

ADVANCES IN MEDICAL PHYSICS

TABLE OF CONTENTS

Title Page and Acknowledgments	iii, iv
Organization	v, vi
Foreword	vii
Preface	viii
List of Contributors	ix

Symposium A, Physical Aspects of Radiobiology

<i>Chairman</i> , Harald H. Rossi	
The Time Factor in Radiobiology, E. W. Emery	1
Statistical Aspects of Radiation Action, A. M. Kellerer	13
Physical Energy Transfer and Biological Effect, C. A. Tobias	28

Symposium B, Measurement of Human Physiological Responses

<i>Chairman</i> , Herbert M. Parker	
Galvanic Cell Transducers for the In-Vivo Measurement of Oxygen Tension, J. S. Clifton	51
Measurement of Human Physiological Responses, H. S. Wolff	65
Some Physical Methods of Skeletal Evaluation, J. R. Cameron	79
Automated Techniques for the Bedside of the Critically Ill, M. H. Weil	91

Symposium C, New Frontiers I

<i>Chairman</i> , Edward W. Webster	
Holography and Its Application to Medical Physics, E. Feleppa	99
Cardiac Assistance by the Intra-Aortic Balloon, A. R. Kantrowitz	112
The Scanning Electron Microscope: Multi-Radiation, Non-focused Imaging, T. L. Hayes	124

Symposium D, Physical Aspects of Nuclear Medicine

<i>Chairman</i> , John S. Laughlin	
New Formulation of Earlier Results in the Kinetic Theory of Tracers. Application to Various Problems in Metabolism, C. Kellershohn ..	141
Modulation Transfer Function and Its Application to Radioisotope Scanning, T. D. Craddock	164
An Image Intensifier Isotope Camera Capable of Pulse Height Dis- crimination, M. M. Ter-Pogossian	179

Symposium E, Ultrasound

<i>Chairman</i> , R. Stuart Mackay	
Ultrasonic Imaging with Image Cameras, Holography and Colour-Phase Representation, C. N. Smyth	185
Ultrasonic Imaging and Decompression Sickness Studies, R. S. Mackay	204

Symposium F, New Frontiers, II

<i>Chairman</i> , Michel M. Ter-Pogossian	
Photochemistry of Excited States of DNA Components, H. E. Johns . .	217
Physical Limits of Manned Underwater Activity, H. R. Schreiner . . .	232
Man's Role in Inner Space, G. F. Bond	255
Presentation on Professional Aspects of Medical Physics and Interdisciplinary Relationships	
Cooperative Programs and the Status of Medical Physics, R. J. Shalek	273

Symposium G, Information Processing

<i>Chairman</i> , Gordon L. Brownell	
Compartmental Modeling, M. Berman	279
Resolution Recovery in Radioisotope Scans, S. M. Pizer	297
Application of Computers to Treatment Plan Radiation Dose Com- putation, J. G. Holt	305
Optical Image Processing, B. J. Thompson	315
Generalized Grayness Profiles As Applied to Edge Detection and the Organization of Chromosome Images, M. L. Mendelsohn	327
A Model of a Computer-Based System for Medical Record-Keeping by Physicians, R. A. Greenes	342

STATISTICAL ASPECTS OF RADIATION ACTION

Albrecht M. Kellerer

Early radiation biology had a particular attraction to the biologist as well as the physicist. The kinetics of radiation action seemed to indicate that in the living cell a single atomic or molecular random event can express itself in a macroscopic result. This induced high expectations into the use of ionizing radiation as an instrument to probe the structure of living matter. Today, some fifty years later, these expectations have been somewhat dimmed. Still the questions asked in the early days of radiation biology have lost little of their interest. It may therefore be useful to point out the directions which radiation biology has been taking after the setbacks encountered by some of the old ideas.

First some of the concepts may be recalled which have shaped quantitative radiobiology in the past. Viruses and certain bacteria show an exponential decrease of survival probability with radiation dose. The exponential relation is characterized by the fact that equal dose increments decrease the fraction of survivors by equal ratios. In analogy to the kinetics of radioactive decay this has been taken as an indication that the radiation damage is not due to continuous accumulation of energy absorption. Instead it was assumed that the effect is brought about in a single critical event. These hypothetical events were not identified by Dessauer (1), who tentatively spoke of 'point heat'. Crowther (2), however, who simultaneously developed the statistical concept of radiation action proposed that the critical events were in fact single ionizations.

The single hit concept was the starting point for a formalism which is based on the assumption that the statistical fluctuations of energy deposition and not the reaction kinetics of the cell determine the survival curve. It was assumed that this is true not only for exponential dose effect curves but also for 'sigmoidal' curves which were considered to be the expression of damage accumulated in a certain number of statistically independent critical random events.

The statistical models of radiation biology have been discussed in a vast literature of their own. Biologists have always been hesitant to accept the highly formal analysis of dose effect relation which has been developed in target theory (3). But it was only in recent years that the weak points in the conventional anal-

ysis have been pinpointed, and that alternative approaches have been indicated (4). There are two essential aspects: First, the conventional formalism is mathematically inconsistent. Secondly, it is wrong to assume that all random factors which express themselves in the survival curve are connected with the fluctuations of energy deposition; additional stochastic factors are equally important.

In order to outline the implications of these facts there will be first some general remarks on the analysis of the random factors involved in radiation action. Then the special problem of the statistics of energy absorption will be discussed. Finally some of the random factors characteristic for the biological processes will be dealt with.

The dose response relation as an expression of statistical processes.

The conventional evaluation of dose effect curves has been based on the assumption that the biological effect, e.g. cell inactivation, is due either to a single energy absorption event or to a succession of random events of energy absorption in the cell. One obtains characteristic dependencies of inactivation probability on dose if one assumes different numbers of events for the critical threshold. There have been various methods to distinguish the resulting curves and to infer the critical threshold values. The mathematical techniques employed have not always been the most efficient ones, but the basic ideas have been the same.

One may summarize the reasoning by the statement that, if the critical number of statistically independent events is n , then one obtains a so-called gamma-distribution of order n as reaction curve. The inactivation probability, $P(D)$, as a function of absorbed dose is then given by:

$$P(D) = \int_0^D k e^{-kD} \frac{(kD)^{n-1}}{(n-1)!} dD \quad (1)$$

where k is the mean number of critical events per unit dose.

Equation (1) is more commonly given in the form:

$$P(D) = \sum_{\nu=0}^{n-1} e^{-kD} \frac{(kD)^{\nu}}{\nu!}$$

It is easy to show that both forms are equivalent. Equation (1) has the advantage that it clearly shows that the differential distribution, $p(D)$, of the inactivation dose is indeed a gamma-distribution:

$$p(D) = \frac{dP(D)}{dD} = k e^{-kD} \frac{(kD)^{n-1}}{(n-1)!} \quad (3)$$

Fig. 1 represents the inactivation curves. Fig. 2 represents the corresponding differential distributions of inactivation dose. Probability theory offers simple ways to distinguish these curves and to infer the characteristic index n . The easiest method is a comparison between the mean inactivation dose and its variance. If few random

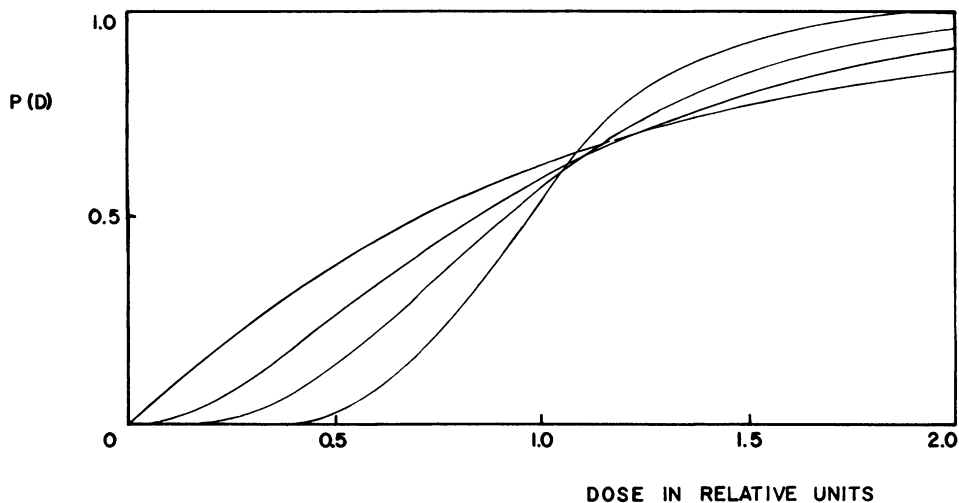


Fig. 1. Reaction curves according to equ. (1) or (2). The curves are integral gamma-distributions of order 1, 2, 4, and 10. The dose is in arbitrary units.

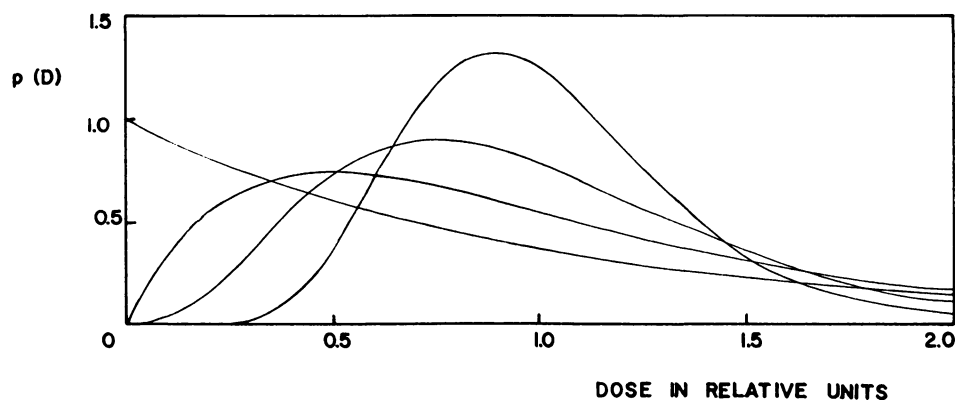


Fig. 2. Probability densities of inactivation dose according to equ. (3). The curves are the derivatives of the curves in fig. (1), i.e. they are gamma-distributions of order 1, 2, 4, and 10.

events are sufficient to bring about the effect, the random nature of the process is strongly expressed, and the response curve instead of being a step-like function must be fairly washed out. If a great number of events is involved in cell inactivation, the random fluctuations are less expressed and one may obtain a curve which is rather steep. Thus the standard deviation, σ , of the inactivation dose as compared to the mean inactivation dose, \bar{D} , indicates the influence of the random factors involved in the process. The mean inactivation dose is defined as:

$$\bar{D} = \int_0^{\infty} D p(D) dD \quad (4)$$

which in the special case of the gamma-distribution leads to:

$$\bar{D} = k \int_0^{\infty} D e^{-kD} \frac{(kD)^{n-1}}{(n-1)!} dD = \frac{n}{k} \quad (5)$$

The variance, σ^2 , is defined as:

$$\sigma^2 = \int_0^{\infty} (D - \bar{D})^2 p(D) dD = \int_0^{\infty} D^2 p(D) dD - \bar{D}^2 \quad (6)$$

and with equation (3) one obtains:

$$\sigma^2 = k \int_0^{\infty} D^2 e^{-kD} \frac{(kD)^{n-1}}{(n-1)!} dD - \frac{n^2}{k^2} = \frac{n(n+1)}{k^2} - \frac{n^2}{k^2} = \frac{n}{k^2} \quad (7)$$

Thus the ratio D^2/σ^2 which measures the *steepness* of the curve is equal to the critical number of events:

$$\frac{\bar{D}^2}{\sigma^2} = n \quad (8)$$

It is remarkable that only the first two moments of a dose-response curve have to be known to obtain the parameter n .

The mean value, \bar{D} , depends on the cross-section constant k (see equ (5)). The absolute position of the curves is therefore irrelevant to the statistical analysis. The curves in fig. 1 and 2 are arbitrarily normalized to the same mean value. The width of the curves indicates how the relative deviations from the mean inactivation dose \bar{D} decrease with increasing value of n .

These considerations have been dealt with in some detail because they have actually been applied, though not always in the most consistent mathematical form, to a number of different fields. The application to the action of ionizing radiation has already been mentioned; an excellent survey of this application has been given by Zimmer (5). Independently the formalism has been used to derive the critical number of light quanta to evoke a light sensation in the human eye (6,7,8,9). If one plots observation frequency as a function of the intensity of a short light flash one should see the influence of the statistical fluctuations of the number of photons striking the retina. By analysing this curve the threshold numbers have been determined by various workers, who have found various values for the critical numbers; an example is given in figure 3. A third application has been in pharmacology where the possibility has been discussed that toxic effects on cellular units are the result of the action of a small number of molecules of the pharmacological agent (10,11,12). One has seriously considered this even in such an extreme case as the killing of the large alga *Nitella* by copper-chloride. It is quite interesting to follow the discussion of this problem for example in Clark's chapter in the handbook of pharmacology (13). Apparently quite similar discussions have been aroused by the respective application in radiobiology, physiology, and pharmacology, but the

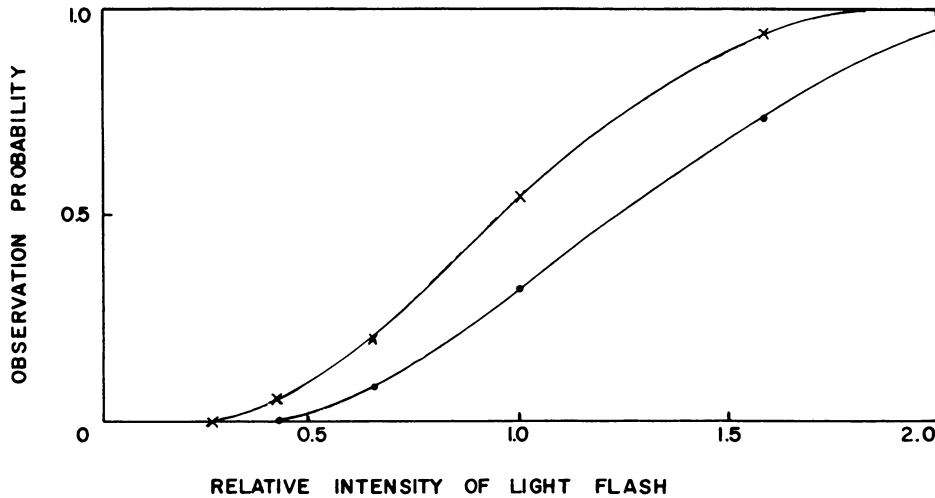


Fig. 3. Observation frequency as function of the intensity of short light flashes. The curves are from data presented by HECHT et al (7).

workers in these different fields seem to have been largely unaware of the parallel approaches.

The weakness of the formal interpretation of the dose-response relations is that it is based on an idealized model. In general—some of the light quanta experiments may be an exception—one had been well aware of this limitation (3,5). Specifically the biological variability of the exposed objects and the possible differences in the hypothetical critical events have been discussed. But more or less tacitly it was assumed that the sum total of all the deviations from the idealized model might cancel out, so that the analysis, though blurred by additional parameters, might still be essentially right. This assumption would imply that if for example the attention of the observer in the light quanta experiments fluctuates this would on the average cancel out. Similarly if the units of an irradiated population responded differently the compound curve would have a shape which corresponds to the mean of the individual hit numbers and threshold values.

This confidence in the mathematical formalism proved to be fundamentally wrong. It has been shown that, far from averaging out, all deviations from the idealized model work in one direction. The general effect is one of broadening the response curve i.e. increasing its relative variance. In other words, the deviations suggest a critical number which is too low. This can be illustrated by the two curves of fig. 3. The parameters n deduced from these curves are 6 and 7. If one pools the data from the two experiments and calculates the parameter n for the averaged curve one does not obtain $n = 6.5$. Instead one finds a value of approximately 3. Thus the relative variance is sharply increased by averaging the two experiments. It is interesting to note that the increase is appreciable even in this case where the difference in the reaction of the test objects is moderate. If one

studies the response of mammalian cells to ionizing radiation in the various phases of their generation cycle one finds differences which are much more expressed. The relative variance of the dose effect relation must then be largely due to the fluctuations in cellular sensitivity. The fact is indeed brought out in studies with synchronized cell cultures. But even with a well synchronized population one can not assume that σ^2 solely reflects fluctuations in energy deposition.

Therefore all one can formally deduce from a dose-response relation is a lower limit for the number of interacting random events. This is true in the light quanta experiments for the threshold of vision, it is clearly true in pharmacological reaction curves, and it applies to radiation biology as well. Formally one can always deduce the order of the reaction kinetics which expresses itself in the dose-response curve. But this is in fact a purely formal index influenced by the full complexity of the reaction of the biological object to an irradiation. It is rather important to keep this in mind as it has too often been overlooked. In the determination of cross-sections one is well aware of the fact that these are in general formal indices which represent only a lower limit for the true physical cross-section. One should be equally aware that the same is true for the reaction order of a survival curve, evaluated on the basis of whatever particular model.

There is little reason to believe that the interplay of fluctuations of energy loss, of biological variability, and of statistical behaviour of the cell can be adequately described either by equation (1) or any of its variations employed in target theory. Curve fitting formulae with or without target theory interpretation have brought little progress to radiation biology. Application of the theory of stochastic processes seems more promising and has already lead to some useful results (4). One may just note one particular finding which is connected to the discussion on the moments of the dose effect curve given earlier in this paragraph. It has been proved in full generality that for an experimentally determined relative variance $V = \sigma^2/\bar{D}^2$ the mean number of statistically independent energy absorption events involved in the effect can not be smaller than V^{-1} . This is a result not without interest. From certain experiments on the x-ray inactivation of mammalian cells in S-phase one can for example deduce that more than 4 energy absorption events (passages of ionizing particles) must on the average be involved in the inactivation process. This is a valuable conclusion, but it is, of course, a rather limited statement as compared to the original idea of deriving an actual 'event number'. The example of the quantum vision experiments makes this particularly clear. The experiment has been designed to show how *few* quanta are needed to evoke a light perception. Formal analysis, however, can only indicate how *many* quanta must be at least involved in the process, the actual number being perhaps much higher. This means that formal analysis has to be supported by additional physical evidence if one wants to bracket the number of interacting random events more closely. In the case of the light quanta experiments one simply has to determine the actual mean number of photons which hit the retina (7,8,9). With ionizing radiations the physical measurements are more complex. In fact they form a discipline of their own for which the name microdosimetry has come into use.

The new approach to the energy absorption problem

A realistic treatment of the statistics of energy absorption has to be based on the analysis of the microscopic spatial patterns of energy deposition with different kinds of ionizing radiation. These patterns are too complicated to be adequately represented by either a Poissonian distribution of statistically independent ionizations or by straight, continuous tracks of charged particles as suggested by the theory of linear energy transfer (LET). A theoretical analysis (4,14) of these patterns involves various factors. Some of these are: track segment length in a sensitive volume or in a region of interaction of free radicals, curled shape of the particle track, varying stopping power and, last but not least, the discontinuous nature of energy deposition along the track. The theoretical analysis of the interplay of these factors has to rely on large scale electronic computing, it must also be based on quantum mechanical data on collision cross sections which are as yet insufficient. It is for this reason that H. H. Rossi and co-workers used a more direct experimental approach when they introduced the concepts of microdosimetry (15,16). They simulated the processes of energy deposition in tissue by employing spherical proportional counters filled with tissue equivalent gas and surrounded by tissue equivalent walls, in order to directly determine the probability distribution of the energy deposited in microscopic volumes. This experimental analysis of the patterns of energy absorption has been performed for various kinds of ionizing radiations and it has lead to very useful conclusions. The resulting probability distributions of specific energy—or, in more naive terminology, local dose—are limited to regions not much smaller than one micrometer. But there seems to be no reason why these distributions cannot also be derived, theoretically or experimentally, for smaller regions. Moreover it appears that in one of the systems of foremost interest to radiation biology, the mammalian cell, the biologically relevant interactions of energy depositions cover distances of the order of magnitude of a micrometer.

The case of a tissue sphere of $0.5\ \mu\text{m}$ diameter exposed to ^{60}Co γ -radiation is taken as an example in fig. 4 to illustrate the probability distributions of specific energy, z , and their dependence on dose D . Without going into the actual definitions of the basic quantities and probability distributions in microdosimetry one may point out a few approaches in order to characterize the possible applications to radiation biology.

One of the most straightforward applications is the one which is used to show that in certain cases exponential survival curves are indeed due to single events. An outstanding example is the exponential survival curve derived for mouse spermatogonia *in vivo* with neutron irradiation (17). Microdosimetry data show that at the 37% survival the mean number of events in the whole cell is near to one. That indicates that with this particular radiation and in this special cellular system exponential survival is indeed an expression of single hit inactivation; it further indicates that a heavy charged particle kills the cell it passes even if it does not penetrate its nucleus. One might still assume that cell-killing in these *in vivo* exper-

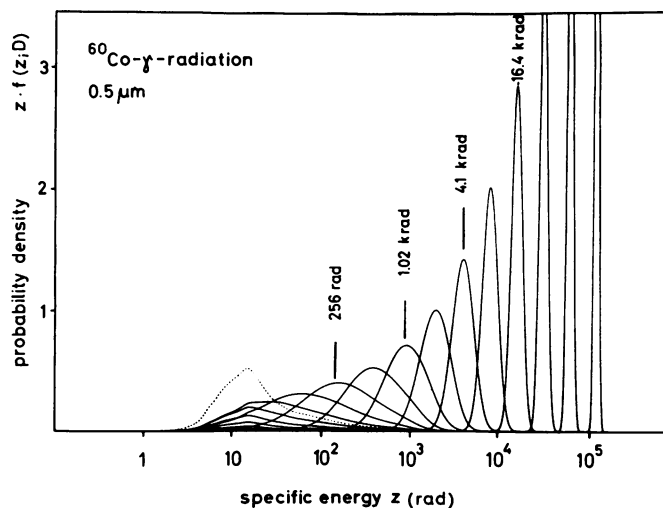


Fig. 4. An example of the distributions of specific energy, z . The curves correspond to the irradiation of a tissue sphere of $0.5 \mu\text{m}$ diameter with ^{60}Co - γ radiation. The dose, D , is given as parameter. For details see (14).

iments is also influenced by humoral factors in the tissue in which the spermatogonia are imbedded. Thus the case for a single hit process is not established beyond all doubt. What microdosimetry data tell, however, is that cell killing in this instance is certainly not due to a multi-absorption event process inside the cell.

One must, of course, be careful not to blindly extrapolate these findings and to conclude that all exponential curves are due to single-absorption event action. This point will be somewhat further discussed in the next section.

A second type of application is somewhat more indirect. It involves the assumption of a well-defined sensitive site in the biological object. Therefore it can only be accepted with the same reservations which one may hold regarding this assumption. An example is the analysis of the mutation frequencies in maize produced by neutrons of different energies (18). The model chosen by Rossi and Smith for evaluation of these experiments is that of a sensitive volume with a certain threshold of absorbed energy for induction of this specific mutation. A comparison of the probability distributions of energy absorption for the different neutron energies with the observed mutation frequencies leads to the conclusion that for a region of diameter $0.3 \mu\text{m}$ and only for this diameter one obtains one and the same critical threshold regardless of radiation quality. There is support for these results in findings which Sparrow, using a different experimental endpoint, has obtained in recent years on various plant species (19). His results suggest that an irradiated plant is killed if a critical amount of energy is deposited in its chromosomes. The notion that the chromosomes are the gross-sensitive structures in radiation effects on plants conforms with the assumption that the critical distances of interaction of energy deposition are of the order of magnitude of a micrometer.

The probability distributions of local energy density can, by the way, also be used to demonstrate that the simple model of a spherical sensitive volume is to be rejected in some cases. An example are studies of lens opacification (20). It is noteworthy that here the analysis leads to the concept of two adjacent sensitive sites or to the assumption that biological effects depend not only on a certain critical energy threshold but also on a certain spatial extension of the energy deposition. This idea is in agreement with models Neary has proposed in recent years (21) to explain the induction of chromosome aberrations in *Tradescantia* by radiation of different ionization density.

Microdosimetry may also be applied in a more general fashion without actual reference to any particular model of radiation action. As was pointed out earlier, the dose response function is not merely a reflection of the energy absorption processes. Other statistical factors may as well be involved. The objects in the irradiated population differ or are in different phases of the cell-cycle. Moreover the reaction chain from the primary steps of energy deposition to the formation of the initial lesion and to the final manifestation in a chromosome aberration or in a mitotic failure involves stochastic elements. This is specifically so if one deals with sigmoidal dose response functions which express a continuous or stepwise accumulation of damage. In these cases it may be appropriate not to try to explain the detailed shape of the curve. Instead one may analyze the relative variance of the observed survival curves on the basis of microdosimetry data. The variance of the dose response curve is only partly due to the fluctuations of energy absorption. In general one cannot split up the observed variance according to the different stochastic factors involved, thus one may merely conclude that the variance due to the energy absorption statistics is equal or smaller than the one which has been experimentally observed. The relative variance of the local energy density distributions is largest for the smallest volumes. It is therefore possible to derive a lower limit for the diameter of the sensitive sites below which the fluctuations of energy absorption alone would exceed the total relative variance of the survival curve (4). From inactivation experiments on mammalian cells with Cobalt gamma radiation and x-rays one concludes in this way that the gross sensitive volume cannot be smaller than $1\text{ }\mu\text{m}$. Otherwise the statistical spread due to the fluctuations of energy absorption would be greater than the observed variance of the dose response curve. This is a finding which shows that microdosimetry can be applied where the classical formalism for the analysis of sigmoidal dose response curves fails.

In order to illustrate this fig. 5 gives two examples of inactivation curves obtained on cultures of isolated mammalian cells. Fig. 6 represents the curves which result if one asks for the probability to exceed a certain value of z at a certain dose. These curves refer to a spherical tissue region of a diameter of $1\text{ }\mu\text{m}$ and to x-rays. The inactivation curves are similar to the distributions of dose, D , necessary to reach a local dose z of about 300 rad in the $1\text{ }\mu\text{m}$ -sphere. This does not imply that one deals in fact with a sensitive site of $1\text{ }\mu\text{m}$ and with a well-defined threshold. The sensitive regions in the cell may be, and probably are, of complicated structure, they may also consist of many separate sites. The comparison of fig. 5 and fig. 6 merely shows that the gross sensitive area cannot

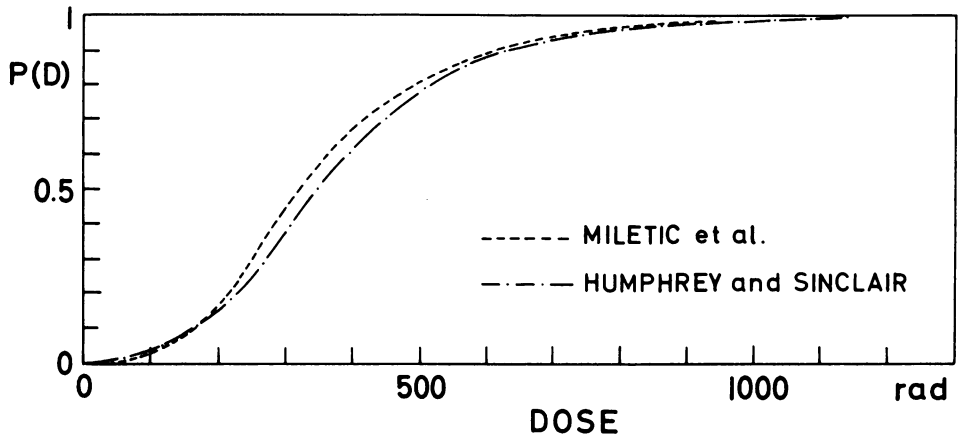


Fig. 5. Two examples of an inactivation curve of mammalian cells with x-rays. No confidence limits are given here; for an estimate of the uncertainty in the curve shape see (22) and (23).

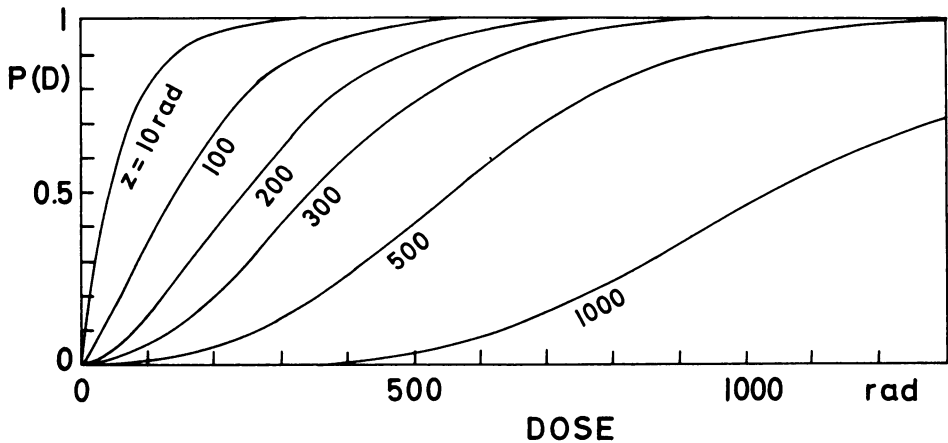


Fig. 6. Probability distributions of the dose necessary to reach certain limits of specific energy, z , in a $1\ \mu\text{m}$ diameter tissue-sphere. The threshold values of z are given as parameters. The curves are based on calculated distributions for 200 keV x-rays (4).

have a diameter less than $1\ \mu\text{m}$. The sensitive structures must be dispersed in the cell in such a way that they cannot be included in a sphere of diameter less than $1\ \mu\text{m}$.

Finally microdosimetry will certainly be of great importance in a more quantitative treatment of radiation chemistry problems. The concept of LET is severely limited even if one deals with the comparatively simple system of a homogeneous aqueous solution, and it has to be substituted by the analysis of the actual distribution of relative distances between tracks and between energy depositions along and around the tracks. Straggling, i.e. the discontinuous energy deposition along the particle track, is in many cases more important than LET (24). This accounts for the fact that the actual mean values of local energy concentration in regions of

roughly 1 μm diameter are very nearly proportional to the empirically determined values of the so-called quality factor while mean values of LET are not (25). Microdosimetry data can certainly lead to a much better understanding of RBE than the LET concept or any refined model based on it.

Randomness due to physiological processes

The cases in which a dose response curve reflects the statistical fluctuations of energy deposition are most characteristic for radiobiology. For this among other reasons, studies with densely ionizing radiations, where one usually obtains exponential dose effect relations, meet particular interest in the field. Conclusions from these experiments must, however, not be generalized to sigmoidal dose response curves without further evidence. It has been mentioned in the preceding paragraph that when a sphere of a diameter of 1 μm is exposed to x-rays, the fluctuations of energy deposition are large enough to explain all of the observed variance of the dose effect relation in the inactivation of mammalian cell cultures. But the fluctuations become much less expressed when one deals with larger volumes. If the whole nucleus of the cell were equally sensitive the statistical variations of energy deposition with x-rays would be rather insignificant. The variance of the dose effect response would then be entirely due to other stochastic factors.

A comparison of the experiments on unsynchronized and synchronized cell cultures shows that biological variability due to different sensitivity of the cell in the various phases of its generation cycle plays an important role. Work with synchronized cultures can at least partly eliminate this factor. It should, however, be noted that the application of the conventional formalism in these experiments may be particularly misleading. It has become more and more common in radiobiology to represent dose effect relations against a logarithmic scale of survival probability. Historically this has developed from certain target theory models. These models have been given up by now. But the use of the logarithmic scale of survival rate has been found practical since it stresses the low survival part of the dose effect curve, and this part is easiest to determine experimentally. For this reason the target theory plot of survival curves is still preferred. It is also preferred because the low survival part of the dose effect relation is specifically relevant, or is thought to be specifically relevant, to radiotherapy. While these may be perfectly good reasons to adhere to the conventional representation of dose effect curves, one should still be aware of the fact that this representation leads to a somewhat biased view of cellular radiation response. Quantities, as for example the so-called extrapolation number, are strongly influenced by small subpopulations of cells with different response. A mathematical analysis based on the moments of the dose response is therefore necessary in order to obtain improved quantitative data on the variations of cellular sensitivity and response characteristics within the generation cycle.

In an ideally synchronized cell culture of a perfectly homogeneous strain one still had to expect stochastic factors in the cellular response. It has been shown that even exponential survival curves may be explained on the basis of the stochastic behaviour of the cell (26,4). In fact, it is characteristic for a multi-component

system that its overall performance rate decreases exponentially if the small breakdown probability of its individual components is increased linearly. Mitosis, specifically in an *in vitro* system, is a critical phase in cell life and as such susceptible to an inherent lability. The spontaneous breakdown probability expresses itself in the limited plating efficiency in cell culturing and in occasional unsuccessful divisions throughout the development of a clone.

Till et al. (27) have introduced the concept that the survival curve may be an expression of the random pattern of successful and unsuccessful divisions within pedigrees of the irradiated cells. They performed Monte Carlo calculations and demonstrated the influence of this factor. The problem can also be solved analytically, it is in fact a well-known example of elementary probability theory, the so-called "gambler's ruin" problem (4). The solutions of this problem show that even with an exponentially decreasing division probability one obtains sigmoidal dose dependence for the probability to reach a certain clone size. This is illustrated by the curves of fig. 7, and it supports the observation that the shape of the survival curve indeed depends on the choice of the experimental end point. From observations on the pedigrees of cells it is obvious that the stochastic aspect of clone development must influence the dose effect relations. Experimental data on mammalian cells are as yet scarce, but detailed pedigrees obtained for yeast cells exposed to UV-radiation (28) agree well with the simple model that the division probability is reduced exponentially with dose (29).

Although extensive Monte Carlo studies have been performed on the basis of the stochastic approach to cellular kinetics (30), the results are still of qualitative rather than quantitative nature. It is not known at present how the observed variance of the various survival curves of mammalian cell systems splits up into the contributions of the different statistical factors involved.

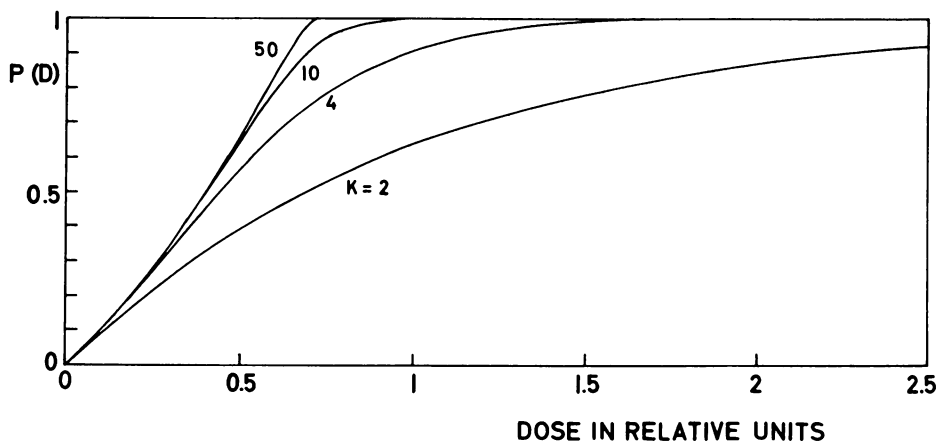


Fig. 7. The probability for failure of a single cell to develop into a clone of at least K cells. The probability is plotted as a function of dose, under the assumption that the division probabilities after irradiation decrease exponentially with dose. For a detailed discussion see (4).

A few concluding remarks on the meaning of the initial slope of the survival curve may demonstrate the ambivalence in possible interpretations. Whatever the detailed shape of the survival curve may be, the initial slope is in general looked upon as a direct indication that the biological effect can be brought about by a single act of energy deposition. This is one of the simplest and most widely accepted assertions concerning the dose effect relation. It can be generalized according to the fact that a dose response curve may be expressed as a power series at zero dose. If $P(D)$ is the fraction of cells which form no visible clone one obtains the following dependence on dose D :

$$P(D) = a_0 + a_1 \cdot D + a_2 \cdot D^2 + \dots$$

Then a_0 is the spontaneous failure rate, a_1 represents the initial slope and is interpreted as single hit probability per unit dose, a_2 is assumed to be the probability that at unit dose two 'sublethal' events occur and interact to inactivate the cell.

A good point can be made for this interpretation. It is in general not easy to obtain accurate figures for the initial slope a_1 . Particularly the corrections for cellular multiplicity as they are generally employed may distort the actual values. But with the help of some additional information as for example provided by Hall and Bedford in their cell inactivation experiments with very low dose rates (31) one can at least obtain estimated values of a_1 . Microdosimetry data show that even with sparsely ionizing radiation there is always a certain probability that high local energy densities are produced in the passage of an ionizing particle. It is then not surprising that there should be a single hit component in the survival curve.

Nevertheless it is unjustified to blindly identify numerical estimates of a_1 and a_2 with event probabilities. Reasonably accurate survival probabilities can at present not be derived with x-ray doses below 50 rad. At a dose of 50 rads however a cell is still penetrated by several hundred ionizing particles. Even the nucleus is passed by roughly a hundred charged particles at this dose. One can therefore not exclude that in this dose range the division probabilities after irradiation are well-defined functions of dose without much influence of the statistical fluctuations. If one assumes that the breakdown probabilities in the first and second generation are q_1 and q_2 D and if higher order terms and cell deaths in later generations are not considered, one obtains the inactivation probability:

$$P(D) = q_1 D + q_2^2 D^2$$

Thus the coefficient in the linear term is interpreted as breakdown probability in the first post-irradiation generation, while the square term represents failure in the second generation. Again the linear term corresponds to the probability that the effect is brought about in a single random event, while the square term reflects the probability for the interaction of two random events, namely the failure of both daughter cells in the second post-irradiation generation. This is yet another example that one and the same formal interpretation of the dose effect relation can be given completely different meaning. It is for this reason that formal analysis must be closely supported by microdosimetric data and by studies on cellular kinetics and its radiation induced disturbances.

Acknowledgement

The author is much indebted to Dr. Otto Hug, who developed many of the ideas expressed in this paper.

REFERENCES

1. Dessauer, F., Über einige Wirkungen von Strahlen I. F, *Z. Physik* **12**, 38 (1922).
2. Crowther, J. A., Some considerations relative to the action of X-rays on tissue cells. *Proc. Roy. Soc. (London)* **B96**, 207 (1924).
3. Timofeeff-Ressovsky, N. M., u. K. G. Zimmer, Das Trefferprinzip in der Biologie. *Biophysik I. Leipzig*: S. Hirzel 1947.
4. Hug, O., and A. M. Kellerer, Stochastik der Strahlenwirkung. Springer, Berlin, New York, 1966.
5. Zimmer, K. G., Studien zur quantitativen Strahlenbiologie. Akad. der Wiss. und Lit., Heidelberg, Abh. Math. Nat. Klasse (1960), Nr. 3.
— Studies on quantitative Radiation Biology. London: Oliver & Boyd 1961.
6. Vavilov, S.I., and T. V. Timofeeva, *J. Phys. U.S.S.R.* **7**, 9 (1943).
7. Hecht, S., S. Shlaer, and M. H. Pirene, *J. Gen. Physiol.* **25**, 819 (1942).
8. Bouman, M. A., and H. A. Van Der Velden, *J. Opt. Soc. Am.* **37**, 908 (1947).
9. Pirene, M. H., Quantum Physics of Vision, Theoretical Discussion. In: *Progr. in Biophysics* **2**, 193-223 (1951). London: Butler and Randall.
10. Arrhenius, S., Quantitative Laws in Biological Chemistry. London: Bells & Sons 1915.
11. Rahn, O., *Symposia Quant. Biol.* **2**, 70 (1935).
12. Jordan, P., Biologische Strahlenwirkung und Physik der Gene. *Physik. u.* **39**, 345 (1938).
— Die Verstärkertheorie der Organismen in ihrem gegenwärtigen Stand. *Naturwissenschaften* **26**, 537 (1938).
— Die Stellung der Quantenphysik zu aktuellen Problemen der Biologie. *Arch. ges. Virusforsch.* **1**, 1 (1939).
— Strahlenbiologie der Bakterien. *Protoplasma* **32**, 464 (1939).
— Zur Quanten-Biologie. *Biol. Zbl.* **59**, 1 (1939).
13. Clark, A. J., Kinetics of Drug Action. In: Handbuch d. exp. Pharmakologie, *Ergänzungsband zu Gen. Pharmacol.* **4**, 96 (1937).
14. Kellerer, A. M., Mikrodosimetrie Grundlagen einer Theorie der Strahlenqualität GSF-Bericht B1, Munich 1968.
— Analysis of Patterns of Energy Deposition, *Proc. Microdosimetry*, Stresa, Euratom (1969).
15. Rossi, H. H., Specification of radiation quality. *Radiation Research* **10**, 522-531 (1959).
16. Rossi, H. H., Energy Distribution in the Absorption of Radiation. Chapter from "Advances in Biological and Medical Physics." Vol. 11, pp. 27-85, Academic Press, 1967.
17. Rossi, H. H., Correlation of radiation quality and biological effect. *Ann. N. Y. Acad. Sci.* **114**, 4-13 (1964).
18. Smith, H. H. and H. H. Rossi, Energy Requirements and Relative Biological Effectiveness for Producing a Cytogenetic Phenomenon in Maize by Irradiating Seeds with X-rays and Monoenergetic Neutrons. *Radiation Research* **28/2**, p. 302 (1966).
19. Sparrow, A. H., A. F. Rogers and S. S. Schwemmer, Radiosensitivity Studies with Woody Plants-I Acute Gamma Irradiation Survival Data for 28 Species and Predictions for 190 Species. *Radiation Botany* **8**:149-186, 1968.
20. Rossi, H. H., The Role of Associated Absorption Events in Lenticular Radiation Injury. *Proc. of Second Panel on Bioph. Aspects of Radiation Quality*, IAEA, Vienna, 161-170, 1968.
21. Neary, G. J., Radiation Quality and RBE for Chromosome Aberrations. *Proc. of Panel on Bioph. Aspects of Radiation Quality*, IAEA, Vienna, p. 58-62, 1965.
22. Humphrey, R. M. and W. K. Sinclair, The relative biological effectiveness of 22-Mevp X-rays, Cobalt-60 gamma rays, and 200-Kv X-rays. VIII. *Radiation Research* **20**, 593-599 (1963).

23. Miletic, B., Petrovic, A. Han, and L. Sasel, Restoration of viability of X-irradiated L-strain cells. *Radiation Research* **23**, 94-103 (1964).
24. Kellerer, A. M., Microdosimetry and the theory of straggling. Proc. of the Conference on Microdosimetry ISPRA, EURATOM, 1967.
25. Kellerer, A. M., Parameters of track structure. Proc. 2nd L. H. Gray Conference, Cambridge, 1969, in press. Brit. Journal of Radiology.
26. Hug, O., and A. Kellerer, Zur Interpretation der Dosiswirkungsbeziehungen in der Strahlenbiologie. *Biophys.* **1**, 20-32 (1963).
27. Till, J. E., E. A. McCulloch, and L. Siminovitch, A stochastic model of stem cell proliferation, based on the growth of spleen colonyforming cells. *Proc. Nat. Acad. Sci.* **51**, 29-36 (1964).
28. Haefner, K., *Photochem. Photobiol.* **5**, 587 (1966).
29. Kellerer, A. M. and O. Hug, Random factors in the survival curve. *Adv. in Biological and Medical Physics*, **12**, p. 353-366, 1968.
30. Paskin, A., B. V. Bronk, and G. J. Dienes, Stochastic models of cell proliferation and cell response to radiation. *Brookhaven Symposia in Biology*, **No. 20**, p. 169-178, AEC, 1968.
31. Hall, E. J. and J. S. Bedford, Dose Rate: Its effect on the survival of HeLa cells irradiated with gamma rays. *Radiation Research* **22**, 305-315 (1964).