CANCER 1

A COMPREHENSIVE TREATISE

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 virtually nothing of the mechanisms involved. Because of its profoundly important theoretical and practical aspects, the phenomenon has been very extensively studied, but most of the information obtained has been of a phenomenological nature.

Between the two extremes of a purely descriptive treatment of a process 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 of the process which permit the formulation of its kinetics. This in turn can furnish clues concerning its mechanism.

The application of radiation biophysics to the phenomenon of carcinogenesis has yielded some insights of this kind. Most of the arguments employed are stochastic, and in the following sections dealing with physics and theoretical radiobiology the influence of random factors is stressed. In a final section, the concepts developed in the previous sections are applied to two types of radiation carcinogenesis.

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Because of practical limitations, much of the information contained in the second section 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 which 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 *directly ionizing radiations* that may originate in external or internal sources, or be generated inside the irradiated matter by *indirectly ionizing 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 which is 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 directly ionizing 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 inverse, the linear absorption coefficient, μ, in water for protons and neutrons 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 the number of particles that arrive at a depth d. The mean free path 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

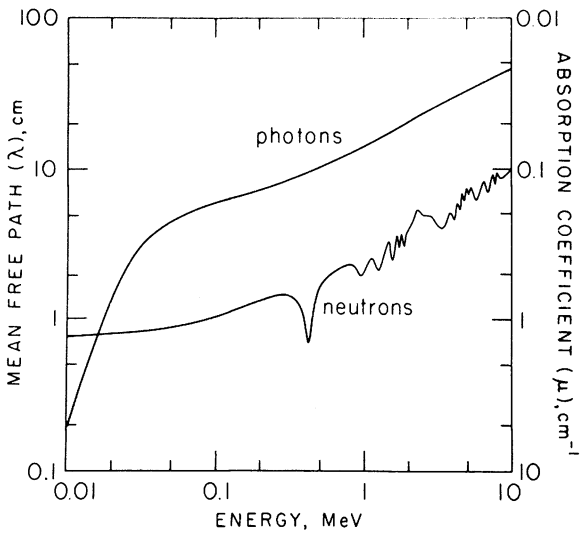


FIGURE 1. Mean free path, $\lambda(E)$, and absorption coefficient, $\mu(E)$, as a function of energy for photons and neutrons in water.

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 energy deposition as a function of depth in irradiated material because in many instances the interactions lead to the production of secondary radiations which have appreciable penetration of their own, with the result that more energy arrives at any given depth than that merely 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 production 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 which 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.

In order to illustrate the far more restricted penetration of directly ionizing radiations, Fig. 2 shows the range of what are perhaps the two most important charged particles in radiobiology, the electron and the proton. In contrast to the indirectly ionizing radiations, which tend to be absorbed exponentially and cannot

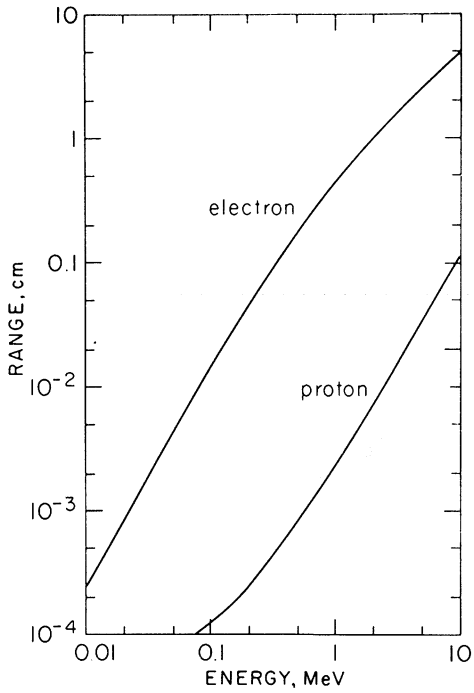


FIGURE 2. Range of electrons and protons in water as a function of energy (ICRU, 1970).

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 of tissue). Figure 3 shows the LET in water of electrons and protons as a function of the energy.

2.2. *Dosimetry*

The physical quantity which 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

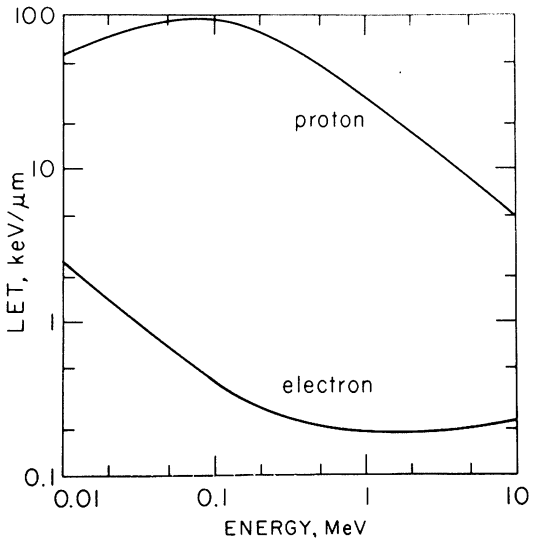


FIGURE 3. Linear energy transfer, LET, for electrons and protons in water as a function of energy (ICRU, 1970).

in the volume element. E is proportional to the product of the number of charged particles traversing the element and to their LET.

In the case of indirectly ionizing 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 directly ionizing radiations released per unit mass in a specified material (here usually tissue). Figure 4 shows this quantity per unit fluence (number of indirectly ionizing particles per unit cross-sectional area) for electromagnetic radiation and neutrons. In irradiated matter, kerma and the absorbed dose frequently have nearly the same numerical value. Because of the short range of charged particles, the energy absorbed per unit mass at some point in the medium is nearly the same as the kinetic energy of the charged particles

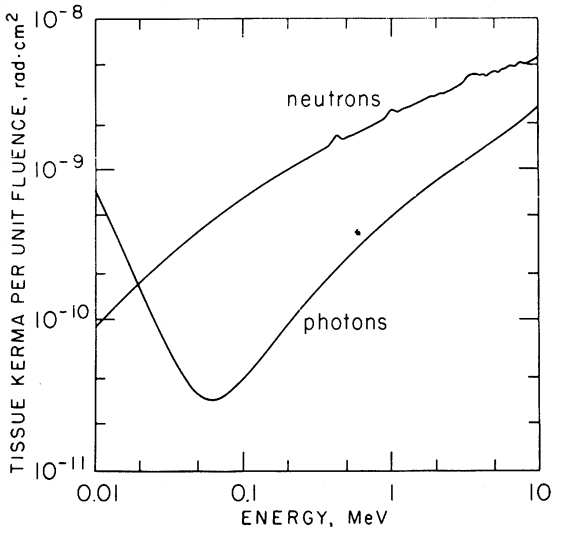


FIGURE 4. Tissue kerma per unit fluence for photons and neutrons as a function of energy. Based on Bach and Caswell (1968).

released. This is the condition known as *radiation equilibrium*. It does not exist when the absorption of indirectly ionizing radiations is comparable to that of the directly ionizing 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 because of the more copious electron emission from the irradiated bone.

The unit generally employed for both absorbed dose and kerma is the *rad*, which represents an energy absorption of 100 ergs per gram of irradiated material. The reason for the magnitude of this unit is largely historical and relates to another quantity, the *exposure*, and its special unit, the *roentgen*. The exposure is a measure of X- and γ-radiations based on their ability to ionize air. Its exact definition is not necessary here, but it may be noted that in almost all cases of interest exposure of tissues to 1 roentgen results in an absorbed dose that is equal to 1 rad within less than 10%. It appears very likely that within a few years these units will be replaced by those of the International System of Units (SI), which has been adopted by virtually all nations. In this system, the appropriate unit for absorbed dose and kerma is the *joule per kilogram* (J/kg), which is equal to 100 rads.

Under well-defined conditions, 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.

2.3. *Microdosimetry*

Many radiobiological phenomena and probably at least one type of radiation carcinogenesis (see Section 5.1) are due to multicellular response to radiation injury. However, in all instances individual cells are injured randomly, and it is consequently the energy absorbed by individual cells that governs all radiobiological phenomena. 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 cellular 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 100 rads (1 J/kg). In the case of γ-rays, the mean number of electrons traversing this volume is near 10; in the case of fast neutrons, the frequency of particle traversals is only of the order of $\frac{1}{10}$. 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* which is represented by the absorbed dose. In the example just quoted, there is no neutron secondary and therefore no energy deposition in nine out of ten cases, but in the remaining one the energy density is typically 10 times larger than

might be expected on the basis of 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 which has a range of values in uniformly irradiated matter. The variability of z is expressed by the distribution function $f(z)$, which represents the probability that the specific energy is equal to z. The width of this distribution depends on three factors:

1. The volume containing Δm. Strictly speaking, this involves both the size and the shape of this volume, 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.

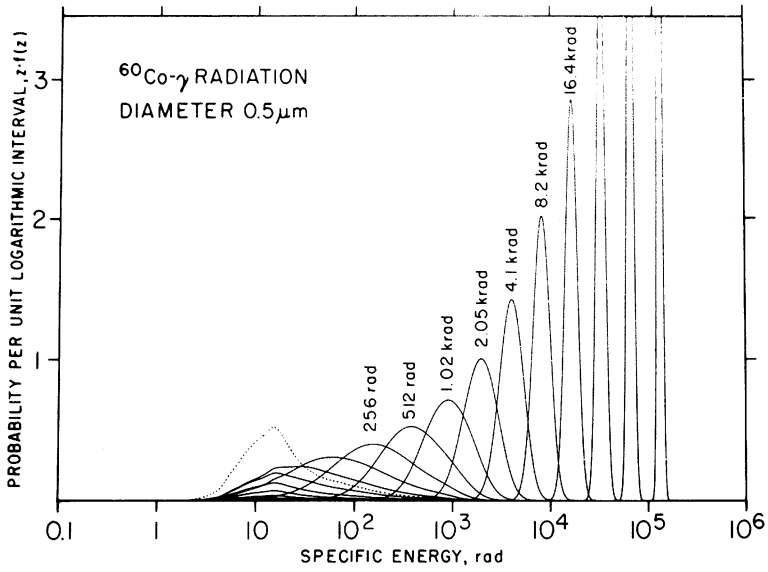
The influence of these factors is illustrated in Figs. 5 and 6, which are logarithmic representations of $f(z)$ vs. z for various absorbed doses of 5.7 MeV neutrons and ^{60}Co-γ-rays for spheres having diameters of 0.5 or 12 μm. Neutrons of energy 5.7 MeV are somewhat more energetic and therefore slightly less densely ionizing than fission neutrons. The average LET is somewhat higher for natural α-emitters and somewhat lower for more energetic neutrons. Electrons produced by ^{60}Co-γ-rays exhibit minimal variance of energy deposition. In the case of X-rays, the statistical fluctuations are somewhat larger.

The curves in Figs. 5 and 6 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 greatly 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 has 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. 5 and 6). A reduction of dose merely results in a decrease of the amplitude of the spectrum with the remainder of the distribution appearing at $z = 0$.

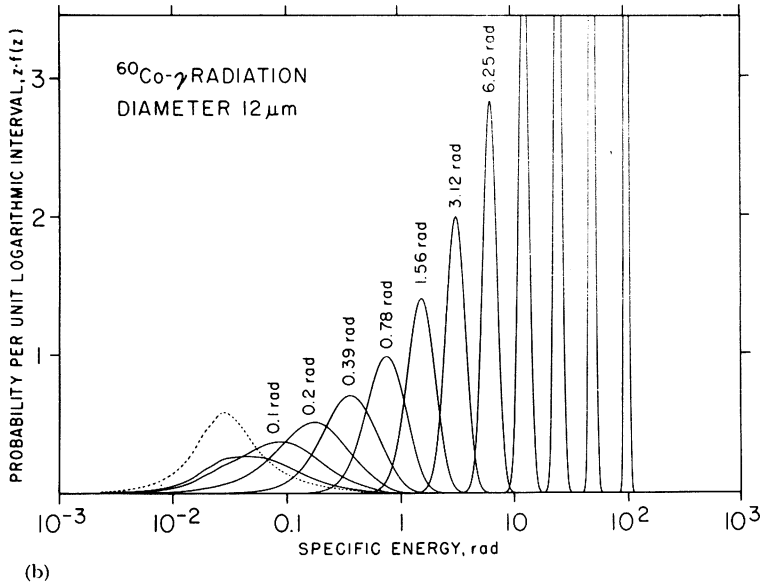
For any distribution, $f(z)$, the mean value of z is defined by

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

In Figs. 5 and 6, and at high doses, it is evident that z is equal to D. 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 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.



(a)



(b)

FIGURE 5. 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.

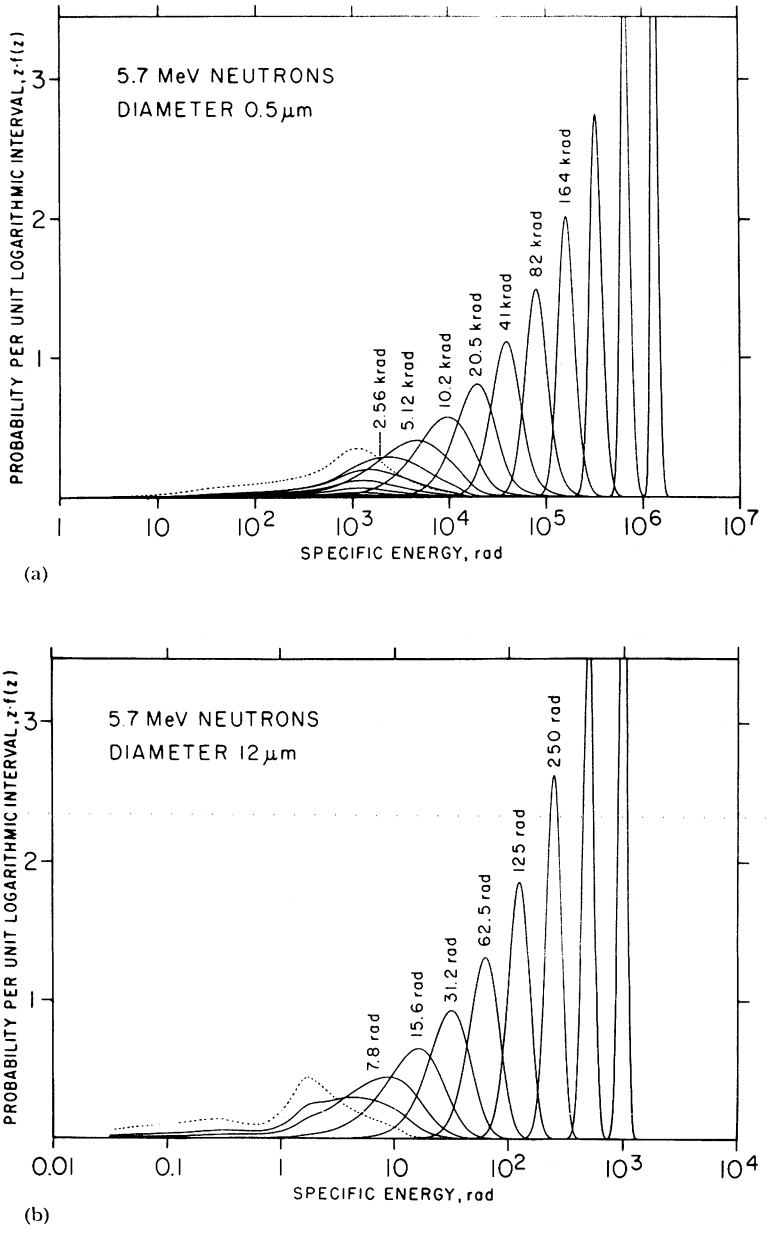


FIGURE 6. Probability per unit logarithmic interval of specific energy, z, at various doses of 5.7 MeV neutrons in a spherical tissue region of diameter (a) 0.5 μm and (b) 12 μm. The distributions of the increments z produced in single events are given as broken lines.

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

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

Comparison of equations (3) and (4) 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 provided that 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 which is not proportional to z but rather to z^2. This statement applies in particular to the two 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.

3. *General Stochastic Considerations*

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

As has been pointed out in the preceding section, the absorbed dose determines only the mean value of the energy absorbed in a cell or in its sensitive nuclear region. The energy actually absorbed in microscopic volumes 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-rays 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 distribution 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 in 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 which 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 which 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 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 which are traversed by a charged particle does not change with decreasing dose. The only change which occurs with decreasing absorbed dose is the decrease in the fraction of cells which 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 which 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 $\phi D \ll 1$, the term $e^{-\phi D}$ can be set equal to 1, and with this simplification one obtains

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

E_1 is the probability for the effect if exactly one event has taken place; 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 ionizing and densely ionizing radiation 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

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

This relation implies that in the action of ionizing radiation on individual 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 a threshold or no 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 which 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 100 mrad/yr. 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 which 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 rad for microscopic regions of various diameters and for different qualities. The largest region included in this table corresponds approximately to the size of a mammalian cell. Various radiobiological studies have shown that for most cellular effects only energy deposition within

TABLE 1
Event Frequencies per rad in Spherical Tissue Regions Exposed to Different Radiations

Diameter of critical region d (μm)	Type of radiation			
	^{60}Co-γ-rays ϕ(rad^{-1})	Neutrons, ϕ(rad^{-1})		
		0.43 MeV	5.7 MeV	15 MeV
12	20	0.55	0.51	0.61
5	3.6	0.042	0.086	0.11
2	0.58	3.9×10^{-3}	1.2×10^{-2}	1.6×10^{-2}
1	0.12	8×10^{-4}	3.2×10^{-3}	3.8×10^{-3}
0.5	0.017	2×10^{-4}	7.3×10^{-4}	9×10^{-4}

the cell nucleus 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; diameters of 1 μm and 2 μm 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 or, in other words, 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 0.05 and 2 rads 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 0.3 and 24 rads 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 fractions of a rad, and for densely ionizing radiations at considerably higher doses, a linear relation must hold if one deals with effects on individual cells. As pointed out, this is because even at the smallest doses appreciable amounts of energy are deposited in those cells which 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 fractions of a rad in the nucleus of the cell for sparsely ionizing radiations and with tens of rads 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 which 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 subsection are valid regardless whether the effect, E, is considered as the probability for a quantal effect, i.e., an effect which 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_v stand therefore for 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 cytogenic 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.

A clarification is also necessary concerning the concepts *sensitive site* and *gross sensitive region*. 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 chromosomal structure. In the next section, it will be shown that various effects on eukaryotic cells can be understood if one postulates sites which are somewhat smaller than the cell nucleus and which 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, and it is therefore useful to apply a concept which is somewhat more general. This is the concept of the so-called gross sensitive region (Rossi, 1964). The term is used to designate that part of the cell which contains all the sensitive structures or all the structures whose sensitivity has to be considered with regard to the experimental end point studied. The concept of a gross sensitive region is not necessarily equivalent to that of a sensitive site since a cell may contain several sites subject to damage produced by ionizing radiations; the gross sensitive region would then include all the different sensitive structures. In many practical cases, it will be a reasonable approximation to assume that the gross sensitive region of the cell is the cellular nucleus.

In the following, a slightly different term will be used: *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 which 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 which 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 which has to be further explained is that of an *energy deposition event*, for brevity *event*. This is defined (ICRU, 1971) 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 which 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 function of absorbed dose.

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

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

$E(D)$ stands for 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, $\bar{n}_E$, in the critical region of those cells which show the effect and the mean event number, $\bar{n}$, in the critical region of the cells throughout the exposed population regardless of whether they show the effect or not:

$$c = \bar{n}_E - \bar{n} \tag{13}$$

This equation holds at any value of absorbed dose. The relation remains valid if a critical region larger than the actual gross sensitive volume 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.

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 $\bar{n}_E$, 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 to

this question 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 which is of significance to the analysis of dose–effect curves at smallest 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 which determines the effect. In the limit where the absorbed dose D (and consequently $\bar{n}$) 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 individual cells first-order kinetics apply at low doses; this corresponds to a value of $c = 1$ when $\bar{n} \ll 1$.

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 $\bar{n}_E$ cannot be less than 1 since the number of absorption events in affected cells must be at least 1. Consequently,

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

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

Studies performed by Vogel (1969) and by Shellabarger *et al.* (1973) on the induction of mammary tumors in Sprague-Dawley rats show a logarithmic slope c of the dose–effect curve for neutrons in the range of very small doses which 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 reflect the action of radiation on individual cells which 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 which 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 which 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 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 sites of the cell has 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 "single breaks." The yield of single breaks is assumed to be proportional to energy absorbed in the cell, and the average number of single 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 single breaks in the cell. A two-break aberration can result from two single breaks which are produced in the same charged particle track, or it can result from the interaction of two single 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-rays or γ-rays, on the other hand, the ionization density in the charged particle tracks is so low that neighboring single 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, recent 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 which turns into 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 7 represents as an example dose–effect relations for the yield of pink mutations in *Tradescantia* (Sparrow *et al.*, 1972. Curves are given for 430 keV

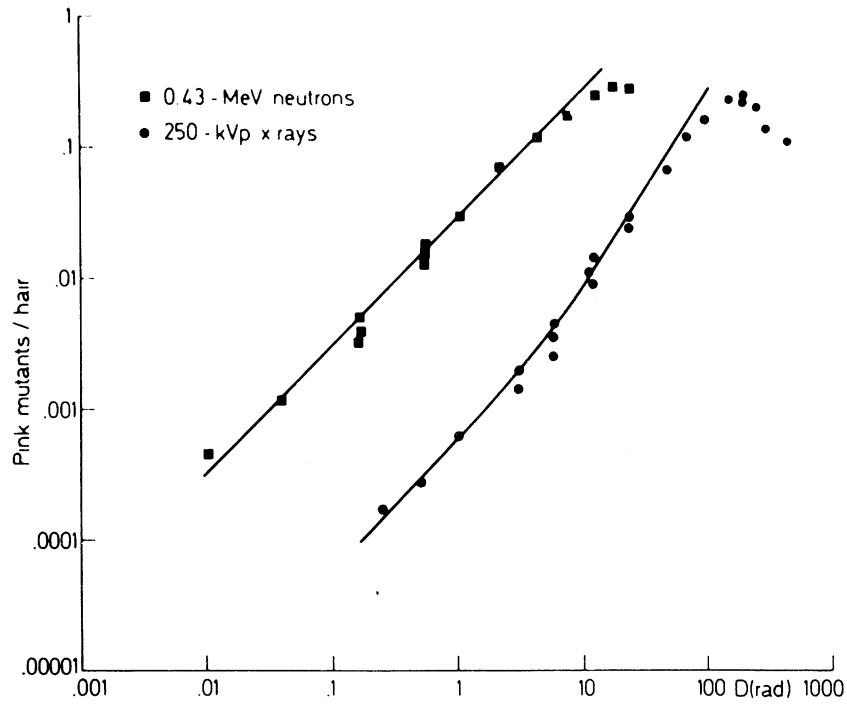


FIGURE 7. Induction of pink mutant cells in the stamen hairs of *Tradescantia* by X-rays and 430 keV neutrons (Sparrow *et al.*, 1972). The spontaneous incidence is subtracted from the observed values.

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 recent study on the transformation of cells *in vitro* (Borek and Hall, 1973) 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 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 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. 7 supports the general conclusions drawn from microdosimetric considerations, 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 effect observed at a certain absorbed dose is obtained by averaging the square of the specific energy in the sensitive sites of the cells over its distribution throughout the irradiated population:

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

It can be shown, and the mathematical details have been given elsewhere (Kellerer and Rossi, 1972) 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:

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

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 D is smaller than ζ, the linear component dominates; 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-rays or X-rays.

Figure 8 represents the value of ζ for different radiation qualities as a function of the diameter of the reference volume. These values are obtained from experimental microdosimetric determinations as well as from theoretical calculations. In the example represented in Fig. 7, one finds that for X-rays the linear component is equal to the quadratic component at a dose of approximately 10 rads. According to Fig. 8, the value of 10 rads 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 this is indeed borne out in Fig. 7, where the initial part of the neutron curve is

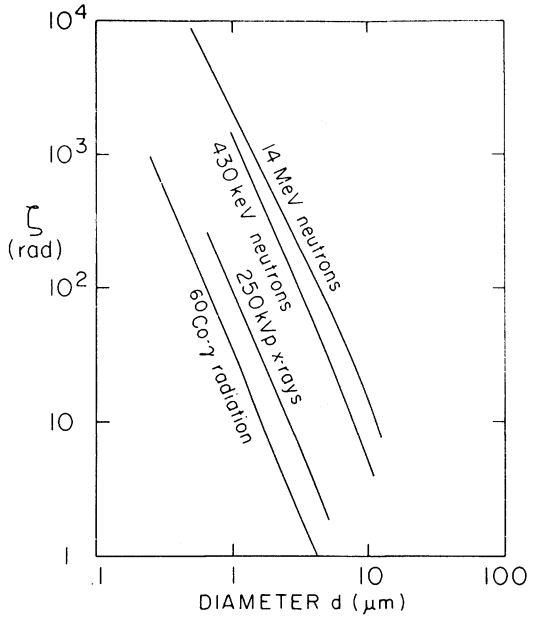


FIGURE 8. Energy mean, ζ, of the specific energy produced in single events by different radiation qualities in spherical tissue regions of diameter d.

shifted vertically by about this factor with regard 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 to values which 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 which 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 which also correspond to site diameters of 1 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 correspond therefore 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 which is linearly related to specific energy in the sensitive sites of the cell remains an open question.

One concludes that in certain cases, and in particular in such cases as cytogenetic effects where the observed experimental end point is closely related to the primary damage in the cell, the quadratic dependence of cellular damage on specific energy can be directly inferred from the dose–effect relations. In other cases, the

situation is more complicated. Particularly this is the case for effects on the tissue level, where the interaction of damaged cells may play a role. Such cases are complicated also because the scale used to measure the effect may often be arbitrary. There are, for example, many ways in which the degree of lens opacification after exposure of the eye to ionizing radiation can be measured. Similar complications arise if in a system, such as the Sprague-Dawley rat, mammary tumors occur with high spontaneous rate so that their incidence is merely accelerated after exposure of the animals to ionizing radiations. In all such cases, the numerical form of dose–effect curves has little absolute meaning. One can, however, assume that complicating factors such as the interaction of damaged cells or the arbitrary construction of the effect scale cancel if one considers equal effects of different radiation qualities.

The relative biological effectiveness (RBE) of radiation B relative to radiation A is defined as the ratio D_A/D_B of the respective absorbed doses for equal effect. It may be expected that this ratio of physical quantities is a measure of the effectiveness of energy distribution on the cellular or subcellular level only.

4.2. Dose–RBE Relations

The preceding considerations have indicated that the dose–effect relation is an expression of the combined influences of primary cellular (or subcellular) lesions and their interactions. If, however, according to the considerations put forward at the end of the last section, only the primary cellular damage depends on radiation quality, the value of the RBE is not determined by interaction processes between damaged cells. Equality of the observed effect for two different radiation qualities then implies equal levels of primary damage.

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

$$k(\zeta_x D_x + D_x^2) = k(\zeta_n D_n + D_n^2) \tag{20}$$

Where ζ_x and ζ_n are the values of ζ for X-rays and neutrons, and D_x and D_n the absorbed doses for X-rays and neutrons. Since the relative biological effectiveness 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 function of either the X-ray dose or the neutron dose. In the following, the relative biological effectiveness 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

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

It is easy to identify certain general characteristics of this dependence of RBE on dose. At very low doses, the linear components are dominant both for neutrons and for X-rays, and RBE must then have a constant value equal to the ratio ζ_n/ζ_x of the values ζ for neutrons and for X-rays. This plateau of RBE corresponds to the region in the example of Fig. 7 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, not easy to obtain meaningful biological data with neutrons at doses which are 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 9, together with Table 2, is a compilation of such results. 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 well with microdosimetric data. It is not surprising that relatively few data are available in the range of extremely small doses, because

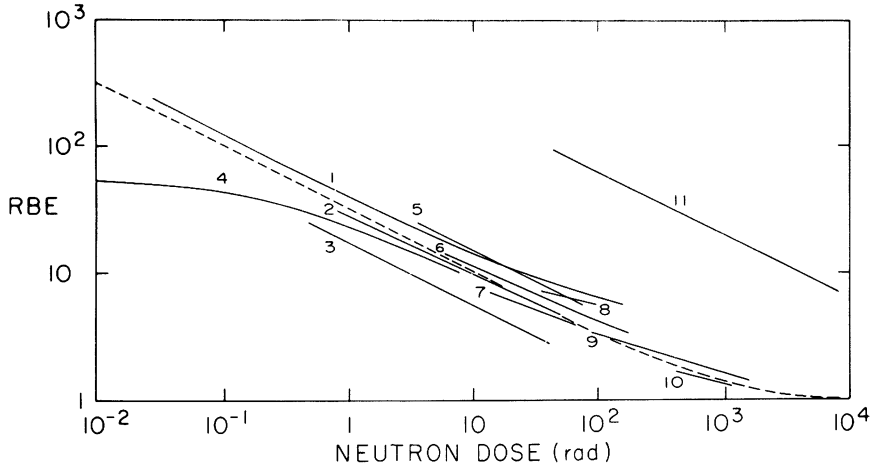


FIGURE 9. Relative biological effectiveness of neutrons as a function of absorbed dose of neutrons for various biological end points. The experimental curves belong to the cases listed in Table 2. The dotted line corresponds to equation (23) with $\zeta_x = 0$ and $\zeta_n = 1000$ rad.

TABLE 2

Author and number of curve in Fig. 9		End point	Neutron energy	Estimate of ζ_n(rad)	Diameter d (μm)
Bateman *et al.* (1972)	1	Opacification of the murine lens	430 keV	150	3
	2	Opacification of the murine lens	1.8 MeV	840	2
	3	Opacification of the murine lens	14 MeV	260	3
Sparrow *et al.* (1972)	4	Mutations of *Tradescantia* stamen hairs (blue to pink)	430 keV	800	1.8
Vogel (1969)	5	Mammary neoplasm in the Sprague-Dawley rat	Fission	2200	1
Biola *et al.* (1971)	6	Chromosome aberrations in human lymphocytes	Fission	1300	1.4
Hall *et al.* (1973)	7	Growth reduction of *Vicia faba* root, aerated	3.7 MeV	600	2
	8	Growth reduction of *Vicia faba* root, anoxic	3.7 MeV	2000	1.3
Field (1969)	9	Skin damage (human, rat, mouse, pig)	6 MeV	1200	1.5
Withers *et al.* (1970)	10	Inactivation of intestinal cryptic cells in the mouse	14 MeV	800	2
Smith *et al.* (1968)	11	Various effects on seeds of *Zea mays*	Fission	400,000	0.15

only few experimental systems permit the necessary statistical accuracy at small doses. It is however, 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 1 to several micrometers diameter, one deals with 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 examples of 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 which extends over an extremely wide range of doses, the studies on the opacification of the murine lens may be presented in detail. Figure 10 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 various other observations which support the assumption that the primary cellular damage is proportional to the square of the specific energy in sites whose diameter is of the order of 1 to several micrometers.

These observations formed the basis of what has been termed the theory of dual radiation action, which is an interpretation in terms of the site concept or in terms of the interaction of pairs of cellular lesions with a characteristic interaction distance of the order of magnitude of micrometers. This is covered in an earlier

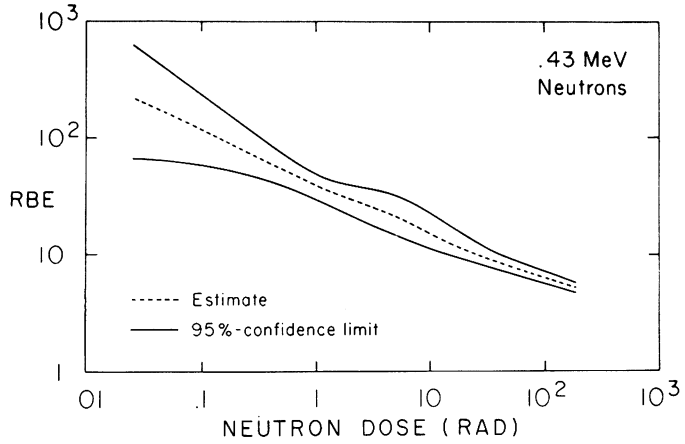


FIGURE 10. RBE. of 430 keV neutrons relative to X-rays for the induction of lens opacification in the mouse (Bateman *et al.*, 1972; Kellerer and Brenot, 1973).

publication (Kellerer and Rossi, 1972). In the next section, the main conclusions of the preceding section will be applied to experimental findings relevant to the etiology of tumors.

5. *Applications to Radiation Carcinogenesis*

The subjects covered in the preceding sections of this chapter are of relatively recent origin. In particular, the theory of dual radiation action was developed only a few years ago. Practical applications are few in number, and the results tend to be notable more because of the proof they adduce for the theory than because of their disclosure of new facts. In each of the two instances where data relating to radiation carcinogenesis were analyzed, other useful results have nevertheless been obtained. In the case of an experimental animal tumor it could be deduced that the observed incidence depends not only on lesions in individual cells but also on radiation-induced changes in several cells or in tissues, and in an analysis of data on the induction of human leukemia conclusions were reached which are of importance to risk estimates.

5.1. *Mammary Neoplasms in the Sprague-Dawley Rat*

Bond *et al.* (1960) and Shellabarger *et al.* (1969, 1974) discovered that moderate doses of γ-radiation or X-rays produce a high incidence of mammary neoplasms in the Sprague-Dawley rat. The majority of the tumors are not malignant (fibroadenomas), but an appreciable proportion are adenocarcinomas. It was found that the incidence of these tumors is approximately proportional to γ- or X-ray dose up to a few hundred rad, where the incidence curve flattens and finally declines when doses in excess of 500 rad are applied. This is a phenomenon that is common in radiation carcinogenesis. It was also concluded that the effect is not abscopal—i.e., it requires irradiation of the tissue in which 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. A related finding is that the incidence of multiple tumors follows Poisson statistics, supporting the view that the neoplasms arise independently from individual foci.

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-rays or X-rays, particularly at low levels of incidence. A number of comparatively large-scale experiments in which Shellabarger employed 0.43 MeV neutrons down to doses as low as about 0.1 rad yielded results of sufficient accuracy to permit the analysis shown in Fig. 11. This shows the dependence of RBE on neutron dose and the confidence limits of this dependence. The broken line indicates the best estimate of the dose–RBE relation. The vertical bars cover those ranges of RBE which can be excluded with statistical certainty exceeding 95%.

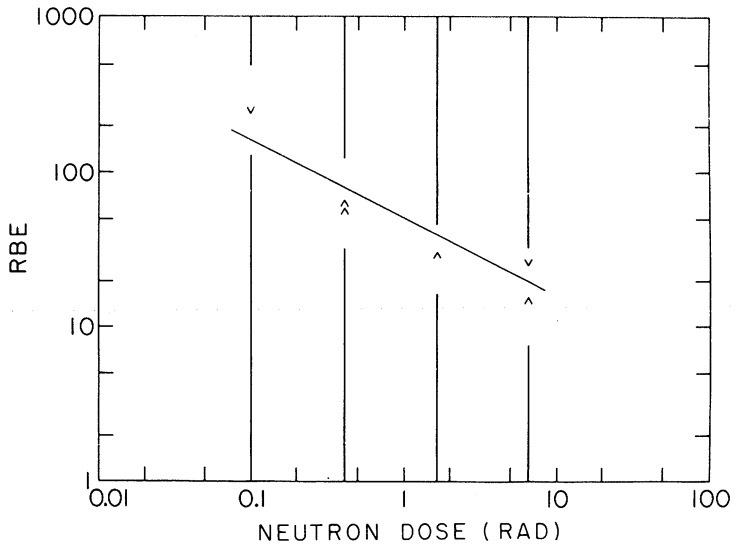


FIGURE 11. RBE of 430 keV neutrons relative to sparsely ionizing radiation for the induction of mammary tumors in the Sprague-Dawley rat (Shellabarger *et al.*, 1974). The vertical bars indicate the ranges of RBE values which are excluded with statistical significance exceeding 95%.

The statistical analysis is based on the direct comparison of the effect of each neutron dose applied in the experiment with that of each X-ray dose. Details of this procedure are described by Kellerer and Brenot (1973). Earlier results obtained by Bond *et al.* (1960) with γ-rays are also utilized in the analysis. As seen from Fig. 11, the slope of $-1/2$ postulated by the theory of dual radiation action applies for RBE values as high as 100, where the incidence approaches levels that are at the border of experimental detection.

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 (although certainly not sufficient) condition is that the time course of incidence be the same. The curves in Figs. 12 and 13 represent the mean number of mammary tumors per animal as a function of time after exposure to neutrons and to X-rays. No systematic differences in the time course of incidence are suggested by these results. Except near the levels of spontaneous incidence, where the dependence appears to be somewhat steeper, the curves seem to be consistent with straight lines of slope 1. Since in these logarithmic plots the abscissa scale has been chosen twice as wide as the ordinate scale, straight lines of slope 1 would correspond to proportionality between the mean number of tumors and the square of the time after irradiation. Such a relation would be obtained if the tumor rate were constant during the interval of observation. Although further experimental studies and a detailed statistical analysis might lead to some modifications, one must conclude that at present there is no evidence of characteristic differences

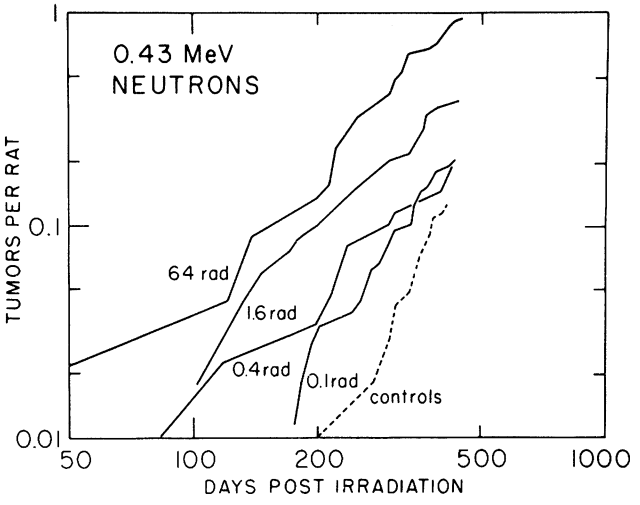


FIGURE 12. Mean number of mammary tumors in the Sprague-Dawley rat as function of time after exposure to different doses of 430 keV neutrons (Shellabarger *et al.*, 1974). The representation is logarithmic; the unit chosen for the abscissa scale is twice as wide as that for the ordinate.

between the neutron- and the X-ray-induced effects. This is further supported by the analysis of the relative frequency of different tumor types produced by the different radiations (Shellabarger *et al.*, 1973).

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

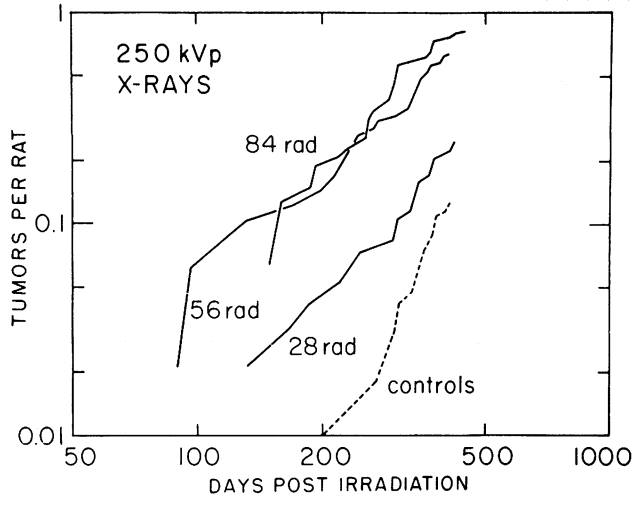


FIGURE 13. Mean number of mammary tumors in the Sprague-Dawley rat as function of time after exposure to different doses of 250 kVp X-rays (Shellabarger *et al.*, 1974). The representation is logarithmic; the unit chosen for the abscissa scale is twice as wide as that for the ordinate.

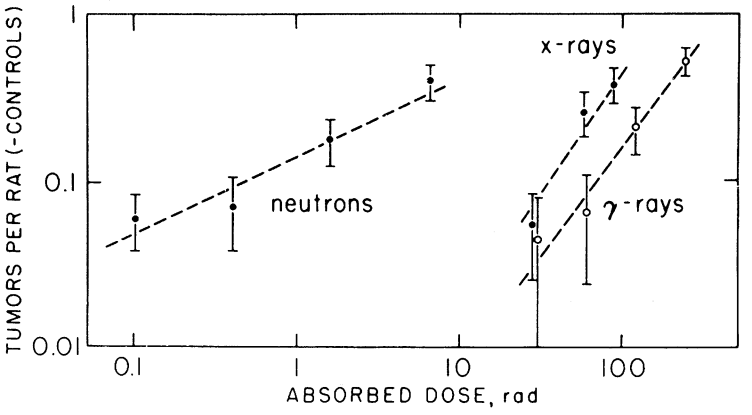


FIGURE 14. Mean number of tumors per rat minus spontaneous incidence 400 days after exposure to neutrons (Shellabarger *et al.*, 1973), to X-rays (Shellabarger *et al.*, 1974), and to γ-rays (Bond *et al.*, 1960). The broken lines have no mathematical significance and are merely inserted to indicate the trend of the data. The vertical bars represent the standard deviations.

the mechanism of tumor inductions. The RBE–dose relation shown in Fig. 11 puts certain constrictions on the dose–effect relations for both X-rays and neutrons, although it does not determine their shape. If dose–effect relations are plotted explicitly as in Fig. 14, it is at once apparent that in this logarithmic representation the slope of the line for neutrons is less than 1, indicating that tumor production is not proportional to dose. This has become especially indicative with the recent availability of the data at very low doses, although even when an earlier analysis (Rossi and Kellerer, 1972) was carried out it was already necessary to conclude that the nonproportionality extends to doses that are so low that multiple traversal of cells is improbable. As mentioned above, the average number of particles traversing a cell nucleus is of the order of 1 for a neutron dose of about 25 rad. The lowest dose used in these experiments was about 0.1 rad, where only one in about 240 cell nuclei experiences the traversal by a neutron secondary; i.e., where the mean number, $\bar{n}$, of events per nucleus is roughly 0.004. If one considers the whole cell, the mean number, n, of events at 0.1 rad 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 vs. dose the slope of the resulting curve can never be smaller than $1 - \bar{n}$, provided that the effect depends only on the lesions in individual cells. Because in the present experiments the slope is considerably less, one must conclude that the observed incidence depends not only on the transformation of individual cells but also on radiation-induced changes in adjacent cells or on dose-dependent changes at the tissue level. At this time, no further statement can be made, but one may consider factors such as virus release by lysed cells with some local saturation or with attendant increase in immune reactions even at low dose levels of the order of fractions of a rad.

For man, the principal somatic hazard of ionizing radiation is carcinogenesis. Although it is now established that radiation can induce a variety of neoplasms in humans, the most frequently observed and the most extensively documented is leukemia. However, even in this disease the available information is insufficient to permit firm conclusions regarding the magnitude of the hazard, especially for doses near the maximum permitted by various recommendations, codes, or laws.

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

Another important aspect of these observations is that they were obtained for two types of radiations. In Hiroshima, a substantial neutron dose was delivered which 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). Dosimetric information for both radiations has been derived for both cities. What is said to be the "dose" or "air dose" is actually the tissue kerma in free air (see Section 2). At any distance from the epicenter of the explosion, the free-air ratio of neutron kerma to γ-ray kerma must have been higher than the ratio of the respective absorbed doses in the blood-forming organs since the overlying body tissues attenuate neutrons more strongly than the prompt γ-radiation emitted by a burst. This necessitates a correction of perhaps a factor of 2 for the absolute value of the RBE but should have a minor effect on relative values.

The availability of data for both neutron and ζ-radiations 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 leukaemia. This is a question of importance to risk estimates, because a dose-dependent RBE makes it impossible that such estimates can be meaningfully carried out by linear extrapolation from high doses for *both* radiations since the shapes of their dose–effect curves must be different.

The establishment of the RBE–dose relation is difficult not only because of considerable statistical uncertainties but also because the neutrons at Hiroshima were accompanied by γ-radiation which in terms of kerma was from about 1.5 to 2.5 times as intense between the inner and the outer perimeters of the zone of interest in this analysis. Consequently, if it is assumed that all of the radiation at Hiroshima was neutrons and all the radiation at Nagasaki was γ-rays, one obtains an underestimate of the neutron RBE which becomes progressively larger at lower values of kerma. Accordingly, one observes less of an increase of RBE at low levels of effect than if one dealt either with a pure neutron radiation or with a constant mixture of neutrons and γ-rays.

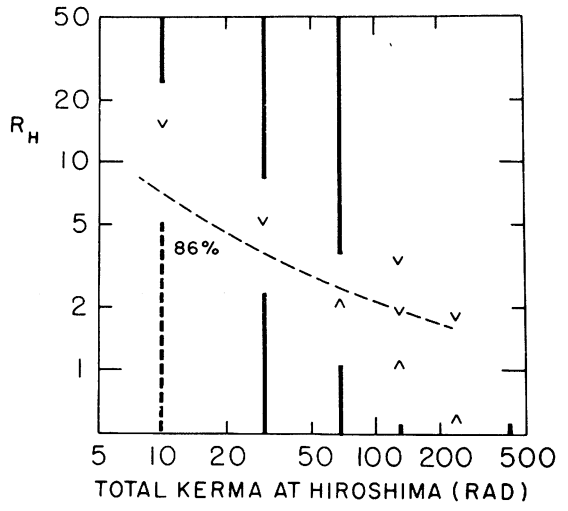


FIGURE 15. Relative biological effectiveness of the radiation in Hiroshima for the induction of leukemia compared to that in Nagasaki as a function of kerma in Hiroshima (Rossi and Kellerer, 1974). The bars indicate those values which can be excluded with 95% confidence; the broken bar stands for a level of confidence of 86%. The broken curve is the result of a least-squares fit.

If one nevertheless analyzes the data with this assumption utilizing the same technique discussed in the previous section, one obtains the relation depicted in Fig. 15.* Although the most crucial of the limits (the lower bound as 10 rad) is established with 86% rather than 95% significance, this value seems sufficiently large, particularly in view of the conservative assumption made. It may thus be concluded that the neutron RBE for human leukemia, like that for all other somatic effects investigated, increases with decreasing level of effect.

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

$$I(K) = I_0 + aK + bK^2 \tag{24}$$

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 most probable value for a turned out to be negative, and only the quadratic component was therefore considered in the further analysis. It thus appears that

* 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. This has 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.

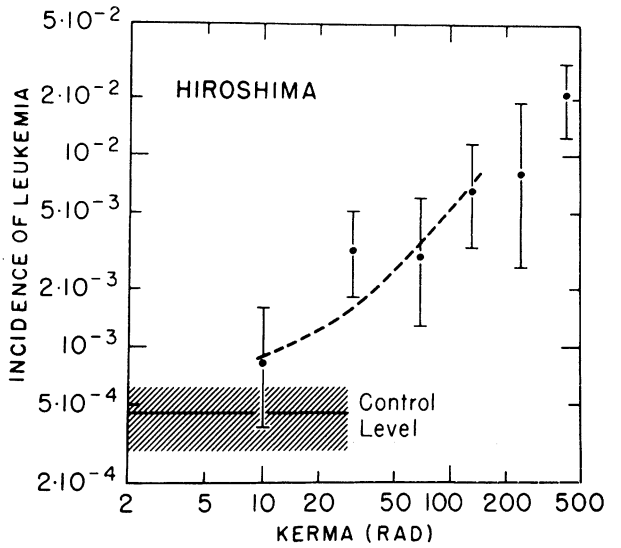


FIGURE 16. Incidence of leukemia for the period from October 1950 to September 1966 vs. kerma at Hiroshima (Rossi and Kellerer, 1974). The bars represent 95% confidence ranges; the shaded area is the 95% confidence region for the unirradiated population of the city. The broken curve is the result of a least-squares fit.

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 incidents 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

$$I = I_0 + aK_N + bK_x^2 \tag{25}$$

where K_N and K_x are the kermas of neutrons and X-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 Figs. 16 and 17 together with the observed incidences and their standard

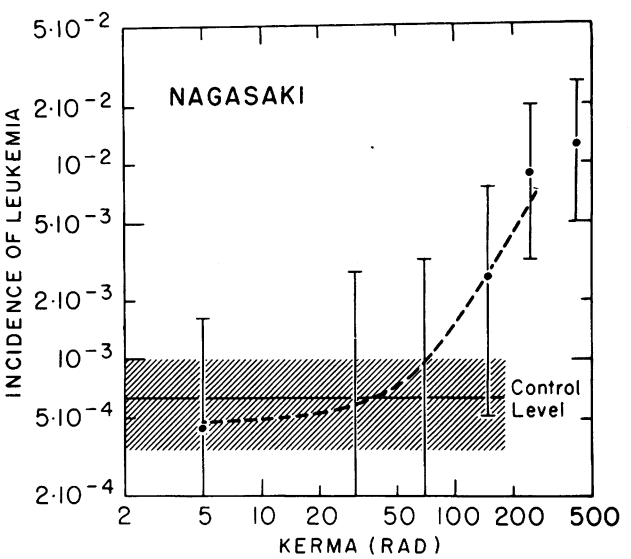


FIGURE 17. Incidence of leukemia for the period from October 1950 to September 1966 vs. kerma at Nagasaki (Rossi and Kellerer, 1974). The bars represent 95% confidence ranges; the shaded area is the 95% confidence region for the unirradiated population of the city. The broken curve is the result of a least-squares fit.

deviations. The resulting values of the estimated parameters are $I_0 = 4.8 \times 10^{-4}$, $a = 2.2 \times 10^{-4}$ (rad^{-1}), and $b = 8.7 \times 10^{-8}$ (rad^{-2}). The data are for leukemias of all types and relate to incidence within the observational period from October 1950 to September 1966. Because of the small numbers involved, a meaningful study of individual types seems impractical.

It is noteworthy that in the two types of carcinogenesis considered in this section the dose–effect relations turn out to be quite different. The question of whether this is because the experimental animal tumors have a high spontaneous incidence while the normal incidence of human leukemia is much lower is intriguing, but it cannot at this time be answered with any certainty.

6. *Appendix*

In the following, a formal derivation will be given of the theorem which 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

$$E(D) = \sum_{\nu=1}^{\infty} p_\nu E_\nu = \sum_{\nu=1}^{\infty} e^{-\phi D}(\phi D)^\nu / \nu! E_\nu \tag{A1}$$

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

$$c = \frac{D}{E(D)} \sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} \left(\frac{(\phi D)^{\nu-1}}{(\nu-1)!} \phi - \phi \frac{(\phi D)^\nu}{\nu!} \right)$$

$$= \frac{\sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} [(\phi D)^\nu / \nu!](\nu - \phi D)}{\sum_{\nu=1}^{\infty} E_\nu e^{-\phi D} [(\phi D)^\nu / \nu!]} \tag{A3}$$

$$= \frac{\sum_{\nu=1}^{\infty} \nu p_\nu E_\nu}{\sum_{\nu=1}^{\infty} p_\nu E_\nu} - \phi D$$

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 which 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 $\sum_{\nu=1}^{\infty} \nu \pi_\nu$ is the mean number of events in those cells which 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 which 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 which is 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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