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A Survey of Approaches to Radiation Biophysics

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ABSTRACT: Current and past lines of reasoning in radiation biophysics are reviewed. The emphasis is on the connection of biological suppositions and microdosimetric data.

Two main fields of inquiry are considered. The first is the non-cumulative action which has first been treated by Lea in terms of the associated volume concept. The second is the cumulative action which underlies radiation effects on eukaryotes. A brief survey over present approaches is given.

The second part of the article contains quantitative criteria for the applicability of absorbed dose, LET, and related concepts. This defines those situations where the use of microdosimetry is essential.

The last section deals specifically with the determination of the combination distance of cellular sublesions. The approaches based on dose-effect relations and on LET-effect relations are contrasted. Microdosimetric data are employed to examine and reject the hypothesis that impairement of the proliferative ability of mammalian cells is due to the formation of individual double-strand breaks in DNA.

INTRODUCTION

The microscopic distributions of energy deposition by charged particles are of great complexity. This complexity may be sufficient to attract the physicist or mathematician to the biophysics of ionizing radiations. However it appears that the physical and mathematical problems have often been treated in detail while necessity and feasibility of the application to radiation biology remained doubtful. Multi-target and multi-hit theory illustrate this situation; these approaches have not led to biophysical insights. One cannot avoid the question whether microdosimetry and its application suffer from similar defects.

A look at the various biophysical approaches to radiation biology may contribute to an answer. A systematic and complete survey would be worthwhile. The present brief outline will fall short of it. Fortunately there have been a number of presentations at these symposia which have dealt broadly with the biological problems. One could mention the contributions by Barendsen (1-4), Fowler (5,6), Neary (7,8), Alper (9), Booz (10), Rossi (11), Hogeweg and Barendsen (12), Elkind and Ben-Hur (13), and Powers (14). A full list would contain additional references. The existence of these studies may justify that the present considerations are focussed on a few essentials.

The first part of this article will deal with the two different problems of radiation biophysics namely the non-cumulative and the cumulative radiation action. Brief reference will be given to some of the relevant studies.

A second section presents criteria which permit a decision whether in a given situation the concept of absorbed dose is adequate or whether the statistical fluctuations of energy

deposition must be taken into account. If the fluctuations of energy deposition must be taken into account one has a number of alternatives. One may either rely on the concept of linear energy transfer (LET), or one may adopt various corrections and modifications of LET, finally one may apply rigorous microdosimetry. The applicability of these alternatives depends on the size of the critical sites and on the type and energy of the charged particles. Relevant quantitative criteria will be presented.

A third section deals in more detail with the nature of the quantitative statements obtained from the biophysical analysis of the cumulative action of ionizing radiations.

GENERAL CONSIDERATIONS

A survey over the contributions to the past symposia and to the present symposium shows a majority of investigations related to the technical aspects of microdosimetry; however it also shows a substantial number of investigations which deal with the application of microdosimetry to radiation biology. These latter studies concern two different situations which may be termed *non-cumulative action* and *cumulative action* of ionizing radiations.

Non-cumulative Action

The term non-cumulative action refers to the case where individual molecular alterations cause the effect. Sparsely ionizing radiations have maximum effectiveness in this case. With densily ionizing radiations a saturation effect occurs, i.e. more than one disturbance is produced in the affected target. Accordingly the relative biological effectiveness

(RBE) decreases with increasing LET. For non-cumulative effects one observes exponential dose-effect relations. However one cannot reverse the statement; an exponential dose-effect relation does not necessarily imply non-cumulative action.

The quantitative treatment of the non-cumulative radiation action has been developed by Lea (15). He introduced to this purpose the concept of *associated volume*. Lea has calculated associated volumes for protons, α-particles, and for x-rays. Katz et al. (16) have dealt with the same problem and have, in effect, derived associated volumes for heavier ions. The validity of the approximation which they have used will be discussed in the second part of this article.

The associated volume is closely linked and is, in fact, proportional to the so-called event frequency, $\phi(o)$, which is one of the essential quantities among the microdosimetric concepts introduced by Rossi (17,18). Values of $\phi(o)$ for much larger regions than those considered by Lea and by Katz et al. can be directly measured; they have also been calculated (19-23).

It had earlier been assumed that non-cumulative action occurs in the inactivation of certain bacteria and generally in the inactivation of virusses and bacteriophages. These biological systems have therefore been usually treated in terms of the associated volume concept. However we know today that one deals with different kinds of damage even in these relatively simple systems. Barendsen (4) has given a useful survey over these matters at the last symposium on microdosimetry. It suffices to say that non-cumulative action appears to apply to single-strand breaks in DNA and to nucleotide damage. It does not apply to double-strand breaks in DNA.

Cumulative Action

The term cumulative action refers to the case where two or more molecular alterations are necessary to produce the effect. These alterations may be produced by the same ionizing particle (intra-track effect); one obtains then linear or exponential dose-effect relations. They may also be produced by separate ionizing particles (inter-track effect); then one finds sigmoidal dose-effect relations. However a common characteristic of both cases is that the RBE goes through a maximum at high values of LET.

An example of the cumulative action is the production of double-strand breaks in DNA. Two molecular alterations in close proximity are necessary to cause a double-strand break. This will, at least in general, require two energy transfers (ionizations) in a proximity of the order of 10 nm. Such neighbouring transfers will frequently occur even in the tracks of sparsely ionizing particles. However at absorbed doses up to a few hundred or even a few thousand rad it is extremely rare that two transfers from two *independent* particle tracks occur in such proximity. This is likely only at much higher doses. Accordingly one will at doses of a few hundred rad obtain linear dose-effect relations (intra-track effect) for this particular example of the cumulative effect. The postulate of Chadwick and Leenhouts (24,25) that individual double strand breaks cause cell lethality is therefore in conflict with the observed sigmoidal survival curves. A quantitative evaluation will be found in the last part of this article.

Another case of cumulative action is the inactivation of eukaryotic cells or the production of dicentric chromosomes. The cellular effect is in this case produced by several sublesions; these sublesions can combine even if produced at distances of fractions of a micrometer to several micrometer.

Such distances are comparable to the typical distances of neighbouring electron tracks at an absorbed dose of a few hundred rad. The sublesions can therefore either be produced in the intra-track mode (densily ionizing radiation) or in the inter-track mode (sparsely ionizing radiation). In the latter case one obtains sigmoidal dose-effect relations.

Cumulative damage to eukaryotes is a much more complicated case than cumulative action to molecular complexes. The reason is twofold. In a site of molecular dimensions one deals with clusters of a few ionizations. This situation is complicated enough, but it is far less complicated than the intricate pattern of track segments which may occur in regions which are comparable to the dimensions of the cell nucleus. The second reason is that the structure of the target is highly variable; for example, it changes markedly in the different phases of the cell cycle. Moreover the reaction of the eukaryotic cell is subject to various stochastic factors which influence the dose-effect relations.

It is therefore not surprising to find a variety of approaches to the problem. It is also not surprising that the mathematical treatment of the cumulative action on eukaryotes is usually either complicated or highly approximative. In some cases it is both, for example in the so-called multi-target or multi-hit models. These models have neither led to the definition of the sublesions nor to an estimate of their spatial separation.

There are nevertheless a number of present approaches which are, at least formally, related to the multi-target theory. These approaches may be called semi-empirical insofar as they use a mathematical expression, generally the so-called multi-target equation, to fit observed survival curves for x-rays and γ-rays. This multi-step inactivation (gamma-kill (26) or β-component (29)) is then,in the case of densily ionizing ra-

diations, mixed with a one-event inactivation mode (ion kill or α-component). The weight factors for the two components are assumed to be certain functions of LET or of related quantities such as velocity and charge of the particles.

The approach is descriptive rather than analytical since the parameters in the multi-target equation are not linked to specified biophysical mechanisms. However dose-effect relations for a mixed radiation field can be calculated once the numerical values of the parameters for the components of the field are chosen. This possibility of curve fitting has found particular interest in connection with attempts to use survival curves for the investigation of the theoretical basis of radiation therapy.

Due to their complexity it will not be possible to deal in detail with these approaches. Instead one may refer to the work of Katz et al. (26-28) and of Wideroe (29). Neufeld et al. (30) have given a survey over these *two-component* theories; they also propose various modifications of the microdosimetric arguments, and occasional lack of microdosimetric arguments, in these approaches.

A different approach which aims at the elucidation of biophysical mechanisms rather than the description of survival curves goes back to the work by Sax (31) and Lea (15). These authors dealt with the production of dicentric chromosomes in plant cells. Considerations of the distance of separate particle tracks in the case of x-rays and of neutrons led to estimates of the order of one micrometer for the exchange distance between chromosome breaks.

This approach is closely linked to a number of later studies (see for example (32-34)). It is similar in character to the work of Neary (8) which is particular impressive as a precise combination of physics and biology. Neary arrived at the con-

clusion that the action of radiation on eukaryotes involves the formation of pairs of sublesions separated by fractions of one micrometer.

The treatment developed by Lea and by Neary was still based on the LET-concept. More recently an accurate analysis of the formation of chromosome aberrations by sparsely ionizing radiations and by neutrons has been performed in terms of microdosimetry (35-36). This analysis has, in essence, confirmed Lea's earlier results. It is particularly remarkable that the microdosimetric analysis has not only led to a quantitative explanation of the RBE for chromosome damage by neutrons as a function of dose (37), but that it has also led to the prediction and apparent confirmation of values of the increased effectiveness of x-rays as compared to γ-rays or fast electrons (38,39). Whether the observed RBE of nearly 2 at small doses applies also to the inactivation of mammalian cells is an open question.

Kellerer and Rossi (40) have applied the microdosimetric analysis to the RBE of neutrons and its dependence on absorbed dose. For a wide variety of effects they found relations which were analogous to the situation observed in the formation of dicentric chromosome aberrations. One might take this as an indication that chromosome aberrations play a wide role in various cellular radiation effects. But this is not more than a hypothesis, and it is possibly in conflict with experimental evidence (41). The essential result is that in the cumulative action on eukaryotes one deals with pairs of sublesions which contribute to the effect even when they are produced at distances of the order of micrometers.

These results are at variance with conclusions by Barendsen et al. (3,12) who have investigated inactivation cross-sections of mammalian cells *in vitro* as a function of LET and who have found that the data agree with the assumption that

cumulative action occurs over distances of approximately 7 nm. Barendsen has pointed out that part of the disagreement may be resolved by the fact that one deals with cumulative action both on the nanometer and the micrometer scale. Further remarks on this problem will be found in the last part of this article.

The approach by Chadwick and Leenhouts (24,25) has already been mentioned. It is formally identical to the other approaches which deal with a quadratic process, however it is based on the postulate that cell death is due to individual double-strand breaks. Microdosimetric data which contradict this postulate are found in the last part of this article.

In view of the variety of approaches to radiation biophysics it is desirable to establish quantitative criteria for the necessity to apply microdosimetry. First one may ask for the applicability of the quantity absorbed dose, secondly one may examine the validity of the approximative treatment in terms of LET or related concepts. These points will be treated in the next section.

NECESSITY FOR THE APPLICATION OF MICRODOSIMETRY

Applicability of Absorbed Dose

Absorbed dose is only a statistical mean. If one considers a small region, if the absorbed dose is small, and the radiation is densily ionizing then the actual value of the spezific energy, z, will fluctuate widely around this mean value. On the other hand, for sufficiently large regions and for sufficiently large absorbed doses the fluctuations may

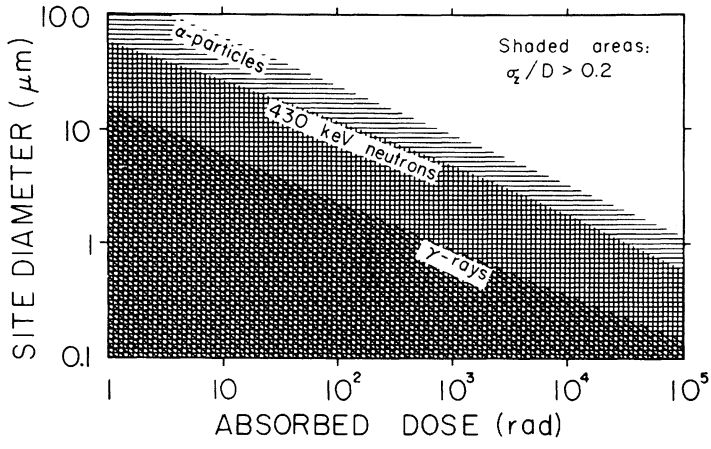

Fig. 1 Diagram of site diameters and absorbed doses for which the specific energy, z, must be distinguished from absorbed dose, D.
For three different radiations those areas where the mean deviation of z from D exceeds 20% are indicated by shading.

be minor and may in fact be disregarded.

In order to obtain a quantitative criterion one may consider the standard deviation of the specific energy from its mean value, the absorbed dose. It is a convenient fact that this standard deviation is a simple function of the dose average, ζ, of z produced in individual events (40):

$$\sigma_z = \sqrt{\zeta\ D}$$

with:

$$\zeta = \int z^2\ f_1(z)\ dz \Big/ \int z\ f_1(z)\ dz$$

Although this is a somewhat arbitrary choice one might consider a fractional standard deviation, σ_z/D, of less than 20% as insignificant. This leads to the criterion that the quantity absorbed dose can be applied without regard to statistical fluctuations if the absorbed dose is larger than 25 ζ. Fig.1 represents this condition for typical radiations. The shaded areas cover those ranges of site diameters and of absorbed doses where the relative fluctuations exceed 20%.

The application of this graph is straightforward. It is therefore unnecessary to exemplify its use. However one may note as a general conclusion that in most radiobiological situations involving structures of cellular dimensions microdosimetry is indeed relevant.

Next one may ask for the applicability of a simplified microdosimetric treatment in terms of LET. This question has been analysed recently; it is therefore sufficient to repeat the main results.

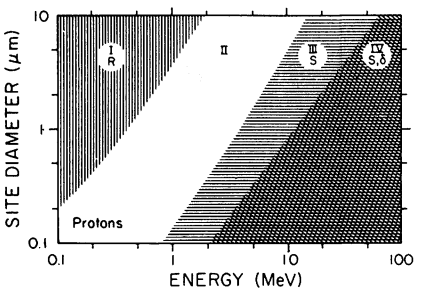

Fig. 2

Diagram of the ranges of site diameters and proton energies where other factors in addition to LET are relevant to energy deposition. The symbols *R*, *S*, and δ identify those domains in which limited particle range, energy-loss straggling, and energy dissipation by delta-rays are pertinent. In region II LET is the only relevant factor.

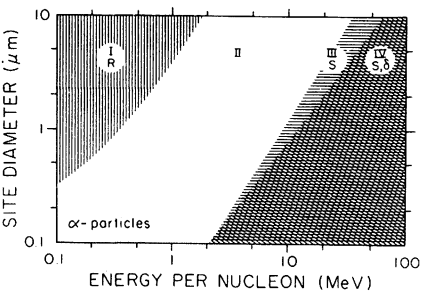

Fig. 3

Diagram of the ranges of site diameters and energies of alpha-particles where other factors in addition to LET are relevant to energy deposition. The symbols *R*, *S*, and δ identify those domains in which limited particle range, energy-loss straggling, and energy dissipation by delta-rays are pertinent. In region II LET is the only relevant factor.

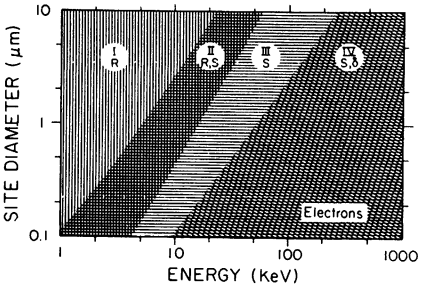

Fig. 4

Diagram of the ranges of site diameters and electron energies where various factors in addition to LET are relevant to energy deposition. The symbols *R*, *S*, and δ identify those domains in which limited particle range, energy-loss straggling, and energy dissipation by delta-rays are pertinent. There is no region where LET is the only relevant factor.

Applicability of LET

The energy which a charged particle deposits in a microscopic region depends on the LET of the particle. But LET is not the only factor. If one deals with a relatively large region then one must account for the finite range of particle which may lead to incomplete traversals. If on the other hand, one deals with very small regions one must consider energy-loss straggling and the dissipation of energy by delta-rays. Usually some of these factors can be neglected, but this depends on the diameter of the spherical region which one considers, as well as on the velocity and charge of the particle.

A recent quantitative analysis (42) has led to the results which are depicted in Figs. (2-4). In these figures the unshaded areas correspond to those ranges of the site diameter and the particle energy where the LET-concept can be directly applied, i.e. where neither the finite range of the particle, nor energy straggling and the structure of delta rays play a role. The shaded areas designate those ranges where other factors must also be considered. The symbols R, S, and δ stand for the factors *finite range* of the particle, *energy-loss straggling*, and radial distribution of energy due to the finite ranges of *delta-rays*. The original article must be consulted for a quantitative definition of the underlying criteria. In the present context we deal only with the essential results. The main conclusion is that there exists a region for protons and for heavier ions where the LET concept is adequate, but that outside this region microdosimetry must be applied. The region which is labelled with R for protons permits the continuous slowing down approximation. It corresponds to the common situation of microdosimetric measurements in neutron fields of moderate energy; it also corresponds to the calculations of Caswell and Coyne (19,22). The other shaded regions are those where track structure in

a real sense is essential. A further important observation is, that with electrons there is no range of energies and site diameters where LET is applicable. Electrons are therefore in all cases an object of microdosimetry.

When H.H.Rossi introduced the concepts of microdosimetry and the experimental techniques of microdosimetry he was led by the recognition that this provided a short-cut through the complexities of track structure. The combined effect of a multitude of factors which were not individually understood could still be determined by direct measurements. Nevertheless it is, as these symposia show, of interest to understand the individual factors, and recent contributions (see for example (43-46)) permit the expectation that a full understanding of track structure may eventually be reached. It is therefore appropriate to consider the problem of track structure in somewhat more detail.

Applicability of the amorphous-track model

One approximation which has been extensively used especially by Katz and his colleagues (16,47) is what one may call the *amorphous track model*. In this approximation the average radial energy distribution around the track is taken into account, but delta-ray structure is otherwise neglected. Individual energy transfers (for example ionizations) are assumed to occur randomly with a probability proportional to the mean energy density at the specified distance from the track core. In other words the track is treated as an amorphous cloud of independent energy transfers. If the actual track resembles a worn-out test-tube brush then the amorphous track looks like the same object rapidly rotating around its axis and rapidly oscillating along it.

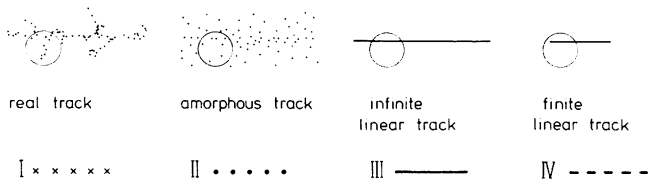

Fig. 5 Schematic diagram of the various approximations of a charged particle track.

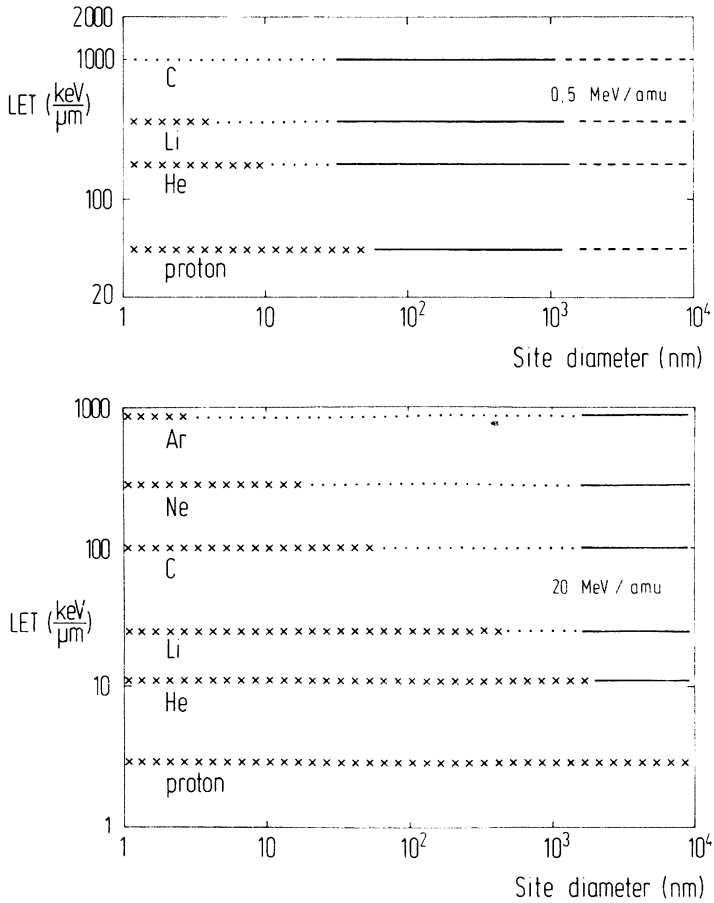

Fig. 6 Applicability of the various approximations of a charged particle track as a function of site diameter. The symbols indicate the four different cases represented in Fig.5.

This amorphous track model is adequate for very densily ionizing tracks where the structure of individual delta rays is unresolvable. On the other hand it leads to event sizes, i.e. to values of $\bar{y}_F$ and $\bar{y}_D$, which are too small if this condition is not fullfilled. A recent theoretical investigation has led to interesting theoretical relations which describe the situation (53). In the present context it is sufficient to indicate the applicability and the limitation of the amorphous track model.

The scheme of Fig.5 illustrates the various approximations. Case I symbolizes the actual track with straggling and explicit delta-ray structure; this corresponds to the microdosimetric treatment. Case II symbolizes the amorphous track model. Case III stands for the situation where the LET-concept is applicable, i.e. where one may assume an infinite linear track. Case IV represents the situation where one can assume a linear track without regard to straggling and radial extension of the track, but where the finite range of the particle must be accounted for.

The diagrams in Fig.6 indicate the site diameters where the individual situations apply. The plots refer to heavy ions of 20 MeV per nucleon and to heavy ions of o.5 MeV per nucleon. These results are based on numerical calculations which have been performed by Chmelevsky (48) on the basis of simulated tracks generated by Paretzke (43,44). The quantitative criterion has been that the individual factors are taken into account when they contribute more than 10% to the value of $\bar{y}_D$. If one assumes a higher critical level, all ranges will be shifted slightly to the left.

The main conclusion is that the amorphous track model is always unrealistic for protons. For heavier ions it has a certain range of applicability. However this range is narrow for particles of 20 MeV per nucleon, and it will be even more nar-

row for particles of higher energy. One must therefore usually apply the precise microdosimetric analysis.

SPECIFIC CONSIDERATIONS

The two previous sections have dealt with a general survey of biophysical approaches and with criteria indicating the applicability of various approximations. The present section will add some specific considerations of important lines of biophysical reasoning. The discussion will in no way be complete, but it may serve to show the interrelation of different approaches.

Linearity at small absorbed doses

The simplest consideration in microdosimetry is also one of the most important: All cellular effects must be linear and must be independent of dose rate at very small doses. The term very small dose refers to the condition that the expected number of events (ionizing particles) in the cell is much smaller than 1. Most cells are then traversed by no particle, a few cells by 1 particle, and the cells traversed by several particles are so few that they need not be considered. Instead of the cell one can often consider the nucleus of the cell as the main target of radiation. The term cellular effect refers to the condition that no interaction between damaged cells occurs. If such interaction occurs one may deal with complicated dose-effect relations even at very small doses (see e.g. (49)).

Fig.7 delineates those ranges of site diameter and absorbed dose where for common types of radiation the mean event num-

ber is smaller than 1. If a combination of absorbed dose and site diameter lies substantially within the shaded area one can predict a linear dose-effect relation, which is independent of dose rate.

Determination of the combination distances of sublesions

One of the main possibilities of the microdosimetric analysis is the determination of site diameters, or, in other words, of the distance over which sublesions can combine in the cumulative action of ionizing radiations. An important observation is that biophysical reasoning leads in general to estimates which must be considered as *lower limits* for this distance. One can understand this by considering the nature of the argument which is commonly applied.

The argument runs as follows. If a dose-effect curve is near to a step function then the critical site must be large. The reason is that a small site is always subject to fluctuations of the specific energy which are so large that it can not react with sharp resolution in absorbed dose. From an observed relative variance (deviation from the step form) of a dose-effect relation and from a knowledge of the variance of z in microscopic sites one can therefore obtain a lower limit of the site diameter. On the other hand, it is possible that part of the observed variance of the effect curve is due to other factors not related to the statistics of energy deposition. The real interaction distance, or site diameter, is then larger than the value obtained in the analysis.

If two valid microdosimetric analyses of the same biological system lead to different site diameters, one should accordingly accept the larger value.

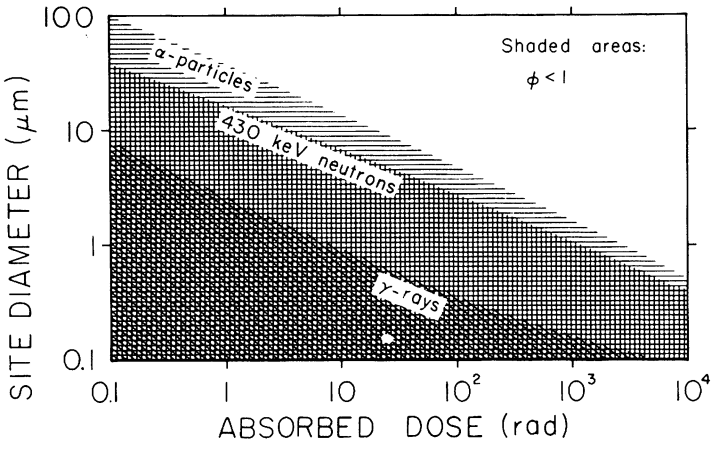

Fig. 7 Diagram of site diameters and absorbed doses for which the mean event frequency, ϕ, is less than 1. The areas with ϕ less than 1 are indicated by shading for three different radiations.

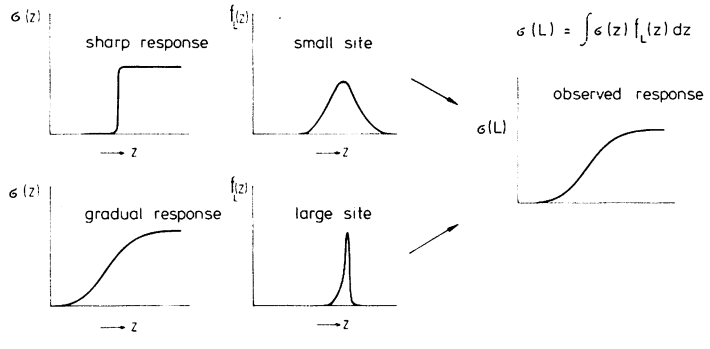

Fig. 8 Schematic representation of the fact that the same LET-dependence of the inactivation cross-section can result from a small target with sharp response and a large target with gradual response.

This important observation applies not only to dose-effect relations, it applies equally to LET-effect relations, for example to those obtained and analysed (3,12,50) by Barendsen and coworkers. The cellular inactivation cross-section is determined as function of LET in these so-called track-segment experiments. One may assume sensitive sites which have an inactivation probability, $\sigma(z)$, which depends on the spezific energy, i.e. on the energy actually imparted to the site. This *response function* could be near to a step function as assumed by Barendsen and coworkers, or it could be different from a step function. The latter possibility, namely a continuous increase with the square of z has been considered by Kellerer and Rossi (40).

What one observes experimentally is the cross-section as a function of LET. This function results from an integration over the product of response function, $\sigma(z)$, and the distribution, $f_L(z)$, at a given LET:

$$\sigma(L) = \int \sigma(z)\ f_L(z)\ dz$$

One can arrive at the same result, $\sigma(L)$, in two different ways. For a steep response function one must invoke a broad z-distribution, i.e. a small site diameter. For a broad response function one must invoke a steep z-distribution, i.e. a large site diameter. This is schematically indicated in the diagram of Fig.8. This type of analysis is therefore not conclusive. The site diameter can certainly not be smaller than the value derived by Barendsen et al. However it may be larger. The observations by Barendsen et al. are therefore not in contradiction to the results which have been obtained by Kellerer and Rossi from experimental data which included the dose-effect relations for sparsely ionizing radiations.

That the consideration of dose-effect relations permits, in this case, sharper estimates of the interaction distance than

the consideration of LET-effect curves has a simple reason. The reason is that in the track segment experiments the mean event size increases approximately linearly with the experimental parameter (LET) both in a small site and in a large site. It is therefore difficult to discriminate the two possibilities. For dose-effect curves of sparsely ionizing radiations, on the other hand, the mean energy increment increases approximately linearly with the experimental parameter (absorbed dose) for large regions while it remains nearly unchanged for very small regions. This permits a sharp discrimination between the two possibilities.

As pointed out earlier there are indications that the cumulative effect over distances of the order of micrometers is accompanied by a cumulative action on the nanometer level (51, 52). It is also possible, as Barendsen has stated, that the cumulative action over short distances is relativly more important for densily ionizing radiations than for sparsely ionizing radiations.

Application of Quantitative Data

It is not the purpose of this general survey to repeat the actual derivation of the combination distance of sublesions. However it will be useful to consider a particularly direct line of reasoning which contradicts the hypothesis that cell death is due to individual double-strand breaks in DNA.

The cumulative action over short distances requires, as pointed out in the beginning of this article, two neighbouring energy transfers. Some of these pairs of neighbouring transfers belong to the same particle track, others to two different tracks. The first type (intra-track effect) leads to linear dose-effect relations, the second type (inter-track

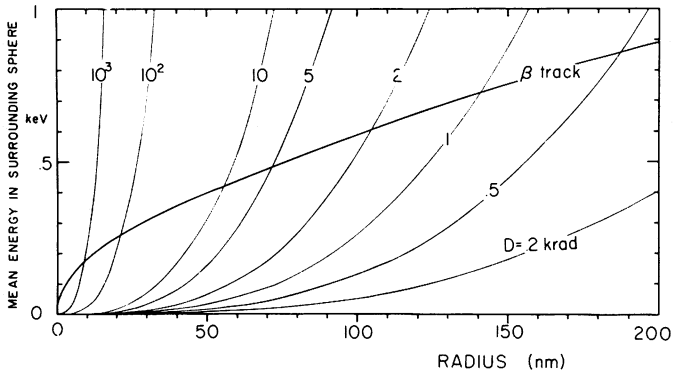

Fig. 9 Expected energy in a spherical region centered around an ionization. The heavy line gives the contribution, ΔE_1, from the same track in the case of a fast electron. The light lines give the contribution, ΔE_2, from other tracks at various absorbed doses.

effect) to quadratic dose-effect relations. One may compare the frequency of these two different types. For this purpose one may simply consider an ionization taken at random in the exposed medium. One can then ask for the expected energy, ΔE_1, within a specified distance which belongs to the same particle track. One may also ask for the expected energy, ΔE_2, due to independent particle tracks. The linear component in the dose effect relation will predominate if ΔE_1 is larger than ΔE_2. The quadratic component will predominate if ΔE_2 is larger than ΔE_1.

Fig.9 presents a quantitative evaluation. The data are from calculations (53) based on electron tracks with simulated delta-rays from the program of Paretzke. The essential arguments have been presented earlier, but the present data are more accurate. The heavy line in the graph gives the quantity ΔE_1 for a fast electron as a function of the radius of the region of reference. The light lines give the quantity ΔE_2 for different absorbed doses. One can see that for regions of the order of 10 nm and for absorbed doses up to many thousand rad the contribution, ΔE_2, of other tracks is much smaller than the contribution, ΔE_1, of the same track The linear component must therefore predominate. In other words, the combination distances of sublesions must be large if the quadratic component is substantial at a few hundred rad. It is apparent that the argument is of sufficiently general nature that the conclusions are valid regardless of the detailed structure of the sensitive sites in the cell.

Microdosimetry permits therefore the conclusion that the inactivation of mammalian cells by sparsely ionizing radiations is not a cumulative action on the nanometer level. This is a definite statement, but leaves room for a variety of questions. Whether one deals with pairs of widely separated *loci*, with a multiplicity of such pairs, or with extended sensitive sites is an object for future microdosimetric studies.

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DISCUSSION:

ALPER

May I first congratulate you, Dr. KELLERER, on a beautiful and clear exposition. You certainly explained a lot of things to me much better than I ever understood them before.

Can I now make a plea: that is, when we are talking about biological effects of radiation, we don't get confused between effects observed as biological endpoints, and those that are observed on individual components, for example DNA double or single strand breaks; enzyme inactivation; or, as another example, the break-up of oligomeric enzymes. We should not confuse such effects with end points which are perhaps, in the context of this meeting, subconsciously of interest; like the mechanisms of lethal effects on cells. I would be quite prepared to include chromosome breaks and certainly mutation induction as true biological endpoints. I would not include chemical observations on constituents of cells in that category, because at the moment we have nothing but our belief in the importance of DNA or any other cell-constituent, to make us regard chemical consequences of irradiation as equivalent to biological endpoints. It is very easy to get confused between the observation of biological end points and observations on certain chemical effects. The slide shows that although you say that the induction of double strand breaks is a cumulative effect of radiation; when you look at the biological effect on DNA, there is a decreasing effectiveness with increasing LET for lethal effects on bacteriophages. This is true both for phages with single-stranded and double-stranded DNA. So when you are looking at the biological end point, the target hypothesis holds true, and you certainly do not have a cumulative effect when it comes to the inactivation of double-stranded phages.

KELLERER

One can only agree with these general remarks made by Dr. ALPER.

They are in fact important. I am interested to see the experimental evidence that the RBE even for double stranded phages can decrease with increasing LET.

ALPER

The conclusion may very well be, and I think this conclusion has been drawn, that double strand breaks do not play a great part in the inactivation of double strand DNA phages.

KELLERER

One may mention in passing that, even on the simpler level for the observation of double strand breaks, there is equivocal evidence, in so far as NEARY has, under certain experimental conditions, observed a decreasing LET-dependence.

GOODHEAD

I understand that primary human cells give an exponential dose response right down to 5 log-decades. It seems, by and large, that they are slightly more sensitive than the normal cultured cells. Would you like to comment on this?

KELLERER

It seems a general finding that the larger the sensitivity of the cell, the closer its survival curve is to the exponential form. This could reflect the fact that in a sensitive cell a single particle, even if sparsely ionising, may produce the effect.

SULLIVAN

Could you explain the relation between microdosimetric qualities, for example, measured LET spectra, and your interpretation of the radiobiological effect?

KELLERER

The quantity which is particularly important in the action of radiation on

eukaryotic cells, is the quantity zeta, i.e. the mean energy increment due to individual charged particles traversing the nucleus of the cell, or a somewhat smaller site within the nucleus. There are cases, as I have indicated, where in fact the LET concept is quite adequate. The experiments performed by BARENDSEN and co-workers on the inactivation of human cells as a function of LET, are a good example of a situation where the LET-concept is valid. There are other cases where the LET concept is rather useless and where microdosimetry will be extremely important. This will apply to the planned experiments at the Bevalac for example.

HARDER

It was very good to hear once more that the concepts of microdosimetry are not limited to the microdosimetric quantities y and z. There is a plurality of approaches and you showed that there is a range of applications where you can apply, for example, the LET.
With reference to the applicability of zeta, I would like to ask whether your fundamental relation, that the reaction probability of a cell nucleus is proportional to z^2, is able to deal with two site - one track effects of the type NEARY has studied.

KELLERER

The relation is applicable if the distance of the two sites is random within the cell nucleus, or within a certain part of it. However, if one were to assume that their distance is fixed, one would have to apply considerations of the type presented by Dr. BURLIN at this Symposium.

KATZ

Well, I have learned a new vocabulary; I have learned that what I called gamma-kill is now called cumulative; what I have called ion-kill is now called non cumulative; that what I called the radial distribution of dose should now be called the amorphous track model. I suppose microdosimetry should now be called a crystalline or non-amorphous model. I am puzzled because I have not yet seen an operation verification of microdosimetry in the biological substances to which it is directed.

I am also puzzled that I did not hear the criteria under which you presented a graph which showed the region in which the amorphous track model was invalid and where we must resort to microdosimetry. I would be most grateful to you if you would enlarge on that curve and explain the circumstances where the amorphous track model is invalid and where we must resort to microdosimetry. I would also like you to show us where the amorphous model has failed and where you have resorted to microdosimetry in order to get objective verification.

KELLERER

The range of applicability of the various treatments has been calculated on the basis of the criteria that individual factors are taken into account when they contribute more than 10% to the value of $\bar{y}_D$. The calculations have been performed by CHMELEVSKY on simulated tracks which have been provided by PARETZKE.

KATZ

That is part one. Now let us have the examples.

KELLERER

The documentation of successes or lack of successes of various microdosimetric approaches is in the literature. I have attempted to survey some of them. As to the validity of the amorphous track model, one may say that a model which does not correspond to physical reality may still serve to fit survival curves. Whether the model corresponds to physical reality was the question I have been addressing myself to.

KATZ

I must insist that you cite at least one case in which microdosimetry has provided an objective answer and where track structure theory has failed.

KELLERER

As far as the applicability of microdosimetry is concerned, I would like to direct your attention to references 8, 10, 35-41 and 52 in my presentation. With regard to your model I am convinced, as I said, that it will not fail to fit all observed survival curves.