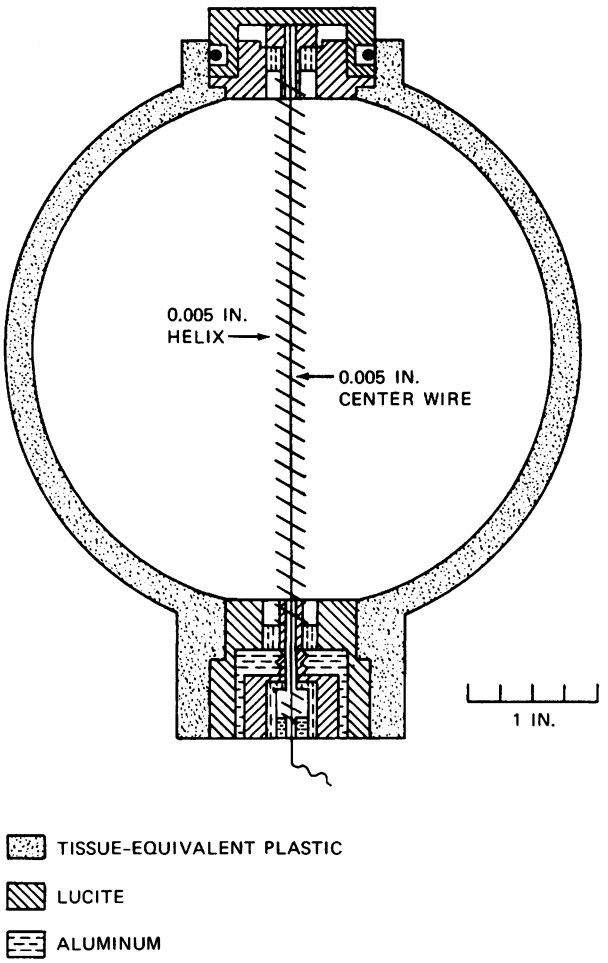

Fig. 4 Diagram of an early type of spherical proportional counter used in microdosimetry.

energy deposition in a spherical volume is determined by making the volume the sensitive region of a proportional counter that can detect energy depositions down to single ion pairs. The diameter of the unit density sphere simulated by the gas volume can be readily changed by changing the pressure of tissue-equivalent gas. Time does not permit a discussion of the details of counter construction or of the gallery of electronic and other equipment associated with the counter. I will simply state that, with limitations to be discussed shortly, the instrument works well enough down to simulated sizes somewhere around 0.25 or 0.5 μm in diameter.

The data obtained can be displayed in a variety of ways. One that is perhaps of immediate theoretical interest is the function $\Phi(y)$ which gives the frequency of events in excess of y per rad of absorbed dose. Figure 5 shows this function for both ^{60}Co gamma rays and 1-MeV neutrons in a 1-μm sphere. Here $\Phi(0)$ is the frequency of events larger than 0, that is, of *any* events. This frequency is about 0.1 for ^{60}Co gamma rays. In other words, if we irradiate tissue with 1 rad of ^{60}Co gamma rays and examine 1-μm-diameter spheres, we find that only about 1 in 10 contains any event at all. The corresponding figure for 1-MeV neutrons is nearly 1 in 1000. However, if we ask the relative frequency with

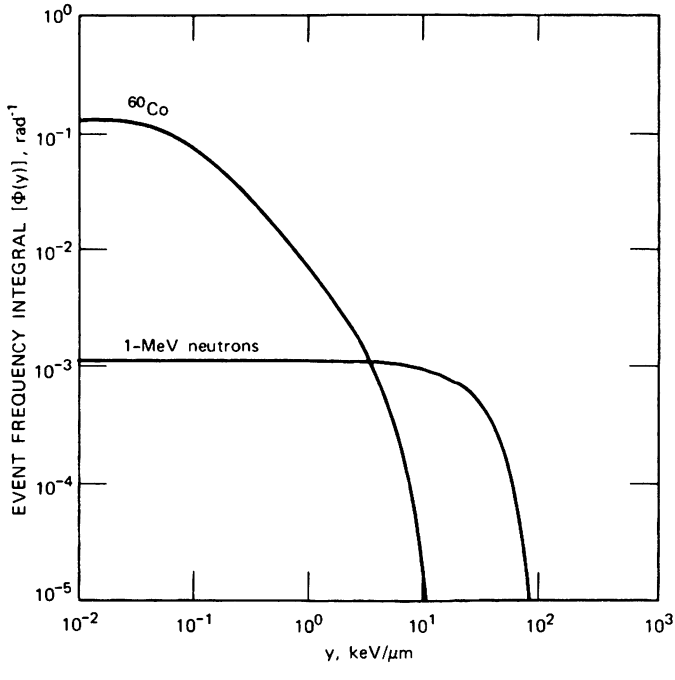

Fig. 5 Event frequency integral vs. lineal energy for ^{60}Co gamma rays and 1-MeV neutrons in a 1-μm sphere.

which energy in excess of 10 kV is deposited, the number is considerably smaller for ^{60}Co gammas than for 1-MeV neutrons. I might mention that, in dealing with very low frequencies, we run into a variety of experimental complications, and after several years we are still not quite certain how the ^{60}Co curve behaves at higher y values. We are continuing our studies in this area because there is a clear indication that these very rare, comparatively large energy depositions are the ones that matter most when cells of higher organisms are exposed to very low doses of gamma radiation.

Several years ago it became apparent that, if the tissue volume of interest is replaced by a gas volume but the surrounding tissue is left solid, certain complications arise. For instance, when the particle traverses the counter volume, its associated delta rays deposit more energy in the instrument than they would in real tissue. Conversely, if a particle does not enter the gas, delta rays are virtually absent although they might be important in the real situation. Because of this effect we had to develop counters in which the sensitive volume and a good deal of the surrounding space are both gaseous. This is a difficult technical problem. We tried a variety of ingenious but only partly workable designs and finally came up with the somewhat pedestrian model shown in Fig. 6. Here the sensitive volume of the counter is separated from the surrounding gas by a delicate spherical grid of tissue-equivalent plastic that permits comparatively free passage of particles but defines the collecting volume rather well. I might mention that, although the physics is simple, the engineering is rather difficult. This counter has a diameter of 0.25 in., and the struts are 0.003 in. thick.

With this type counter we are now verifying that delta rays can carry energy an appreciable distance from a particle track. Figure 7, from a recent study by W. Gross (personal communication), was obtained by irradiating a wall-less counter with alpha particles. On geometrical ground we would expect the pulse-height spectrum to be a triangle. It is distorted at high pulse heights because of statistical fluctuations in energy loss. The principal cause of these fluctuations is the existence of delta rays, which make their appearance at the low end of the spectrum in those instances where alpha particles have missed the counter altogether but have ejected delta rays into it. This evidence of track diameter can be expected to become far more important as we look at smaller sizes or perhaps at more rapidly moving nuclei. It demonstrates once more that the concept of a linear track ultimately becomes a poor representation of reality. The concepts of microdosimetry have been deliberately tailored to ignore this approach.

THEORY OF DUAL RADIATION ACTION

Figure 1 illustrates the general observation that constitutes the principal basis of the theory of dual radiation action. It is found that, for somatic effects of radiation on higher organisms, the curve relating the logarithm of RBE to the

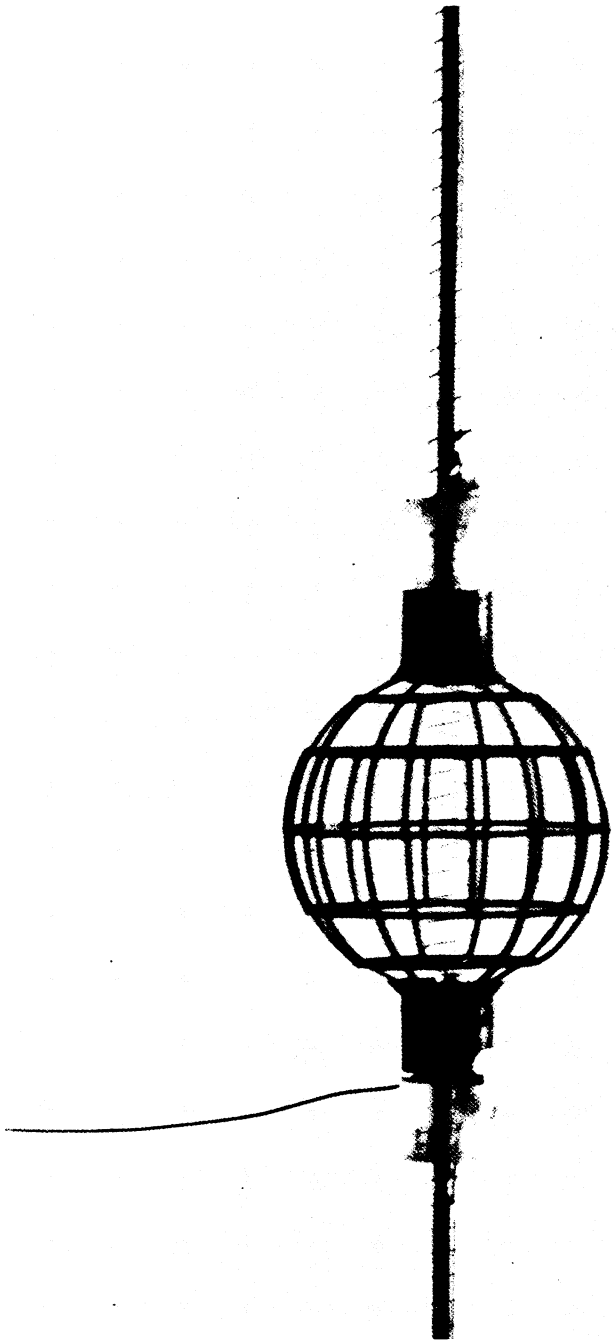

Fig. 6 Wall-less proportional counter used in microdosimetry.

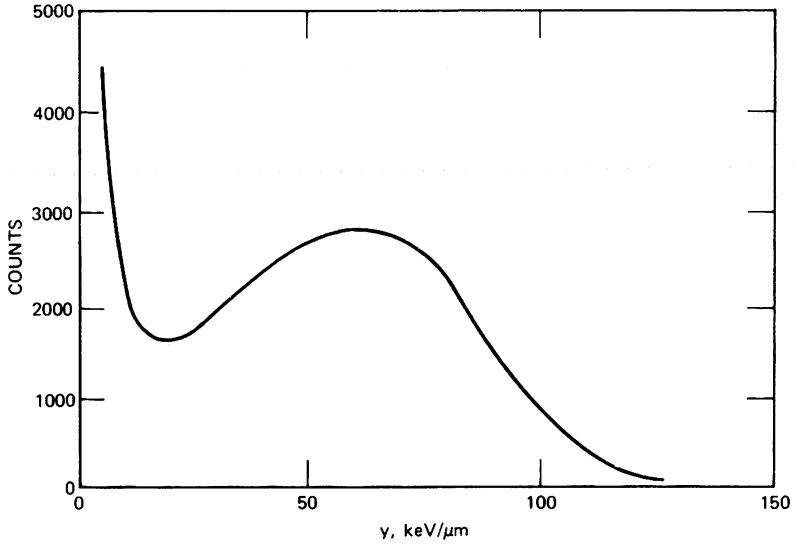

Fig. 7 Lineal energy distribution for ^{241}Am alpha particles in a 1-μm sphere.

logarithm of dose of high-LET radiation never* has a slope outside the range 0 to -0.5 and that for low doses and high LET the latter value is closely approached. Cellular effects of high-LET radiation are first-order processes, as indicated by an exponential decrease with dose of the nonaffected units of an irradiated population. It can also be shown on the basis of elementary microdosimetric data that the cellular effect must be due to single charged particles. Thus the relation between dose, D_n, and the nonaffected fraction, S,

$$-\ln S = \alpha D_n \tag{1}$$

must apply below neutron doses† of the order of 100 rads because single secondaries (usually protons) cause the effect. Other factors that can be invoked to account for this response (such as an exponential distribution of radiosensitivity in the cell population or an exponential response function of individual cells) might be operative as well, but they may be expected to have little if any relation to radiation quality and therefore are irrelevant if dose–RBE relations are considered.

*There is a possible exception to this in certain types of mutations in *Tradescantia*.

†The high-LET radiation usually employed is neutrons. However, experiments with heavy ions, particularly recent experiments with nitrogen ions, yield essentially the same results.

Effects on cells and the various, often intricate, biophysical responses under consideration (e.g., skin damage) should depend on the primary injury in complex ways. Accordingly we can obtain dose–response relations for densely ionizing radiations that deviate from the simple linear or exponential laws. The factors responsible for these complexities are generally independent of radiation quality, however, and we can expect them to cancel each other out in the RBE analysis. Therefore we use the concept of elementary cellular lesions which, because the lesions are proportional to small and intermediate doses of densely ionizing radiation, eliminates the various complicating factors. Thus $S = \Phi(\epsilon)$, where ϵ is the yield of elementary lesions and Φ is a function that depends on neither dose nor radiation quality. For densely ionizing radiation

$$\epsilon = \beta D_n \tag{2}$$

The coefficient β depends on the radiation quality and on the experimental end point being considered. Even if the lesions causing the effect are subject to saturation (e.g., if a more correct formulation were $\epsilon = 1 - e^{-\beta D_n}$), the function involved would cancel if different radiation qualities are compared.

In a broad dose range, we find (see Fig. 1)

$$\ln \text{RBE} = c - 0.5 \ln D_n \tag{3}$$

or, with $c_1 = \ln c$,

$$\text{RBE} = c_1 D_n^{-0.5} \tag{4}$$

Since RBE is defined as the ratio of the equivalent X-ray and neutron doses, D_x/D_n,

$$D_x^2 = c_1^2 D_n \tag{5}$$

If this relation is to hold for equal survival over a wide range of doses, we obtain from Eq. 2

$$\epsilon = kD_x^2 \qquad \left(\text{for } k = \frac{\beta}{c_1^2}\right) \tag{6}$$

The physical quantity relevant in cellular inactivation or other cellular effects is not the absorbed dose. Absorbed dose is merely the expectation value of the ratio of absorbed energy and mass (because of the statistics of energy deposition, it can be considerably different from this ratio in any particular microscopic region). The pertinent quantity must be the specific energy, z, which is the actual value of the energy density in whatever cellular region, or site, where energy deposition contributes to the effect. The quantity z is a

random variable, and, at a fixed dose D, we can only give a probability distribution, f(z;D), of the possible values of z. The distribution f(z;D) depends not only on dose but also on radiation quality and on the size of the microscopic reference volume.*

The basic rule governing cellular radiation response must be the dependence of ϵ on z. The connection between ϵ and the absorbed dose is less fundamental. It is determined by the integral of $\epsilon(z)$ over the probability distribution f(z;D) of all possible values of z at a given D and therefore depends on radiation quality:

$$\epsilon(D) = \int_{z=0}^{\infty} \epsilon(z)\, f(z;D)\, dz \tag{7}$$

Since we cannot observe $\epsilon(z)$ directly in the experiment, it is the object of microdosimetric analysis to determine the probability distributions f(z;D) for all radiation qualities of interest and to deduce the basic relation $\epsilon(z)$ from observed dose relations $\epsilon(D)$. In principle Eq. 7 can be inverted so that $\epsilon(z)$ can be derived if $\epsilon(D)$ and the probability distributions f(z;D) are known. Particularly, we can ask for the dependence $\epsilon(z)$ leading to an approximately linear dose relation $\epsilon(D)$ for densely ionizing radiation and to an approximately quadratic dependence on dose for sparsely ionizing radiation. The result is that we must deal with a quadratic dependence of ϵ on z:

$$\epsilon(z) = kz^2 \tag{8}$$

This can be seen when the dose dependences corresponding to this relation are derived. To obtain the dose dependence for a given radiation quality, we must average the quantity kz^2 over the probability distribution of z at a given dose:

$$\epsilon(D) = k\overline{z^2} = k \int_0^{\infty} z^2\, f(z;D)\, dz \tag{9}$$

Evaluating this formula, we find an expression that depends merely on dose and on the expectation value of z and z^2 in single events.† If $f_1(z)$ designates the probability distribution of the values of z induced by single events in the reference volume, we can show that

$$\overline{z^2} = \int_0^{\infty} z^2\, f(z;D)\, dz \tag{10}$$

can be transformed to

$$\overline{z^2} = \frac{\int_0^{\infty} z^2\, f_1(z)\, dz}{\int_0^{\infty} z\, f_1(z)\, dz} D + D^2 \tag{11}$$

*For a formal definition of microdosimetric quantities see Ref. 9.

†An energy-deposition event is the deposition of energy in the region of interest by an ionizing particle and/or its secondaries (see Ref. 9).

The detailed derivation of this important relation has been given elsewhere.[10] If the ratio of the integrals is abbreviated by ζ,

$$\overline{z^2} = \zeta D + D^2 \tag{12}$$

The quantity ζ is formed analogously to a dose-average LET (see Ref. 11), but it has the dimension of a dose and can be considered as the average increment of z due to single events in the reference volume. Inserting Eq. 12 into Eq. 9, we obtain the dose dependence of the primary lesions:

$$\epsilon(D) = k(\zeta D + D^2) \tag{13}$$

The quantity ζ_X for X rays is considerably smaller than the corresponding quantity ζ_n for neutrons. For this reason the linear term can be neglected for X rays, and the quadratic term can be neglected for neutrons over a wide dose range. There should, however, be a region of linear dependence on dose when $D_X \ll \zeta_X$. Similarly, for neutrons there should be a quadratic dependence of effect on dose when $D_n \gg \zeta_n$. Under these conditions, at very low doses the RBE should reach the constant value ζ_n/ζ_X and at very large doses should approach the value 1. The general dependence shown in Fig. 8 for various values of ζ_n/ζ_X is in good agreement with the curves of Fig. 1, where the curve for $\zeta_X = 0$ is shown as a dashed line.

The slope -0.5 in the dose–RBE relation is reached in the region where we can neglect the quadratic component for neutrons and the linear component for X rays:

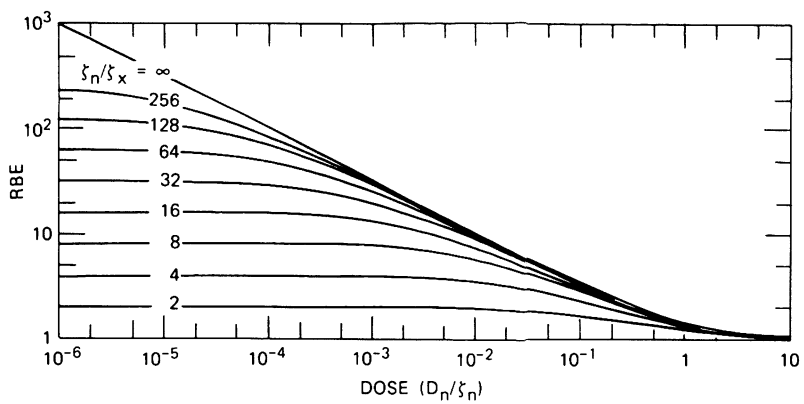

Fig. 8 Relation between RBE and dose. The dose is given as a multiple of the quantity ζ_n; the parameter of the curves is the ratio of ζ_n to the corresponding quantity ζ_X of the reference radiation.

$$\epsilon_n = k\zeta_n D_n$$
$$\epsilon_x = kD_x^2 \tag{14}$$

Therefore, for the RBE = D_x/D_n in this region, we obtain

$$\epsilon_x = \epsilon_n = kD_x^2 = k\zeta_n D_n$$
$$\text{RBE} = \left(\frac{\zeta_n}{D_n}\right)^{1/2} \tag{15}$$

Thus straight-line extrapolation of the part of the dose–RBE curve that has the slope -0.5 must intersect the abscissa at the value ζ_n. From the curves in Fig. 1, we find that ζ_n has values of the order of 1000 rads. Since ζ_n as a function of the site diameter, d, is known for all the neutron energies involved, we can determine the value of d for the various effects. It turns out to be roughly 1 to 2 μm for all cases represented in the figure except for the dry seeds (curve 11), where it is an order of magnitude less.

From microdosimetric measurements we obtain a value of roughly 40 for the ratio ζ_n/ζ_x, which should be the limit value of RBE for very low doses. An RBE close to this value has indeed been observed in the one experiment where the data reach down to sufficiently low doses to show the initial plateau of RBE; this is the case of the induction of pink mutations in *Tradescantia* (Fig. 1, curve 4). In another case where RBE values have been obtained for very low doses, i.e., in the opacification studies on the murine lens with 430-keV neutrons (Fig. 1, curve 1), a higher value, possibly exceeding 100, is found. This could be due to the fact that, even for equal values of z, in the sensitive site the distribution of energy on the nanometer scale is not the same for neutrons and X rays and that therefore the factor k could be larger for neutrons than for X rays. This is also indicated because the RBE seems to approach a higher value than 1 for large doses in this case (see Fig. 9). If k_n is larger than k_x, the dose–RBE curve is simply shifted vertically by the factor k_n/k_x. That such a shift has thus far been found only in the lens opacification studies could be because the lens is a hypoxic system.

The seemingly well-established conclusions represented by Eqs. 8 and 13 can be added to by plausible but not rigorously proven further deductions. The quadratic dependence of the primary cellular lesions, together with the approximate equality of k_n and k_x, leads to the notion that two loci must be impaired if a site is to be inactivated. A locus that is impaired with about equal probability regardless of LET must be a small region (e.g., a base of DNA). It appears that traversal of the site by a high-LET particle can frequently lead to the impairment of at least two loci but that, on the other hand, traversal by a low-LET electron is unlikely to impair even one locus. If this is so, loci must occupy a limited fraction of the site volume. Finally we may conjecture that the

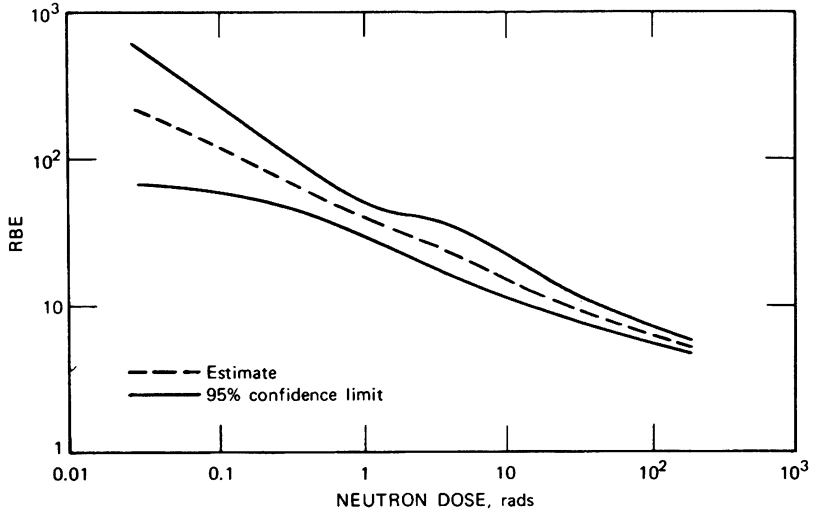

Fig. 9 The RBE of 0.43-MeV neutrons relative to X rays for the induction of lens opacification in the mouse as a function of neutron dose.[1,15]

site as a volume in which interaction of impaired loci occurs with unit probability is merely an abstraction and that the sensitive volume involved is the nucleus of the cell in which loci interact with varying probability according to their separation (interactions may depend on other factors also).

In a more general formulation, we can say that the primary lesions in the cell are caused by a second-order reaction that may involve one or a number of dual targets in the nucleus and that the interaction distances are of the order of 1 to 2 μm. The linear term in the dose-dependence equation (Eq. 13) represents intratrack action (i.e., interaction of loci affected by the same charged particle), and the quadratic term represents the intertrack action (i.e., interaction of loci affected by separate charged particles).

The quadratic dependence of the cellular effects on energy density within a sensitive site and the resulting linear–quadratic dose dependence was found earlier in the case of radiation-induced chromosome aberrations (see, for example, Refs. 12 and 13). Equation 13 is the exact formulation of a result obtained in various semiquantitative forms by different investigators for this special case.

For comparison with the results of earlier works, it is useful to give the equation that corresponds to Eq. 13 if we approximate the microdosimetric quantities by the LET concept. In this case we obtain (see Ref. 11):

$$\epsilon(D) = k(22.9 \frac{\bar{L}_D}{d^2} D + D^2) \qquad (16)$$

where $\bar{L}_D$ is the dose-average LET (keV/μm) and d is the site diameter (μm); d can also be considered as an effective interaction distance of single breaks or, in the more general case, of affected loci. For single-event inactivation the linear term in Eq. 16 implies proportionality of the effect to $\bar{L}_D$. This is equivalent to the quadratic dependence of the cross section on $\bar{L}_D$ found by Powers, Lyman, and Tobias.[14]

There are strong indications that DNA is the important target in the cell. The similarity in the quadratic dependence of cellular lesions on energy density for various experimental end points lends further support to this assumption. It must, however, be stressed that similarity of the dependence on z does not prove that we are dealing always and exclusively with damage to DNA or with chromosome aberrations.

The preceding remarks summarize the basic arguments underlying the theory of dual radiation action. A more-detailed analysis, given elsewhere,[10] includes a treatment of the dependence of RBE on neutron energy; a discussion of the saturation effect, which becomes important for particles with a stopping power exceeding 100 keV/μm; and an inquiry into the oxygen effect, which still presents considerable unsolved problems.

One of the most important additional aspects is the resulting treatment of the dose-rate problem. The linear term in Eq. 13 represents the intratrack action (i.e., the synergism of sublesions produced in one and the same particle track) and is therefore independent of dose rate. The quadratic component, on the other hand, represents the interaction of sublesions produced by independent charged particles. The preceding discussion did not take into account that sublesions can have a finite lifetime, e.g., in chromosome breaks, where recovery times of the order of 20 min are found, or in cellular inactivation, where, in the so-called Elkind phenomenon, recovery of sublethal damage within hours is observed. The finite lifetime of sublesions implies that the interaction probability of sublesions is reduced when they are formed at a temporal separation. We can show that because of recovery during the irradiation time T, the quadratic component D^2 in Eq. 13 is reduced to the term $q(T)\,D^2$, where the reduction factor is simply the integral over the distribution h(t) of time intervals t between dose elements and the recovery function $r(t)$, which describes the reduction of the interaction probability after time t:

$$q(T) = \int_0^T r(t)\, h(t)\, dt \qquad (17)$$

The temporal distributions h(t) and the resulting reduction factors in the quadratic component of the effect have been derived for the typical temporal distributions of dose applied in radiation biology and radiation therapy.[10] Specific experimental tests of the results and their application to therapy will be one of the important objects of future work.

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