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RADIATION RESEARCH 56, v–vii (1973)

CONTENTS OF VOLUME 56

NUMBER 1, OCTOBER 1973

NUMBER 2, NOVEMBER 1973

NUMBER 3, DECEMBER 1973

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VOLUME 56, NUMBER 1, OCTOBER 1973

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Volume 56, Number 1, October 1973

CONTENTS

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Nonparametric Determination of Modifying Factors in Radiation Action[1]

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Modifying factors such as the oxygen-enhancement ratio or the relative biological effectiveness of different radiation qualities are commonly derived from the comparison of dose-effect curves. In this approach, the statistical analysis is frequently made difficult or impossible by the uncertain influence of the scale of effect and of the method of curve fitting. To avoid this disadvantage, a method is used to establish the dose dependence of modifying factors and their confidence limits without the intermediate step of constructing dose-effect curves. The method is based on the comparison of effect levels in pairs of irradiated samples. The statistical tests suitable for this comparison of effect levels in typical experimental situations are considered, and the method is illustrated by its application to experimental studies of the opacification of the murine lens and to growth-reduction studies of *Vicia faba*.

I. INTRODUCTION

Although the study of dose-effect relations is fundamental in radiation biology, it is frequently controversial due to the element of uncertainty which is brought in by interpolation and curve fitting according to more or less hypothetical models and equations. As far as the dose-effect relation itself is concerned, this need not lead to serious difficulties as long as the theoretical models do not bias the data selection and as long as the focus is on the experimental data rather than on the analytical expression used to fit these data. Difficulties can, however, arise in the derivation of modifying factors, such as the relative biological effectiveness (RBE), the oxygen enhancement ratio (OER), the time factor, or related quantities. Conventionally, such factors are derived from the comparison of dose-effect curves, but this often involves interpolation or extrapolation of experimental data, and the resulting inaccuracies cannot always be clearly judged. In the following it will be shown that the construction of dose-effect curves as an intermediate step in the derivation of modifying factors can be avoided. This eliminates

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computational difficulties. The direct analysis which does not invoke dose-effect functions is also to be preferred because quantities such as RBE are often independent of physiological factors which enter the dose-effect relation but cancel when two radiation qualities are compared. Accordingly dose-RBE relations are often simpler than dose-effect relations (*1*, *2*), and their derivation need not be based on the latter.

II. THE CONCEPT OF MODIFYING FACTOR

The following considerations will apply equally to RBE, OER, and similar quantities. In order to avoid repetition of the arguments, it will, therefore, be useful to define formally the concept of a *modifying factor* which can stand for each of these ratios. The following definition will be used in this paper. A modifying factor is the ratio of doses D_A and D_B necessary to produce equal effect under two different irradiation conditions A and B. The difference between A and B can be one of radiation quality, one then speaks of RBE; it can be a difference in oxygen concentration, then one deals with OER; or it can be a difference in the temporal distribution of the dose, then one deals with the time factor. It is important to note that in all these cases one must consider the modifying factor as a function either of the effect level or of one of the doses, D_A or D_B. If one considers the modifying factor as a function of either D_A or D_B, one has the advantage that one deals exclusively with physical quantities, namely the ratio of two absorbed doses as a function of absorbed dose. This makes the analysis applicable to all those cases where there is no natural scale of effect. For example, in the study of skin reaction or lens opacification (*3*), effect relations have little absolute meaning. This is so because the establishment of a numerical scale for the effect may involve complex procedures or even an element of subjectivity. In such cases, the individual observer may be able to reproduce his own results, but two dose-effect relations established by two independent observers may not be comparable. In spite of the differences between their effect scales, the two observers will, however, obtain the same dose-RBE relation.

In the following, the modifying factor D_A/D_B will be designated by F, and the condition A will be termed the reference condition. If one deals with RBE the reference radiation is usually x-rays, and D_A, therefore, stands for the x-ray dose, while D_A/D_B is the RBE of the comparison radiation, for example neutrons, and D_B is the dose of this comparison radiation, e.g., the neutron dose. Another example is OER. In this case, the reference condition is hypoxia. Therefore, D_A stands for the absorbed dose in the absence, or partial absence, of atmospheric oxygen, D_B is the absorbed dose necessary to produce the same effect at normal atmospheric pressure of oxygen, and OER $= D_A/D_B$ is the oxygen enhancement ratio.

III. DETERMINATION OF MODIFYING FACTORS AND THEIR DOSE DEPENDENCE

Determination of a modifying factor is usually based on a set of experiments in which groups of biological specimens are exposed to various doses under the two different conditions, A and B. Assume that D_A is one of the doses applied in condi-

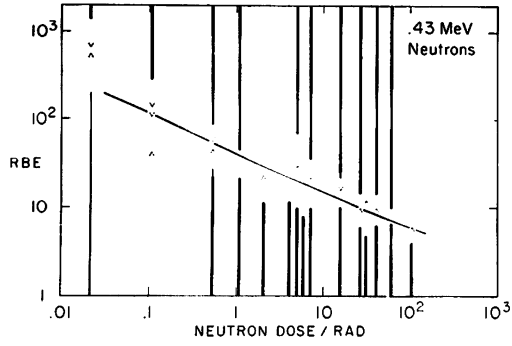

FIG. 1. RBE of 430 keV neutrons relative to x-rays for the induction of lens opacification in the mouse as function of neutron dose (*3*). The solid bars indicate the ranges of RBE values which, according to the comparison of x-ray and neutron doses, are excluded. Broad bars, significance exceeding 99%; narrow bars, significance exceeding 95%; wedges, nonsignificant differences. The solid curve corresponds to a theoretical relation discussed elsewhere (*3*, *4*).

tion A and E_A is the effect at this dose, while D_B and E_B are one of the doses applied in condition B and the corresponding effect level. If E_A is larger than E_B, then at the absorbed dose D_B the modifying factor F is smaller than D_A/D_B. If E_A is smaller than E_B, then F is larger than D_A/D_B. Accordingly, one can exclude certain ranges of values of F, depending on the outcome of the comparison. Figure 1 gives an example, namely the RBE dependence on neutron dose for the opacification of the murine lens (*3*). If in the comparison of an x-ray dose, D_x, and a neutron dose, D_n, the effect is found to be different with statistical significance of at least 95%, then that region of RBE which can be rejected is marked by a vertical bar. The results of those comparisons of effects produced by two doses, for which the effect levels have been found different but with less than the required 95% statistical significance, are merely marked by wedges.

The dose-RBE relation must pass between the two sets of vertical bars which extend downwards from the top of the figure and upwards from the bottom of the figure. The wedges which point upwards or downwards indicate that, according to the comparison of the two doses, the RBE should be higher or lower; but, since the results of these comparisons have not been found statistically significant, the curve may in a certain number of cases actually pass on the wrong side of the wedges. In Fig. 1 a dose-RBE relation has been inserted which corresponds to a theoretical equation for RBE discussed elsewhere (*3*). It is found that a curve of the required shape can be drawn without intersecting any of the vertical bars. The fact that the curve passes on the wrong side of some of the wedges is expected on statistical grounds and does not indicate that the theoretical model is invalid.

It may be useful to illustrate the procedure in somewhat more detail. Figure 1 is the combined result of four groups of experiments which were performed over the course of several years (*3*). One of these groups of experiments may be considered as an example. In this study, samples of 40 lenses each were examined after exposure to x-ray doses of 70, 160, 260, 400, and 600 rad and neutron doses

TABLE I

COMPARISON OF NEUTRON AND X-RAY DOSES IN EXPERIMENT G OF THE LENS OPACIFICATION STUDIES[a] AND THE RESULTING INEQUALITIES FOR THE NEUTRON RBE

x-Ray dose (rad)	*Neutron dose (rad)*					
	7.2	*16*	*27*	*41.3*	*61.2*	*104*
600	<83.3	<37.5	<22.2	<14.5	<9.8	(>5.8)
400	<55.5	<25	<14.8	(>9.7)	>6.5	>3.8
260	<36.1	(<16.2)	(>9.6)	>6.3	>4.2	>2.5
160	(<22.2)	>10	>5.9	>3.9	>2.6	>1.5
70	>9.7	>4.4	>2.6	>1.7	>1.1	>0.67

[a] Ref. (*3*).

of 7.2, 16, 27, 41.3, 61.2, and 104 rad. The 40 scores of each x-ray group 34 weeks after exposure were compared to the 40 scores of each neutron group. The statistical test employed for the comparison of the two groups of 40 scores each is the Mann-Whitney rank order test; details of the test are described in Section IV. At this point it is sufficient to consider the result. As one deals with 5 x-ray doses and 6 neutron doses, the total number of possible comparisons is 30. In Table I the result of each comparison is indicated by a greater-than or less-than symbol in front of the ratio of the x-ray dose to the neutron dose. The greater-than symbol indicates that the neutron dose is found to be more effective than the x-ray dose, and that, therefore, the RBE is higher than the ratio of the x-ray dose D_x and the neutron dose D_n. The less-than symbols have the opposite meaning. Whenever the result of the comparison has not been found statistically significant on the 95% level, the terms are set in parentheses. From the results of each column in the rectangular scheme, one obtains an inequality for the RBE at the corresponding neutron dose. These inequalities find their graphic representation in the high dose region of Fig. 1; the terms in parentheses correspond to the wedges, and the other terms determine the position of the vertical bars. One may note that only a fraction of the total number of comparisons, namely those which define the closest range of RBE at a given neutron dose, are relevant to the result. In the present case, 14 of the 30 comparisons are redundant.

IV. COMPARISON OF TWO EFFECT LEVELS

In the preceding section, it was shown how one can obtain a modifying factor and its dose dependence from a series of comparisons of doses applied under the two conditions which are considered. How the individual comparisons are performed has, however, not yet been discussed. It is clear that the comparison of the effect level in two exposed groups will involve different statistical tests depending on the experimental endpoint considered; in the following, a survey of some of the possible statistical procedures will be given.

One can distinguish two common ways in which effect levels in an irradiated population are measured. In one case, each unit within the exposed sample can

show a continuous range of effect. In this case, it is common to use the mean effect level within the population to measure the effect. Specifically, this is done if one deals with dose-effect curves. The concept of a mean is, however, problematical if one considers effects which do not have a natural scale. The study of lens opacification is an example where one is forced to adopt an arbitrary numerical scale and where averaging over the observed indices has very little meaning. It is important to realize the fact that certain effects are measured on a scale which is unique only up to a monotonic transformation, i.e., a scale which establishes a larger-smaller relation, while apart from this order relation the numerical values are irrelevant. Such numerically arbitrary scales are sometimes called *ordinal* scales, and this term will also be used in the present context. For an ordinal scale, mean values have only very limited meaning, since they may change in complicated ways with monotonic transformations of the scale. One could instead use the median values as the basis for the comparison of effect levels in two groups, but one achieves a much sharper comparison by using the full sets of observed values in all the elements of the two irradiated groups. In the following section, nonparametric procedures which are useful in this context will be discussed. Such nonparametric procedures can not only be applied if one deals with ordinal scales; they are quite generally useful when one deals with gradual effects and wants to avoid hypothetical assumptions on the distribution of the experimental variable.

The second way in which effect levels are measured in an irradiated population occurs with the so-called all-or-nothing effects. Examples are the inactivation of a certain fraction of cells within an irradiated population or the induction of leukemia in an exposed human population. In this case, the effect levels must be compared on the basis of the observed incidences and the sizes of the two experimental groups. Statistical tests applicable under this condition will be discussed in a separate section.

Comparison of the Effect Level for Gradual Effects

In the case of effects measured on ordinal scales, the analysis must be based on a nonparametric test. A particularly powerful test is the Mann-Whitney rank-order test, also known as the U test. The details of this test which is essentially equal to a test proposed by Wilcoxon can be found in the literature [see, for example, (*5*)], but a description of the essential points may be useful in the present context.

Assume that one deals with two irradiated groups, group A containing N_A elements, group B containing N_B. The null hypothesis is that the effect in both groups has the same distribution. The alternative hypothesis is that the effect in group A is stochastically larger or smaller than in group B. To apply the U test, one combines the observations of both groups and ranks them in order of increasing values. The smallest observation is given the rank 1, the second smallest the rank 2, etc., up to the largest observation which is given the rank $N_A + N_B$. Tied observations are assigned the average of the tied ranks.

The sum, R_A, of the ranks assigned to the elements of group A is calculated

and the U statistic is obtained from the definition:

$$U = R_A - N_A(N_A + 1)/2. \tag{1}$$

Tables of the critical values of this statistic are usually given in terms of U, or in terms of $N_A N_B - U$ if U exceeds the value $N_A N_B/2$. If the larger group contains at least 20 elements, one can approximate the distribution of U under the condition of the null hypothesis by a normal distribution with mean $\bar{U} = N_A N_B/2$ and with the variance:

$$\sigma_U^2 = N_A N_B(N + 1)/12, \tag{2}$$

where $N = N_A + N_B$ is the total number of elements in the two groups. If ties occur, a corrected value of σ_U must be used:

$$\sigma_U^2 = \frac{N_A N_B}{N(N-1)}\left(\frac{N^3 - N}{12} - \sum T\right), \tag{3}$$

where T is defined in terms of the number, t, of observations tied for a given rank

$$T = (t^3 - t)/12 \tag{4}$$

and $\sum T$ is obtained by summing the terms T over all groups of tied observations. The null hypothesis can be rejected on the 95% significance level if the quantity

$$Z = (U - \bar{U})/\sigma_U \tag{5}$$

is either larger than 1.95 or smaller than -1.95.

If one deals with small sample sizes, one must use tables of the distribution of U given in the literature.

The application of the U test may be illustrated by an example from the experiment discussed in the preceding section and represented in Table I. In these studies, the degree of opacification in each lens is characterized by an index between 0 and 14. The first two rows in Table II represent the number of lenses assigned to the different indices when examined 34 weeks after exposure to 70 rad of x-rays or 7.2 rad of neutrons. In this case both the number, N_A, of elements in the x-ray group and the number, N_B, of elements in the neutron group are 40. Due to the limited number of index values and the large sample sizes, the number of ties is large. In the third row of the table the mean rank for each class is given, the fourth row represents the contribution of each class to the rank sum, R_A. The resulting value of U is 378, while the expectation value, $\bar{U}$, is 800. From Eq. (3) one obtains $\sigma_U = 101.7$ if one uses the value of $\sum T$ resulting from the fifth row in Table II. It is of interest to note that, even in the present example where the number of ties is large, the correction term is quite unimportant; it will, therefore, be usually sufficient to derive σ_U from the uncorrected Eq. (2). The resulting value of Z is -4.15. One concludes that the effect of the x-ray dose is smaller than that of the neutron dose. Because Z is much less than -1.95, the result is statistically significant on a level considerably higher than 95%.

In most applications the determination of the confidence region for the dose-RBE dependence or the dose dependence of some other modifying factor will

TABLE II

APPLICATION OF THE U TEST TO THE COMPARISON OF THE LENS OPACIFICATION PRODUCED BY 70 RAD OF X-RAYS AND 7.2 RADS OF NEUTRONS[a]

	Opacification index												
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	
Number of lenses Group A, 70 rad x-ray	—	3	8	13	6	6	3	1	—	—	—	—	$N_A = 40$
Number of lenses Group B, 7.2 rad neutrons	—	—	2	—	16	13	5	1	1	1	—	1	$N_B = 40$
Mean rank	—	2	8.5	20	37.5	58	71.5	76.5	78	79	—	80	
Contribution to R_A	—	6	68	260	225	348	214.5	76.5	—	—	—	—	$R_A = 1198$
$T = (t^3 - t)/12$	—	2	82.5	182	888.5	590	42	0.5	—	—	—	—	$\Sigma T = 1764.5$

Resulting values: $U = 378$; $\bar{U} = 800$; $\sigma_U = 101.7$; $Z = -4.15$.

[a] Ref. (*3*).

require so many comparisons that the statistical evaluation must be performed on the computer; this is the case particularly if the experimental groups are as large as in the example of the lens opacification studies (*3*) where each comparison includes 80 irradiated lenses. Computer evaluation presents no problem since the algorithms for the U test or for similar rank order tests are commonly available.[3]

Comparison of Effect Levels for Quantal Effects

If one deals with an all-or-nothing effect, such as the inactivation of cells, the induction of specific mutations, the production of chromosome aberrations, or the radiation-induced incidence of leukemia, the effect level in two irradiated groups must be compared merely on the basis of the observed incidence rates. What type of statistical test is best suited will then depend on the size of the two samples and on the observed incidence frequencies. In the following, the most important cases will be distinguished, and basic formulae applicable to these cases will be compared.

Assume that N_A and N_B are the number of objects in the two irradiated groups and n_A and n_B are the number of affected elements in these groups. If n_A and n_B as well as $(N_A - n_A)$ and $(N_B - n_B)$ are sufficiently large, the effect levels can be compared on the basis of the χ^2 test with 1 degree of freedom and with the following relation for χ^2:

$$\chi^2 = \frac{(n_A - rN_A)^2}{rN_A} + \frac{(n_A - rN_A)^2}{(1-r)N_A} + \frac{(n_B - rN_B)^2}{rN_B} + \frac{(n_B - rN_B)^2}{(1-r)N_B}, \tag{6}$$

where r is the observed incidence rate averaged over both groups:

$$r = (n_A + n_B)\,(N_A + N_B). \tag{7}$$

In certain cases, for example in mutagenesis or leukemogenesis, but not in the study of cell lethality, the incidence rates are small, i.e., r is small compared to 1, while n_A and n_B are still sufficiently large. Then Eq. (6) simplifies to

$$\chi^2 = \frac{(n_A - rN_A)^2}{rN_A} + \frac{(n_B - rN_B)^2}{rN_B}. \tag{8}$$

These equations can be modified by a correction for continuity which in the case of small incidences improves the power of the test; the details are described in the literature (*6*).

The values of χ^2 which result from Eq. (6) or (8) are compared with the distribution of χ^2 for 1 degree of freedom at the predetermined level of significance. If the 95% level is chosen, the difference of effect is considered statistically significant if χ^2 exceeds the value 3.84.

The situation is more complicated if not all the numbers n_A, n_B, $(N_A - n_A)$, and $(N_B - n_B)$ are large compared to 1. In this case, the most practical test for homogeneity of the two groups is Fisher's "exact probability test" [see (*7*)].

[3] See for example IBM, Scientific Subroutine Package, H20-0205-3 (1968).

This test is based on the derivation of the sum of the probabilities for all those combinations ν_A and ν_B for which $\nu_A + \nu_B$ is equal to the observed sum $n_A + n_B$, where $\nu_A \leq n_A$ if $n_A/N_A < n_B/N_B$, or $\nu_A \geq n_A$ if $n_A/N_A > n_B/N_B$; i.e., one considers all possible outcomes with the same total incidence for which the difference in incidence rate between the two groups is at least as extreme as the observed difference. It appears somewhat doubtful whether it is justified that in Fisher's test only those potential outcomes are considered which have the same sum of observed events, but an extensive discussion of this problem in the literature has not lead to a suitable alternative to Fisher's test.

If the null hypothesis holds, i.e., if the effect probability for the elements in the two samples is the same, one obtains the following probability for finding ν_A affected elements in the first and ν_B in the second group:

$$p(\nu_A, \nu_B) = \binom{N_A}{\nu_A}\binom{N_B}{\nu_B}\Big/\binom{N}{\nu} = \frac{N_A!}{(N_A-\nu_A)!\,\nu_A!}\,\frac{N_B!}{(N_B-\nu_B)!\,\nu_B!}\,\frac{(N-\nu)!\,\nu!}{N!}, \quad (9)$$

where the abbreviations $N = N_A + N_B$ and $\nu = \nu_A + \nu_B$ are used.

The acceptance probability of the observed occurrence is therefore

$$p = \sum_{\nu} p(\nu, n-\nu) \qquad \text{with} \qquad n = n_A + n_B. \quad (10)$$

In this expression, ν is taken from 0 to n_A if $n_A/N_A < n_B/N_B$ and from n_A to n, otherwise. If the resulting value of p is lower than a preset significance level, the null hypothesis of equal underlying event frequencies in both groups must be rejected; the difference in the observed effect rates n_A/N_A and n_B/N_B is then statistically significant.

A correction for continuity can be applied (*8*) to the Fisher exact probability test similar to that applicable to the χ^2 test.

Frequently the situation is considerably simplified because the observed frequencies are small while the sample sizes N_A and N_B are large. In this case, one obtains the following acceptance probability:

$$p = \sum_{\nu} \binom{n}{\nu} \rho^{\nu}(1-\rho)^{n-\nu}, \quad (11)$$

where ρ and n are defined as

$$\rho = N_A/(N_A + N_B) \qquad \text{and} \qquad n = n_A + n_B \quad (12)$$

and where the range of summation of ν is the same as in Eq. (10). One can derive this formula from Eq. (9) by going to the limit of large N_A and N_B. One can also understand the terms in Eq. (11) directly as the binomial probabilities that out of the $n_A + n_B$ events ν occur in group A and $n-\nu$ in group B.

An example for the application of Eq. (6) is the inactivation of cells at intermediate survival levels. An example for the applicability of Eq. (8) are cellular inactivation data at very low survival levels or epidemiological observations, such as the Japanese leukemia data (*9*), where in large exposed groups the incidences are in excess of approximately 10. An example for the applicability of Eq. (10)

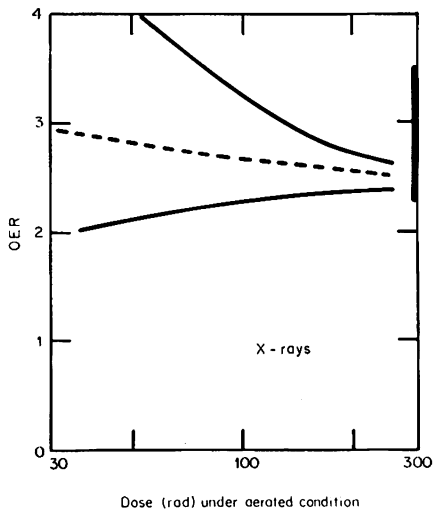

FIG. 2. OER for the growth reduction of *Vicia faba* (*11*) and its 95% confidence region. The bar at the right ordinate indicates the confidence interval derived in the earlier analysis (*11*) under the assumption of a constant OER.

are survival studies with small numbers of animals; and finally Eq. (11) has been applied in the analysis of the RBE-dose dependence for leukemia induction in Hiroshima and Nagasaki in all those cases where the absolute incidences were small (*9*).

V. EXTENSION AND CONCLUSION

The method described here has been found useful in the determination of RBE-dose relations in various systems (*3*, *9–12*). It has also been applied to the investigation of the possible dependence of OER on dose (*11*); this latter study may serve as an example to explain a further step which can in certain cases improve the analysis.

Figure 2 shows the 95% confidence region for the OER-dose dependence for growth reduction of *Vicia faba* by x-rays. For comparison the confidence range which results from a conventional analysis based on the assumption of constant OER is indicated by a bar on the right ordinate. This figure is a somewhat more detailed representation of a result given previously (*11*); it indicates that the data do not permit the conclusion that OER varies with dose; it does however also indicate that this may merely be due to the limited accuracy of the data at low doses. Although, at this point, no definite statement on the dependence of OER on dose is possible, the explicit analysis is superior to the conventional analysis as it leads to a much narrower confidence range of OER at high doses.

In Fig. 2 the confidence region is delimited by solid lines instead of being defined as a region between individual bars, such as in Fig. 1, which exclude certain ranges of the modifying factor. This has been achieved by first using the *U* test (see section IV) for the comparison of the growth increments of bean roots

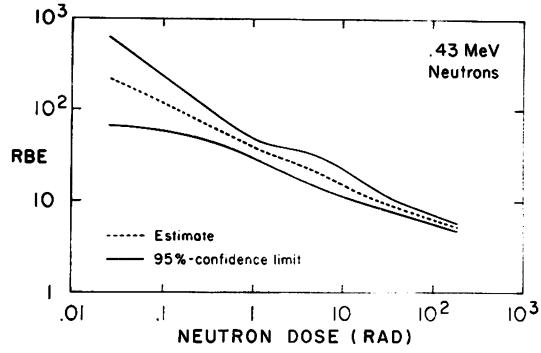

FIG. 3. RBE of 430 keV neutrons relative to x-rays for the induction of lens opacification in the mouse.

irradiated in oxygenated and hypoxic conditions and performing the analysis outlined in section III. In this way, a grid of values of the statistic Z [see Eq. (5)] has been established over the plane of the two variables OER and dose applied under aerated condition. From this grid, interpolated values of Z between the grid points were derived, and the lines which delimit the 95% confidence range were obtained as those lines where the interpolated Z values had the value $+1.95$ or -1.95 which correspond to a 5% acceptance probability of the null hypothesis. The interpolation procedure has been possible in this particular case because the analysis is based on a comparatively large number of groups of specimens exposed to different doses. In this particular case 16 groups have been exposed in anoxic and 15 groups in aerated condition. Thus the individual rank order comparisons yield the values of Z on a grid of 240 points in the OER-dose plane.

Interpolation, particularly if one performs such algorithms as the Lagrange-Aitken interpolation on the computer, can, however, also be applied in cases where the total number of irradiated groups is somewhat smaller. Figure 3 shows the result for the lens opacification studies with 430 keV neutrons which derives by interpolation from the data depicted in Fig. 1. The interpolation procedure is justified in all cases where one deals with a large number of different doses. In the general case, however, the representation of the type which is employed in Fig. 1 has the advantage that one can more directly judge the influence of the individual results at the different dose values.

In conclusion, one may state that the use of nonparametric techniques affords the determination of modifying factors directly, as long as appropriate doses have been applied; dose-response curves are unnecessary for the calculation of modifying factors. This eliminates difficulties of curve fitting and interpolation, and it permits the application of statistical tests which are not merely based on the comparison of mean values and their variances. It may be possible to modify certain experimental radiobiological approaches in such a way that maximum use can be made of the more refined statistical treatment. In cellular inactivation studies, for example, the dose dependence of RBE or OER may be determined with greater statistical accuracy by modifications of the experimental criteria

which may include the determination of distribution of clone sizes at a certain time after irradiation instead of the mere determination of the fraction of clones which exceed a critical size at this time. Another example are studies of tumor incidence. RBE-dose relations have been investigated (*12*) for the induction of mammary tumors (*13*, *14*) on the basis of total incidences at a given time after irradiation; one can hope to improve these results by basing the analysis on the full temporal pattern of incidence and not merely on the total incidences at a fixed time after irradiation. Such improvements in statistical precision are of special importance in studies which relate to the effectiveness of radiation at small doses.

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