MICRODOSIMETRIC DATA AND THE BIOLOGICAL EFFECTIVENESS OF NEUTRONS*

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There is a variety of reasons for the considerable attention which is presently being given to the radiobiology of neutrons. Some, and not the least important, reasons are of purely practical nature. The increasing spread of nuclear technology has led to an intense discussion of the potential hazards of small doses of neutrons as they may occur in the vicinity of nuclear power stations, or of acute exposures in nuclear accidents. The recent introduction of neutron therapy in various centers around the world is equally important, and it poses a related problem, with the difference that the interest is in the effects not of small but of high absorbed doses of neutrons.

Apart from these practical reasons the radiobiology of neutrons is of fundamental importance for an understanding of the primary mechanisms in the action of ionizing radiation on the cell. Neutrons offer a particularly powerful tool for radiobiological investigations on the tissue level because they enable the experimenter to apply a homogeneous dose of densely ionizing radiation to objects of macroscopic dimensions. Primary fast charged particles do not offer the same advantage because they have either high linear energy transfer (LET) and very short ranges, or at ranges sufficient to cover more than cellular distances they have only low LET. Sources of very heavy ions will have similar advantages as neutrons. However, they are not as yet generally available. Moreover their use introduces a complication which is absent in the case of neutrons, namely the fact that one deals with two entirely different components of the energy deposition, the extremely dense ionization in the core of the particle tracks and the very sparsely ionizing contribution of the δ-ray tracks which at the required relativistic speeds of the ions extend far from the core of the ion track. Microdosimetry will be essential in all attempts to understand the action of such heavy ions. In the case of neutrons the situation is somewhat simpler; the rather slow recoil particles have very short δ-ray ranges and in a first approximation one can therefore apply the LET concept. Neutrons offer therefore a convenient example of a radiation for which the microdosimetric analysis can be understood in close analogy to the more familiar LET concept.

In this contribution an attempt will be made to survey our understanding of neutron RBE. Particular reference will be given to data obtained at the Radiological Research Accelerator Facility in Brookhaven.

This will make the comparison of results easier; it does not imply that the results obtained at other laboratories are of less importance.

1. The linear-quadratic dependence of cellular damage on absorbed dose.

It has long been known that sparsely ionizing radiations, such as x-rays or γ-rays or fast electrons, affect the cell by a stepwise accumulation of damage. A great number of particles are, in general, necessary to kill a cell. After its nucleus has been traversed by about 2,000 fast electrons a mammalian cell still has an equal chance of death and survival. The cell therefore is able to tolerate small doses of sparsely ionizing radiation quite well. The effectiveness of such radiations increases only as one approaches doses which are sufficiently high that the sublesions produced by a number of ionizing particles can interact. Accordingly one obtains the well-known sigmoidal dose-effect curves for sparsely ionizing radiations.

The situation is entirely different in the case of neutrons because neutrons produce such densely ionizing recoils that one such particle traversing the nucleus of a mammalian cell has a very substantial chance to inactivate this cell, or to produce one or several chromosome aberrations. No interaction between different particles is therefore necessary to produce the effect, and accordingly one obtains a linear dependence of the cellular effect on absorbed dose. In other words the effectiveness of the radiation per unit absorbed dose is independent of the dose.

The relative biological effectiveness of neutrons relative to x-rays is defined as the ratio of an x-ray dose to the equivalent neutron dose. With this definition it is immediately apparent from the shape of the response curves that the RBE of neutrons is largest at the lowest level of effect or of absorbed dose. At higher doses the relative difference in the effectiveness of neutrons and x-rays must decrease. This fact which has sometimes been overlooked is of greatest practical importance. It accounts, at least partly, for the widely varying values of RBE which are found in the literature. Section II will deal in detail with this matter.

While the general characteristics of the dose dependence for sparsely and for densely ionizing radiation are easily understood, one cannot as readily make quantitative statements on the precise mathematical form of the dependences. It is therefore a priori uncertain whether in

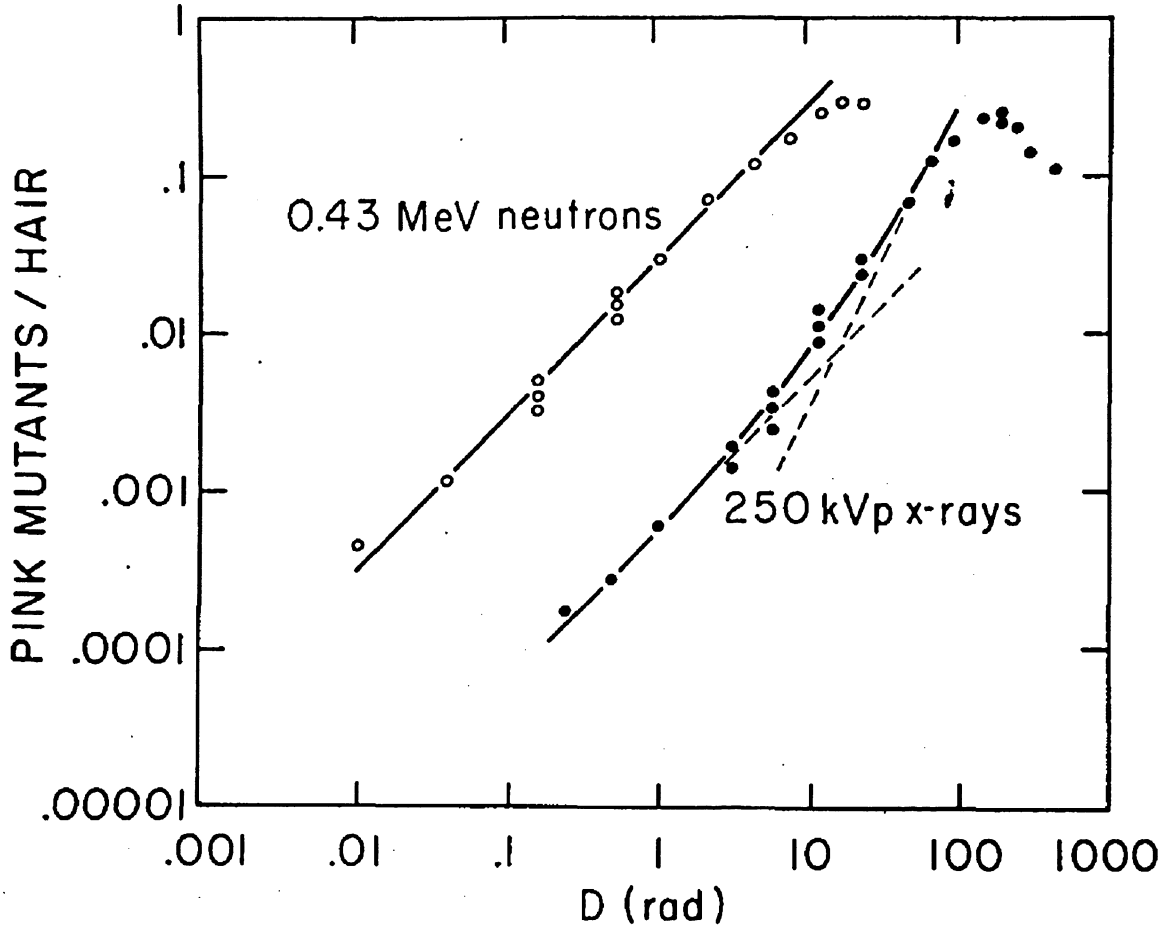

Fig. 1 Induction of pink mutant cells in the stamen hairs of Tradescantia by x rays and 430 keV neutrons (33). The spontaneous rate is subtracted. The solid line for the neutron irradiation corresponds to a linear dose-effect relation; the solid line for x rays corresponds to the linear-quadratic dose effect relation. At a dose of 16 rad the linear and the quadratic component are equal.

the action of sparsely ionizing radiation on the cell one has a true threshold of the effect, a parabolic dependence, or a superposition of a small linear component and a higher order term in absorbed dose. However, recent experiments have indicated that one deals in many important instances with the last of the three cases. This will be the object of the remainder of this section. In the following section it will be shown that the finding is supported by the analysis of neutron RBE as a function of dose.

In the special case of two-break chromosome aberrations it has been realized long ago (29,21) that one deals with a dependence of the effect on the square of the energy locally deposited in the cell. From this it follows that there must be a linear component which reflects the interaction of chromosome breaks produced by the same particle track, and a quadratic term in absorbed dose due to the interaction of single breaks produced by different primary particles. In the case of neutrons the linear component is dominant because at doses up to a few hundred rad the tracks of the heavy recoils are so widely separated that the interaction of sublesions occurs mostly within individual tracks. In the case of electrons the quadratic component is dominant because the primary ionizations are more widely spaced along the electron track and the distance from one to the next particle track is much smaller at a given dose. However, one should note that, particularly at low doses of sparsely ionizing radiation, one must also deal with a linear component which is due to the clustering of energy deposition events within individual particle tracks and particularly within δ-rays.

One example where these characteristics can be seen clearly are the studies of pink mutations in Tradescantia (33). Fig.1 shows the dose dependence of these mutations for 430 keV neutrons and for x-rays. If one disregards the saturation at high doses which is probably connected with cell killing, one finds a linear dependence for neutrons and a nearly quadratic dependence for intermediate doses of x-rays. At the lowest x-ray doses a linear component appears, as expected on theoretical grounds.

The study of dicentric chromosomes involves much greater experimental difficulties than the scoring of mutations in Tradescantia, accordingly the statistics are less accurate. However, Bauchinger and Schmid et al. (4,5,6,30,31) have recently succeeded in determining the

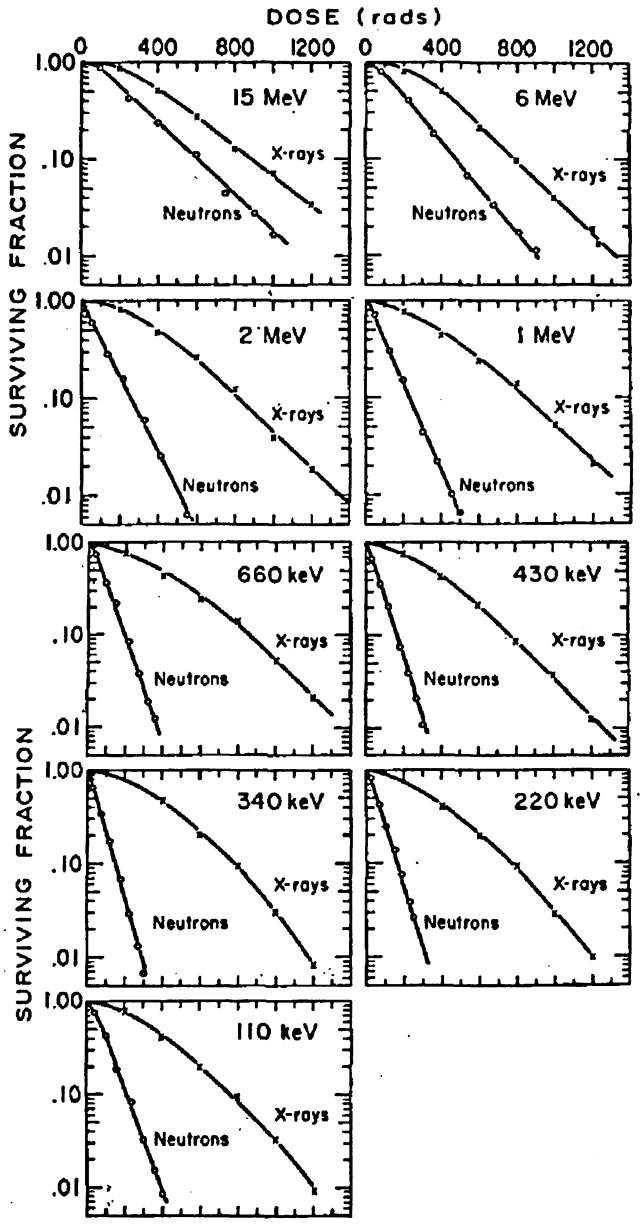

Fig. 2 Survival of cultured V79-hamster cells after exposure to monoenergetic neutrons of various energies (14). The data for x-rays are included for comparison.

corresponding curves for 15 MeV neutrons, for x-rays and for electrons. Their results correspond closely to the characteristic form of the curves in Fig. 1.

Recent investigations performed with V-79 hamster cells (14) lead to the conclusion that survival curves obtained with x-rays and with monoenergetic neutrons of various energies show very similar behavior. It has been found that in all these cases the logarithm of the survival probability can be represented by a linear-quadratic function of absorbed dose:

$$-\ln S(D) = \alpha D + \beta D^2 \qquad (1)$$

For neutrons the linear term is dominant. For x-rays the two terms are comparable at doses of a few hundred rad. The agreement between the observed data and the theoretical expression is particularly good in an x-ray survival curve for V-79 cells synchronized in late S-phase (13).

Fig. 2 represents the survival curves obtained with neutrons of various energies for non-synchronized V-79 cells; the results for x-rays are included for comparison. These observations and various similar results obtained on higher organisms indicate that the quadratic dependence of the cellular effect on energy concentration may apply not only to the production of dicentric chromosomes. Accordingly it is of interest to derive the dose dependences which result for different radiation qualities under this assumption. Essential in such a consideration is the distinction between the absorbed dose, D, which is only a mean value and the microdosimetric quantity, z, which measures the actual energy deposition in a microscopic region (24,25).

The specific energy, z, is the random variable which corresponds to absorbed dose, it is defined as energy deposited divided by a mass of the reference region (16,18). In the same way as absorbed dose the specific energy can be measured in rad or in J/kg. If one merely considers energy deposition due to single particle tracks, another closely related quantity is frequently used. This quantity, the lineal energy, y, is defined as the energy deposited in a microscopic region divided by the mean diameter of the region. If one expresses the specific energy in rad, the lineal energy in keV/μm, then one has the following relation between the two quantities for a spherical region of diameter d (in μm):

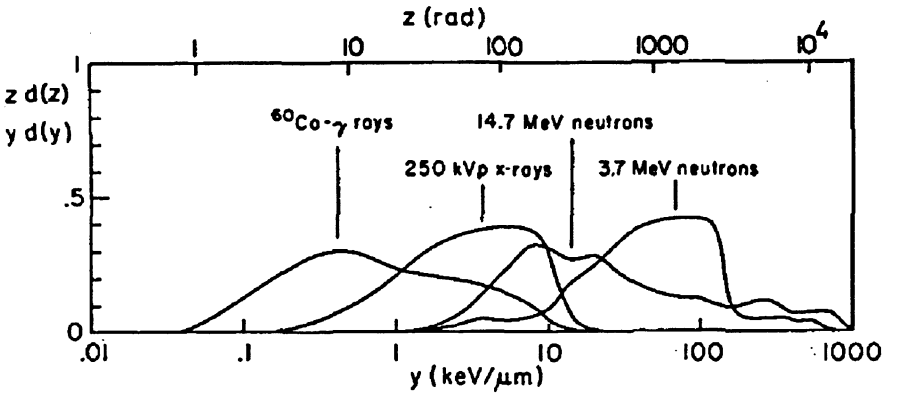

Fig. 3 Distribution of dose in y and z for single events in spherical tissue regions for various radiation qualities. The curves are based on experimental (7,8) and theoretical data (15); they refer to a diameter of 1 μm.

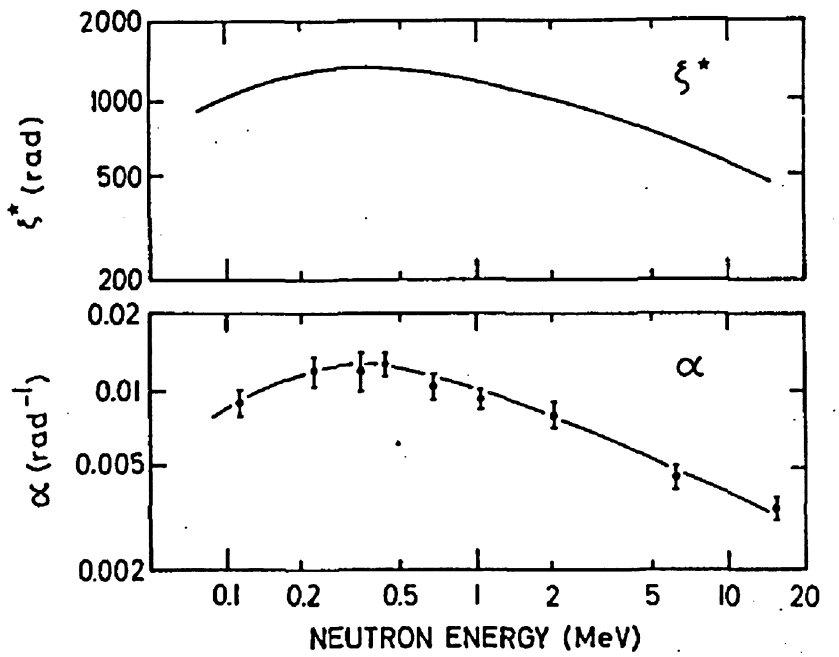

Fig. 4 Mean increment, ζ^*, of specific energy in single events for various neutron energies, and the coefficient α of the linear term in Eq.(1) for the survival of V79-cells at various neutron energies (14).

$$z = 20.4\ y/d^2 \qquad (2)$$

y is the microdosimetric analogon to LET.

Fig.3 represents typical probability distributions of the increments z or y produced by various radiation qualities in microscopic tissue regions of the diameter 1 μm. It is apparent from these curves that neutrons produce vastly larger increments of specific energy than sparsely ionizing radiations. It is also apparent that these increments can vary by orders of magnitude even for a given type of radiation. If one considers the random variable, y, one can compare the distributions to the LET distributions which belong to neutrons. However, one must note that due to the short ranges of many of the recoil particles and due to the variation of LET along the particle tracks the distributions of y can be substantially different from the distributions of LET.

Since it is possible to measure or to calculate the microdosimetric distributions for any neutron energy and for any region of interest (see e.g. (7,8,9,11)), one can obtain the mean value of the square of the specific energy. If the cellular effect is indeed proportional to the square of the energy deposition in critical sites, then the average observed effect at a given dose must be proportional to the mean of the square of specific energy at this dose:

$$E(D) = k\overline{z^2(D)} \qquad (3)$$

This mean value of the square of the specific energy can be readily calculated on the basis of elementary properties of the microdosimetric distributions. One finds the following surprisingly simple relation (19):

$$\overline{z^2(D)} = \zeta D + D^2 \qquad (4)$$

where ζ is the dose average of z in individual events. Accordingly one obtains the linear quadratic dependence of the cellular effect on absorbed dose:

$$E(D) = k(\zeta D + D^2) \qquad (5)$$

By using the relation between y and z one can obtain the analogous relation in terms of y, and by equating the dose average of y with the dose average, $\bar{L}_D$, of LET one obtains the approximate relation in terms

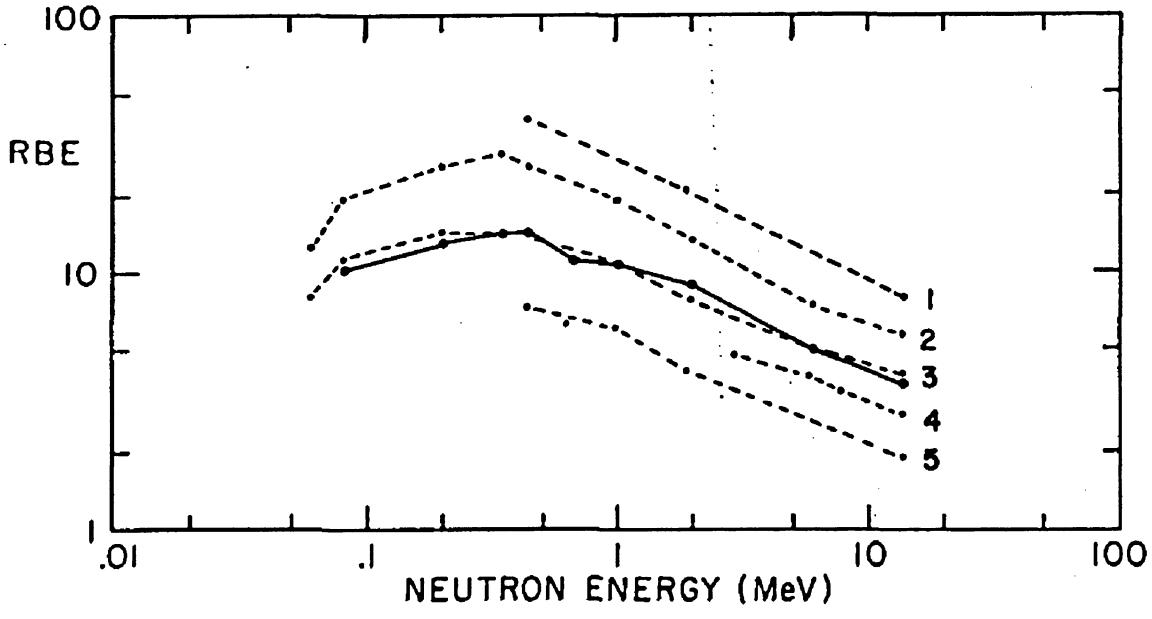

Fig. 5 Observed dependence of RBE on neutron energy: Solid line: Limiting RBE at low doses for inactivation of aerated V79-cells (14). Broken lines:

1: Lens opacification at x ray dose 40 rad (3)

2,3: 50% growth reduction of Vicia Faba (anoxic, and oxygenated (12)

4: Cellular inactivation (initial part of the survival curves) (1)

5: 37% depletion of spermatogonia (2)

of LET.

One can test Eq.(5) by comparing the energy dependence of the microdosimetric quantity, ζ, for neutrons with the dependence of the linear coefficient, α, of the curves in Fig.2 on neutron energy. Such a comparison is given in Fig.4. With regard to this figure one must note one further complication; this is the fact that the microdosimetric quantity, ζ^*, plotted in the graph is corrected for the saturation effect which occurs when a charged particle has such high stopping power that it deposits more energy in the cell than is necessary to inactivate it. Details of this correction are described elsewhere (19), the fact that one deals with the corrected quantity is indicated by the star. Up to energies of about 600 keV the correction is of minor importance. For higher neutron energies it is substantial due to the presence of very densely ionizing heavy recoils.

One concludes from the comparison that the experimental data are in essential agreement with the microdosimetric data. The highest biological effectiveness is reached for neutrons of energy between 220 keV to 430 keV. Both at lower energies and at higher energies the biological effectiveness decreases.

The same remarkable agreement is found in a variety of other effects on higher organisms as can be seen from the compilation of data in Fig.5. The absolute values of the RBE in this representation are uncertain due to the statistical uncertainty in the determination of the linear component of the response for x-rays in the various experimental systems. However, this uncertainty does not affect the shape of the curves in the logarithmic plot, and this shape is in essential agreement with the microdosimetric data.

Another interesting aspect of Eq.(5) is the comparison of the magnitude of the linear component in absorbed dose with that of the quadratic component. According to the relation the linear and the quadratic components are equal at an absorbed dose, ζ. One can use this result to obtain the diameters of the sites in the cell over which sublesions interact. In Fig.1, for instance, the linear and the quadratic component for x-rays are equal at about 16 rad. This value of ζ for x-rays corresponds to a site diameter of about 2 μm. The value of ζ for 430 keV neutrons for the same site diameter is approximately 450 rad, i.e. the

linear component should be about 30 times larger in the case of the neutrons than in the case of the x-rays. This is indeed observed in the experiment.

II. RBE as a function of neutron dose

The determination of precise dose-effect relations is not usually as simple as in the mutation induction in Tradescantia. Moreover dose-effect relations can be, and usually are, influenced by factors which are not related to the statistics of energy deposition and which are independent of radiation quality. The dose-effect relation itself is therefore not always indicative of the primary mechanisms of radiation action. As first proposed by Rossi (26) it is useful in such cases to consider the RBE of neutrons as a function of neutron dose. In this way one can avoid arbitrary scales of effects which must be adopted for tissue effects, such as the induction of lens opacification or the production of skin damage. One can also hope to eliminate in this way those factors which are common to the two radiations which are being compared.

If one considers the intermediate dose range in which the effect of x-rays is proportional to the square of the dose, while the effect of neutrons is proportional to dose, one can derive a simple relation for the RBE of neutrons as a function of dose. From the equality of the effect $E_x(D_x)$ of x-rays and the effect $E_n(D_n)$ of neutrons:

$$E_n(D_n) = k\zeta_n D_n = E_x(D_x) = kD_x^2 \qquad (6)$$

one obtains:

$$RBE = D_x/D_n = \sqrt{\zeta_n/D_n}$$

This means that, at least in an intermediate dose range, the RBE of neutrons is inversely proportional to the square root of the neutron dose. One obtains a straight line of slope minus one-half if one plots the logarithm of RBE vs. the logarithm of the neutron dose. Extending this line to its intersection with the abscissa, RBE = 1, one obtains the value ζ.

In a great variety of effects on higher organisms the RBE of

neutrons follows this equation (19). The values of ζ for neutrons which have been obtained in these experiments are such that the corresponding site diameters are close to one or several micrometers.

Figs.6 to 9 contain examples of RBE dose-relations. In all these examples the estimated RBE dose-relation is given by a curve or straight line and vertical bars mark those ranges of RBE which can be excluded with statistical certainty. Wedges indicate that the RBE should be lower or higher according to the comparison of the effect of a pair of neutron and x-ray doses, but that the result of the comparison has less than 95% statistical certainty. Details of the non-parametric statistical approach which is used in this analysis are described elsewhere (17).

The case of the opacification of the murine lens is particularly remarkable among these examples for the extremely wide range of neutron doses, from less than 100 mrad up to hundreds of rads, over which the RBE has been determined. Both in this case and in the recently performed investigation of the induction of mammary tumors in the Sprague-Dawley rat (32) one obtains very high values of the RBE at the lowest doses. These values are in fact higher than expected on the basis of the microdosimetric data. They indicate therefore that the effectiveness of neutrons may not only be determined by the long-range interaction of sublesions which exhibits itself in the quadratic dependence of the primary cellular effect on specific energy, but that it may in addition be influenced by the energy density in much smaller regions in the vicinity of the particle tracks. Microdosimetry has not yet contributed to the solution of this question, since neither the experimental methods nor the theoretical tools were available to obtain probability distributions of energy concentration in regions of molecular size. This situation is, however, changing. Various new experimental approaches are now being tested, and theoretical approaches, such as the ones followed here in Neuherberg (22,23), are equally important.

Figs.8 and 9 deal with two further examples of the dependence of neutron RBE on dose. Both examples are characterized by the fact that they are of great practical importance, but also by the fact that they are limited by the statistical accuracy of the data. For the leukemia induction in the survivors of the atomic bomb explosions in Japan only rough estimates of the RBE have been given earlier. Such RBE values

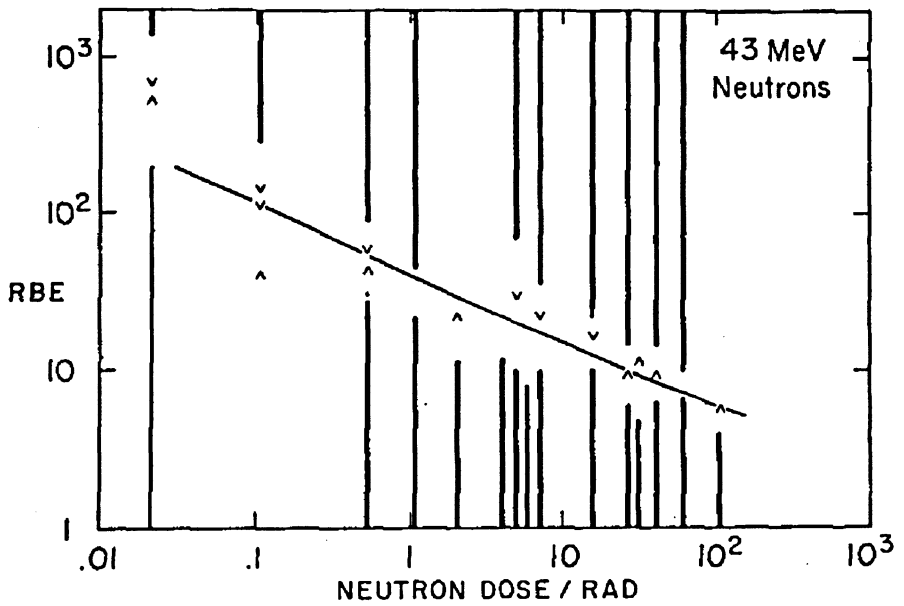

Fig. 6 RBE of 0.43 MeV neutrons relative to x-rays for the induction of lens opacification in the mouse as a function of neutron dose.

The vertical bars indicate the ranges of RBE values which, according to the comparison of x-ray and neutron doses are excluded. Broad bars: significance exceeding 99%; light bars: significance exceeding 95%; wedges: nonsignificant differences. The solid line corresponds to a formula given by Kellerer and Rossi (17).

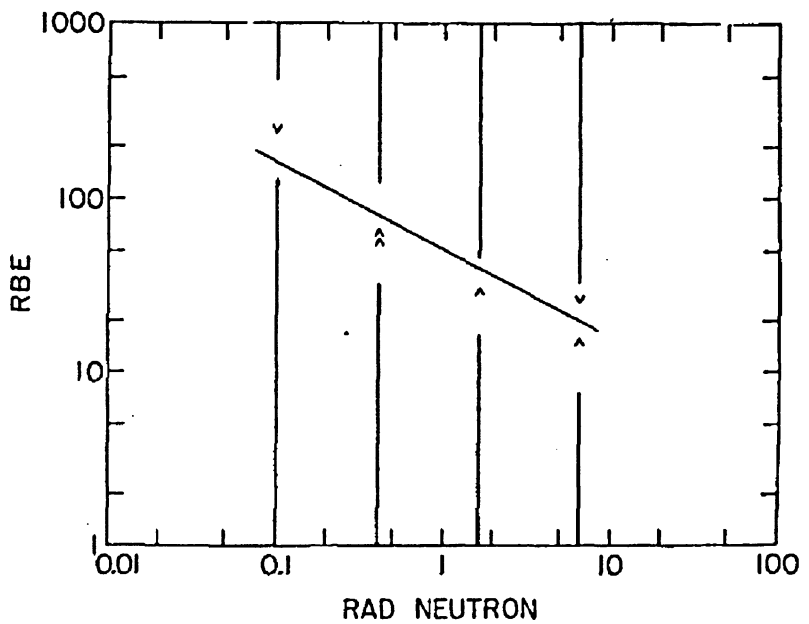

Fig. 7 The dependence of RBE for the induction of mammary tumors in the Sprague-Dawley rat on neutron dose (32). Vertical bars indicate the ranges of RBE that are excluded on a level of statistical significance exceeding 95%, wedges indicate non-significant differences.

have to be considered because the radiation in Hiroshima consisted primarily of neutrons and the radiation in Nagasaki has been primarily x-rays. It has only been in a recent re-analysis of these data (28) that the characteristic dependence of RBE on neutron dose has been found. As a final example the RBE dose-dependence is given for the experiments on mammalian cells which have already been discussed in Section 1. If one plots the RBE as a function of neutron dose from the interpolated curves shown in Fig.2, one obtains the curve segments in the graphs. The results of the explicit statistical analysis which are represented by the vertical bars, show however that insufficient information is available at low doses. From the dose-effect curves one estimates maximal RBE values for intermediate neutron energies and for low doses which are close to 12 (see Fig.5). However, the values are by no means precise. Considerable statistical uncertainties are unavoidable, in the conventional cell culture technique, because small changes in the survival rate at low doses are masked by the fluctuations in plating efficiency and in the number of plated cells. One must also consider that these data refer to non-synchronized cells and that, as shown by various authors, in an inhomogeneous population one may obtain complicated RBE dose-relations. It is however, of interest that even in this experimentally difficult system one observes the same characteristic relations which are found in a wide range of other effects on higher organisms.

There is considerable need for further improvement of the statistical accuracy of the experimental data, and a similar need exists for the improvement of microdosimetric data. Particularly at neutron energies above 15 MeV, only few microdosimetric measurements have been performed, and our knowledge of the relevant nuclear cross sections is very limited. The results obtained at present make it, however, clear that microdosimetric data can be useful in our attempts to understand the cellular action of widely varying types of ionizing radiation.

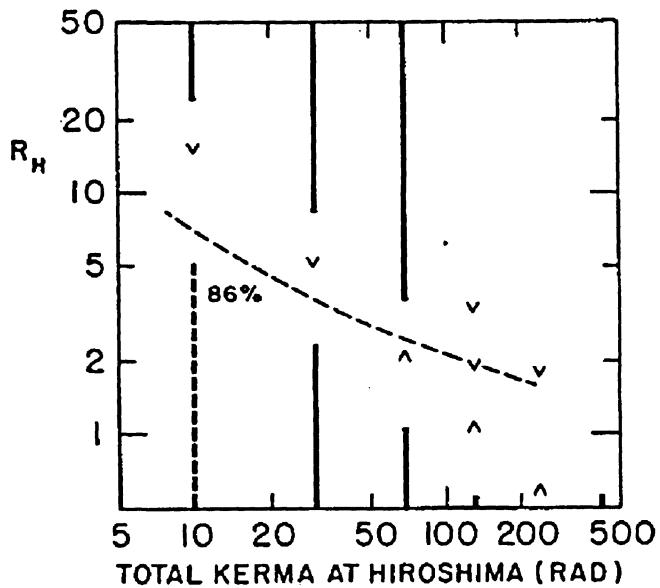

Fig. 8 Relative biological effectiveness of the radiation in Hiroshima compared to that in Nagasaki as a function of kerma in Hiroshima. The vertical bars represent those values which can be excluded with 95% confidence; the broken bar stands for a level of significance of 86% (28).

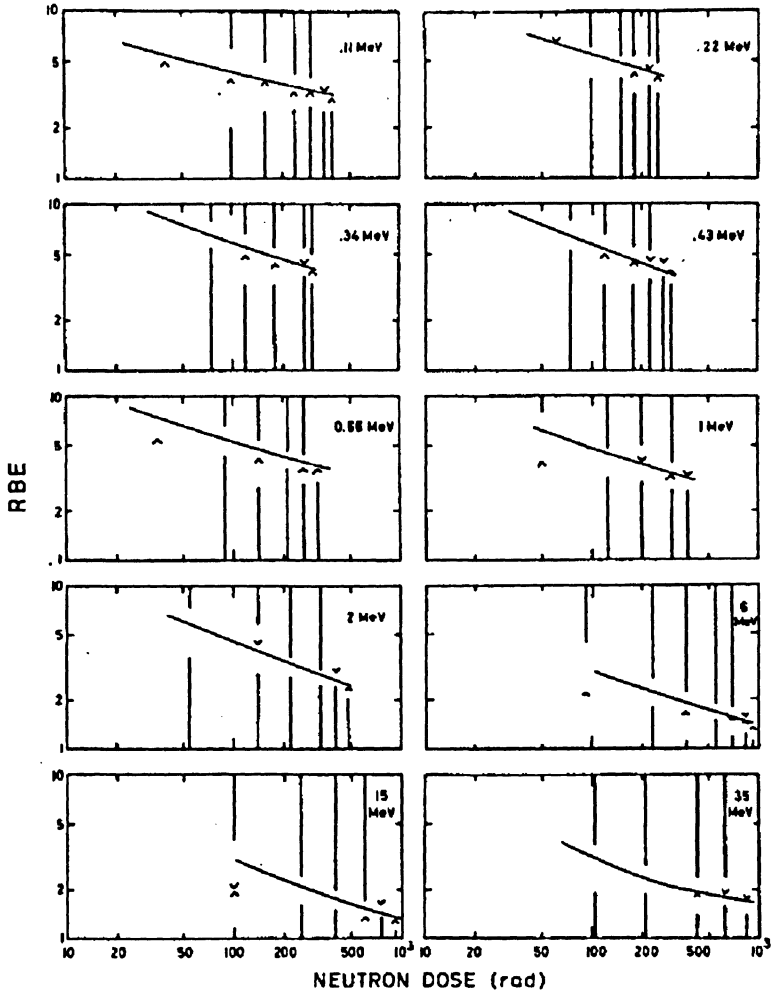

Fig. 9 RBE of monoenergetic neutrons for the inactivation of non-synchronized, aerated V79-hamster cells (14). The vertical bars represent those values which can be excluded with 95% confidence.

The curves result from a least squares fit of the data in Fig.2 to Eq.(1).

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