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RBE as a Function of Neutron Energy[1]

II. Statistical Analysis

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KELLERER, A. M., HALL, E. J., ROSSI, H. H., AND TEEDLA, P. RBE as a Function of Neutron Energy. II. Statistical Analysis. *Radiat. Res.* 65, 172–186 (1976).

An experimental study of the relative biological effectiveness of neutrons of various energies for the inactivation of hamster cells has been reported previously; the present article contains a detailed statistical analysis of the data.

Repeated determination of X-ray survival curves made it possible to assess the statistical fluctuations of survival rates. Within individual experiments the statistical variations are close to those expected on theoretical grounds. However, between experiments substantial variations in the reaction to X rays occur; these fluctuations can be approximately described by dose-modifying factors. An idealized survival curve for X-irradiation has been derived.

The survival curves for neutrons have been compared to the linear–quadratic dose–effect relation and the two coefficients in this equation have been derived together with their joined confidence regions. The linear coefficient for neutrons reaches a maximum for neutron energies of several hundred thousand electron volts; the coefficient of the quadratic component is increased at neutron energies below 1 MeV. The dependence of RBE on neutron energy and on neutron dose is derived from the estimated dose–effect relations. In addition, the more rigorous nonparametric method is used to derive confidence intervals for the RBE of neutrons.

INTRODUCTION

An extensive experimental study of the colony-forming ability of V-79 hamster cells after neutron and X-irradiation has been reported earlier (*1*). Survival curves have been presented for various neutron energies and for X rays, and the relative biological effectiveness (RBE) of neutrons as a function of their energy has been given. The results were obtained without the need of detailed statistical analysis.

However, the experiments have been conducted in such a way as to permit an explicit analysis of the statistical errors in the survival curves. The essential point

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is that a separate X-ray survival curve has been established simultaneously with each neutron experiment. This makes it possible to analyze the fluctuations in cellular sensitivity over an extended period, which, in the present case, spanned about 12 months. Furthermore, the determination of simultaneous neutron and X-ray curves has made it possible to apply the nonparametric statistical analysis (*2*) to the determination of the RBE of neutrons as function of neutron dose.

The statistical fluctuations within individual experiments and between separate experiments are dealt with in the first part of this article. The assessment of statistical errors is often critical for the interpretation of data obtained from irradiated cell cultures; the statistical analysis is therefore presented in considerable detail. The analysis of RBE as a function of neutron dose is dealt with in the last part of the article.

ANALYSIS OF THE X-RAY DATA

Fluctuations of Survival Rates within Individual Experiments

Each point of the 11 survival curves for neutron irradiation and of the simultaneously established X-ray curves that have been presented in the preceding publication (*1*) has been obtained by exposing four vials filled with cell suspension to a specified neutron or X-ray dose. If the only statistical fluctuations are those due to the finite number of surviving colonies, then the standard error should on the average, in accordance with the properties of the Poisson distribution, be equal to the square root of the number of surviving colonies. If, on the other hand, there are systematic variations between the individual vials the standard deviation of the survival rates in each group of four vials will on the average exceed the standard deviation of the survival rates that is predicted according to Poissonian statistics. Whether this is the case has been tested on the basis of the χ^2-distribution. The test has been performed for all individual X-ray doses, but in addition also for all neutron doses, in each experiment. Among the 159 values of χ^2 that were obtained for the 159 groups of four vials there were 15 values that exceeded the theoretical value of χ^2 on the 95% level. This is a significantly larger number than the eight cases expected on statistical grounds. Accordingly, one concludes that the actual standard deviations exceed the theoretical deviations. It has not been found that the cases of increased standard deviation occur mainly at high doses. This indicates that the increase in the statistical fluctuations is not due to the fact that different vials that were assigned the same absorbed dose were in fact exposed to slightly different absorbed doses. In other words, it does not appear that the increase of the statistical fluctuations is due to inaccuracies in dosimetry.

As an additional test the sum of the χ^2 values for all points of each individual survival curve, i.e., the χ^2-values pooled for all doses in each individual experiment, have been compared to the theoretical values. It is found that for 5 out of the 22 survival curves the resulting χ^2 exceeds the theoretical values on the 95% significance level. This test, based on the pooled χ^2-values, therefore confirms the conclusions from the analysis of the individual points.

To obtain an estimate of the ratio by which the actual fluctuations exceed the theoretical fluctuations, the total sums of χ^2 pooled from all doses and all survival curves have been computed.

For the X-ray experiments the total observed value is $\chi^2 = 401$, while the 95% level of χ^2 is $\chi_{95}^2 = 346$. At the large number of 296 degrees of freedom this difference is highly significant. The high statistical significance is due to the large amount of data; it does not in itself imply that the actual statistical fluctuations exceed the theoretical values greatly. Indeed it can be seen that the excess is small. To obtain the ratio of the actual standard deviations to the theoretical standard deviations one may consider the theoretical median value, χ_{50}^2. This median value is equal to the number of degrees of freedom, and the ratio of the actual standard deviations to the theoretical standard deviation for the X-ray experiments is therefore

$$\rho_x = (\chi^2/\chi_{50}^2)^{\frac{1}{2}} = (401/296)^{\frac{1}{2}} = 1.164. \tag{1}$$

Thus the statistical fluctuations are increased by a factor of only 1.16 relative to the fluctuations that are expected on theoretical grounds. For many practical purposes this factor is negligible. With the experimental technique employed in the present study it is therefore a good first approximation to give the theoretical values of the standard deviations, or of the confidence intervals. Naturally one cannot generalize this conclusion, and it may be necessary to perform similar analyses if one deals with different experimental conditions.

The finding for the X-ray data is supported by those for neutron irradiations. If all neutron experiments are pooled one obtains the value $\chi^2 = 427.9$ and the theoretical 95% value $\chi_{95}^2 = 356$ at 314 degrees of freedom. This again is a highly significant difference. But, as in the case of X rays, the ratio of the actual to the theoretical fluctuations is not far from 1. The numerical value is

$$\rho_n = (\chi^2/\chi_{50}^2)^{\frac{1}{2}} = (427.9/314)^{\frac{1}{2}} = 1.167. \tag{2}$$

It has been mentioned earlier that no correlation has been observed between the increased fluctuations and the dose, and that this indicates that dosimetric inaccuracies are not the cause of the increased statistical errors. The fact that the two values ρ_x and ρ_n are nearly equal confirms this conclusion. If dosimetric inaccuracies were responsbile for the increased fluctuations one would expect this influence to be different for the two different radiation types. It appears likely, therefore, that the small increase of the observed statistical fluctuations over the theoretical values is due to other systematic factors that influence the survival probability in the individual vials. We cannot at present identify these factors, but they are of minor influence, and in the next section it will be seen that the fluctuations from experiment to experiment are considerably more important.

Fluctuations of Sensitivity between Experiments

The findings described above permit no conclusions on the extent of systematic fluctuations between different experiments. However, one can assess such syste-

TABLE I

Comparison of the Observed Total χ^2 for the Pooled Data from All X-Ray Experiments with the 95% Value, χ_{95}^2

Absorbed dose (rad)	χ^2	χ_{95}^2	*Degrees of freedom*
0	275.5	120.7	97
200	37.1	40.1	27
400	139.5	53.1	38
600	356.9	50.7	36
800	240.3	54.3	39
1,000	349.9	53.1	38
1,200	821.4	67.2	50
1,500	6.94	12.6	6

matic fluctuations by determining the χ^2 values for the pooled data from all successive experiments at each individual X-ray dose. Accordingly, all data at each individual value of the absorbed dose of X rays were put into the same group, so that the obtained χ^2-value represents the deviations from the *common* mean value. This is in contrast to the analysis in the preceding section where the χ^2-values were related to the individual averages of the survival rates for each quadruplet of vials that results in one data point. Table I contains the results that are obtained if all survival rates from the different experiments at each individual value of absorbed dose are combined. One finds that in the majority of the cases, and especially at larger absorbed doses, the statistical fluctuations between different experiments are considerably larger than theoretically expected. Figure 1 illustrates this fact. It is a superposition of all individual data points

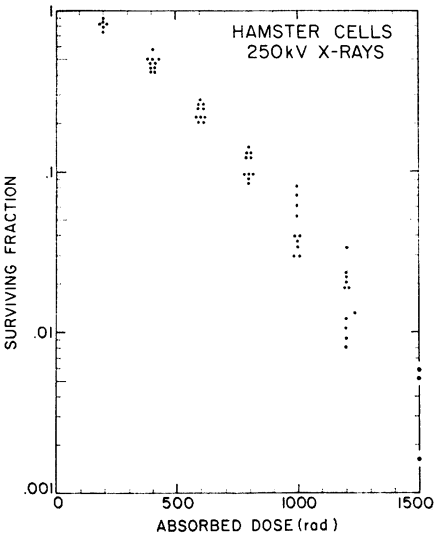

FIG. 1. Superposition of survival rates of Chinese hamster cells obtained in 10 different X-ray experiments over a period of several months. The data correspond to X-ray survival curves presented in the earlier article (*1*). Each curve is normalized relative to its own controls.

TABLE II

Spearman Rank Order Correlation Coefficient for Survival Rates in Eight Separate Survival Curves[a]

Absorbed dose (rad)	*0*	*400*	*600*	*800*	*1,000*
400	0.66				
600	0.38	0.83			
800	0.50	0.73	0.90		
1,000	0.39	0.86	0.86	0.80	
1,200	0.50	0.92	0.83	0.73	0.97

[a] Each coefficient measures the correlation of the two survival rates at two doses for all X-ray experiments. Except in the comparisons involving controls all values exceed the 95% confidence level of 0.643. This implies that within one experiment survival rates at all doses tend to deviate in the same direction. Only those X-ray experiments have been used that in addition to the control data have data at 400, 600, 800, and 1000 rad.

obtained in the various X-ray experiments. In view of the large number of points no attempt is made to identify points belonging to the same curves; the separate curves have been presented in the earlier publication (*1*). The theoretical standard deviation for the individual points is typically $\pm 4\%$; the actual deviations are obviously much larger. Even in this simplified form the plot therefore demonstrates that the fluctuations between experiments exceed greatly the random fluctuations due to the finite number of colonies. The results confirm the necessity to derive the X-ray survival curve repeatedly in successive experiments. Only in this way can reliable comparisons between neutron survival curves and X-ray survival curves be achieved. The findings also make it clear that parameters of a survival curve cannot always be considered as absolute, constant characteristics of a cell line.

To obtain an understanding of the nature of the observed fluctuations, one may ask whether the differences of survival rates in two experiments at different doses are correlated. This question has been analyzed on the basis of the Spearman rank correlation coefficient [see, for example, (*3*)] for the data from all experiments at each pair of absorbed doses. The result is that there is a highly significant correlation for all pairs of absorbed doses. However, no significant correlation is found between the differences at a given absorbed dose and the differences between the control groups. It appears therefore that the statistical fluctuations are not linked to fluctuation of the plating efficiency. Numerical results are given in Table II.

These findings imply that systematic fluctuations of the radiation sensitivity of the cells occur between individual experiments. It is not clear whether these differences are due to the fact that the cells for different experiments are taken from different stock solutions, or whether they are due to other factors, such as trypsination, that may not be completely controlled. Estimates of the magnitude of sensitivity fluctuations are derived in the next section.

Idealized Survival Curve for X rays

In view of the fluctuations of survival rates between experiments it is appropriate to relate each neutron survival curve to the simultaneously established X-ray curve. This approach is followed in the next section However, it is also of interest to determine an idealized X-ray survival curve. Various ways to arrive at such an idealized curve could be considered. One, and perhaps the simplest, way would be to average all survival rates at each dose. However, this procedure is questionable because it is not clear what type of average would have to be employed. One might use an arithmetic or a geometric mean; since the fluctuations are substantial these averages do not coincide.

There are various alternatives to the unsatisfactory averaging of survival rates. However, any method must be arbitrary insofar as it requires assumptions on the nature of the systematic fluctuations. As long as these fluctuations are incompletely understood one can only achieve an approximate treatment. In the following such a treatment is attempted. It is based on the simplifying assumption that the variations between separate experiments are due to a change in sensitivity of the cells that corresponds to a simple dose-modifying factor. One must then search for those correction factors that, applied to the individual experiments, lead to a set of X-ray survival curves that are in closest agreement. After establishing these factors one may apply them to the neutron survival curves that have been established simultaneously with the individual X-ray survival curves. This, too, is tentative insofar as it is not certain that the same dose-modifying factor should apply to different radiation qualities. However, in the absence of better information the treatment is at least a useful approximation.

The dose-modifying factors can be obtained by a least-squares method applied to the individual survival curves. However, such a statistical procedure is relatively complicated and therefore in the following we present a simpler method for the analysis of the results. This method is based on the determination of the mean inactivation dose, $\bar{D}$, for each of the survival curves and on the comparison of the resulting values with their average for all X-ray survival curves.

The mean inactivation dose is a useful parameter of the survival curve because, unlike the initial slope or the parameter D_0, it measures the overall reaction of the cell population over the whole dose range. It is the statistical mean that belongs to the survival curve if the latter is considered as the distribution of survival probabilities as a function of absorbed dose. The definition of $\bar{D}$ is [for a more detailed discussion see (*4*)]

$$\bar{D} = \int_0^\infty D \frac{dS(D)}{dD} dD = \int_0^\infty D \, dS(D). \tag{3}$$

By partial integration one finds that this is equal to

$$\bar{D} = \int_0^\infty S(D) dD. \tag{4}$$

TABLE III

The Mean Inactivation Dose, $\bar{D}$, for the Individual X-Ray Survival Curves[a]

Neutron energy used simultaneously (MeV)	*Mean Inactivation dose, $\bar{D}$, for the X-ray survival curve (rad)*	*Dose-modifying factor f*
50	442	0.95
35	447	0.94
15	466	0.90
6	416	1.01
2	424	0.99
1, 0.66	437	0.96
0.43	396	1.06
0.34	394	1.065
0.22	391	1.075
0.11	400	1.05
	$\bar{D}_A = 422 \pm 23$	

[a] The factor f is the ratio of the average value $\bar{D}_A$ of the mean inactivation dose and the mean inactivation dose $\bar{D}$ obtained in an experiment; this factor can be considered as a dose-modifying factor that expresses fluctuations in sensitivity.

According to this formula the mean inactivation dose, $\bar{D}$, is equal to the area under the survival curve in linear representation. This area has been determined for all X-ray survival curves. The resulting values are listed in Table III. The average of the values for the 10 separate survival curves is 422 rad, and the standard deviation is 23 rad, i.e., 5%.

Other parameters of these curves, such as the extrapolation number or the pseudothreshold number, have been quoted in an earlier article (*5*); they exhibit substantially larger fluctuations than $\bar{D}$. The parameter $\bar{D}$ is therefore more meaningful. However, it must be noted that the values $\bar{D}$ are subject to interpolation errors in the determination of the area under the survival curve.

One can use the values $\bar{D}$ and their average, $\bar{D}_A$, to rescale the individual survival curves by a dose-modifying factor. The dose-modifying factor for a particular experiment is $f = \bar{D}_A/\bar{D}$, where $\bar{D}$ is the mean inactivation dose for this experiment. The result is given in Fig. 2; here each point is plotted at the dose $f \cdot D$, where D is the absorbed dose at which it has been obtained, and f is the dose-modifying factor listed in Table III.

Some fluctuations of the survival rates between experiments remain. This is to be expected, since the correction procedure is only an approximation. But the graph defines the idealized form of the X-ray survival curve much more closely than the unadjusted superposition of the data in Fig. 1.

It is of interest to compare the idealized survival curve for X rays with the linear–quadratic equation

$$S(D) = \exp(-\alpha D - \beta D^2) \tag{5}$$

for the colony-forming ability, $S(D)$, as a function of absorbed dose, D. The equation has earlier been applied by Sinclair (*6*) and microdosimetric analysis

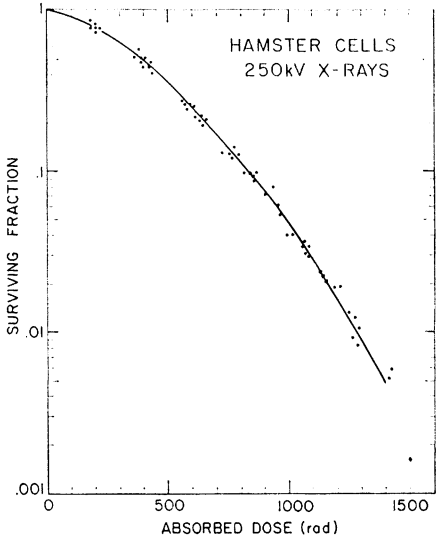

FIG. 2. The data from Fig. 1 rescaled with the factors f in Table III that account for fluctuations of the sensitivity of the cells between individual experiments. The solid curve corresponds to Eq. (5) with $\alpha = 0.99 \times 10^{-3}$ rad^{-1}, $\beta = 2.07 \times 10^{-6}$ rad^{-2}.

has led to the conclusion (7) that it corresponds to a proportionality of cellular damage to the square of specific energy in sensitive sites of the cell with dimensions of fractions of a micrometer or more. In this interpretation the linear term results from the interaction of sublesions formed in the same particle track while the quadratic term results from interaction of sublesions formed by separate charged particles.

If Eq. (5) is valid for synchronous cell populations, then it cannot be strictly valid for a mixed cell population. As Gillespie *et al.* (9) point out, the deviations from Eq. (5) are relatively small for an exponentially growing population. Nevertheless they can be readily detected in the very extensive X-ray data represented in Fig. 2. In fact, a statistical test of the overall fit of the data to the linear–quadratic equation results in rejection of the fit. However, one can achieve a close fit of the data if one disregards the range of lowest survival where the influence of the less sensitive moiety of the cells causes the greatest distortion. The solid line in Fig. 2 is the result of such a partial fit; it corresponds to the parameters $\alpha = 0.99 \times 10^{-3}$ rad^{-1} and $\beta = 2.07 \times 10^{-6}$ rad^{-2}. Since these estimates are the result of only a partial fit, and since it is difficult to assess the validity of the rescaling that has been applied to the data, no confidence limits are given for α and β.

RELATIVE BIOLOGICAL EFFECTIVENESS OF NEUTRONS

Analysis of the Survival Curves

Survival curves have been established for nine different neutron energies and for the broad energy distributions at the Texas A & M Variable Energy Cyclotron

TABLE IV

Least-Squares Estimates of the Parameters α and β in Eq. (5) for the Neutron Survival Curves and the Modified Parameters α' and β' Obtained on the Basis of the Modifying Factors f Given in Table III

Neutron energy (*MeV*)	α (10^{-3} rad^{-1})	β (10^{-6} rad^{-2})	α' (10^{-3} rad^{-1})	β' (10^{-6} rad^{-2})
50	2.65	2.91	2.79	3.22
35	3.8	2.9	4.04	3.28
15	3.45	0.8	3.83	0.99
6	4.55	0.7	4.51	0.69
2	8.1	1.7	8.18	1.73
1	9.45	1.7	9.84	1.84
0.66	10.0	6.8	10.42	7.37
0.43	12.8	6.0	12.08	5.34
0.34	12.8	12.1	12.02	10.67
0.22	12.2	9.6	11.35	8.31
0.11	8.6	8.7	8.19	7.89

(TAMVEC) and at the Naval Research Laboratory (NRL) Cyclotron. The data have been presented in the preceding publication (*1*) and a comparison with earlier investigations of the RBE of neutrons (*10–16*) has been made. In the following, a more rigorous quantitative analysis of the neutron data is performed. In the present section the neutron survival curves are related to the linear–quadratic equation. In the following section the results are compared to the X-ray data.

For all neutron survival curves a least-squares fit to Eq. (5) has been performed. As stated in the preceding section the linear–quadratic equation cannot be strictly valid for nonsynchronized populations. The χ^2-test indicates significant deviations from the equation for the data obtained with 15 and with 6 MeV neutrons, but not for the data obtained at other neutron energies. As an alternative to the linear–quadratic equation one could use an analytical expression with three free parameters. However, such parameters would have little biophysical meaning, and it was therefore felt that the simple treatment is to be preferred even if it is only an approximation. The least-squares estimates of the coefficients α and β are listed in Table IV. The fit has been performed with proper weighting factors of the individual observed points according to their observed variance. The estimates of α and β together with their joint elliptical regions of variation are depicted in Fig. 3. To obtain greater clarity of this graph the ellipses have been drawn with axes half the size of those for the 95% significance level. The ellipses can therefore be considered as areas of standard deviation. Details of the statistical analysis have been described earlier (*8*).

The estimated values of α and β for X rays are inserted in Fig. 3 for comparison. As pointed out in the preceding section no confidence region has been derived for these values; however, the data for X rays are much more extensive than the values for neutrons and it can therefore be assumed that the fractional un-

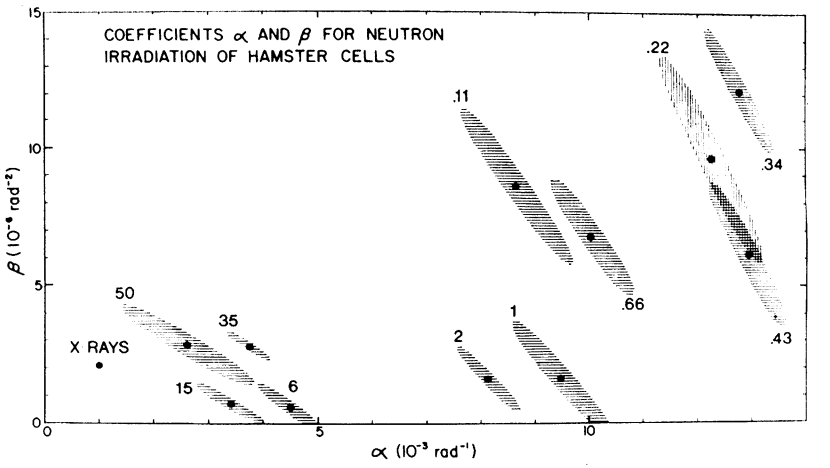

FIG. 3. Least-squares estimates of the linear and the quadratic coefficient in Eq. (5) for the survival curves obtained with neutrons. The shaded ellipses are the joint regions of standard deviations of the two coefficients. The ellipses for the 95% confidence limit are double the size of the ellipses shown in the graph. The isolated dot marks the estimated values of the parameters α and β for X rays. The numbers refer to the neutron energy in MeV.

certainty of the coefficients α and β for X rays is considerably smaller than that for neutrons.

The diagram of the regions of standard deviation of the linear and quadratic coefficients at the different neutron energies may be misleading insofar as the ellipses that belong to larger values of the coefficients, i.e., to the more effective neutron energies, are considerably larger. However, this is a consequence of the linearity of the diagram. The relative error is proportional not to the absolute size of an ellipse but to the ratio of its axes and the coordinates of its center. It is not convenient to choose a logarithmic representation because the ellipses would then be distorted to a complicated shape.

A second point should be noted in connection with the plot of the confidence regions of the coefficients. This is the fact that the statistical analysis does not account for the systematic fluctuations between experiments that have been discussed in the earlier sections of this paper. For X rays the fluctuations between experiments were described by dose-modifying factors with a standard deviation of 5% from unity. For an approximative treatment one may assume that the systematic fluctuations are the same in the case of the neutron irradiations. One can then apply the correction factors obtained for X rays (see Table III) to the neutron data. In this way the modified coefficients in the last two columns of Table IV are obtained. The adjusted value of the linear coefficient is α/f; the adjusted value of the quadratic coefficient is β/f^2. The corrected coefficients are used in Fig. 4, in which theoretical curves for the different neutron energies are compiled. The X-ray survival curve from Fig. 2 is added as a broken line for comparison. The original data points are not inserted in this figure; they have been presented in separate plots in the preceding publication (*1*).

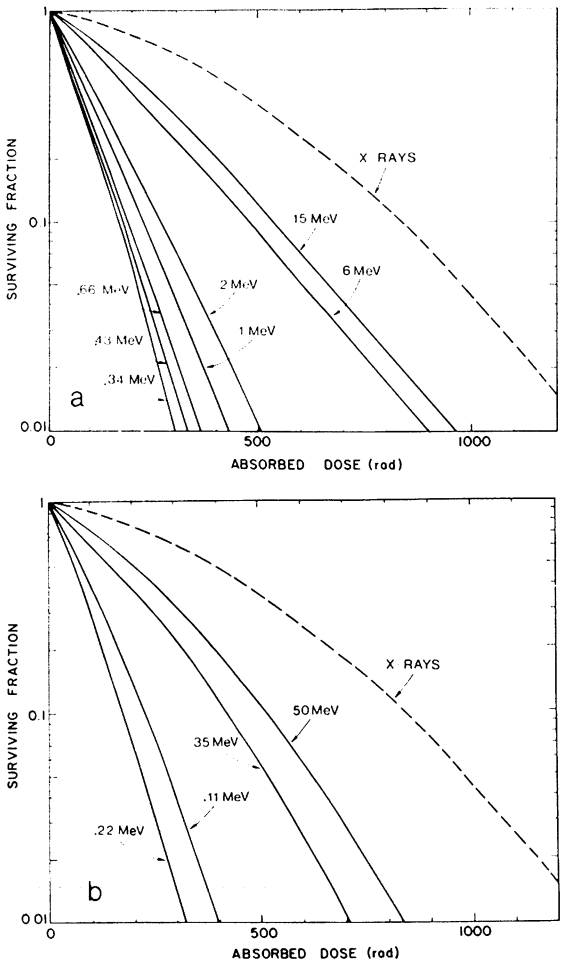

FIG. 4. Theoretical approximation of the survival curves obtained with different neutron energies according to the values α' and β' in Table IV. The theoretical survival curve for X rays from Fig. 2 is inserted for comparison.

From Fig. 4 one obtains the dependence of the RBE of neutrons on neutron energy at different effect levels. The results, depicted in Fig. 5, can be compared to the analogous plot (*1*) that has been obtained directly from the data without statistical analysis. Confidence limits for the values of RBE are not inserted but the magnitude of the statistical errors can be estimated from Fig. 3. Confidence intervals for the RBE are obtained from the nonparametric analysis of RBE that is presented below.

The statistical uncertainties in the coefficients α and β are, as seen in Fig. 3, substantial. However, it is apparent that the value of α reaches a maximum at neutron energies between 200 and 400 keV and that it decreases at higher and at lower energies. A second observation is that the quadratic component increases at neutron energies below 1 MeV. In view of the statistical uncertainties it is

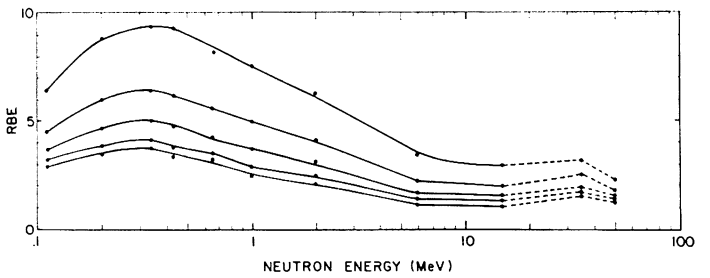

FIG. 5. Dependence of the RBE of neutrons on neutron energy according to the theoretical curves in Fig. 4. The individual curves belong, in the order of decreasing RBE values, to the survival levels 0.8, 0.37, 0.1, 0.01, and 0.001. Data are plotted at 35 and 50 MeV; however, these are the maximum energies for broad energy spectra.

difficult to quantify this increase; accordingly the estimated values in Table IV must be considered as tentative. However, even the qualitative finding is of considerable importance; it corresponds to earlier observations [see (*17*)] that the RBE of neutrons remains larger than 1 at high doses.

As pointed out in the earlier article (*1*) the absorbed-dose rates were smallest and the irradiation times were largest for the lowest neutron energies. This may have led to some recovery of sublethal damage and may therefore have caused a decrease of the values of β. One must therefore assume that the actual values of β at the lowest neutron energies may be even higher than the observed values.

Nonparametric Determination of the RBE of Neutrons

The confidence limits of the values of RBE of neutrons as a function of neutron dose can be obtained by the nonparametric method that has been described earlier (*2*). This method is based on the direct comparison of pairs of data points for X rays and neutrons obtained in the same experiment. Figure 6 represents the results. The vertical bars in these graphs cover those ranges of RBE that, according to the results of the nonparametric comparison of an X-ray dose with a neutron dose, can be excluded with statistical certainty exceeding 95%. The wedges indicate that, on the basis of the comparison, the RBE should be higher or lower, but that this conclusion is not significant at the 95% level.

It can be concluded from these results that the dependence of the RBE on the neutron dose is in all cases consistent with the assumption (see (*7*)) that the RBE of neutrons is inversely proportional to the square root of the neutron dose in an intermediate dose range, while it reaches an asymptotic value at higher doses that may at least in some cases be larger than 1. It is also apparent that the statistical accuracy is not sufficient to determine the maximum value of RBE that can be reached at low neutron doses. This difficulty is inherent in the experimental technique, since the statistical accuracy of cellular survival curves at very low doses is limited by the unavoidable fluctuations of plating efficiency.

The nonparametric analysis is rigorous in the sense that it is free from assumptions concerning models for the survival curves. Its limitation in the present context is that it does not take into account the information gained from the re-

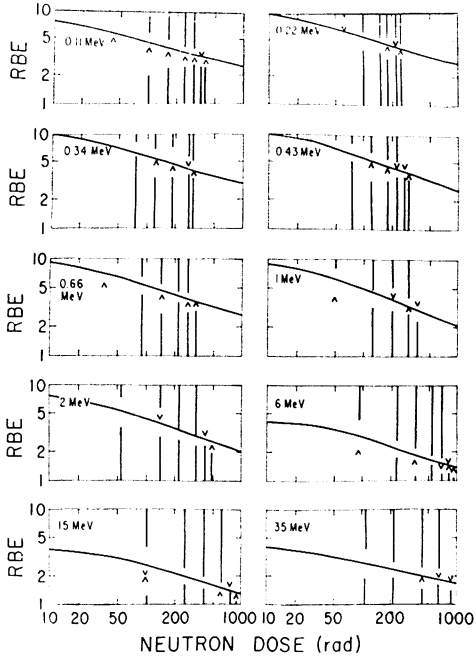

FIG. 6. The RBE of neutrons as a function of neutron energy. The vertical bars cover those ranges of RBE that according to the result of the nonparametric statistical analysis can be excluded with statistical certainty exceeding 95%. The wedges indicate results that are not significant on the 95% level. The solid curves are the theoretical relations according to the values α' and β' in Table IV and the value $\alpha = 0.99 \times 10^{-3}$ rad^{-1} and $\beta = 2.07 \times 10^{-6}$ rad^{-2} for X rays. No diagram is given for the 50 MeV neutron beam because the X-ray data for this experiment are incomplete.

peated assessment of the survival curves for X rays. For this reason curves have been inserted into the graph that correspond to the theoretical survival curves in Fig. 5.

CONCLUSIONS

The statistical fluctuations within a series of X-ray survival curves for non-synchronized Chinese hamster cells have been examined, and an idealized survival curve for X rays has been derived. An approximate analytical form of this survival curve is

$$S(D) = \exp\left[-\frac{D}{1001} - \left(\frac{D}{697}\right)^2\right], \tag{6}$$

where the absorbed dose, D, is expressed in rads. This idealized survival curve for X rays is compared with survival curves obtained for a series of different neutron energies.

It has been found that the statistical fluctuations of survival rates within a given experiment are only slightly larger than the theoretical fluctuations that have to be expected due to the finite number of surviving colonies. However, the

fluctuations of survival rates between separate experiments exceed the theoretical fluctuations substantially. In a first approximation one can consider the fluctuations as the result of sensitivity fluctuations that express themselves in a constant dose-modifying factor for each experiment. The standard deviation of this modifying factor from unity is 5%. Such dose-modifying factors may appear to be relatively insignificant; however, as seen from the comparison of Figs. 1 and 2, they can correspond to substantial differences in survival at higher doses.

A least-squares fit to the linear–quadratic equation has been performed for the survival curves obtained at the various neutron energies. In addition the regions of the joint standard errors of the two parameters have been obtained for the individual curves; these regions are ellipses in the plane of the two coefficients α and β. The results confirm the observation that neutrons are most effective at energies between 200 and 400 keV. The values of RBE have been derived as a function of neutron energy and of the absorbed dose of neutrons.

It has been found that the coefficient β of the quadratic term in the absorbed dose is increased at neutron energies below 1 MeV. This finding is important for the interpretation of cellular survival curves in terms of microdosimetric data. An earlier analysis of various effects of ionizing radiations on eukaryotic cells (*7*) had led to the conclusion that the primary cellular damage, ϵ, depends on the absorbed dose according to the linear–quadratic equation

$$\epsilon(D) = k(\zeta D + D^2). \tag{7}$$

It has been shown that this equation holds if the cellular effect is proportional to the square of the specific energy, z, produced in a sensitive site of the cell. The quadratic dependence on energy deposition in the cell results if pairs of sublesions combine to produce lethal damage. It has furthermore been shown that the quantity ζ can be understood as the mean specific energy produced in the sensitive site by a single charged particle. The value of ζ is considerably larger for the more densely ionizing neutrons than for X rays; in the original analysis it has therefore been assumed that the increased RBE of neutrons is solely due to the larger linear component in the linear–quadratic equation. From this assumption it would follow that at high doses, where the quadratic component dominates both for X rays and neutrons, the RBE of neutrons should approach the value 1. There have been several observations, however, that indicate that the asymptotic value of RBE of neutrons at high doses is larger than 1. As pointed out by Rossi (*17*) this would imply that the coefficient, k, in Eq. (6), which depends on the efficiency of the production of sublesions, is larger for neutrons than for X rays. The present results confirm this conclusion for neutrons below 1 MeV, i.e., for those neutrons that produce the most densely ionizing proton recoils.

The linear–quadratic equation for the survival curves must be considered as an approximation, particularly since the experiments have been performed with nonsynchronized cultures. For this purpose a more direct nonparametric determination of the dependence of neutron RBE on absorbed dose has been performed. The results are consistent with the conclusions based on the analytical model;

however, they demonstrate that the present experiments give no definitive experimental evidence concerning the maximum value of RBE at low doses.

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