The importance of microdosimetry for radiation biology and radiation protection.

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THE IMPORTANCE OF MICRODOSIMETRY FOR RADIATION BIOLOGY AND RADIATION PROTECTION

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ABSTRACT

In this contribution several features will be discussed of relations between biological effects produced by ionizing radiations and the spatial distributions of energy deposition of these radiations. Effects produced by high-LET radiations are generally found to be less dependent on dose-rate, dose-fractionation and on the influence of cellular conditions and of various compounds in the cell environment, than effects of low-LET radiations.

Relations between the relative biological effectiveness of ionizing radiations and the distributions of local energy densities will further be shown to provide information which is required to test various hypotheses about radiobiological mechanisms. This is especially of importance for the extrapolation of experimental results obtained at high doses and dose-rate to the low-dose rates and low doses of interest in radiation protection.

It is finally pointed out that measurements of energy deposition patterns for different radiations may provide the basis for selecting the types of radiation which should be compared in order to obtain information about differences in dose-effect relations for those biological endpoints, e.g. tumour induction and genetic effects, that can only be investigated in experiments with large numbers of animals and long observation periods.

I. INTRODUCTION

The aim of radiobiological investigations is to obtain knowledge concerning various sequences of events, which are initiated by the absorption of energy from ionizing radiations in biological material. The primary physical interaction processes result in ionizations and excitations of atoms and molecules in cells and tissues, which give rise to a variety of physico-chemical, biochemical and biological reactions and these finally produce such diverse observable effects as chromosome aberrations, cell death, tumour induction, lethality of animals and hereditary changes. Depending on the nature of specific radiobiological studies, attention may be focussed on either of the processes in the various sequences of reactions, but it will be clear that for a complete understanding of radiobiological phenomena, knowledge about the various steps must be integrated.

This symposium will be concerned mainly with physical processes and their spatial distributions in irradiated biological material, but in this introduction I will discuss some of the relations between these physical aspects and the subsequent biological effects, and point out some of the difficulties which are encountered in applying results of microdosimetry to the elucidation of radiobiological problems.

Basic interest in the comparison of biological effects of different radiations stems from the well-known fact that for many biological systems the degree of damage produced by a given dose depends on the radiation quality (1, 2, 3). Without specifying radiation quality in detail* it can be stated that for many types of damage in living cells and organisms high-LET radiations are per unit dose more effective than low-LET radiation, despite the fact that the latter radiations give rise to a higher charged particle fluence per unit dose. A simple interpretation of this phenomenon can be based on

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*The reader is referred to the paper in this symposium, entitled "Microscopic distribution of radiation energy" by Dr. H.H. Rossi.

the assumption that a more efficient production of high local energy concentration in some sensitive structure or molecule by high-LET radiation is directly related to a more effective induction of biological damage.

For a comprehensive discussion of the differences in effectiveness between various radiations it is obviously necessary to have an adequate characterisation of radiation quality as well as sufficiently accurate dose-effect relations for various biological end-points of interest (4). Certainly with regard to the latter type of information it must be stated that insufficient experimental data are available. Only a few systems have been investigated with a variety of radiations e.g. survival of bacterial spores, survival of cultured mammalian cells, induction of lens opacities in mice. For some of the most important effects, e.g. tumour induction and genetic effects in mammals, only limited data are available. Results of investigations of these latter effects are particularly difficult to interpret, because the end-point is observed only at very long intervals after irradiation and consequently many intermediate reactions complicate the final responses. Correlations of radiation quality with the efficiency for the production of these effects are of special interest because they offer the possibility of directly relating differences in the initial physical energy deposition patterns with the biological end-point, without exact knowledge about the intermediate sequences of events.

In addition to the fundamental studies of radiobiological phenomena, microdosimetric data are of importance for the discussion of hazards of exposure of man to ionizing radiation. Radiation protection problems are, except in the cases of radiation accidents, mainly concerned with low doses and low dose rates. The assessment of risks from occupational exposure involves a number of difficulties. First the dose-effect relations for many radiobiological effects are generally investigated with doses and dose rates greatly in excess of the levels relevant to health physics problems. Consequently, in order to arrive at the probability per unit of

dose for certain effects, e.g. tumour induction or hereditary changes, after exposure to low doses of less than one rad or at low dose rates of less than 1 mrad per hour, it is necessary to extrapolate from the data at high doses and dose rates. This extrapolation can only be carried out, however, if adequate knowledge of the dose-effect relationship is obtained that can be expressed by some mathematical formula, derived from insight in the mechanism by which damage is induced. Furthermore in radiation protection problems, the relevant RBE values on which the choice of QF values has to be based are frequently inadequately known. Again the assessment of the hazards of low doses of different ionizing radiations, such as produced by high energy accelerators, must be based on an extrapolation from data obtained at high doses and this extrapolation will be particularly difficult in cases where dose-effect relations are dissimilar for different types of radiations (5).

II. GENERAL ASPECTS OF DIFFERENCES BETWEEN BIOLOGICAL EFFECTS PRODUCED BY VARIOUS IONIZING RADIATIONS.

It is generally assumed that the properties of ionized and excited molecules produced by radiations are to a first approximation independent of the velocity, mass and charge of the ionizing particles. Consequently, differences between effects of various ionizing radiations must be due to differences between the spatial distributions of ionizations in the irradiated material. It cannot be concluded, however, that due to the similarity of primary effects of different radiations, final biological end-points must also be qualitatively the same. Although it has often been pointed out that biological effects produced by one type of ionizing radiation can also be produced by any other type of radiation, some restrictions must be made. A number of important differences between biological effects produced by different ionizing radiations in cultured cells will be discussed to illustrate this point.

In figure 1 a number of survival curves are presented, obtained by irradiating cultured cells of human kidney origin with 250 kVp X-rays or with 3.4 MeV α-particles. The techniques employed have been described in detail elsewhere (6, 7, 8). The fraction of cells which have retained the capacity for unlimited proliferation is plotted on a logarithmic scale as a function of the dose on a linear scale. Curve b_1 in figure 1, measured after exposure of cells to different doses of 250 kVp X-rays, shows an initial region of low slope, frequently referred to as "shoulder-region", followed by a more rapid decrease of the surviving fraction with increasing dose. This enhanced effectiveness of 250 kVp X-rays with increasing dose implies that at least part of the cell killing is due to accumulation of damage. By inference sub-effective or sub-lethal damage is produced at low doses, that contributes to lethality only if a subsequent dose is administered. Studies with doses fractionated with intervals of a few hours have shown further that sub-lethal damage persists in the cell for only a limited time (9, 10). This is demonstrated by curves b_2 and b_3 of figure 1, which show that cells recover within six hours from a previous exposure and subsequent doses of radiation are then no more effective than the first dose, i.e. the cells respond to the second dose and to the third dose as if they had not been irradiated at all. The type of sub-lethal damage illustrated by these experiments has not been observed for high-LET radiation e.g. 3.4 MeV α-particles at an average LET_∞ of about 140 keV/μ of unit density tissue. Curve a shows that the surviving fraction decreases exponentially with the dose and no sub-effective damage is produced. Consequently no effect of fractionation is observed either. The complete absence of sub-lethal damage has been demonstrated also in experiments in which a dose of α-radiation was given first, followed immediately by various doses of 250 kVp X-rays. The X-ray survival curve of cells which have survived the dose of α-radiation

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LET, measured for damage to the reproductive capacity of cultured

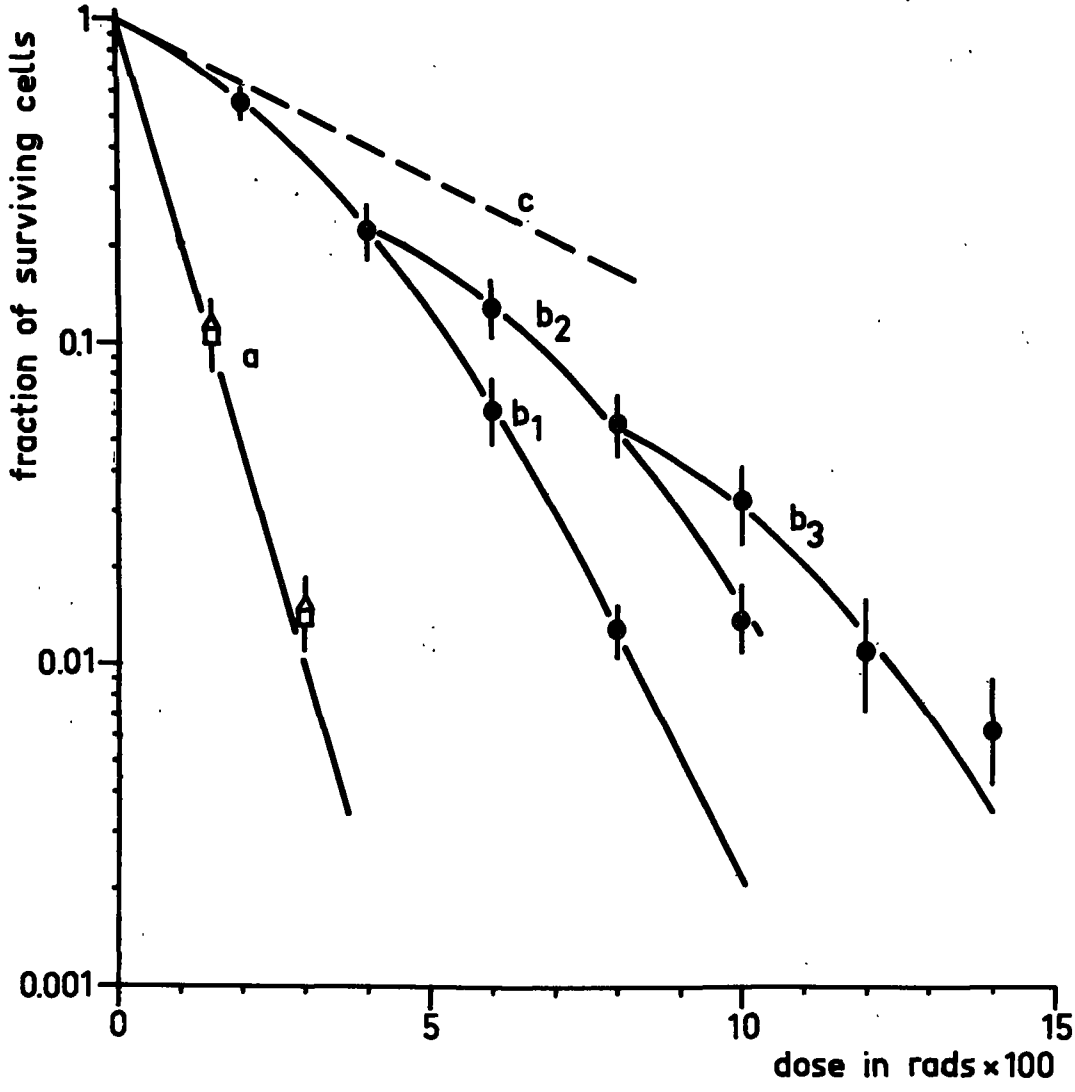

Figure 1. Dose-survival curves of cultured T-1_g cells in equilibrium with air, irradiated with single and fractionated doses of α-radiation at an LET∞ of 140 keV/μ or 250 kVp X-rays.

Curve a: α-radiation; □: single exposures; △: total doses fractionated in three equal fractions with intervals of 6 hours.

Curve b: 250 kVp X-rays; b_1: cells exposed to single doses; b_2: cells exposed to a first dose of 400 rads, followed by different doses after an interval of 6 hours; b_3: cells exposed to a first dose of 400 rads, after a 6 hours interval followed by a dose of 400 rads, after another interval of 6 hours exposed to different doses.

Curve c: tangent to curve b at dose zero.

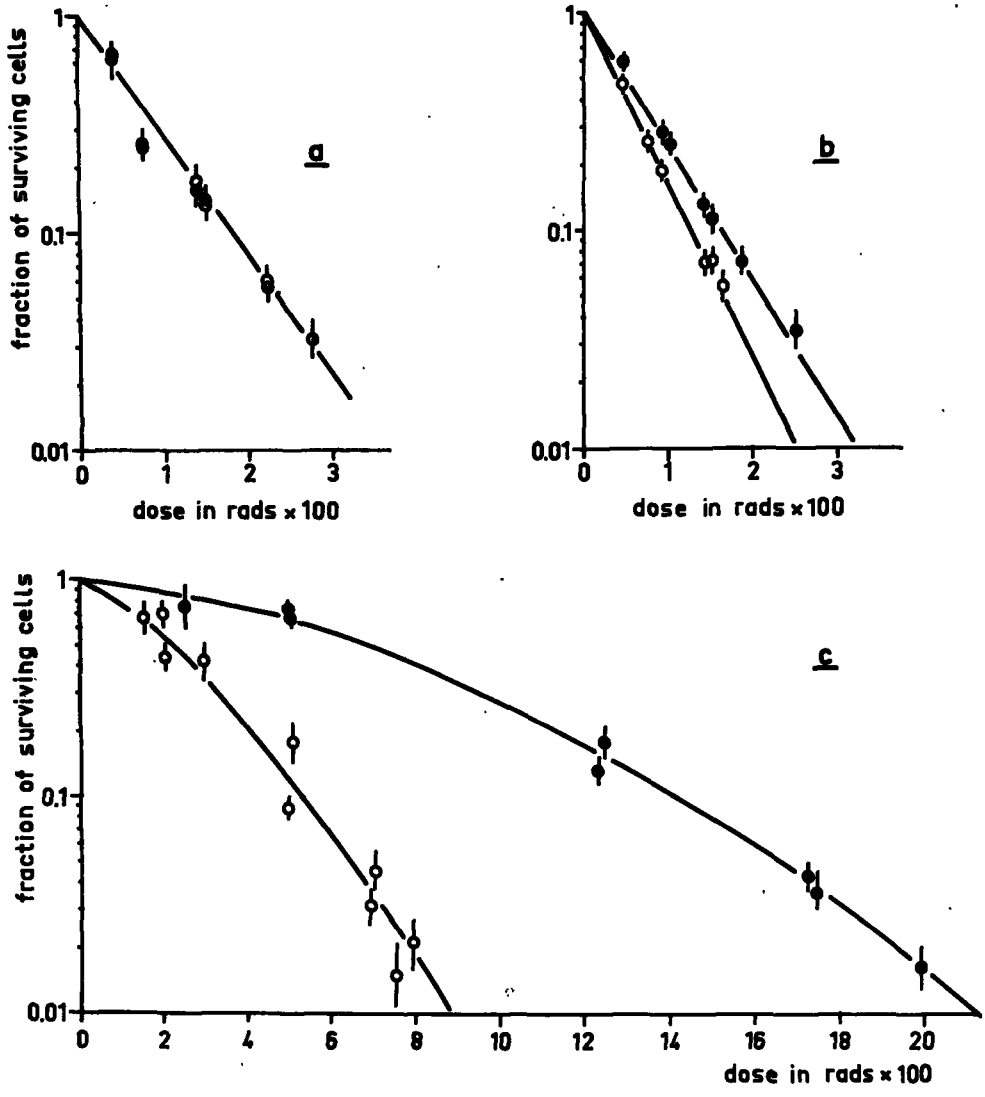

Figure 2. Dose-survival curves for cultured cells of human origin in equilibrium with air and nitrogen respectively: open symbols, air; filled symbols, nitrogen. (a) 2.5 MeV α-particles, LET_∞ = 166 keV/μ, OER = 1.0 + 0.1. (b) 4.0 MeV α-particles, LET_∞ = 110 keV/μ, OER = 1.3 ± 0.1. (c) 14.9 MeV deuterons, LET_∞ = 5.6 keV/μ, OER = 2.6 ± 0.3.

was found to have a shoulder identical to that of unirradiated cells. It may be concluded that high-LET radiation does not produce sub-effective damage that renders subsequent doses of X-rays more effective (7). It may further be noted that the possibility of repair of sub-effective damage is directly related to the dose-rate effects which have been demonstrated for low-LET radiation and have not been detected for 3.4 MeV α-particles (11, 12, 13).

Another type of qualitative difference between biological effects of different radiations is observed if environmental conditions of the irradiated objects are varied. A notable example is the well-known oxygen effect. In figure 2 survival curves are presented of cells irradiated in the presence and in the absence of oxygen. With 14.9 MeV deuterons an oxygen enhancement ratio (OER) of about 2.6 is observed. This value is equal to the OER of 250 kVp X-rays. This difference implies that part of the cell killing is produced by a mechanism which is oxygen dependent. In the case of 4.0 MeV α-particles however the oxygen enhancement ratio is much smaller, namely 1.3, and with 2.5 MeV α-particles at an LET_{∞} of 165 keV/μ no oxygen effect can be detected. It may be concluded that 2.5 MeV α-particles do not produce oxygen-dependent cell killing (3).

In addition to oxygen, many other factors in the cell environment, e.g. temperature, protective agents and sensitizing compounds, are known to modify the effects of ionizing radiations on biological systems. In general it is found that the effectiveness of low-LET radiations e.g. γ-rays, can be varied to a greater extent than the effectiveness of high-LET radiations e.g. α-particles with energies of few MeV. In figure 3 a few examples are presented for cultured cells treated with different concentrations of BUDR, which is incorporated in the DNA, because it is an analogue of thymidine. Cells were incubated during four generation cycles in the presence of different concentrations of BUDR and subsequently irradiated and cultured in medium without BUDR. It will be clear that damage

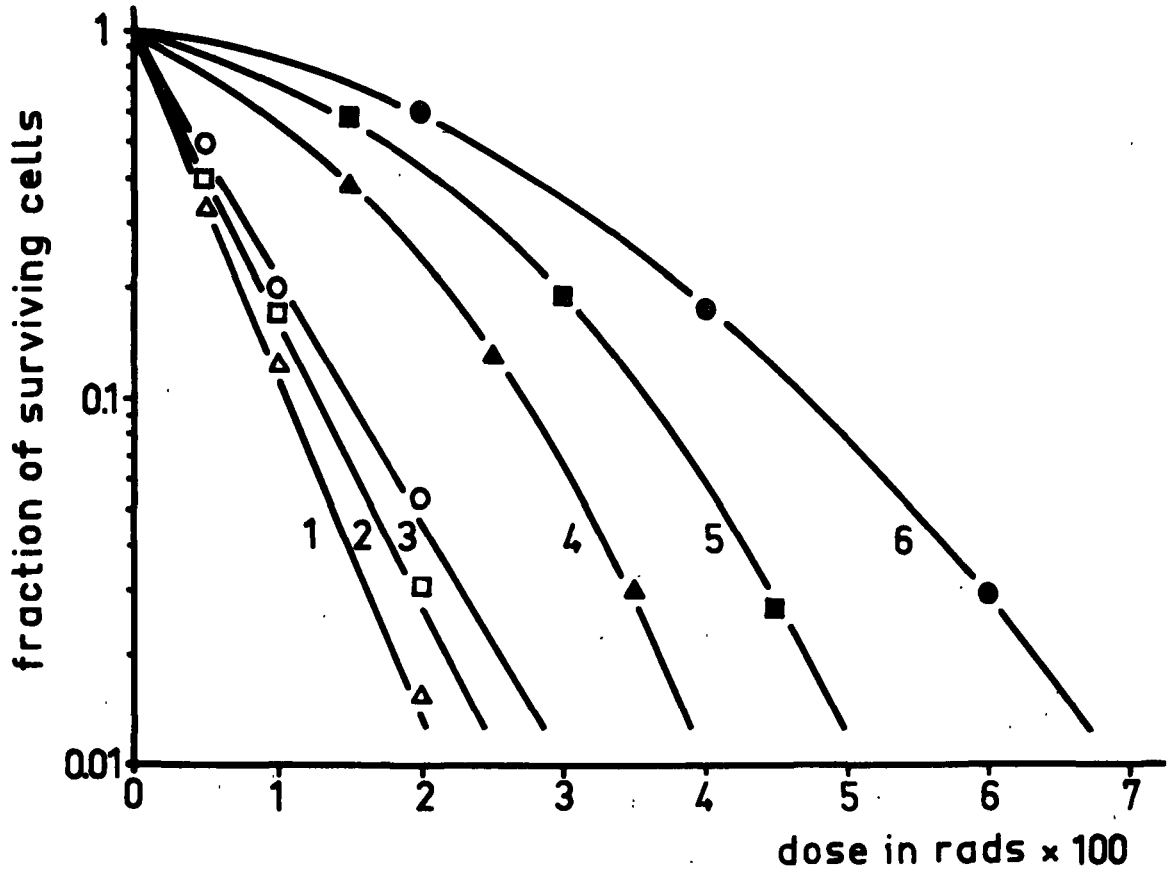

Figure 3. Survival curves for T-1_g cells, cultured before irradiation in media containing different concentrations of BUDR, 8 µg/ml for curves 1 and 4, 2 µg/ml for curves 2 and 5 and 0 µg/ml for curves 3 and 6. Curves 1, 2 and 3 were obtained with 3.4 MeV α-radiation at an LET_∞ of 140 keV/µ. Curves 4, 5 and 6 were obtained with 250 kVp X-rays.

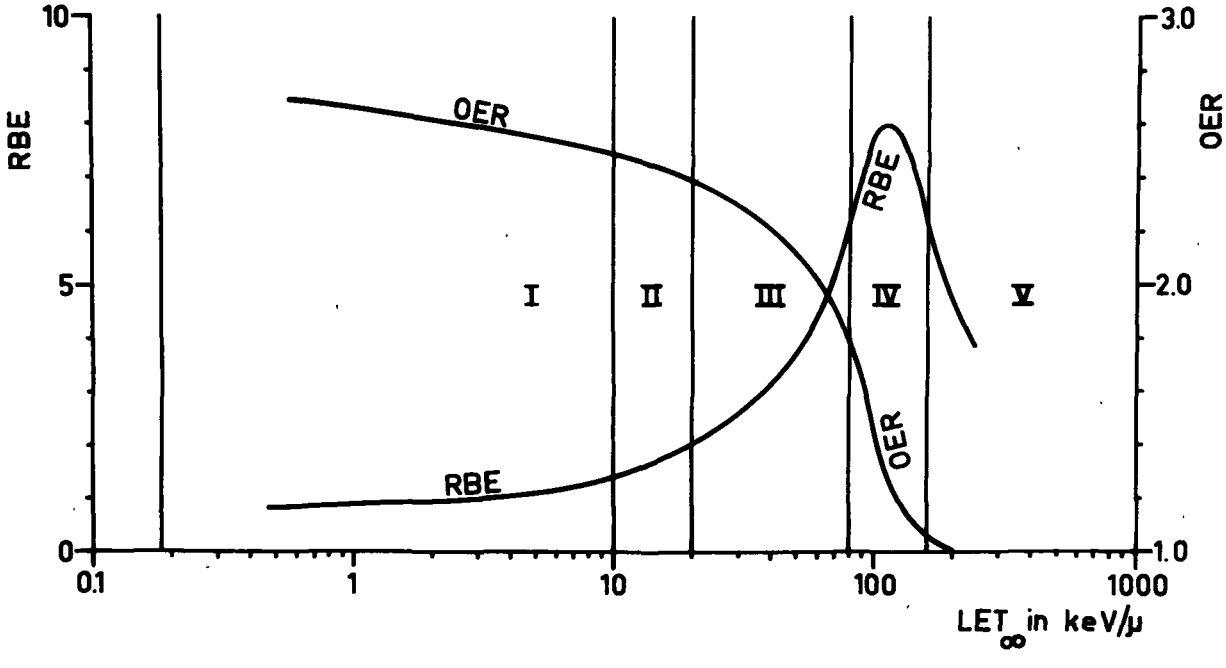

Figure 4. The relations of RBE and OER with LET_∞ in different regions of LET, measured for damage to the reproductive capacity of cultured cells of human origin (T-1_g cells).

produced by low-LET radiation is modified to a greater extent than damage produced by high-LET radiation (13). At very high-LET's no modification of radiation induced damage is observed, i.e. no modifiable damage is produced at all (14).

III. QUANTITATIVE ASPECTS OF DIFFERENCES BETWEEN BIOLOGICAL EFFECTS PRODUCED BY VARIOUS RADIATIONS.

The experiments which were referred to in the previous section to illustrate qualitative aspects of differences between damage produced by radiations with different spatial distributions of energy deposition in cells, concern impairment of the reproductive capacity of single mammalian cells. This experimental system has the advantage that irradiations can be carried out with parallel beams of monoenergetic heavy charged particles, in conditions where the cells are traversed by short selected portions of the tracks of the particles. With this track segment method relatively narrow LET distributions are obtained, although the spread may still be considerable (15). Using these techniques it has been possible to obtain quantitative relations of the relative biological effectiveness (RBE) and the oxygen enhancement ratio (OER) as a function of the LET of the radiations employed (16). These relations are presented in figure 4. On the absciss the LET shown is the LET_{∞}. This is undoubtedly not an adequate parameter, since part of the energy of the primary heavy ions is dissipated through energetic δ-rays. These δ-rays may dissipate at least part of their energy at a considerable distance from the tracks of the primary particles. If this distance is large enough, the effects must be assumed to be incapable of interaction with effects produced in the track core. However, even an approximate correction for the contribution of δ-rays would require knowledge about the distance along which interactions may occur, about the sizes of the critical structures in the cell, about the ranges of the low energy electrons and

about their own effectiveness for producing damage. It is important to note in this respect that very little experimental data have so far been published about the microscopic distribution of energy deposition by heavy ions of different energies which would allow a better interpretation of the biological experiments on cultured cells (16).

Notwithstanding these shortcomings, which cause some uncertainty about the significance of the absolute values of the LET given on the absciss, it is possible to distinguish five main regions of LET, which correspond to different characteristics of the biological damage produced.

In the low-LET range below 10 keV/μ, denoted I in figure 4, the energy dissipated can contribute to cell lethality only through accumulation of damage. This corresponds to a survival curve which has a shoulder followed by an increasing slope at higher doses. The effect of a given dose of radiation corresponding to this LET range, is strongly dependent on dose-fractionation and dose-rate. As shown in figure 4 the RBE is relatively constant in this region, varying from about 0.8 to 1.3. Furthermore the damage induced by a given dose may be modified to a large extent by various experimental conditions, as exemplified by the relatively high values of OER of about 2.6.

In the region denoted III in figure 4, ranging from 20 to 80 keV/μ, damage is predominantly produced by traversals of single particles through a critical structure or target in the cell, resulting in exponential survival curves and the absence of effects of dose-fractionation and variations in dose-rate. The RBE corresponding to a surviving fraction of 0.8 increases with LET_{∞} in this region from about 2.5 to about 6. It should be pointed out, however, that the damage produced by particles having LET_{∞}'s in this range can be modified considerably by various factors, as exemplified by the OER values in this region which range from about 2.4 to 1.7. In region V corresponding to LET_{∞} values in excess of 160 keV/μ, damage is produced by traversals of single particles, resulting in exponential sur-

vival curves. There is no effect of dose-fractionation and variations in dose-rate. The RBE decreases in this region with increasing LET_∞ due to a saturation effect (17). This implies that the effectiveness per particle is independent of LET_∞ in this region. Furthermore the damage produced cannot be modified by various conditions as exemplified by the OER which is equal to 1.0. Between the regions I, III an V, regions II and IV represent transition regions in which some of the characteristics discussed change rapidly.

IV. IMPLICATIONS OF DOSE-EFFECT CURVES AND RBE-LET RELATIONS WITH RESPECT TO RADIOBIOLOGICAL MECHANISMS AND FUNDAMENTAL ASPECTS OF RADIATION PROTECTION PROBLEMS.

In the preceding sections some characteristic differences between dose-effect relations for damage to the reproductive capacity of mammalian cells obtained with various radiations, have been discussed. Dose-effect curves for different radiations have also been obtained for a number of other biological effects, e.g. chromosome aberrations, opacification in lenses of mouse eyes and induction of malignancies. Investigations of these effects are of very great importance both for scientific and practical reasons, but fewer and less accurate results have so far been obtained as compared with data on cell killing. One factor in this respect is that instead of single cells, multicellular systems have to be irradiated, necessitating the use of radiations with sufficient penetrating power, e.g. γ-rays, fast electrons or fast neutrons. The LET-distributions of these radiations are more complex and extend over a wider interval of LET than those of mono-energetic heavy charged particles traversing single cells whereby the track-segment technique can be employed. A second factor which hampers the determination of accurate dose-effect relations, is the complexity of the responses investigated, involving the observation of large numbers of animals and of long latent periods before the effect can be

observed. One way in which microdosimetric studies may contribute to these latter problems is to provide a basis for selection of the types of radiations to be compared with a view of investigating significant differences between effects of various radiations. If two types of radiations, e.g. ^{60}Co γ-rays and 500 MeV protons, were found to produce energy deposition patterns which are closely similar, independent of the size of the critical structure considered, then little new information can be expected from a comparison of dose-effect curves of these radiations for any biological effect.

In addition to this possibility to predict which radiations are most likely to yield significant differences in biological effects, detailed information about the local energy density distributions will have to provide the basis for a quantitative interpretation of experimental results in terms of radiobiological mechanisms. In principle three possibilities can be explored, depending on the type of experimental data available, leading to a comparison of:

a. Differences between characteristics of dose-effect relations for a particular end-point, measured with only a few types of radiation.

b. Characteristics of RBE-LET relations, derived from dose-effect curves measured for a variety of radiations.

c. Differences between RBE-LET relations obtained for different biological effects in the same biological system.

No attempt will be made to present a general discussion of these possibilities and the difficulties in the interpretation of available data, but a few examples will be discussed in order to point out some of the problems involved.

a. Characteristics of dose-effect relations

Characteristics of dose-effect relations have been used extensively to derive information about the mechanism by which damage is produced and

to infer a correlation with the patterns of energy dissipation of various radiations. It is necessary to note in this respect that in addition to insufficient knowledge of the physical parameters, uncertainties in the biological data as well as about variability of the biological material, may present serious difficulties. Even for some of the simple systems which have been studied extensively, such as survival of mammalian cells, a statement made by Rossi a few years ago is still relevant: "dose-effect curves are generally unsatisfactory sources of information because instead of a curve one deals usually with a few points, having such limited statistical accuracy that it is possible to fit several mutually exclusive theoretical curves through them". Since furthermore the absorbed dose is not a sufficient characterisation of the energy deposition, Rossi concludes: "A dose-effect curve is typically a collection of a few uncertain points on a plot relating a variety of complex responses with a physical parameter of secondary relevance"(4).

As an example of these complications it is of interest to discuss some characteristics of mammalian cell survival curves. As mentioned before, with high-LET particles exponential survival curves are obtained which may be interpreted by the assumption that cell lethality is due to traversals of single particles through some critical site in the cell. With low-LET radiations, however, the curves generally exhibit a pronounced curvature in the low dose region between surviving fractions of 1.0 and 0.1, followed at higher doses by a region where curvature is smaller and sometimes insignificant. The general implication of this shape of the curve is that at least for part of the cell killing damage must be accumulated.

These dose-survival curves have been discussed extensively in the past years and attempts have been made to describe them quantitatively in terms of multi-hit and multi-target models. From these studies it has become apparent that an adequate fit of all the data obtained for various cell lines cannot be obtained by theoretical dose-survival curves derived from either multi-hit or multi-target models with single values of the number of hits or targets in ex-

cess of 1 (7, 18, 19, 20, 21). This is mainly due to the fact that in the low dose range, corresponding to surviving fractions between 1.0 and 0.8, the curve has an initial slope different from zero (indicated by curve c in figure 1). This can be explained only by assuming that at least part of the cell killing by low-LET radiations, is produced according to first order kinetics (7, 8, 12, 22). It has been suggested that this part of the cell killing is due to the fact that all radiations of low mean LET, dissipate part of their energy at high local energy densities through low energy electrons and δ-rays (3, 8, 23). Thus the shape of the survival curve might be interpreted by assuming a direct correspondence with the spatial pattern of energy absorption. The increase in the slope of the curve at doses in excess of about 100 rads may be assumed to be due to accumulation of damage due to multiple traversals of ionizing particles through a critical structure in the cell. Indeed, Fowler has shown that good agreement between the multi-hit model and the experimental data measured for various cell lines, can be obtained for a distribution of hit numbers extending from 1 to about 5 (21, 24). This interpretation is of interest because the model would allow an extrapolation to the very low doses relevant to radiation protection considerations, where experimental data cannot be obtained. The model would imply that no threshold dose exists with low-LET radiations due to the component of first order kinetics of cell killing. If it is assumed further that the local energy concentrations required for other types of injury, which are non-lethal to the cell but may result in hereditary effects and consequently are more relevant to health hazards, are equal to or less than those required for cell killing, then it may be concluded that for these other types of cellular damage no threshold would exist either (23).

The considerations and conclusions about shapes of survival curves have usually been based on the assumption that the cell populations investigated, are homogeneous with respect to radiosensitivity. Experiments with synchronized cells have shown variations in radiosensitivity with the age of

the cells in the cell cycle. It has been pointed out by many authors that shapes of survival curves obtained for mixed populations of cells, with different fractions having different sensitivities, may not reflect at all the shapes of the survival curves for each of the sub-populations. Consequently, with random populations of mammalian cells, the characteristics of survival curves cannot be related directly with the pattern of energy deposition. Further accurate experiments on synchronized populations, irradiated with low doses, are required to assess the relative contributions of inhomogeneity of the cell populations and of the spatial distributions of energy deposition, to the characteristics of the shape of a survival curve.

Investigations with high-LET radiations provide an important advantage, because the interpretation of the results frequently presents less uncertainty. The dose-effect relations have frequently a more simple shape. Accumulation of damage plays a smaller part, environmental conditions do not modify the responses to a large extent and variation in sensitivity with age in the cell cycle is smaller though not absent. However, quantitative interpretation of the results depends on adequate knowledge about the energy dissipation patterns. Most investigators who have made measurements of microscopic distributions of energy deposition, have till now concentrated on measurements of X-rays, γ-rays, fast neutrons and mixed beam of various particles which all have wide distributions of dose in LET. Data on mono-energetic heavy charged particles would be of great value for the interpretation of radiobiological data obtained with heavy ions during the past ten years.

b. Characteristics of RBE-LET relations

If sufficient data are available for a biological effect investigated with a variety of radiations, it is possible to derive a RBE-LET relation. These relations show in general an increase in RBE with LET to a maximum, followed by a subsequent decrease at very high-LET's. The absolute value of this maximum as well as the LET at which it is attained are obviously of great interest

not only in radiobiology but for radiation protection considerations as well. For reproductive death of bacterial cells and yeast cells the maximum RBE-values observed are of the order of 2 to 3. For mammalian cell killing the maximum is definitely higher, but depends on the level of damage considered as shown in figure 5, although the maxima occur at approximately the same LET_{∞} values. This indicates that the energy requirements for induction of lethality in these various objects are different. Quantitative interpretations of these differences are hampered by the fact that the influence of the spread in the distributions of energy deposition, even in experiments employing the track segment method, is difficult to assess, as long as no sufficient data on microdosimetry are available. A more detailed analysis of differences between RBE-LET relations measured for single cell systems will be presented later in this symposium.

For the more complicated biological effects such as carcinogenesis and hereditary changes in mammals, sufficient experimental results are not yet available to derive RBE-LET relations. The effects cannot be produced by irradiating single cell populations and for irradiation of tissues and whole organs, radiations with sufficient penetrating power have to be employed, which all have wide distributions of dose in LET. Consequently determinations of the spatial distributions of energy deposition are an absolute requirement for the interpretation of the data available and the testing of various hypothesis.

c. Qualitative differences between RBE-LET relations obtained for different effects in the same cell or organism

These differences merit a brief discussion, because in certain cases they can lead to fairly unambiguous conclusions about radiobiological mechanisms. Figure 6 shows two theoretical relations of RBE versus LET which differ significantly in shape. Where such differences are observed between RBE-LET relations for two types of effects produced in the same cell or the same organism, it may be concluded that different fundamental mechanisms are involved. An example may serve to illustrate this point. In experiments

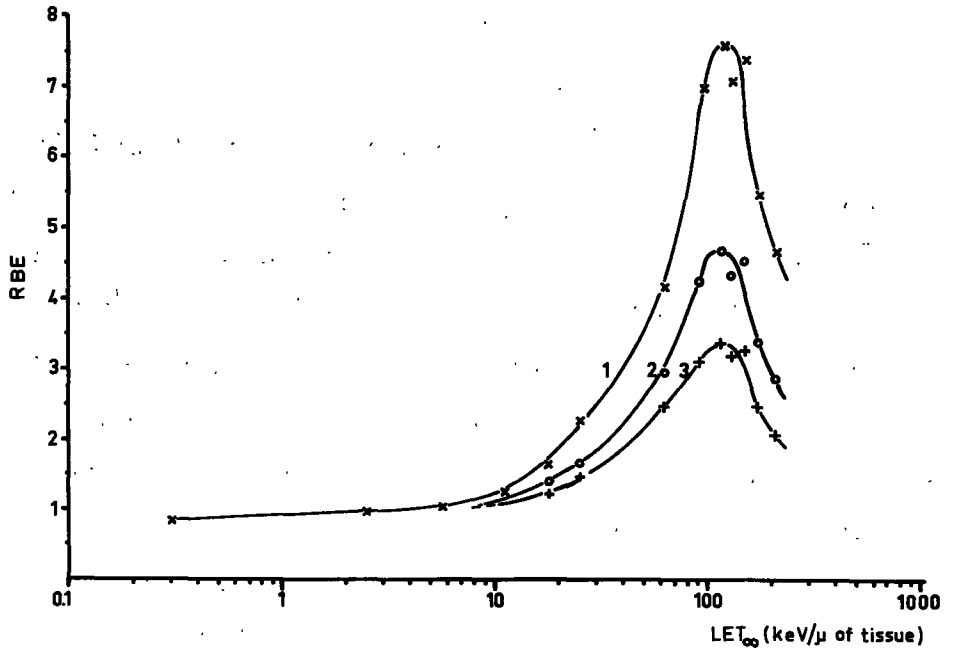

Figure 5. RBE-LET relations for impairment of the proliferative capacity of cultured cells of human origin by monoenergetic charged particles. Curves 1, 2 and 3 have been derived from survival curves for cultured cells of human origin irradiated with 250 kVp X-rays, deuterons and α-particles of different energies. They correspond to fractions of surviving cells of 0.8, 0.1 and 0.01 respectively.

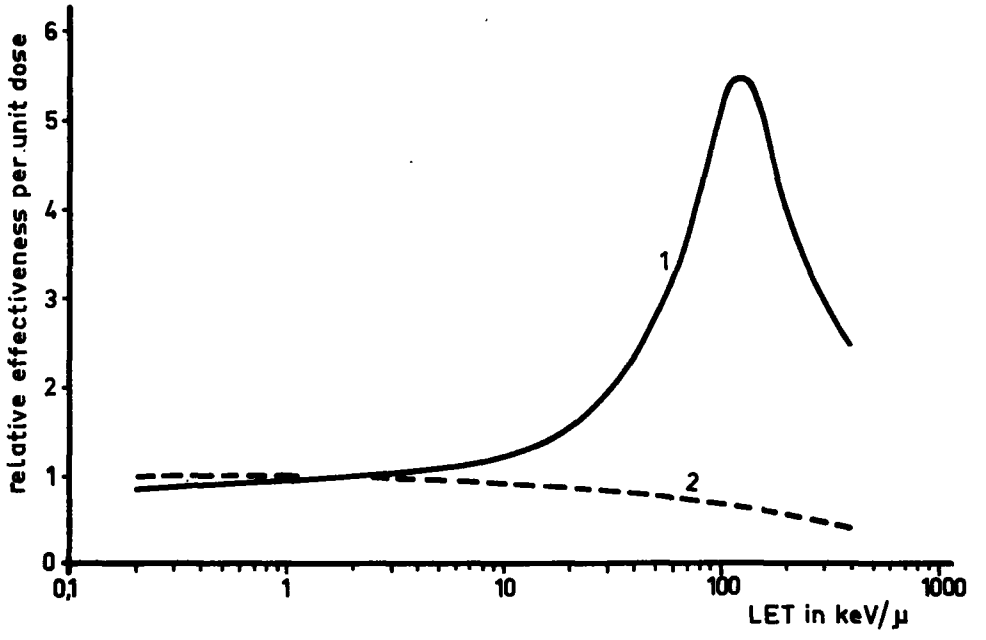

Figure 6. General aspects of possible relations between the Relative Effectiveness of ionizing particles and their LET's, concerning changes induced in different systems. Curve 1 is characteristic for various biological effects on cells, curve 2 is characteristic for inactivation of enzymes and phages.

with cultured mouse fibroblasts, Smith has measured a RBE of 0.25 for 3 MeV α-particles relative to X-rays with respect to reduction of DNA-synthesis (25). Although he did not measure survival curves for the same cells, all evidence obtained for a variety of cell lines indicates that for impairment of the reproductive capacity of mammalian cells the RBE of α-particles of this energy is in excess of 3. This indicates that it is unlikely that reduction of DNA synthesis in mammalian cells is related to impairment of the reproductive capacity.

It is obvious that studies of RBE-LET relations can provide an important tool for the testing of hypotheses, which imply relations between biochemical effects of ionizing radiations and different biological effects such as cell killing, hereditary changes and the induction of malignancy. If such differences can be properly interpreted on the basis of exact data of the microscopic distribution of energy density, radiobiology could make a significant contribution to the solution of these problems. The insights obtained in this way will be of great importance not only for the assessment of the risks of exposure of radiation workers and of the world population, but also for the elucidation of mechanisms involved in tumour induction and hereditary aberrations.

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D I S C U S S I O N

Mr. KASTNER

I am curious about your curve of RBE versus oxygen enhancement effect; is there any real relationship, is one really the differential of the other or is that just a coincidence ?

Mr. BARENDSEN

Well, I think the OER-LET curve is not an exact differential of the RBE-LET curve, but there is some relation of course. As I discussed in my paper, at very high LET, the RBE decreases with increasing LET and we might interpret this as being due to a saturation effect. This implies that a particle passing through some critical structure will dissipate more energy in this structure than is required for damage. Now, if cells are irradiated in anoxic conditions, part of the energy dissipated is rendered ineffective as compared to oxygenated conditions. If, however, a particle dissipates e.g. twice the amount of energy required in a critical structure to kill a cell, than the elimination of e.g. a fraction of 0.25 of the primary damage will not result in a decrease in the effectiveness per particle to kill the cell. Thus at very high LET the oxygen effect will be absent. At lower LET values, when no excess of damage is produced by a single incident particle, the oxygen effect becomes important. There is no complete coindidence of the two curves in figure 4 however. A more direct relation is observed if the effectiveness per particle or effective cross section is compared with the OER.

Mr. NEARY

I agree with Dr. Barendsen that this is a possible interpretation of those results, but I think I have an opportunity tomorrow, but I would just like to mention it now, that there is another possible interpretation, a purely physical chemical explanation that in a high LET-track there is a production of oxygen by reaction between radicals and that the falling dependence of oxygen, on the external oxygen in the

system is purely a sort of incidental consequence of this radiation chemical production of oxygen in situ.

Mr. BARENDSEN

Well, this is an interesting explanation, but I feel a number of arguments can be raised against it. One of the main objections is that if a purely physico-chemical effect produced the decrease of OER with LET, than this decrease would be expected to occur always at the same LET, independent of the system investigated. Since your own results on chromosome aberrations in tradescantia show a sharp decrease in OER between 10 and 20 keV/μ and my data on mammalian cells show a sharp decrease in OER between 60 and 100 keV/μ, I feel that the oxygen production in the tracks of high LET particles cannot explain the decrease in OER with LET.

Mr. FOWLER

Can I ask you, Dr. Barendsen, about the last figure, where RBE was plotted against LET. Would you please say more about the different interpretation of the rising and falling curve with the peak of RBE, as compared with the dotted curve of RBE which falls with increasing LET?

Mr. BARENDSEN

Yes, I used this figure to exemplify a situation where we have data concerning two different effects of radiation in the same experimental system e.g. a cell. It is a hypothetical curve since very little data on different effects in the same cell are available. If such data were available however, it is possible to conclude that the two types of effects, e.g. cell killing and reduction of DNA synthesis measured in the same cell, would have little in common. Thus one might conclude that reduction of DNA synthesis is not instrumental in producing cell killing.

Mr. FOWLER

In terms of energy deposited, are you willing to say that curve 2 (the falling curve of RBE vs LET) requires less energy to produce the damage

than curve 1 (the curve with a peak of RBE) ?

Mr. BARENDSEN

Yes, I think that is the most obvious interpretation.

Mr. FOWLER

Are you willing to say anything about the dimensions of the volume within which this energy must go ?

Mr. BARENDSEN

Yes, as you know I have made some approximate calculations which indicate that for mammalian cell-killing an amount of energy of about 500 keV is required within a volume with dimensions of about 100 Å. If for instance for yeast cells the RBE starts to rise at a lower LET, as compared with the curve for mammalian cells than one might deduce that either a lower energy density is required or the same density is required in a smaller volume. I will come back to this point in my other contribution to this symposium.

Mr. FOWLER

But can you distinguish between those two ?

Mr. BARENDSEN

That would depend in part on the accuracy of the experimental data but I feel it would be very difficult to give a definite answer.

Mr. FOWLER

So the left hand curve that begins to rise sooner is either more energy in the given volume, or it might be a bigger volume requiring a given energy. Are you talking about this also in your later paper ?

Mr. BARENDSEN

Yes.

Mr. BOOZ

You have shown us a survival curve measured for a LET value of 5.6 keV per micron which had an initial slope that was exponential. From this you have drawn the conclusion that all survival curves measured at low LET radiations show this effect. Do you want to include gamma and X-rays too?

Mr. BARENDSEN

Yes.