CANCER 1

A COMPREHENSIVE TREATISE

SECOND EDITION

ETIOLOGY: Chemical and Physical Carcinogenesis

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Biophysical Aspects of Radiation Carcinogenesis

ALBRECHT M. KELLERER AND HARALD H. ROSSI

1. *Introduction*

Although radiation carcinogenesis was recognized some 75 years ago, we still know little about the mechanisms involved. Because of its profoundly important theoretical and practical aspects, the subject has been very extensively studied, but most of the information obtained has been of a phenomenological nature. It seems unlikely that a complete step-by-step description of radiogenic cancer induction will be possible in the foreseeable future. Merely the first purely physical process—that of energy deposition by charged particles—is highly complex and difficult to quantitate. There is every reason to believe that the ensuing physicochemical, biochemical, intracellular, intercellular, and systemic processes are at least as complex and that many of them are unknown at this time.

Between the two extremes of a purely descriptive treatment of a phenomenon and the detailed knowledge of the causal chain of events responsible for it can be intermediate levels of understanding. Sometimes these can be based on generally observed or otherwise deduced basic features that permit the formulation of basic kinetics. This in turn can furnish clues concerning its mechanism. An example of this kind of understanding are the laws of Mendelian genetics, which formulated the basic laws of inheritance before the underlying cytogenetic and molecular mechanisms were recognized.

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The application of radiation biophysics can provide certain limited insights of this kind to the subject of radiation carcinogenesis. This approach is comparatively recent and has not been widely adopted. It remains in a state of continuing development, as indicated by substantial modifications in this chapter as compared with its version in the previous edition. However, this reflects further development rather than change and the basic conclusions drawn previously are confirmed and extended rather than altered.

Since the arguments are based on energy deposition in irradiated tissues and especially on the stochastic nature of this process, it is necessary that its principal features be considered. However, the presentation is condensed and simplified. General literature references have been provided for more exhaustive study.

2. *Interaction of Radiation and Matter*

2.1. *Mechanisms*

Radiation is termed "ionizing" when its interactions are so energetic that they remove electrons from the atoms that constitute the irradiated matter. In the case of many materials—including tissues—this leads to permanent changes that are produced with far greater efficiency than is obtained with radiations that merely induce electronic or molecular excitations.

In nearly all cases of practical interest, ionization occurs through the agency of electrically charged particles that may be high-speed electrons or nuclear constituents such as protons and α-particles. These are *charged particle radiations* that may originate in external or internal sources or may be generated inside the irradiated matter by *uncharged particle radiations.* The latter include high-frequency electromagnetic quanta (or photons) such as X- and γ-rays and electrically neutral particles such as neutrons.

Although the energies of ionizing particles can vary by an enormous factor of at least 10^{20}, the energies of principal practical importance range roughly from 0.1 to 10 MeV. In this energy interval, the range of directly ionizing particles is generally much less than the dimensions of the human body or even the dimensions of organs of small animals. Consequently, irradiation by charged particles arising from external sources is of limited significance, but it is important in the case of radioactive substances that are deposited within the irradiated tissues by physiological processes. Examples include location of ingested or injected radium in bone and concentrations of radioactive iodine isotopes in the thyroid. With a few exceptions (such as the presence of water containing tritium, the radioactive isotope of hydrogen), internal irradiations tend to be quite nonuniform. More or less uniform irradiation of organs of whole animals usually occurs when the more penetrating indirectly ionizing radiations are applied.

It may be useful to provide numerical indications of the degree of penetration of some of these radiations. Figure 1 depicts the mean free path, λ, and its reciprocal, the linear *absorption coefficient,* μ, in water for protons and neutrons

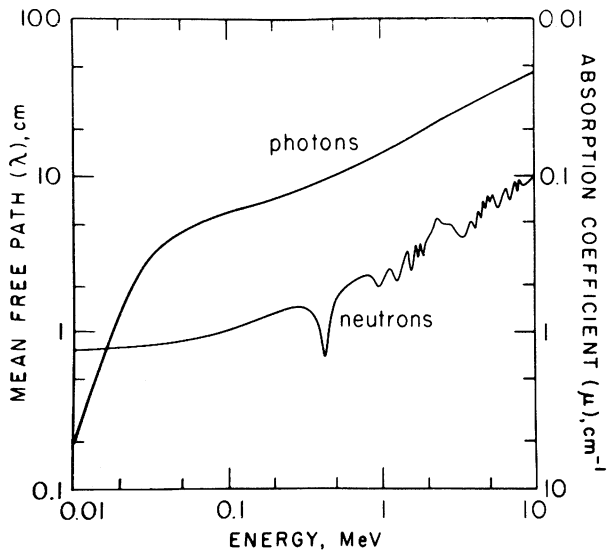

FIGURE 1. Mean free path, $\lambda(E)$, and absorption coefficient, $\mu(E)$, as a function of energy for photons and neutrons in water.

of energies between 10 keV and 10 MeV; μ is defined by the equation

$$N = N_0 e^{-\mu d} \tag{1}$$

where N_0 is the number of incident particles and N is the number of particles that arrive at a depth d. The *mean free path* (or *attenuation length*), λ, is equal to $1/\mu$. When d is equal to $1/\mu$, the fraction of particles that have not interacted is e^{-1}, which is approximately 0.37. For example, μ for 1-MeV photons is approximately 0.07/cm, which means that a thickness of about 1/0.07 or approximately 14 cm of water will transmit 37% of incident 1-MeV photons without interactions.

It must be noted that these curves cannot be used to derive immediately the energy deposition as a function of depth in the irradiated material because in many instances the interactions lead to the production of secondary radiations that have appreciable penetration of their own, with the result that more energy arrives at any given depth than merely that carried by the primary radiation. In the case of photons, the three principal types of interaction reflected in Fig. 1 are the *photoelectric effect*, the *Compton effect*, and *pair* (and to some extent *triplet*) *production*. The first of these processes is of importance only at the low end of the energy scale and results in the ejection of a photoelectron and of fluorescent radiation, both of which are locally absorbed. Pair production, which occurs only at the upper end of the energy scale, results in the generation of an electron–positron pair. Following the annihilation of the positron, about 1 MeV of the original photon energy appears as the shared energy of two new photons that have appreciable penetration. The main section of the photon curve in Fig. 1 is due to the Compton effect, in which varying fractions of the incident photon energy appear in the form of scattered photons, particularly near the low end of the energy scale.

In the case of neutrons, by far the most important reaction responsible for the shape of the curve in Fig. 1 is *elastic scattering* (principally by hydrogen), in which the neutron can retain a substantial fraction of its energy. Thus, also in this case appreciable radiation energy can penetrate beyond the site where primary radiation has been absorbed.

To illustrate the far more restricted penetration of charged-particle radiations, the range of perhaps the two most important charged particles in radiobiology, the electron and the proton, is shown in Fig. 2. In contrast to the uncharged-particle radiations, which tend to be absorbed exponentially and cannot be characterized by a well-defined range of penetration, charged particles have as a rule a reasonably well-defined distance of penetration.

The principal process determining the range of charged particles is electronic collision. The electrons of atoms located in the vicinity of the particle trajectory are subject to electrical impulses that excite them, or eject them from their parent atom with varying energy. To a good first approximation, the interaction is proportional to the square of the charge of the incident particle and inversely proportional to the square of its velocity. Both the electron and the proton carry unit charge, but because of its far greater mass a proton moves much more slowly than an electron of equal energy. This results in a much higher rate of energy loss and consequently a much shorter range for the proton.

The rate of energy loss of charged particles is known as the *linear energy transfer* (LET), and it is usually specified in terms of kiloelectron volts per micrometer in the medium of interest (usually water or tissue). Figure 3 shows the LET in water of electrons and protons as a function of their energy.

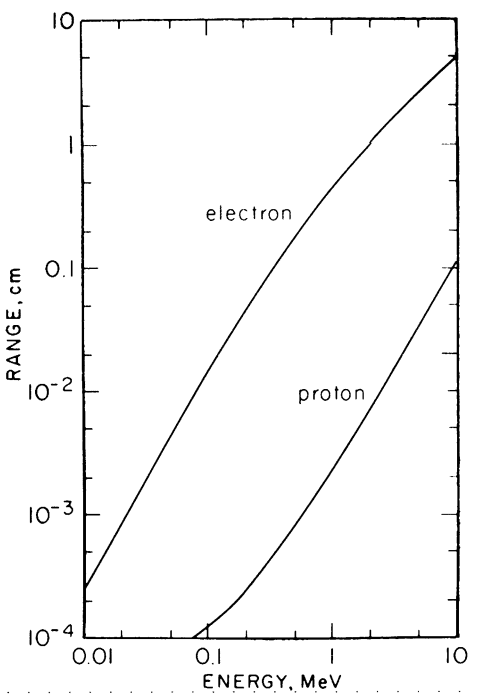

FIGURE 2. Range of electrons and protons in water as a function of energy (ICRU, 1970).

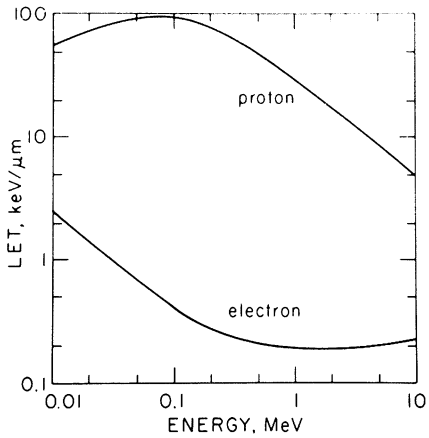

FIGURE 3. Linear energy transfer, LET, for electrons and protons in water as a function of their energy (ICRU, 1970).

The LET is only an average characterizing a complicated process. The electrons resulting from ionizing collisions are ejected with initial kinetic energies that generally have a very wide range (and highly skewed) distribution. The term *δ-rays* is applied to these electrons if their energy is sufficient such that they can in turn ionize, and there may be several generations of such secondaries produced. The track of an ionizing particle therefore, consists of *primary ionizations* produced in close proximity to the geometrical trajectory and *secondary ionizations* that surround this path up to highly variable distances. This pattern has been referred to as the *inchoate energy distribution*, and the locations where the primary and secondary particles undergo energy losses are termed *transfer points* (Kellerer

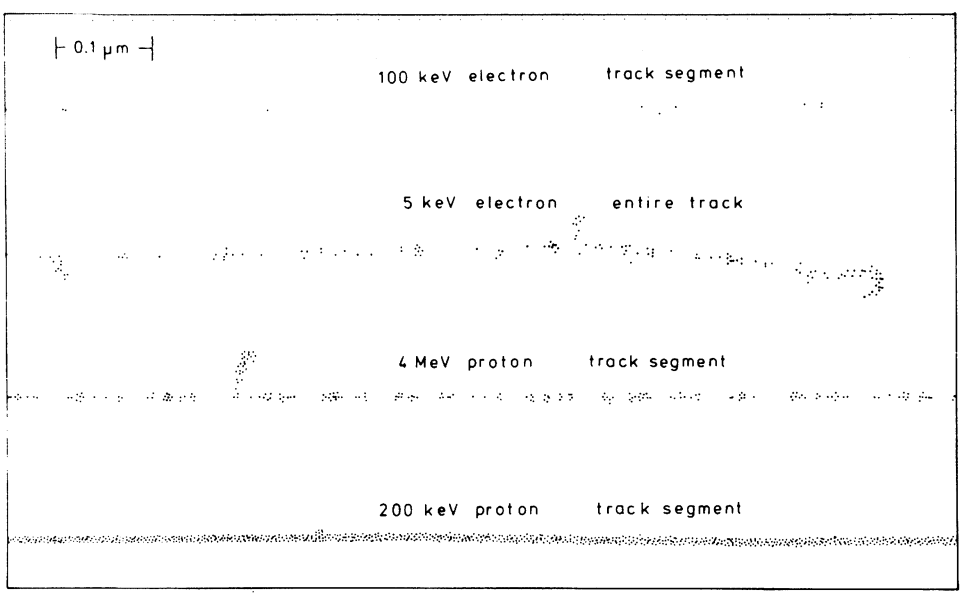

FIGURE 4. Two-dimensional projections of track segments in tissue of electrons and protons of different energies. The dots represent ionizations; the lateral extension of the track core is somewhat enlarged in the diagrams in order to resolve the individual energy transfers. The length of the track segments is 1 μm, i.e., a fraction of the dimension of the nucleus of a mammalian cell.

and Chmelevsky, 1975). Figure 4 gives two-dimensional projections of the inchoate energy distributions generated in small sections of the trajectories of charged particles of different LET.

2.2. *Dosimetry*

The physical quantity that is of central importance in radiobiology is the *absorbed dose, D,* which is defined as

$$D = E/m \tag{2}$$

where E is the energy deposited in a volume element and m is the mass contained in the volume element. E is proportional to the product of the number of charged particles traversing the element and to their LET. It should be noted that this statement as well as the definition [equation (2)] hold only if the number of particles that deposit E is large since D is the mean or expectation value of the specific energy (defined below).

In the case of uncharged-particle radiation, the absorbed dose evidently depends on the fraction of the incident energy that is transformed into kinetic energy of charged particles. A useful quantity in this connection is the *kerma,* which is the kinetic energy of charged particles released per unit mass in a specified material (here usually tissue). Figure 5 shows this quantity per unit fluence (number of uncharged particles per unit cross-sectional area) for photons and neutrons. In irradiated matter, kerma and the absorbed dose frequently have nearly the same numerical value. The equality exists if the range of charged particles is short compared to the attenuation length of uncharged particles. In this condition, the energy absorbed per unit mass at most locations in the medium

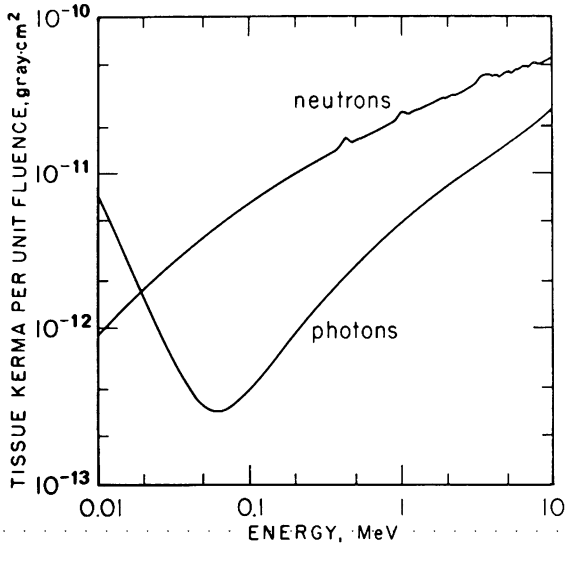

FIGURE 5. Tissue kerma per unit fluence for photons and neutrons as a function of energy. Based on Bach and Caswell (1968).

is nearly the same as the kinetic energy of the charged particles released. This is the condition known as *radiation equilibrium.* It does not obtain when the absorption of uncharged-particle radiations is comparable to that of the charged-particle radiations or when one is near interfaces of different materials. For example, in the case of X-irradiation, soft tissues in proximity to bone receive a higher dose than those more distant due to the greater electron emission from the irradiated bone, while the tissue kerma does not depend on the nature of any material that may surround the point of interest.

Under well-defined conditions, absorbed doses can be measured and often also calculated within an accuracy of a few percent. However, in some instances, and in particular those relating to human carcinogenesis, doses must often be determined retrospectively on the basis of incomplete information. Under these conditions, major uncertainties arise.

According to the widely accepted Système International d'Unités (SI), the quotient of energy by mass when expressed in base units is square meters per second squared. An acceptable and more usual formulation is in terms of joules per kilogram. In radiological physics this unit has been given the special name *gray* (Gy). Until a few years ago and during the current interim period (which is to end in a few years), the *rad* has been the unit of absorbed dose and quantities of the same dimension (such as kerma). One rad (100 ergs/g) is equal to 0.01 Gy.

2.3. Microdosimetry

Many radiobiological phenomena and at least one type of radiation carcinogenesis (see Section 5) are due to tissue response to radiation injury. However, in all instances individual cells are injured randomly, and consequently the energy absorbed by individual cells is a relevant quantity. It appears to be established that virtually all of the radiation sensitivity of the eukaryotic cell resides in its nucleus, and it is quite probable that the ultimate target is DNA. The biological effect of ionizing radiations is therefore determined by energy concentrations in domains of subcellular dimensions.

As explained above, radiation energy is deposited by discrete, directly ionizing particles. Its concentration is therefore subject to statistical fluctuations. These fluctuations can be appreciable in small volumes for doses that are sufficiently large to produce marked biological effects. Consider, for example, a region with a diameter of 1 μm in tissue that receives an absorbed dose of 1 Gy. In the case of γ-rays, the mean number of electrons traversing this volume is more than 10; in the case of fast neutrons, the frequency of particle traversals is only of the order of 1/10. Any radiation effects are, of course, determined by the energy actually deposited, and it is plain that this can differ greatly from the *mean* or *expectation value* that is represented by the absorbed dose. In the example just quoted, there is no neutron secondary and therefore no energy deposition in 9 out of 10 cases, but in the remaining one the energy density is typically 10 times larger than the absorbed dose. Such fluctuations are the principal subject of microdosimetry.

The central variable in microdosimetry is the *specific energy*, z, which is defined as

$$z = \Delta E/\Delta m \tag{3}$$

where ΔE is the energy actually deposited in the region of mass, Δm.

Unlike the absorbed dose, the specific energy is a *stochastic* quantity that has a range of values in uniformly irradiated matter. The variability of z is expressed by the probability distribution $f(z)$, which represents the probability that the specific energy is equal to z. The width of this distribution depends on three factors:

1. The mass Δm of the region. Strictly speaking, this involves both the size and the shape of the region, but as a rule, shape is of secondary importance and it is usually assumed that the volume is at least approximately spherical and that it can therefore be characterized by its *diameter, d.*
2. The absorbed dose.
3. The LET of the charged particles traversing Δm.

The influence of these factors is illustrated in Figs. 6 and 7, which are logarithmic representations of $f(z)$ vs. z for various absorbed doses of ^{60}Co γ-rays and 5.7-MeV neutrons for spheres having diameters of 0.5 or 12 μm. Neutrons of energy 5.7 MeV are somewhat more energetic and therefore less densely ionizing than fission neutrons. The average LET is higher for natural α-emitters and lower for more energetic neutrons. Electrons produced by ^{60}Co γ-rays have minimal LET; in the case of X-rays, the LET is somewhat greater.

The curves in Figs. 6 and 7 have common characteristics. At high doses the number of particles is large, particularly for γ-radiation and the larger diameter. Consequently, statistical fluctuations are small and z is unlikely to differ from D. As the dose is reduced, fluctuations become greater because the number of particle traversals is correspondingly lessened. At low doses, a distribution is observed that has a shape largely independent of dose but an amplitude proportional to dose. This occurs when the average number of events is less than 1. In this case, one is dealing with the energy-deposition spectrum generated by single particles (indicated by the broken lines in Figs. 6 and 7). A reduction of dose merely results in a decrease of the amplitude of the spectrum with the remainder of the distribution appearing at $z = 0$ (which is not shown on these logarithmic graphs).

Microdosimetric distributions are determined experimentally with spherical gas-filled proportional counters. It is sufficient to obtain the energy-deposition spectra generated by individual particles; the other distributions can then be readily computed.

Figure 8 permits a comparison of single-event spectra for different radiations in a spherical tissue region of diameter 1 μm, and illustrates in more quantitative terms than Fig. 4 the extremely broad range of event sizes that can be produced by the sparsely ionizing fast electrons, on the one hand, and the slow, heavy neutron recoils, on the other hand.

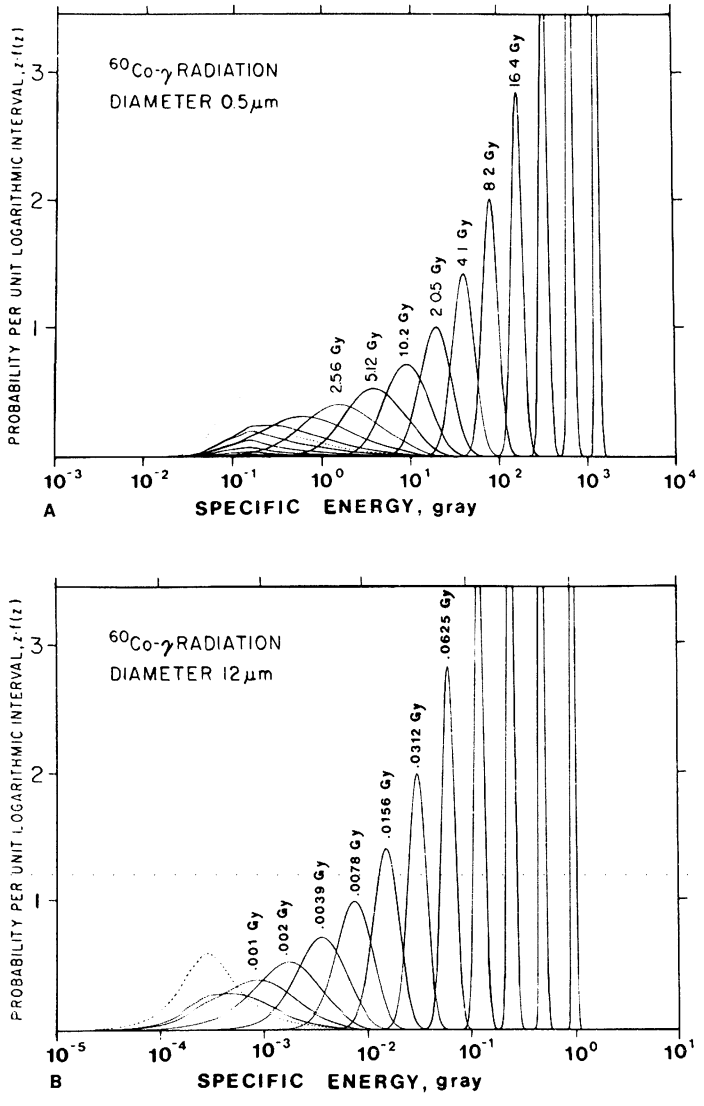

FIGURE 6. Probability per unit logarithmic interval of specific energy, z, at various doses of ^{60}Co γ-rays in a spherical tissue region of diameter (a) 0.5 μm and (b) 12 μm. The distributions of the increments of z produced in single events are given as broken lines.

For any distribution, $f(z)$, the mean value of z is defined by

$$\bar{z} = \int_0^\infty z f(z)\, dz \tag{4}$$

In Figs. 6 and 7, it is evident that $\bar{z}$ is equal to D at high doses. Although the shape of the distribution for finite energy losses does not change with decreasing dose when only single events are of importance, the decreasing

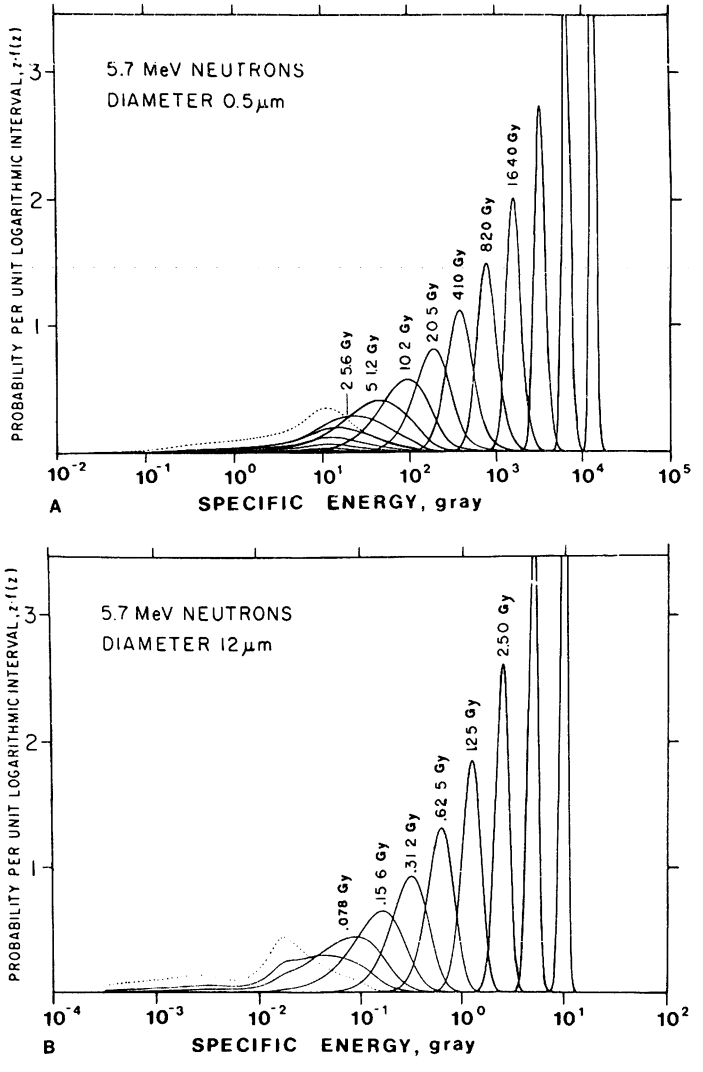

FIGURE 7. Probability per unit logarithmic interval of specific energy, z, at various doses of 5.7-MeV neutrons in a spherical tissue region of diameter (a) 0.5 μm and (b) 12 μm. The distributions of the increments of z produced in single events are given as broken lines.

frequency of events and the corresponding increase of instances in which there is no event result in equality between $\bar{z}$ and D at all doses.

It can be assumed that the biological effect of radiation on the cell is due to deposition of energy in one or several sensitive sites. Consider one of these sites and denote the probability of it being affected by $E(z)$. Any dose D then produces corresponding distributions $f(z)$ and $E(D)$. The effect produced by this dose is given by

$$E(D) = \int_0^\infty E(z)f(z)\,dz \tag{5}$$

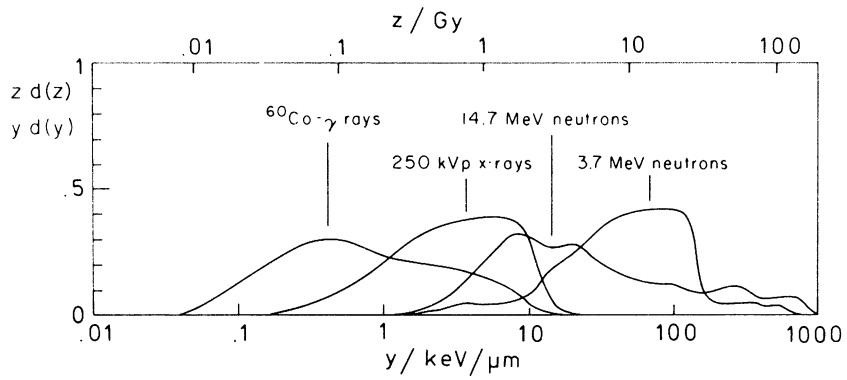

FIGURE 8. Probabilities of event sizes per unit logarithmic interval of linear energy y or specific energy z for different radiations in a spherical tissue region of 1-μm diameter.

Comparison of equations (4) and (5) indicates that if

$$E(z) = kz \tag{6}$$

i.e., if the effect probability is proportional to z, then

$$E(D) = k\bar{z} = kD \tag{7}$$

Thus, $\bar{z}$ and therefore also the absorbed dose are meaningful averages of specific energy if the effect probabilities are proportional to z. As will be seen in the next section, most, if not all, somatic radiation effects on higher organisms are characterized by a dependence that is proportional not to z but rather to z^2. This statement applies in particular to the few instances where the induction of malignancies by ionizing radiation could be studied in adequate detail. This nonlinear dependence is the ultimate reason for the need to employ microdosimetry in the analysis of the primary steps in radiation carcinogenesis.

The assumption that cellular injury or death is due to energy concentration in one or several sites in the cell is a reasonable first approximation that will largely be employed in the following discussions. It appears, however, that a more realistic picture is one in which the radiation-sensitive material in the cell (presumably the nuclear DNA) is distributed in a highly nonuniform pattern throughout a larger volume (presumably the nucleus). This pattern has been termed the *matrix*. As will be explained below, there are strong indications that the lesions responsible for cellular impairment are due to the combination of pairs of altered molecular configurations in the matrix. Since the combination probability of such pairs may be expected to depend on their separation, it is essential for any theoretical analysis to know the distance distribution of pairs of transfer points in the irradiated medium. The *proximity function*, $t(x)$, has been developed for this purpose. According to its definition, $t(x)\,dx$ is the mean energy deposited by the same particle at a distance x to $x+dx$ from randomly selected energy transfers in the medium. Figure 9 gives $t(x)$ for neutrons of

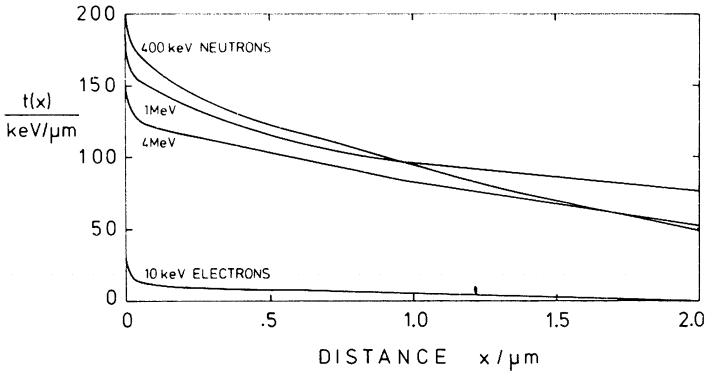

FIGURE 9. Computed proximity functions $t(x)$ for neutrons (F. Zieker, personal communication) and for 10-keV electrons (Chmelevsky *et al.*, 1980).

different energies and for 10-keV electrons, i.e., for inchoate distributions of the type illustrated in Fig. 4.

3. *General Stochastic Considerations*

3.1. *The Linear Dose–Effect Relation at Small Doses*

There is a well-known distinction in radiobiology between effects due to injury of individual cells and effects due to the collective response of irradiated cells. It is, however, also important to determine whether the response of a given cell is independent of the irradiation of other entities (cells, medium, etc.). When this independence exists, the cell may be termed *autonomous.* In the following, cells will be understood to be autonomous if not stated otherwise.

As has been pointed out in the preceding section, the absorbed dose determines only the mean value of the specific energy absorbed in microscopic volumes which may widely deviate from this mean value. It has also been concluded in the preceding section that the statistical fluctuations in energy deposition play no role if the cellular damage is proportional to the specific energy, z; in this case, the average effect observed at a given absorbed dose is proportional to this absorbed dose.

In all effects on higher organisms, one finds, however, that densely ionizing radiations are more effective than sparsely ionizing radiations, such as X- or γ-rays. All commonly employed ionizing radiations work by the same primary physical processes, namely by electronic excitations and by ionizations. The unequal biological effectiveness of different types of ionizing radiations can therefore only be explained by the different spatial distributions of absorbed energy on a microscopic scale. Specifically, the increased biological effectiveness of densely ionizing radiations must be due to the high local concentration of absorbed energy near the tracks of heavy charged particles. Accordingly, one

concludes that the dependence of cellular damage on specific energy, z, is steeper than linear. The actual form of the nonlinear dependence, $E(z)$, will be considered later. One can, however, draw certain important conclusions that follow from microdosimetry and are valid regardless of the actual form of $E(z)$. Such conclusions will be dealt with in the remainder of this section.

One general conclusion that follows from microdosimetry is that in the limit of small absorbed doses, the average cellular effect is always proportional to dose. Such a linear relation between observed cellular effect and absorbed dose must be expected regardless of the dependence of cellular effect on specific energy; it is due to the fact that even at the smallest doses, finite amounts of energy are deposited in a cell when this cell is traversed by a charged particle. The energy deposited in such single events does not depend on the dose; accordingly, the effect in those cells that are traversed by a charged particle does not change with decreasing dose. The only change that occurs with decreasing absorbed dose is the decrease in the fraction of cells that are subject to an event of energy deposition. This can be treated quantitatively, and microdosimetry can furnish conclusions as to the range of absorbed doses in which the statement applies for different radiation qualities.

The effect probability $E(D)$ at a given dose D is equal to the sum of all products of the probabilities for various numbers ν of events (charged-particle traversals) in the sensitive sites and the effect probabilities E_ν under the condition that ν events occur:

$$E(D) = \sum_{\nu=1}^{\infty} p_\nu E_\nu \tag{8}$$

The equation is written in the form that does not include the spontaneous incidence, E_0; i.e., it is assumed that $E(D)$ is corrected for the spontaneous incidence and that the latter need therefore not be considered.

Because energy-deposition events are by definition statistically independent, their number follows Poisson statistics; i.e., the probability p_ν that exactly ν events occur is

$$p_\nu = e^{\phi D}(\phi D)^\nu / \nu! \tag{9}$$

The term ϕD is the mean number of events per site. Event frequencies, ϕ, for various radiation qualities and site sizes will be given below.

It will in the present context not be necessary to evaluate equation (8) in its complete form. Instead, it will be sufficient to consider the case of small event frequencies, ϕD, which occurs at small doses especially of densely ionizing radiations.

In order to evaluate the case where the number, ϕD, of events is small compared to 1, equation (9) can be expanded into a power series. Because it is assumed that $D \ll 1$, the term $e^{\phi D}$ can be set equal to 1, and with this simplification one obtains

$$E(D) = E_1 \phi D + E_2 (\phi D)^2 / 2 + \cdots \tag{10}$$

where E_1 is the probability for the effect if exactly one event has taken place

and E_2 is the effect probability if two events have taken place. The probability E_2 will normally exceed E_1, but if ϕD is sufficiently small, the quadratic term and higher terms can be neglected in comparison with the linear term.

A possible objection to this conclusion is that E_1 may be zero while E_2 is not zero; i.e., one could assume that the effect cannot be produced by a single charged particle, while it can be produced by two particles. However, this assumption is inconsistent with microdosimetric evidence. It has been found that for both sparsely and densely ionizing radiations, there is a broad distribution of the increments of specific energy produced in single events. There is always a probability, although it may be small, that the same amount of energy deposited in two events can also be deposited in one event. One can therefore quite generally state that in the limiting case of small absorbed doses, the cellular effect is proportional to dose. If, as pointed out above, the spontaneous incidence is eliminated by subtraction from the observed effect, one has the simple linear relation

$$E(D) = E_1 \phi D \quad \text{for} \quad D \ll 1 \tag{11}$$

This relation implies that in the action of ionizing radiation on autonomous cells there is no threshold as far as absorbed dose is concerned. The probability E_1 may be small if one deals with sparsely ionizing radiation, but ultimately in the limiting case of very small absorbed doses the effect must be proportional to dose. It is important to realize that this is the case whether there is or is not a threshold in the dependence of the cellular effect on specific energy z. The absence of a threshold with regard to absorbed dose is merely due to the fact that even at the smallest doses some of the cells receive relatively large amounts of energy when they are traversed by a single charged particle.

The preceding considerations apply only to objects that are small enough that at the lowest doses of practical interest, the number of absorption events is small. That this is the case for cells or subcellular units but not for multicellular organisms can be seen from the following example. The exposure to environmental radioactivity and to cosmic radiation leads to absorbed doses of the order of 1 mGy/year. This background exposure corresponds to a large number of events for a multicellular organism. For man, several charged-particle traversals occur per second. For a smaller animal, such as a mouse, a few events may occur per minute. For a single mammalian cell, however, only a few events per year will occur, and if one considers only the nucleus of the cell, less than one event per year will take place. These are the frequencies that result mainly from sparsely ionizing radiations, such as the γ-component of the environmental radiation or the relativistic mesons from the cosmic radiation. If one were to consider the densely ionizing radiation, event frequencies would be considerably lower.

Table 1 gives event frequencies per gray for microscopic regions of various diameters and for different qualities. The largest region included corresponds to the typical size of a mammalian cell. Various radiobiological studies have shown that for most cellular effects, only energy deposition within the cell nucleus

TABLE 1
Event Frequencies (ϕ) per Gray in Spherical Tissue Regions Exposed to Different Radiations

Diameter of critical region, d (μm)	Type of radiation			
		Neutrons		
	^{60}Co γ-rays	0.43 MeV	5.7 MeV	15 MeV
12	2×10^{-1}	5.5×10^{-3}	5.1×10^{-3}	6.1×10^{-3}
5	3.6×10^{-2}	4.2×10^{-4}	8.6×10^{-4}	1.1×10^{-3}
2	5.8×10^{-3}	3.9×10^{-5}	1.2×10^{-4}	1.6×10^{-4}
1	1.2×10^{-3}	8×10^{-6}	3.2×10^{-5}	3.8×10^{-5}
0.5	1.7×10^{-4}	2×10^{-6}	7.3×10^{-6}	9×10^{-6}

is relevant; therefore, a region of 5-μm diameter, which corresponds approximately to the cell nucleus, is included in Table 1. In Section 4, evidence will be given that for most effects in eukaryotic cells, the effective site diameter is somewhat less than the size of the nucleus; smaller diameters are therefore also of interest.

One can generally state that the linear component in the dose–effect relation must be dominant whenever the event frequencies are substantially below 1; i.e., if the absorbed dose is considerably smaller than $1/\phi$. This defines the dose region in which proportionality between effect and absorbed dose can be assumed. For the whole cell, the value of $1/\phi$ is approximately 0.5 and 20 mGy for γ-rays and 5.7-MeV neutrons, respectively. If one considers only the nucleus of the cell as the sensitive region, the values of $1/\phi$ are approximately 3 and 240 mGy for these two radiation qualities. As mentioned earlier, a recent analysis (see Section 4) has shown that the actual sensitive sites in the cell are somewhat smaller than the cellular nucleus, and one deals therefore with even larger values of $1/\phi$. It is a very important result for all considerations regarding radiation protection that below about 1 mGy, and for densely ionizing radiations at considerably higher doses, a linear relation must hold if one deals with effects on autonomous cells. As pointed out, this is because even at the smallest doses appreciable amounts of energy are deposited in those cells that are subject to an event of energy deposition. The mean specific energy produced in a single event in the cell or in its sensitive site is equal to the reciprocal, $1/\phi$, of the event frequency; i.e., one deals with doses of about 1 mGy in the nucleus of the cell for sparsely ionizing radiations and with a few tenths of a gray for densely ionizing radiations, such as neutrons. In Section 4, it will be shown that the effective event size produced in single events is even higher because the relevant average of the specific energy produced in single events is larger than the frequency average, which corresponds to the values of ϕ.

3.2. Dose–Effect Relation and the Number of Absorption Events

The considerations in this section are of a more abstract nature and require a certain amount of mathematical formalism. The essential result that links the

logarithmic slope of the dose–effect relation with the number of absorption events in the cell can, however, be understood and applied without detailed knowledge of the mathematical derivation. This derivation is therefore given in the Appendix, and only the main conclusions are discussed in this section. A practical application of the result will be dealt with in Section 5. For the purpose of the present discussion, rigorous definitions of some of the quantities involved are necessary.

The considerations in the preceding section are valid regardless whether the effect, E, is considered as the probability for a quantal effect, i.e., an effect that either takes place or does not take place in the cell, or whether it is considered as the average value within the irradiated cellular population of a gradual effect. The following considerations will be restricted to the former case; i.e., only the occurrence or nonoccurrence of certain cellular effects will be considered. The coefficients $E(D)$, $E(z)$, and E_ν therefore represent probabilities and can only take values between 0 and 1. Examples of such quantal effects, which include the survival of irradiated cells or the occurrence of certain cytogenetic alterations, are of great practical importance in quantitative radiobiology. Another example is transformation of irradiated cells, which underlies carcinogenesis. This latter case will be further discussed in Section 5.1.

It is also necessary to provide a clarification of various terms that can be applied to the geometrical configuration of the radiosensitive elements in the cell. As already stated, the space occupied by this material is termed the *matrix.* The matrix must be assumed to have a complex shape and it seems indeed likely that it does not form a connected body. The term *gross sensitive volume* (gsv) (Rossi, 1964) has been employed for that part of the cell that contains the matrix. A more specific definition is that the gsv is the smallest convex volume containing the matrix. However, in many cases it is sufficiently accurate to consider the gsv to be either the smallest sphere that meets this condition or to simply consider the nucleus to be the gsv.

The concept of a *sensitive site* has frequently been invoked in biophysical models of radiation-induced cytogenetic alteration such as chromosome aberrations (e.g., see Lea, 1946; Wolf, 1954; Savage, 1970); however, it is not confined to radiation effects on chromosome structure. In the next section it will be shown that various effects on eukaryotic cells can be adequately understood if one postulates sites that are somewhat smaller than the cell nucleus and that are affected with a probability dependent on the square of the energy actually deposited in these sites. In such considerations it is not necessarily implied that the cell contains only one of these sites, but it is merely implied that the response of the cell can often be interpreted in terms of the energy concentration in volumes that are smaller than the gsv. The radius or a mean diameter of a site is thus the average distance over which individual energy deposits interact, although the site does not have physical reality.

In the following, a slightly different concept will be used, namely that of a *critical region.* The reason for introducing yet another term is that it will be convenient in the following considerations to deal with a reference volume that

contains all the sensitive structures of the cell but may be even larger than the gross sensitive region itself. The concept is useful, first, because it can be applied to a population of irradiated cells that are not all equal or are not all in the same stage of the generation cycle. In such an inhomogeneous population, the gross sensitive region and its size may vary from cell to cell; however, their critical region can be chosen in such a way that it is equal for all irradiated cells. Furthermore, it is convenient to obtain certain conservative estimates in the absence of precise knowledge concerning the gross sensitive region; this can be done by equating the critical region either with the cell nucleus or with the whole cell.

Another concept that has to be further explained is that of an *energy-deposition event* (for brevity, *event*). This is defined (ICRU, 1980) as energy deposition by a charged particle or by a charged particle together with its associated secondary particles in the region of interest. Two ionizing particles that pass the region are counted as separate events only if they are statistically independent. Usually, for example in the case of neutron irradiation, one can identify an absorption event with the appearance of a charged particle in the reference region.

These definitions are of interest in connection with an important theorem concerning the number of absorption events in the cell and the slope of the dose–effect curves in a logarithmic representation of effect probability as a function of absorbed dose.

Assume that c is the slope of the dose–effect relation in a logarithmic representation*; then

$$c = \frac{d \ln E(D)}{d \ln D} \tag{12}$$

where $E(D)$ is the effect probability at dose D; it is assumed that this probability is corrected for spontaneous incidence, which need therefore not be considered.

It can be shown, and the detailed derivation is given in the Appendix, that the slope c is equal to the difference of the mean event number, n_E, in the critical region of those cells that show the effect and the mean event number, n, in the critical region of the cells throughout the exposed population regardless of whether they show the effect or not:

$$c = n_E - n \tag{13}$$

This equation holds at any value of absorbed dose. The relation remains valid if a critical region larger than the actual gsv is considered. The sole condition is that energy deposition outside the critical region does not affect the cell. As pointed out above, it is often sufficient to identify the critical region with the nucleus of the cell. It is also important to note that biological variability, e.g., the variation of sensitivity throughout the cellular population, does not invalidate the result.

* It should be noted that a (fully) logarithmic graph is different from the semilogarithmic plots that are usually employed to display the (log of) cell survival as a function of absorbed dose.

The theorem is fundamental for the application of microdosimetry to the analysis of dose–effect relations. If, for certain values of the absorbed dose, the effect probability $E(D)$ and the slope c of the dose–effect curve in logarithmic representation are known, one can derive the minimum size of the critical structure. Although n_E, the frequency of traversals in the affected cells, may not be known, it is evident from equation (13) that it cannot be less than c. One can therefore ask how large the sensitive structure must be so that at the dose D the cell is traversed by at least c charged particles with a probability $E(D)$. The answer is given by microdosimetric data for various radiation qualities and for different sizes of the critical region. In this way, one can derive lower limits for the dimensions of the sensitive structures in the cell and for the interaction distances of elementary lesions in the cell.

Equation (13) contains as a limiting case a statement that is of significance to the analysis of dose–effect curves at very small doses. The relation implies that in the region of small doses, the slope c of the effect curve in the logarithmic representation is equal to the order of the reaction kinetics that determine the effect. In the limit where the absorbed dose D (and consequently n) approaches 0, this fact may appear obvious. According to the considerations in the previous section, it is to be expected that at least in the case of radiation action on autonomous cells, first-order kinetics apply at low doses; this corresponds to a value of $c = 1$ when $n \ll 1$.

In connection with basic aspects of radiation carcinogenesis, it is of interest to determine whether c can in fact be less than 1. The degree to which this can occur is limited by the fact that n_E cannot be less than 1 since the number of absorption events in affected cells must be at least 1. Consequently,

$$c > 1 - \phi D \tag{14}$$

This inequality follows directly from the more general relation expressed in equation (13).

Studies performed by Vogel (1969) and by Shellabarger *et al.* (1974, 1980) on the induction by neutrons of mammary tumors in Sprague–Dawley rats show that in the range of very small doses, the logarithmic slope c of the dose–effect curve is considerably less than 1. This fact will be further discussed in Section 5 and it will be concluded that in these experiments the observed tumor frequencies in the irradiated animals cannot merely reflect the action of radiation on autonomous cells that give rise to the observed tumors without mutual interaction or interference.

4. The Quadratic Dependence of the Cellular Effect on Specific Energy

4.1. Dose–Effect Relations

It has been pointed out in the preceding sections that the dependence, $E(z)$, of the cellular effect on specific energy is not identical to the observed dependence,

$E(D)$, of the cellular effect on absorbed dose. This would be the case only if the cellular damage was a linear function of specific energy. In the preceding section general statements have been derived that are valid regardless of the actual form of the dependence of effect on specific energy. Particularly, it has been pointed out that at very low doses, the cellular effect must always be linearly related to absorbed dose. It has also been possible to derive a relation that connects the mean number of charged particles traversing the affected and unaffected cells with the slope of the dose–effect curve in the logarithmic representation. In the present section, the actual dependence of cellular effect on specific energy will be analyzed. It will be seen that dose–effect relations, as well as relative biological effectiveness (RBE)–effect relations for higher organisms, point to a quadratic dependence of the primary cellular damage on specific energy.

As far as the production of two-break chromosome aberrations is concerned, a quadratic dependence of the yield of the observed effect on energy deposited in sensitive cells had been postulated as early as in the works of Sax (1938, 1941) and in numerous other studies, particularly those by Lea (1946). In this case, the quadratic dependence is merely due to the fact that two-break chromosome aberrations are assumed to result from the interaction of two "chromosome breaks." The yield of breaks is assumed to be proportional to energy absorbed in the cell, and the average number of breaks per cell is therefore simply proportional to dose. Statistical fluctuations in energy deposition in the cell are, however, highly relevant if the probability for the production of a two-break aberration depends on the square of the concentration of breaks in the cell. A two-break aberration can result from two breaks that are produced in the same charged-particle track, or it can result from the interaction of two breaks produced by independent particle tracks. In the former case, one expects a linear relation to absorbed dose; in the latter case, one expects a quadratic dependence on absorbed dose. For densely ionizing radiations, such as neutrons or α-particles, the increments of specific energy produced in the critical sites of the cell are so large that the linear component is dominant. For sparsely ionizing radiations, such as X- or γ-rays, on the other hand, the ionization density in the charged-particle tracks is so low that neighboring breaks are usually produced by independent particle tracks. One must therefore expect the quadratic component to be dominant in the latter case. This characteristic difference between densely ionizing radiation and sparsely ionizing radiation has been borne out by experimental results.

While the quadratic dependence of the yield of the chromosome aberrations on absorbed dose is approximately valid for sparsely ionizing radiation, it must be concluded from microdosimetric data that at very small doses the dose–effect relation must be linear even for such radiations. Until recently, it has not been possible to assess the magnitude of this linear component because of limitations in the statistical accuracy of the experimental data. However, work performed in different laboratories (see Brewen *et al.*, 1973; Schmid *et al.*, 1973; Brenot *et al.*, 1973) with X-rays and with fast electrons has indeed shown a linear relation at small doses of X-rays and a quadratic dependence only at somewhat higher

doses. These studies thus confirm the predictions made on general microdosimetric principles. As will be shown, the relative contributions of the linear and quadratic components can be accounted for on the basis of microdosimetric data. In the following it will be seen that such considerations apply also to other radiation effects on eukaryotes. Furthermore, the quantitative relation of the site diameter, the radiation quality, and the ratio between linear and quadratic components of the cellular damage will be discussed.

Figure 10 illustrates dose–effect relations for the yield of pink mutations in *Tradescantia* (Sparrow *et al.*, 1972). Curves are given for 430-keV neutrons and for X-rays. For the purpose of the present discussion, the saturation and the ultimate decline of the yield in the range of higher doses will not be considered. This latter effect may be connected to cell killing, but as a study on the transformation of cells *in vitro* (Borek and Hall, 1973; see Section 5.1) has indicated, it may involve a complex interrelation between the observed cellular alterations and cell killing.

It should be pointed out that a logarithmic representation has been used for these curves in order to represent the experimental data in the range of low doses and small observed yields of mutations with sufficient accuracy. The logarithmic representation has the further advantage that proportionality of the effect to a power, n, of the absorbed dose expresses itself in the slope, n, of the effect curve. In their initial parts, both the curve for neutrons and the curve for X-rays have the slope 1; i.e., effect and absorbed dose are proportional in both cases. The slope of the X-ray curve approaches the value 2 at somewhat higher

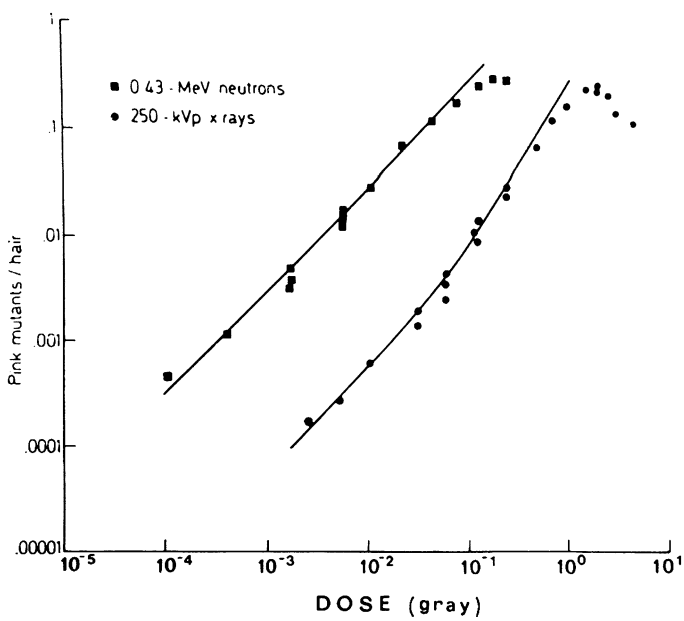

FIGURE 10. Induction of pink mutant cells in the stamen hairs of *Tradescantia* by X-rays and 430-keV neutrons (Sparrow *et al.*, 1972). The spontaneous incidence is subtracted from the observed values.

doses, and the observations are therefore consistent with the statement that in an intermediate dose range, the yield of mutations produced by X-rays is proportional to the square of the absorbed dose. The accuracy with which the linear component in the dose–effect curve for X-rays has been established in this experiment is due to the fact that this particular experimental system permits the scoring of extremely large numbers of irradiated cells in the stamen hairs of *Tradescantia.*

While the example given in Fig. 10 supports the general conclusions drawn from microdosimetric consideration, it remains to be seen whether these results are also quantitatively in agreement with predictions based on microdosimetry. For this reason the quadratic dependency of cellular damage on specific energy and the resulting dose–effect relation will be analyzed in detail.

If one assumes that the degree of cellular damage or the probability for a certain effect in the cell is proportional to the square of specific energy

$$E(z) = kz^2 \tag{15}$$

then the average effects observed at a certain absorbed dose are obtained by averaging the square of the specific energy in the sensitive sites of the cells over its distribution throughout the irradiation population:

$$E(D) = k \int_0^\infty z^2 f(z) dz \tag{16}$$

It can be shown [see Kellerer and Rossi (1972) for the mathematical details] that the integral in equation (16) has a simple solution. One finds that this integral, which is the expectation value of z^2, is equal to the square of the absorbed dose plus the product of absorbed dose and the energy average, ζ, of the increments of specific energy produced in single events in the site:

$$\bar{z}^2 = \int_0^\infty z^2 f(z)\, dz = \zeta D + D^2 \tag{17}$$

Accordingly, one has

$$E(D) = k(\zeta D + D^2) \tag{18}$$

The ratio of the linear component to the quadratic component is therefore equal to the ratio ζ/D of the characteristic increment ζ of specific energy to the absorbed dose. If the absorbed dose is larger than ζ, the quadratic component dominates; if the absorbed dose is equal to ζ, both components are equal. The value of ζ is determined by the size of the site and by the type of the ionizing radiation. It is largest for smallest site diameters, and it is considerably larger for densely ionizing radiation than for sparsely ionizing radiation, such as γ- or X-rays.

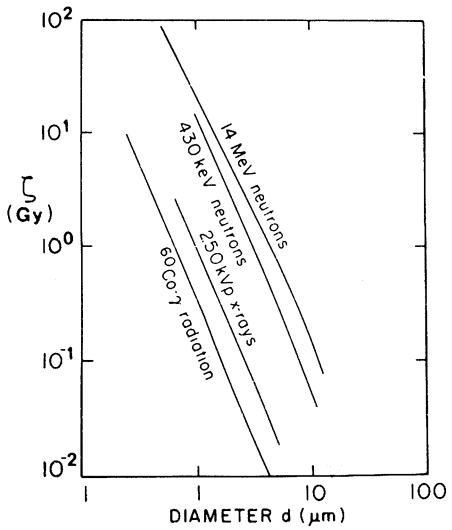

FIGURE 11. Energy mean, ζ, of the specific energy produced in single events by different radiation qualities in spherical tissue regions of diameter d.

The value of ζ for different radiation qualities as a function of the diameter for the reference volume is shown in Fig. 11. These values are obtained from experimental microdosimetric determinations as well as from theoretical calculations. In the example represented in Fig. 10, one finds that for X-rays the linear component is equal to the quadratic component at a dose of approximately 0.1 Gy. According to Fig. 11, the value of 0.1 Gy for ζ corresponds to a site diameter of approximately 2 μm. According to the microdosimetric determinations, the quantity ζ for neutrons should be approximately 35 times larger than for X-rays, and the initial part of the neutron curve in Fig. 10 is, in fact, shifted vertically by about this factor relative to the initial part of the X-ray curve.

The analysis of dose–effect relations for two-break chromosome aberrations (Kellerer and Rossi, 1972; Schmid *et al.*, 1973; Brenot *et al.*, 1973) has led to somewhat larger values of ζ, namely values that correspond to site diameters of approximately 1 μm.

Survival curves for mammalian cells *in vitro* can, to a good approximation, be represented by an exponential that contains a linear and a quadratic term in dose:

$$S(D) = S_0\, e^{-k(\zeta D + D^2)} \tag{19}$$

where $S(D)$ is the survival at dose D and S_0 is the survival at zero dose. If one uses this equation, which has earlier been invoked by Sinclair (1968), one obtains values that also correspond to site diameters of one to several micrometers for cells in S phase. For cells in G_1 and G_2 and in mitosis, the initial linear component is more pronounced, and the values of ζ therefore correspond to smaller site diameters of only a fraction of a micrometer. Whether this latter observation corresponds to a more condensed state of the DNA in these stages of the generation cycle of the cell [see observations of Dewey *et al.* (1972) and earlier results by Cole (1967)] or whether the initial linear component in the survival curve is partly due to a type of cellular damage that is linearly related to specific energy in the sensitive sites of the cell remains an open question.

The theory of dual radiation action attributes the quadratic dependence of biological effects on specific energy to a mechanism in which the former are due to the production of *lesions* that are produced by the interaction of pairs of *sublesions*. Sublesions are presumably alterations of DNA that are produced at a rate that is proportional to specific energy, and they can combine over a distance that is equal to the mean diameter of the site. It may be expected that the interaction probability for a pair of sublesions depends on their initial separation, and this has in fact been shown in *molecular ion experiments* (see Rossi, 1979; Colvett and Rohrig, 1979; Bird, 1979; Kellerer *et al.*, 1980). In these studies pairs of charged particles traverse the cell at varying separation and it is found that the linear portion of the dose–effect curve decreases markedly if the mean separation of the particles is increased. The experiments disclose a highly skewed dependence of sublesion combination on initial separation and support the conclusion that the "site" is in fact the entire nucleus.

Additional complications are that the biological effectiveness cannot indefinitely increase with energy concentration and that for very heavily ionizing particles, energy is wasted in individual cells, leading to a saturation effect that results in declining effectiveness per unit of dose. Another very likely, and possibly related, phenomenon is that the production of sublesions also depends on energy concentration in distances that are of the order of nanometers. Yet another effect is diffusion of energy from the inchoate distribution prior to the induction of sublesions.

The treatment given above in terms of the site model does not take account of the manner in which a given amount of energy is distributed in a fixed nominal interaction volume. In view of these complicating factors, it must be considered to be an approximation. It is, however, often adequate and also didactically useful. It should also be noted that more realistic treatments merely change the physical interpretation of the factors k and ζ in equation (18). None of the complications cited above cause any change of the basic dependence of $E(D)$ on two terms that respectively are linear and quadratic in absorbed dose.

4.2. Dose–RBE Relations

It has already been pointed out that the theoretical considerations based on microdosimetry must be restricted to autonomous cells. Thus, equation (19) represents a survival curve based on the assumption that the survival probability of cells in tissue cultures decreases exponentially with the mean number of lesions in the cells. The same assumption is also justified for effects other than cell death, particularly for somatic mutations and genetic effects in general.

It can, however, not be assumed that such comparatively simple kinetics obtain in radiation carcinogenesis, which is a highly complex process. The dose–effect curves for the induction of cancers in experimental animals can have a great variety of shapes, and an example will be given in Section 5 of an instance where it can be proven that tumors do not arise simply as a result of radiation-induced transformations of autonomous cells.

Although the dose–effect curve is usually the most important quantitative expression of radiobiological effects, another important relation is the dependence of RBE on dose. The RBE of radiation A relative to radiation B is defined as D_B/D_A, where D_B and D_A are respectively the absorbed doses of the two radiations that produce the same biological effect. The RBE is usually found to depend on the degree of effect and therefore on the absorbed dose of either radiation. This dependence is not only of theoretical interest, but also of considerable pragmatic importance because it is vital to any attempts to evaluate late radiation effects. This will in Section 5.3 be shown for the observation on A-bomb survivors in Hiroshima and Nagasaki. The bomb that exploded over Nagasaki released γ-radiation, but almost no neutron radiation whereas at Hiroshima the neutron component was significant in terms of absorbed dose and very likely dominant in terms of biological effect.

The importance of the dose–RBE relation arises from the possibility that it may be the same for autonomous cells and for interacting cell systems. This may be expected to be the case if the interaction process is the same regardless of the quality of the radiation that caused the primary cellular damage. It would appear that this condition is met, or at least adequately approximated, in many instances.

In the following example, neutrons and X-rays will be used, but the considerations are equally valid for any two types of radiation. From the quadratic dependence of cellular damage on specific energy, one derives the condition for equal effectiveness of X-rays and neutrons:

$$k(\zeta_X D_X + D_X^2) = k(\zeta_n D_n + D_n^2) \tag{20}$$

where ζ_X and ζ_n are values of ζ for X-rays and neutrons, and D_X and D_n are the absorbed doses for X-rays and neutrons. Since the RBE of neutrons relative to X-rays is defined as the ratio of the X-ray dose to the equivalent neutron dose,

$$\mathrm{RBE} = D_X/D_n \tag{21}$$

one can express the RBE as a function of either the X-ray dose or the neutron dose. In the following, the RBE of neutrons will be expressed as a function of the neutron dose. Inserting equation (21) into equation (20), one obtains

$$\zeta_X \cdot \mathrm{RBE} \cdot D_n + \mathrm{RBE}^2 \cdot D_n^2 = \zeta_n D_n + D_n^2 \tag{22}$$

or

$$\mathrm{RBE} = \frac{2(\zeta_n + D_n)}{\zeta_X + [\zeta_X^2 + 4(\zeta_n + D_n)D_n]^{1/2}} \tag{23}$$

This relation is shown in Fig. 12 for various values of ζ_n/ζ_X. It is easy to identify certain general characteristics of the dependence of RBE on dose. At very low doses the linear components are dominant both for neutrons and for X-rays,

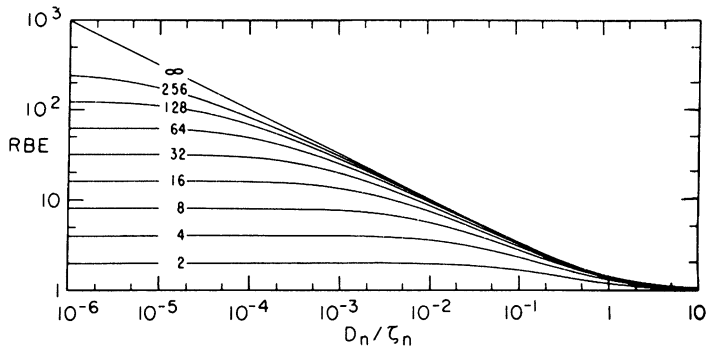

FIGURE 12. Relation between RBE and neutron dose (D_n) according to equation (23). The neutron dose is given in multiples of ζ_n; the parameter of the curves is ζ_n/ζ_X.

and RBE must then have a constant value equal to the ratio ζ_n/ζ_X of the ζ values for neutrons and for X-rays. This plateau of RBE corresponds to the region in the example of Fig. 10 where the initial part of the X-ray curve runs parallel to the neutron curve. In the range of intermediate doses one can neglect the linear component for X-rays, while the linear component for neutrons is still dominant. In this case the RBE of neutrons is inversely proportional to the square root of the neutron dose; in a logarithmic plot of RBE vs. neutron dose, one obtains curves of slope $-1/2$. At the high doses, finally, one should expect that RBE tends toward the value 1. It is, however, often not easy to obtain meaningful biological data with neutrons at doses large enough that the linear component can be neglected.

The dose–RBE relation expected on the basis of a quadratic dependence of primary cellular damage on specific energy has been compared with the experimental observations for a wide spectrum of radiation effects on mammalian cells. Figure 13 is a compilation of such results for neutrons having an energy of 0.43 MeV and for fission neutrons that have about the same effective energy. One must draw the general conclusion that in the intermediate dose range in which the available data are most complete, the observed dose–RBE relations are in agreement with the dependence theoretically predicted. In the example of the mutations in *Tradescantia*, it has been possible to find the plateau of the values of RBE at low doses, and this value agrees with microdosimetric data. It is not surprising that relatively little data are available in the range of extremely small doses, as few experimental systems permit the necessary statistical accuracy at small doses. However, it is remarkable that in two experimental systems, namely in the lens opacification studies and in the system for induction of mammary tumors in the rat, extremely high values of the RBE of neutrons have been found at low doses. These values exceed the predictions made on the basis of microdosimetric data, and they may be taken as evidence that, in addition to the quadratic dependence of the effect on energy concentration over regions of the order of magnitude of one to several micrometers' diameter, one deals with the already mentioned differences in effectiveness of sublesion production. This

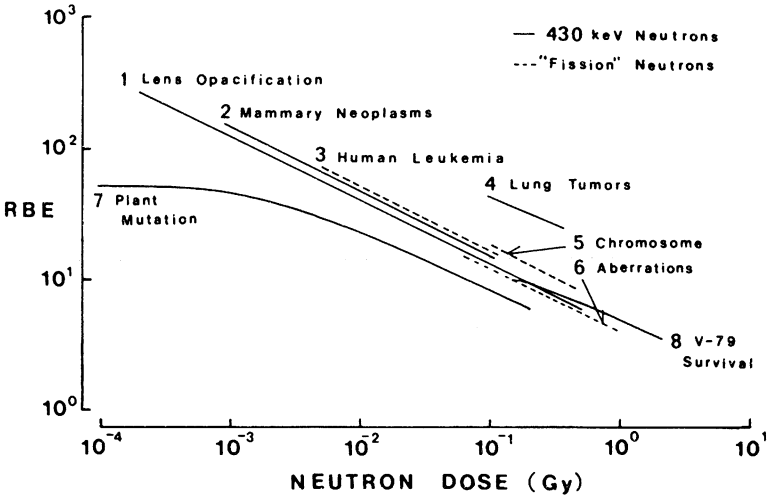

FIGURE 13. Logarithmic plot of the RBE of low-energy neutrons vs. neutron dose. (1) Bateman *et al.* (1972); (2) Shellabarger *et al.* (1974); (3) Rossi (1977); (4) Ullrich *et al.* (1979); (5) Awa (1975); (6) Biola *et al.* (1971); (7) Sparrow *et al.* (1972); (8) Hall *et al.* (1973).

implies a dependence of the effectiveness of ionizing radiation on the distribution of energy in regions of the order of only a few nanometers. Formally, this would correspond to a dependence of the coefficients k in equation (18) on radiation quality.

The induction of mammary tumors by neutrons and X-rays and the study of leukemia incidence after neutron irradiation and exposure to γ-rays will be discussed in the next section. As an example of a dose–RBE relation that extends over an extremely wide range of doses, the studies on the opacification of the murine lens may be considered. Figure 14 contains this relation together with its 95% confidence limits. One should note that the inverse relationship between the RBE of neutrons and the square root of the neutron dose extends over more than 4 orders of magnitude of the neutron dose in this example. These results obtained in a multicellular system are therefore in good agreement with the

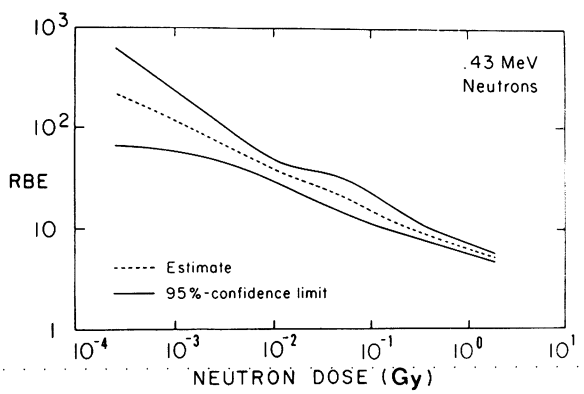

FIGURE 14. RBE of 430-keV neutrons relative to X-rays for the induction of lens opacification in the mouse (Bateman *et al.*, 1972; Kellerer and Brenot, 1973).

various other observations supporting the assumption that the primary cellular damage is proportional to the square of the specific energy in sites of the order of one to several micrometers.

5. *Applications to Radiation Carcinogenesis*

Many of the subjects covered in the preceding sections are of relatively recent origin. In particular, the theory of dual radiation action was developed only a few years ago and is, as are many concepts of microdosimetry, subject to further development. Practical applications are still few in number since few radiation carcinogenesis studies are broad enough to permit a detailed analysis of dose–effect relations and of their dependence on radiation quality. However, in the few instances where data relating to radiation carcinogenesis were analyzed, they could be interpreted in terms of the theory and they have also lead to other results. In the case of mammary neoplasms in the rat, it could be deduced that the observed incidence depends not only on lesions in autonomous cells, but is codetermined by radiation-induced changes in several cells or in tissue. Furthermore, in an analysis of data on the induction of human leukemia, conclusions were reached that are of importance to risk estimates.

The findings on the induction of mammary neoplasms demonstrate a complexity of the process of radiation carcinogenesis that is also reflected in the variety of often complicated dose–effect relations observed in other experimental animal studies. In at least some instances radiation does not cause cancer by mere transformation of individual cells that subsequently proliferate regardless of any radiation injury to other cells. One is thus not dealing with the relatively simple autonomous response of a single cell. Consequently, theoretical predictions cannot be made with respect to the dose–effect relations, although for reasons given above it may be expected that the theoretical dose–RBE relations should apply. However, even in the transformation of what would appear to be autonomous cells, the theoretical dose–effect relation is at least markedly modified by processes that are at this point only poorly understood.

5.1. *Transformation*

While the development of radiogenic cancer cannot be fully accounted for if only isolated cells are considered, it appears plausible that it is initiated by changes (probably cytogenetic) in individual cells, and this kind of change may well be exhibited by cells in culture that can be scored as having undergone an *oncogenic transformation* by their altered morphology. Cells in tissue culture ordinarily grow in an orderly manner and in a single layer until they are subject to contact inhibition. However, fresh explants of embryonic fibroblasts, as well as a few established cell lines, can produce transformed clones when exposed to chemical and physical agents. This change can be readily recognized by a densely stained,

piled-up appearance and by the random crisscross pattern of cells at the periphery of the clone. Cells from such clones satisfy many of the criteria of malignancy and produce fibrosarcomas when injected in large numbers into immunosuppressed animals.

It was shown by Borek and Sachs (1966) that transformations can be induced by radiation in cultures of explants of hamster embryo cells. The dose–effect curves for X- and neutron irradiations are shown in Fig. 15 (Borek *et al.*, 1978). It should be noted that in this logarithmic representation, the ordinate represents the fraction of observed clones that are transformed; it thus represents the transformation frequency in cells that survived the irradiation. The decline of the X-ray curve at high doses cannot, therefore, be explained as simply due to killing of transformed cells by high doses. It would appear that one must be dealing with differences in sensitivity to killing. These may be either a greater sensitivity of those cell types in the (heterogeneous) population that can be transformed, or cells containing the lesions responsible for transformation are more sensitive than those that do not.

There are considerable difficulties in the assay of transformations. The maximum fraction of clones that can be transformed is less than 1%, and at low doses the fraction of transformed cells is much smaller. The resultant statistical uncertainties can accommodate a wide range of curve shapes. The straight lines drawn through the rising portions in Fig. 15 are certainly not inconsistent with the data. However, there are two considerations that indicate that even in this dose range one is dealing with complex mechanisms that may be responsible for a more complicated curve shape. Fractionation at doses of less than 1.5 Gy results in an unusual *increase* in effect, and a complex shape has in fact been demonstrated in another transforming cell system.

Miller *et al.* (1979) in experiments on transformable mouse fibroblasts ($10T\frac{1}{2}$ cells) obtained the dose–effect relations shown in Fig. 16, wherein what appear

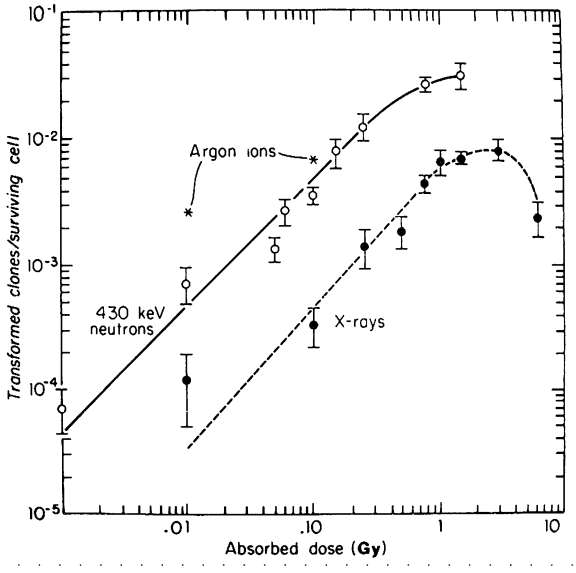

FIGURE 15. Dose–response curves for cell transformation by X-rays, neutrons, and argon ions. The data *points* plotted are the means of replicate experiments, and the error *bars* represent the S.E. or the error expected from the number of clones counted, whichever was larger (Borek *et al.*, 1978).

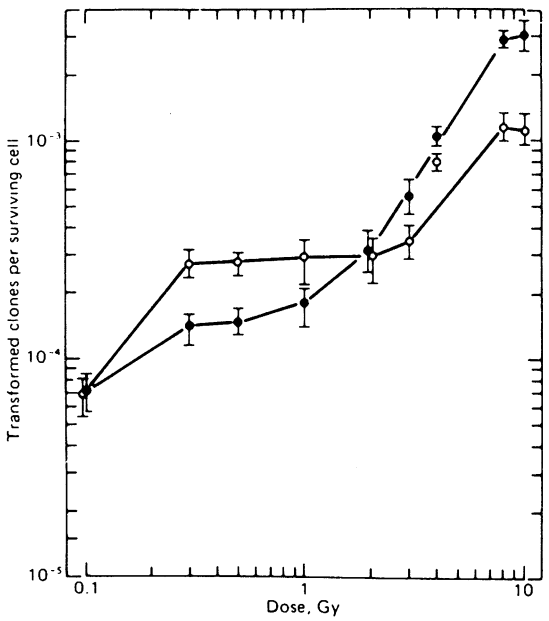

FIGURE 16. Pooled data from many experiments for the transformation rate for single (●) and split (○) doses of X-rays. The time interval between split doses was 5 h (Miller *et al.*, 1979).

to be linear and quadratic rises are separated by a plateau in which the transformation rate per surviving cell changes little over a threefold range of dose. An additional peculiarity is that fractionation of the dose into two increments separated by 5 h decreases the transformation rate at high doses, but increases it at low doses. The same dependence was found for the hamster embryo system by Borek (1979), and in mouse 3T3 cells by Little and Terzaghi (1976).

The fractionation effect shown in Fig. 17 can be accounted for by the simple assumption that there is no interaction between doses given in different fractions. In terms of dual radiation action, this means that sublesions produced by the first fraction are eliminated (probably by repair processes) before the second fraction is applied. In this case the effect of a fractionated dose, D, should be twice the effect of a single dose, $D/2$. This means that when the curve rises

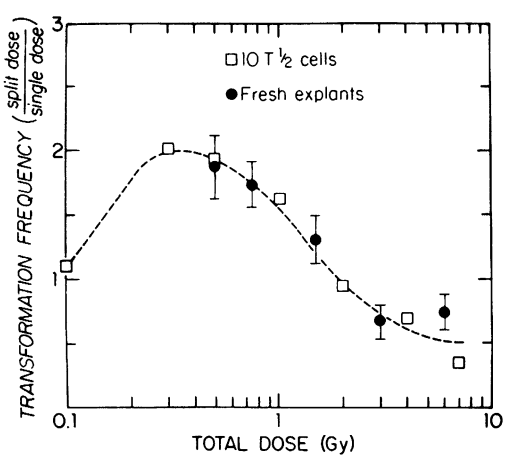

FIGURE 17. Comparison of the data for C3H/10T½ cells and fresh explants of hamster embryo cells. The ratio of the transformation frequencies for split and single doses is plotted as a function of total dose (Miller *et al.*, 1979).

linearly, fractionation should not change the number of transformations observed, that in the quadratically rising portion fractionation should lessen the transformation rate, and that at zero slope (the plateau) fractionation should double the rate. The data are in substantial agreement with this algorithm, which is equivalent to the requirement that either of the curves results from a 45° shift of the other on a logarithmic plot.

Although the fractionation effects can thus be explained on the basis of the unusual shape of the dose–effect curve, the reason for this shape is not obvious. It would appear that the linear and quadratic components of dual radiation action instead of adding in the usual way are separated because the linear component is saturating before the quadratic component is significant; the quadratic component then levels out as well at high doses. In a homogeneous cell population, the implied saturation cannot be due to a reduction of the cells available for transformation because the curve levels first at a low frequency ($\sim 10^{-4}$) and subsequently rises by more than a decade.

It is evident from microdosimetric considerations that the initial portion of the curve must be due to transformations produced by individual electrons with a frequency of about 10^{-6} per cell, but there is no reason evident why this process should stop after only about 1 cell in 10,000 has been transformed. A possible explanation is competition between transformation and other effects for available sublesions. If dual radiation action is to be a general mechanism, it follows that the nature of any radiation effect induced depends on the identity of the sublesions that form the relevant lesions. It would seem entirely possible that a given sublesion may contribute to the formation of lesions associated with various effects depending on the nature of the other sublesions. This would seem particularly plausible if the radiation-induced lesion is considered to be a rearrangement of substantially linear DNA molecules that result when the four terminations produced by two double-strand breaks (sublesions) rejoin in an altered pairing.

If this hypothesis is correct, the initial portion of the curve can be interpreted to indicate that single electrons can in an initial step produce a pair of initial sublesions (and the lesions resulting from their combination) causing transformation. While the number of cells so affected increases linearly with dose, subsequent electron events cause a much more copious production of sublesions which are more likely partners for either of the two initial sublesions. The second part of the curve can then be interpreted to correspond to a process in which the two critical sublesions are produced by separate electrons and competition operates against each of them. This qualitative explanation would appear to account for observations, but a numerical justification has yet to be provided.

5.2. Mammary Neoplasms in the Sprague–Dawley Rat

Bond *et al.* (1960) and Shellabarger *et al.* (1969, 1974) discovered that moderate doses of γ- or X-rays produce a high incidence of mammary neoplasms in rats

of the Sprague–Dawley strain. The majority of the tumors are not malignant (fibroadenomas), but an appreciable proportion are adenocarcinomas. It was found that the incidence of these neoplasms is approximately proportional to the γ- or X-ray dose up to a few gray, where the incidence curve flattens and finally declines when doses in excess of 5 Gy are applied. This is a phenomenon common in radiation carcinogenesis. It was also concluded that the effect is not *abscopal*, i.e., it requires irradiation of that part of the tissue where the neoplasms are to arise, and it was moreover demonstrated that the effect can be produced by *in vitro* irradiation of excised mammary tissue when it is subsequently grafted onto unirradiated animals.

Vogel (1969) as well as Shellabarger *et al.* (1974) investigated the effectiveness of neutrons for this phenomenon and found it to be high in relation to that of γ- or X-rays, particularly at low levels of incidence. On the basis of this experience and in view of the microdosimetric considerations that have been described in the preceding section, a large-scale experiment was performed by Shellabarger *et al.* (1980) employing 0.43-MeV neutrons down to doses as low as 1 mGy. This experiment is of particular interest to the analysis of dose–effect relations at small doses and for the understanding of the RBE of neutrons vs. X-rays. It will therefore be described in some detail.

Groups of Sprague–Dawley rats received, at age 60 days, a single dose of neutrons or X-rays. The neutron doses were 1, 4, 16, or 64 mGy; the X-ray doses were 0.28, 0.56, or 0.85 Gy. Subsequent to irradiation, the animals were examined for mammary neoplasms; when neoplasms were noted, they were removed surgically and the animals were returned to the experiment.

A conventional analysis based on the *total incidence* of neoplasms throughout life without correction for mortality leads to the dose–effect curves of Fig. 18. It must be noted that the dose scales for neutrons (upper scale) and for X-rays (lower scale) differ by a factor of 10; the dose ratio for equal effect is therefore 10 times larger than would appear from the graph. One deduces from these dose–effect relations RBE values for the neutrons of 100 or more at small doses

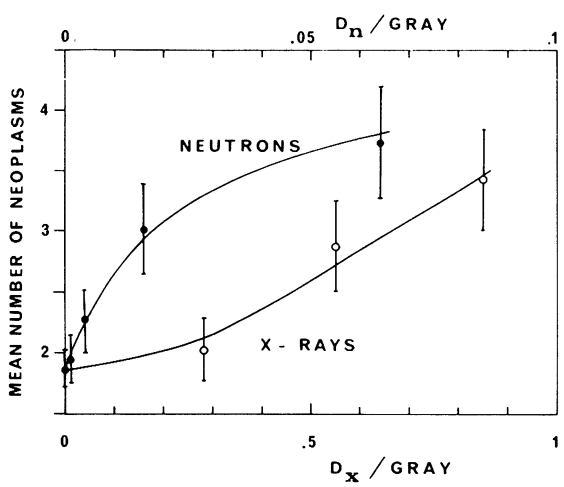

FIGURE 18. Mean number of mammary neoplasms throughout life per animal in Sprague–Dawley rats irradiated by 400-keV neutrons or X-rays. The scales for the neutron dose and the X-ray dose differ by a factor of 10 (Shellabarger *et al.*, 1980).

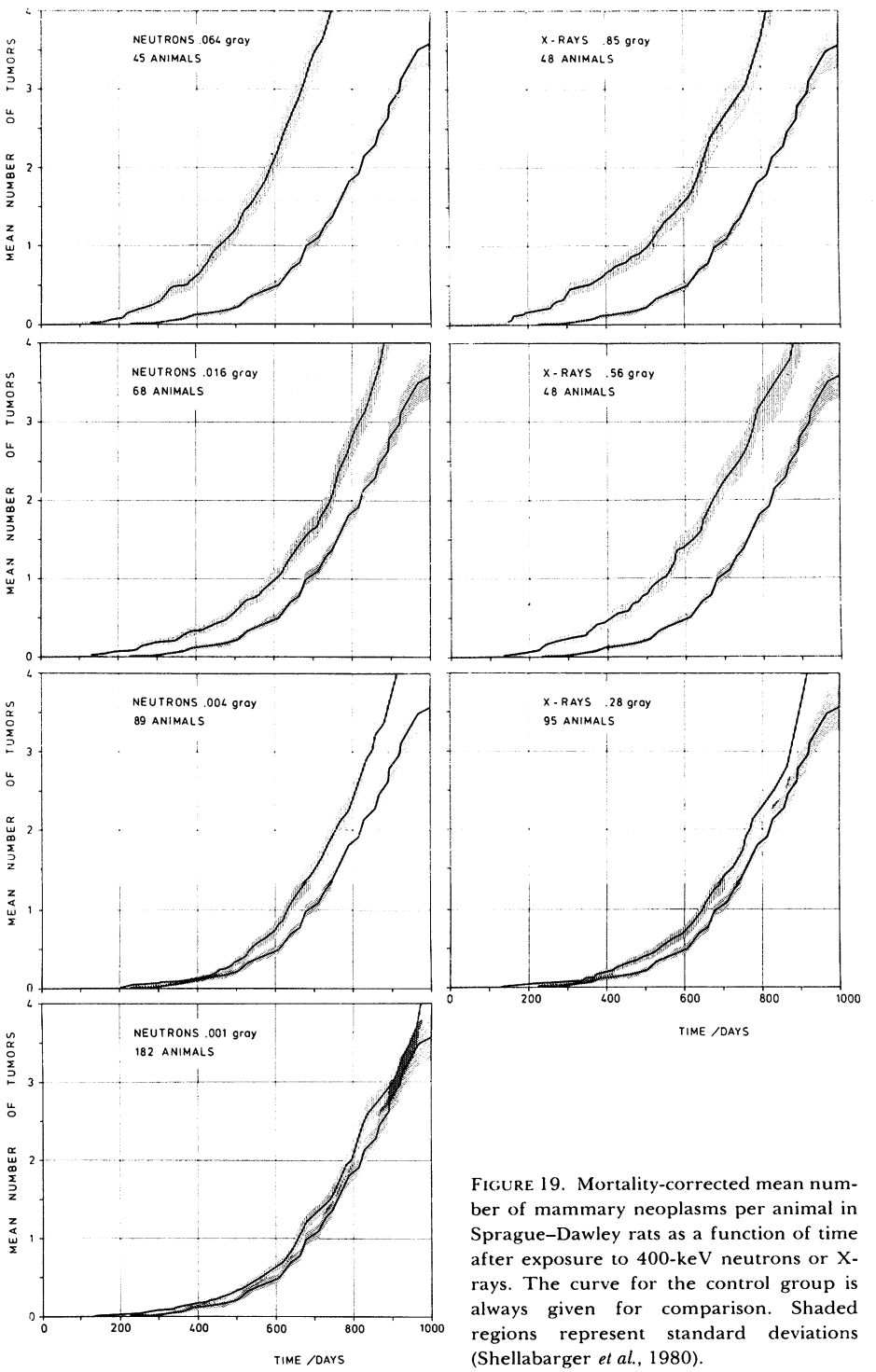

FIGURE 19. Mortality-corrected mean number of mammary neoplasms per animal in Sprague–Dawley rats as a function of time after exposure to 400-keV neutrons or X-rays. The curve for the control group is always given for comparison. Shaded regions represent standard deviations (Shellabarger *et al.*, 1980).

and still in excess of 10 at higher neutron doses of the order of 0.1 Gy. Furthermore, it is striking that the dose–effect relation for neutrons is *sublinear*, i.e., that a graph relating effect to dose has negative curvature.

The general characteristics of these observations are readily apparent from the simple analysis based only on total incidence; however, it is desirable to consider also the more rigorous actuarial analysis that indicates the actual time course of the appearance of radiation-induced neoplasms and that permits the identification of possible qualitative differences in the effect produced by neutrons and by X-rays.

A suitable quantity in the numerical analysis is the *cumulative tumor rate* that can also be considered as the mortality-corrected mean number of tumors per animal as a function of time after irradiation. Figure 19 gives the time course of the cumulative tumor rate for all mammary neoplasms in the different irradiated groups. The shaded areas correspond to the standard deviations, and the lower curve repeats in each plot the time course for the unirradiated controls.

It is apparent from these data that Sprague–Dawley rats are subject to a high spontaneous rate of mammary neoplasms during the later phases of their life. It is also apparent that the effect of irradiation can be described as a forward shift of the spontaneous occurrence in time. A point of particular interest is that a single dose of 1 mGy of neutrons produces a significantly increased incidence or, alternatively expressed, a significantly earlier occurrence of neoplasms. The time shift for this very low neutron dose is readily recognized to be approximately 30–40 days. The high RBE values of neutrons versus X-rays at low doses are also evident.

It is one of the major assumptions of the theory that, beginning with the production of elementary lesions, the series of steps leading to the effect under observation is the same regardless of the radiation type involved. The validity of this assumption is difficult to assess. However, one necessary (also certainly not sufficient) condition is that the time course of incidence be the same. The curves in Fig. 19 suggest no systematic differences in the time course of incidence for neutrons and for X-rays. All the curves are well in agreement with the assumption that the spontaneous incidence is merely shifted forward in time. A more systematic analysis indicates that the cumulative tumor rate, $R(t)$, i.e., the mortality-corrected mean number of tumors per animal, can be represented by the equation

$$R(t) = 7.5 \times 10^{-10}[(t - \Delta t)/\text{day}]^{3.3} \tag{24}$$

for all times t exceeding a *latent time* Δt that depends on absorbed dose. One concludes that there is no evidence of characteristic differences between the neutron- and the X-ray-induced effects. This is further supported by the analysis of the relative frequency of different types of neoplasms produced by different radiations. For the control group, one obtains the value $\Delta t = 106$ days. For the group exposed to 1 mGy of neutrons, $\Delta t = 142$ days; this corresponds to a forward shift of the incidence of neoplasms by 36 days. The time shifts for all doses are plotted in Fig. 21 together with other dose–effect relations.

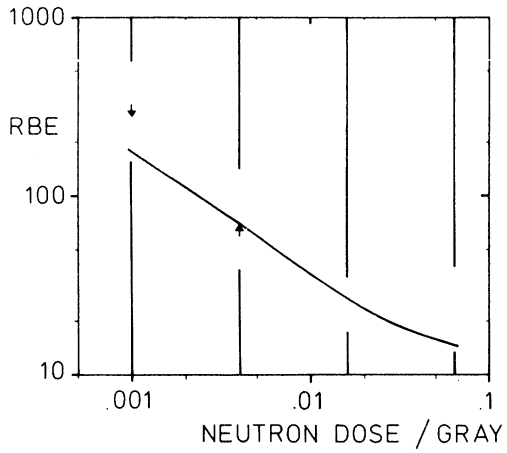

FIGURE 20. Dose dependence of the RBE of 400-keV neutrons for the induction of mammary neoplasms in Sprague–Dawley rats. Vertical bars indicate the ranges of RBE that are excluded on a level of statistical significance exceeding 95%; arrows represent differences with lower level of significance. The solid curve is an estimate consistent with the different types of dose–effect relations that can be constructed (Shellabarger *et al.*, 1980).

The experimental data indicate clearly the high RBE values of neutrons at low doses, but the actual dependence of RBE on neutron dose has to be deduced by a more detailed analysis. The result of such an analysis is shown in Fig. 20. This shows the dependence of RBE on neutron dose together with confidence limits of this dependence obtained by nonparametric comparison of groups of animals exposed to different doses of neutron or γ-rays. The vertical bars cover those ranges of RBE that can be excluded with statistical certainty exceeding 95%. The analysis is based on the data in the experiment of Shellabarger *et al.* (1980), but also on earlier results obtained by Bond *et al.* (1960) with γ-rays. Details of the nonparametric procedure to obtain the curve are described by Kellerer and Brenot (1973). The RBE–dose dependence and the very large RBE values are in general agreement with the theoretical considerations and are also consistent with the earlier experimental results in the lens opacification studies. However, the RBE values at low neutron doses exceed, as stated in Section 4.2, the predictions made on the basis of microdosimetric data; and the data also indicate that RBE may level out at high neutron doses at values larger than unity. As stated earlier, this may reflect the increased effectiveness in sublesion production.

The information presented thus far corroborates the postulates of the theory. However, there is another aspect of the results that is of significance with regard to the mechanisms of tumor induction. The RBE–dose relation shown in Fig. 18 puts certain constraints on the dose–effect relations both for X-rays and for neutrons, although it does not determine their shapes. As pointed out, the data in Fig. 18 indicate a dependence that may be approximately linear for X-rays while it is *sublinear* for neutrons. However, Fig. 18 is based on data that are not corrected for mortality and it represents only one of many possible ways to construct dose–effect relations. It is therefore desirable to ascertain the findings by comparing the dose–effect relations constructed in different ways. Such dose–effect relations are given in logarithmic form in Fig. 21. In this presentation straight lines correspond to proportionality of the effect to a power of the

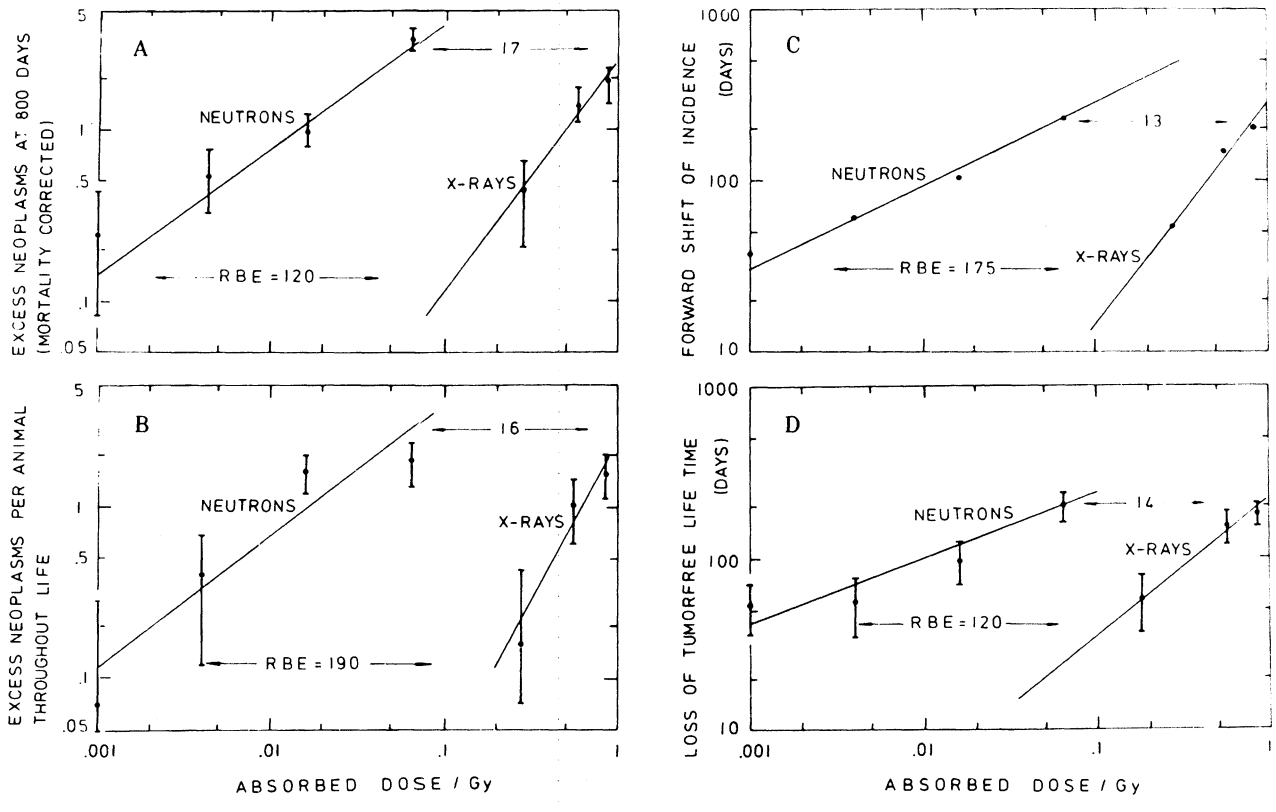

FIGURE 21. Dose–effect relations constructed in different ways for the induction of mammary neoplasms in Sprague–Dawley rats by 400-keV neutrons and X-rays. The straight lines in the logarithmic representation are rough fits to the data, and the estimated RBE values at the lowest and highest neutron doses correspond to these lines. Vertical bars are standard deviations. (Data from Shellabarger *et al.*, 1980.) (A) Total excess incidence of mammary neoplasms per animal; this corresponds to the data in Fig. 18. (B) Mortality-corrected excess incidence of mammary neoplasms per animal at day 800 postirradiation. (C) Estimated forward shift of the occurrence of mammary neoplasms due to irradiation. (D) Average loss of tumor-free life of the animals due to irradiation.

absorbed dose; the slope of the line is numerically equal to the power of the absorbed dose.

Panel A gives the data from Fig. 18, i.e., the uncorrected total incidence. Panel B gives the mortality-corrected excess number of neoplasms at day 800 after irradiation. Panel C gives the forward shifts in time that are listed in Table 1, and Panel D gives the average loss of tumor-free life span of the animals. It is evident that all of these various ways of constructing dose–effect relations lead to dose exponents for neutrons that are substantially below 1 and may be equal to 0.5. For X-rays the data may be consistent with proportionality to absorbed dose, i.e., with a dose exponent of 1. The solid lines through the data are rough fits that indicate the general character of the dose–effect relations. The RBE values estimated from these relations at the highest and lowest neutron doses are indicated in the individual panels. Although there are differences due to statistical uncertainties, it is apparent even from these rough fits, regardless of the details of the analysis, that one obtains sublinear dose–effect relations for neutrons and large RBE values at small neutron doses.

It had already been concluded in an earlier analysis (Rossi and Kellerer, 1972) that the sublinearity of the incidence of neoplasms versus neutron dose extends to doses that are so low that multiple traversals of cells are highly improbable. As mentioned earlier, the average number of particles traversing a cell nucleus is of the order of 1 for a neutron dose of 250 mGy. The lowest dose used in these experiments was 1 mGy where only 1 in about 250 cell nuclei experiences a traversal by a neutron secondary, i.e., where the mean number n of events per nucleus is roughly 0.004. If one considers the whole cell, the mean number n of events at 1 mGy of 430-keV neutrons is about 0.05. In Section 3.2 it has been concluded that, in a logarithmic plot of the effect probability (corrected for control incidence) versus dose, the slope of the resulting curve cannot be smaller than $(1-n)$ if one deals with autonomous cells. Because in the present experiment the slope is considerably less, one must conclude that the observed incidence is determined not only by transformations of individual cells, but also by radiation-induced processes in adjacent cells or by dose-dependent changes at the tissue level. The observed decrease of the yield of neoplasms per unit dose cannot, at any rate, be due to a depletion of a critical cell population. At this time, no further statement can be made, but one may consider factors such as virus release by damaged cells with some local saturation or an induced increase of immune reactions even at low doses of the order of 1 mGy.

The induction of mammary neoplasms in the Sprague–Dawley rats offers a remarkable illustration of the considerations given in the earlier theoretical sections. This is, however, at present the only study that permits a detailed analysis that includes the dose–effect relations, the RBE–dose dependence, and the time dependence of the induction of neoplasms by sparsely and densely ionizing radiations. It is, therefore, important to ascertain that these findings are not merely specific to the Sprague–Dawley rat with their high spontaneous incidence of mostly benign mammary neoplasms.

Shellabarger (private communication) has recently performed a further experiment on the induction of mammary neoplasms by X-rays and 430-keV neutrons in rats of the ACI strain that exhibit a low spontaneous incidence of mammary neoplasms, but show a pronounced incidence of adenocarcinomas after treatment with the synthetical hormone diethylstilbestrol (DES).

Data for non-DES-treated animals have been obtained at neutron doses between 0.045 and 0.36 Gy, and the neutron–RBE values in this dose range are somewhat in excess of 10, both for fibroadenomas and for adenofibromas. This is, as seen from Fig. 20, consistent with the data for the Sprague–Dawley rats.

For the DES-treated animals data have been obtained for neutron doses between 0.01 and 0.09 Gy. One deals in this case exclusively with adenocarcinomas, and the incidence is high even for the unirradiated animals. The RBE shows the expected decrease with neutron dose, but at a specified neutron dose the RBE values exceed those obtained for the Sprague–Dawley rats. An RBE of about 200 is obtained at a neutron dose of 0.01 Gy. There is at present no explanation why the neutron RBE is markedly enhanced in this experiment that involves the synergism of DES and ionizing radiation. However, the observation is of obvious importance.

A further significant result is that the DES-treated animals show a dose–response relation for neutrons that is as clearly sublinear as the dose–response of the Sprague–Dawley rats.

Apart from its biophysical implications, the sublinear dose–response is important from the standpoint of risk estimates for small doses. It could lead to an increased effect if a dose is split into two parts separated by a time interval that is long enough that the effects of the two doses are independent and simply additive. If such a possibility exists for low doses of densely ionizing radiations, it becomes doubtful whether linear extrapolations from observations at high dose rates and high doses to low dose rates and low doses are always conservative.

The analogy to the split-dose experiments with cell transformations will be noted (Borek and Hall, 1974). However, these experiments were performed at high doses and with sparsely ionizing radiation. An increased effect of lower dose rates with densely ionizing radiation has been found in the induction of osteosarcomas in α-irradiated mice (Müller *et al.*, 1978), and it has earlier been demonstrated for the induction of osteosarcomas in patients subjected to injections of short lived radium-224 (Spiess and Mays, 1973). However, the doses were relatively high in these instances.

Various other examples can be given that indicate the variety and complexity of dose–effect relations for radiation carcinogenesis. For one case, namely the high spontaneous frequency of reticulum cell sarcomas in RFM mice, it has actually been shown that sparsely ionizing radiation causes a substantial decrease of the incidence (Ullrich and Storer, 1979); this appears to be not a real decrease of the neoplastic response, but a shift toward an increased incidence of thymic lymphoma. Another, contrasting example of a well-established dose dependence is the induction of ovarian tumors in mice by γ-radiation (Yuhas, 1974). In this case a purely quadratic dose dependence has been established for absorbed doses

down to 0.2 Gy, and a marked recovery effect is found with decreasing dose rates.

Finally, one may mention studies by Ullrich *et al.* (1979) on the induction of lung tumors in RFM mice by X-rays and fission neutrons. These observations are interesting because a threshold-type dose–effect relation is found for X-rays and a dose relation with positive curvature is obtained for neutrons. However, even in this case the RBE–dose dependence agrees with the inverse relation between RBE and the square root of the neutron dose.

In view of the complexities of the dose–effect relations, it is unlikely that general statements can be made on the dose dependence for radiogenic cancer in man. However, it also appears that the inverse relation between RBE and the square root of the neutron dose remains valid. This will be considered for the most important set of human epidemiological data for late somatic effects.

5.3. Radiation Leukemogenesis

The epidemiological data on the induction of cancer by ionizing radiation are commonly so limited that it is impossible to determine the shape of the dose–effect curve with even moderate confidence. The data cannot usually be shown to be inconsistent with a variety of relations including a simple straight line. It has, therefore, often been decided to adopt the "linear hypothesis," i.e., the assumption that the risk is proportional to dose. It is generally agreed that this is a "prudent" assumption in that it is likely to lead to overestimates rather than underestimates. This belief is, at least in part, due to findings of experimental radiobiology. Curves relating cancer incidence in animals to dose can, as shown in Section 5.2, have a variety of shapes, but at least in the case of low-LET radiations, one usually observes a positive curvature at low doses.

The acceptance of linearity permits the formulation of various concepts employed in radiation protection. Thus, the *population dose* (usually measured in *man-rem*) has been defined as the sum of the doses received by persons in a given group, and it is assumed that within wide limits the total number of cancers induced by a given population dose will be the same regardless of the distribution of individual doses in the population. The assumption of linearity is commonly applied to both low- and high-LET radiations with the implied further assumption that the RBE is a constant. It should be noted that this constancy is assumed not only for the low doses of interest to radiation protection, but also for the much higher doses where the significant effect frequencies are observed, which determine the slope of the linear relations derived.

The postulate that the RBE is independent of dose at moderate or high doses (of the order of 1 Gy) is obviously at variance with theoretical considerations and with experimental data on nonhuman systems. It seems plausible that man should not be an exception and that risk estimates based on constant RBE must either overestimate the hazard of low-LET radiation or underestimate the hazard of high-LET radiation. A determination on whether this is so is of fundamental importance to radiation protection.

The first attempt at such an analysis employed data on leukemia mortality. Among the various types of cancer that can be induced by radiation, leukemia has the highest potential utility for statistical analysis because it is one of the most frequently induced malignancies and because its natural incidence is relatively low.

The most important sources of information on radiation-induced leukemia are the data obtained from studies on survivors of the Japanese cities exposed to nuclear weapons at the end of World War II. Not only are the populations involved far larger than those in other studies, but special efforts have also been made by the Atomic Bomb Casualty Commission to achieve maximum follow-up, to select optimum control populations, and to determine as accurately as possible the doses received by individuals.

The most important aspect of these observations is, however, that they were obtained for two types of radiations. In Hiroshima, a substantial neutron dose was delivered that was primarily responsible for the biological effects observed. In Nagasaki, the relative neutron dose was very low and virtually negligible at greater distances from the epicenter of the explosion (Ishimaru *et al.*, 1971). The availability of data for both neutron and γ-radiation thus provides an opportunity to address the question of whether the dose dependence of RBE regularly found in other systems can be shown to apply also to human leukemia.

Earlier assessments of the Japanese leukemia data were based on "dose" (also "T-65 dose" or "air dose"), which is actually the tissue kerma in free air (see Section 2). The fraction of this kerma that was due to neutrons varied somewhat with distance from the epicenter, but for the population of interest, it was between 20 and 25% at Hiroshima and essentially negligible at Nagasaki.

An early analysis (Rossi and Kellerer, 1974) first addressed the question whether the effectiveness of radiation at Hiroshima compared with that at Nagasaki was the same at all levels of leukemia mortality. Since the comparison is based on R_H, the kerma ratio, rather than the ratio of absorbed doses in the bone marrow, it does not constitute a determination of the RBE (which will be shown to be much higher). However, the ratio between dose and kerma does not depend on either of these quantities, and consequently, the dependence of RBE on dose is a multiple of the dependence of R_H on kerma.

Utilizing the nonparametric techniques discussed in the previous section, the relation depicted in Fig. 22 is obtained.* Although the most crucial of the limits (the lower bound at 10 rads) is established with 86% rather than 95% significance, this value seems sufficiently large, particularly in view of the conservative assumptions made. It may thus be concluded that the neutron RBE for human leukemia, like that for virtually all other somatic effects investigated, increases with decreasing level of effect.

* In this as well as in the other analysis, the information for the highest kerma level at Nagasaki and the two highest kerma levels at Hiroshima has been ignored, for two reasons. One is that survivors in these categories must represent a highly selected and uncertain group because LD_{50} levels are approached or even exceeded. The other reason is that, in accord with all other experience with radiation carcinogenesis, it must be expected that the dose–effect curve should at such high doses saturate or even decrease.

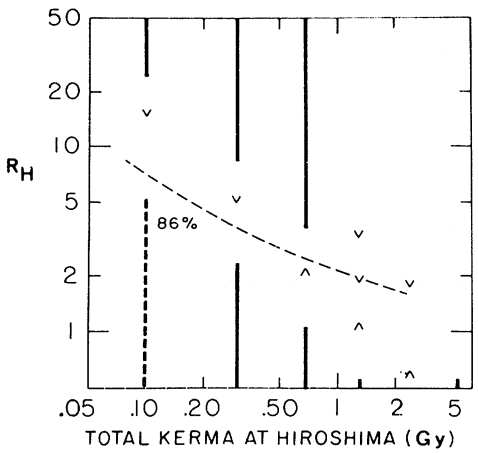

FIGURE 22. Relative biological effectiveness of the radiation in Hiroshima for the induction of leukemia compared to that in Nagasaki as a function of kerma in Hiroshima (Rossi and Kellerer, 1974). The solid bars indicate those values that can be excluded with 95% confidence; the broken bar indicates a level of confidence of 86%. The broken curve is the result of a least-squares fit.

While this finding is of interest, it is of course even more desirable to determine the shapes of the dose–effect relations. In particular, the very important question arises of whether, as in the case of mammary neoplasms, the neutron dose–effect relation rises with a power of the dose that is less than 1 or whether the power is 1 or exceeds 1. Linear extrapolations would in the former case underestimate the neutron hazard, but in the latter case overestimate the γ-ray hazard.

In order to gain information on this point, it was assumed that for both cities the dose–effect can be approximated by

$$I(K) = I_0 + aK + bK^2 \tag{25}$$

where I is the incidence and I_0 its control level, K is the total kerma, and a and b are constants. Utilizing a statistical treatment described elsewhere (Kellerer and Brenot, 1974), it was established that for Hiroshima the quadratic component has to be rejected and only a linear component need be assumed. For Nagasaki, the least-squares estimate of a turned out to be negative, and only the quadratic component was considered in the further analysis. It thus appears that at Hiroshima, where the biological effect of the neutrons was dominant (because of their higher RBE), radiation induced leukemia at a rate proportional to kerma, while at Nagasaki, where neutrons could be all but neglected, the leukemia mortality increased with the square of kerma.

In a final step, a more accurate treatment was utilized by assuming that in both cities the incidence could be expressed by

$$I = I_0 + aK_n + bK_\gamma^2 \tag{26}$$

where K_n and K_γ are the kermas of neutrons and γ-rays and the parameters I_0, a, and b are the same for both cities. The least-squares fit obtained on this basis is shown in Fig. 23a and b together with the observed mortalities and their standard deviations.

When the factors relating marrow dose to kerma became available (Kerr, 1978), it was possible to derive the leukemia mortality to dose of neutrons or

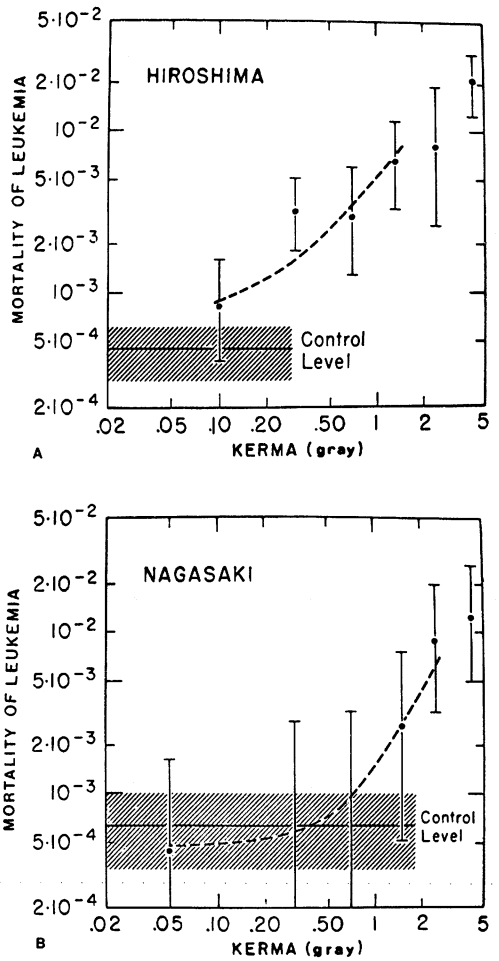

FIGURE 23. Mortality of leukemia for the period from October 1950 to September 1966 vs. kerma at Hiroshima (Rossi and Kellerer, 1974). The bars represent 95% confidence ranges; the shaded area is the 95% confidence region for the unirradiated population of the city. The broken curve is the result of a least-squares fit.

γ-radiation. These are about 8.5×10^{-2}/Gy for neutrons and 3×10^{-3}/Gy^2 for γ-radiation. These relations are shown in Fig. 24. It should be noted that they apply for doses that are less than about 1 Gy and more than about 0.1 and 0.01 Gy, respectively, of γ- and neutron radiations. It should also be noted that they apply to the mortality from all types of leukemia. It has been claimed (Mole, 1975) that there are different RBE relations for different types of leukemia.

Employing a different, somewhat simpler approach, Rossi and Mays (1978) essentially confirmed these findings. They are also, at least in part, supported by others (Jablon, 1979; Ishimaru *et al.*, 1979), and there is now increasing acceptance of the opinion that the risk of leukemia is substantially proportional to neutron dose and that it increases at least approximately as the square of the dose of low-LET radiation.

The induction of other types of neoplasms in the atomic bomb survivors has been too low to permit an accurate analysis. Significant conclusions, however, are possible if mortality from malignant neoplasms is considered in toto. Figure 25, based on a survey by Beebe *et al.* (1977), details the mortality per year as a

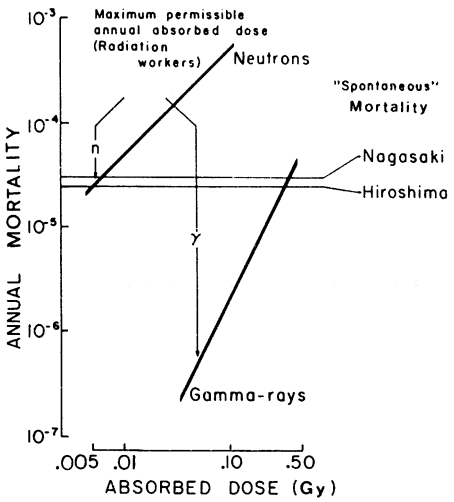

FIGURE 24. Annual mortality of leukemia vs. absorbed dose to the bone marrow as deduced from the Japanese data. The graph also shows the "natural" mortality at Hiroshima and Nagasaki and the maximum permissible annual absorbed doses for radiation workers (Rossi, 1977).

function of kerma in Hiroshima and Nagasaki. The straight line fitted to the data from Hiroshima ignores again the two highest kerma points, as it is difficult to account for selection effects in the survivors and resulting uncertainties in the dose estimates at these high exposure levels that include a substantial neutron component. Furthermore, it should be noticed that the curves are fitted to different control values in the two cities, and that the assumption of a common control value would lead to markedly increased RBE values for the radiation in Hiroshima compared to that in Nagasaki.

High RBE values are indicated by the data in Fig. 25 even without a rigorous analysis. However, using information on the relation between kerma and absorbed doses for neutron and γ-radiation (Jones, 1977), an estimate of the radiation-induced annual mortality from all malignant neoplasms from 1950 to 1974 is

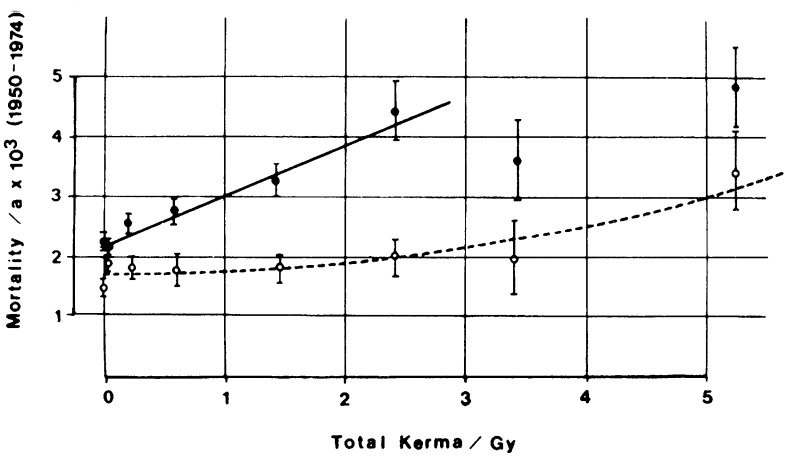

FIGURE 25. Annual mortality from all malignant neoplasms (averaged over 24 years) in individuals exposed to various values of total kerma at Hiroshima and Nagasaki.

$1.3\times10^{-2}(D_n/\mathrm{Gy})$ and $1.7\times10^{-4}(D_\gamma/\mathrm{Gy})^2$, where D_n is the neutron dose to the bone marrow and D_γ the γ-ray dose.

Various uncertainties limit the reliability of the numerical risk estimates. In any case, these estimates should not be applied to low-LET absorbed doses of 10 mGy or less, as a more refined statistical analysis or an augmentation of the observations to a broader sample of the exposed population might disclose a linear component in the data from Nagasaki. However, despite its statistical limitations the information contained in Fig. 25 is of high pragmatic and fundamental interest.

The pragmatic conclusion is that the relation between mortality due to cancer in general and dose of high-LET radiation is substantially linear at doses in the range from roughly 10 mGy to 100 mGy, but the relation is nonlinear for doses of low-LET radiation that range from perhaps 50 mGy up to several gray. It follows that risk estimates for low-LET radiation obtained by linear extrapolation from high doses are too high. It also follows that while the hazard from low-LET radiation is thus likely to be less than given by most current risk estimates, the hazard of neutron radiation is greater than implied by a Q (quality factor) of 10.

The basic scientific conclusion is that data on human cancer are consistent with the theory of dual radiation action. Radiation carcinogenesis is therefore very likely to be a process initiated by lesions that in turn are due to the interaction of pairs of sublesions. It seems reasonable to speculate that these sublesions are double-strand breaks of DNA, but any further or more assured notions must await the availability of additional pertinent information.

6. *Appendix*

In the following, a formal derivation will be given of the theorem that is expressed in equation (13) of Section 3.2. As exemplified in Section 5.1, this theorem can be utilized to decide whether an observed dose–effect relation is compatible or incompatible with the assumption that the effect is due to independent alterations in individual cells.

Let E_ν be the probability of observing the effect in a cell after exactly ν energy-deposition events have occurred. As pointed out earlier, an event is energy transfer to the critical region of the cell by a charged particle and/or its secondaries. The cells are assumed to belong to an irradiated population in which no interaction of cellular damage occurs; i.e., energy deposition in one cell does not influence the effect probability for another cell.

Energy-deposition events are by definition statistically independent; their number is therefore distributed according to Poisson statistics. According to equation (8), the effect probability at dose D is

$$E(D)=\sum_{\nu=1}^{\infty}p_\nu E_\nu=\sum_{\nu=1}^{\infty}\left[e^{-\phi D}(\phi D)^\nu/\nu!\right]E_\nu \qquad \text{(A1}$$

It is important to note that this equation holds even for an inhomogeneous population. The sole condition is that the critical regions for the individual cells are chosen to be of equal size. Without this condition, Poisson statistics would not apply. Since the critical regions can be larger than the sensitive sites of the cells or even than the cells themselves, the condition of equality of critical regions can always be met even for a population of unequal cells. It is, furthermore essential to note that the coefficients E_ν do not depend on absorbed dose. This is the case because, by definition, energy deposition outside the critical region does not influence the fate of the cell; the effect is determined solely by the number of events taking place within the critical region and by the amount of energy imparted by these events.

The slope of the dose–effect relation in the logarithmic representation is

$$c = \frac{d \ln E}{d \ln D} = \frac{D}{E} \frac{dE}{dD} \tag{A2}$$

If one inserts equation (A1) into this expression, one obtains

$$\begin{aligned} c &= \frac{D}{E(D)} \sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} \left[\frac{(\phi D)^{\nu-1}}{(\nu-1)!} \phi - \phi \frac{(\phi D)^\nu}{\nu!} \right] \\ &= \frac{\sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} [(\phi D)^\nu / \nu!](\nu - \phi D)}{\sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} [(\phi D)^\nu / \nu!]} \\ &= \frac{\sum_{\nu=1}^{\infty} \nu p_\nu E_\nu}{\sum_{\nu=1}^{\infty} p_\nu E_\nu} - \phi D \end{aligned} \tag{A3}$$

The term

$$\pi_\nu = \frac{p_\nu E_\nu}{\sum_{\nu=1}^{\infty} p_\nu E_\nu} \tag{A4}$$

can be understood as a conditional probability, namely as the fraction of cells with exactly ν events among those cells that are affected. From equations (A3) and (A4),

$$c = \sum_{\nu=1}^{\infty} \nu \pi_\nu - \phi D \tag{A5}$$

This form of the equation for the logarithmic slope of the dose–effect curves has a highly interesting interpretation. The sum $\Sigma_{\nu=1}^{\infty} \nu \pi_\nu$ is the mean number of events in those cells that show the effect; one can symbolize this mean value by $\bar{n}_E$. On the other hand, the mean number of absorption events throughout the cell population, regardless of whether the cells will show the effect or not,

is equal to ϕD; this latter quantity can, therefore, be symbolized as $\bar{n}$. Thus, the difference of the mean event numbers in those cells that show the effect and in the cells throughout the population is equal to the slope of the dose–effect curve in the logarithmic representation at the particular value of the absorbed dose considered. This is the theorem discussed in Sections 3 and 5:

$$c = \bar{n}_E - \bar{n} \tag{A6}$$

A somewhat more general formulation of the relation can be found elsewhere (Kellerer and Hug, 1972).

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