

INTERNATIONAL JOURNAL OF
RADIATION
ONCOLOGY
BIOLOGY • PHYSICS

VOLUME 8, NUMBER 9

SEPTEMBER 1982

Sponsored by

THE AMERICAN SOCIETY OF THERAPEUTIC RADIOLOGISTS
THE INTERNATIONAL SOCIETY OF RADIATION ONCOLOGY
CIRCULO DE RADIOTERAPEUTAS IBERO-LATINOAMERICANOS



Published by

PERGAMON PRESS New York • Oxford • Toronto • Paris • Frankfurt • Sydney

● Oncology Intelligence

DEPENDENCE ON NEUTRON ENERGY OF THE OER AND RBE

ERIC J. HALL,* ALBRECHT M. KELLERER† AND HERBERT FRIEDE†

INTRODUCTION

Trials of neutrons as an alternative to x-rays for the radiotherapy of human cancer are underway in Great Britain, the United States, Continental Europe and Japan.³ The investment of time, effort and resources is considerable and from the outset the need has been recognized for cooperation between centers and across national boundaries.

A major obstacle to the pooling of clinical data and to the comparison of results is the (unfortunate) fact that the various machines used for neutron therapy include a wide range of different neutron energies with consequent variations in biological effectiveness. Various efforts have been made to make biological intercomparisons between the various neutron beams with a variety of biological systems and the early results have already been published.^{5,6,8,9,10,15} The present report summarizes the results of a new series of biological intercomparisons based on measurements at facilities around the world using an established line of cells cultured *in vitro*. The principal characteristics of the facilities visited are listed in Table 1.

MATERIALS METHODS AND RESULTS

Chinese hamster V79 cells were used for all of the experiments described, cultured using standard techniques and grown in GIBCO F10 medium supplemented with 10% fetal bovine serum and antibiotics.

RBE intercomparisons

The RBE experiments were designed to take advantage of the fact that, with cells in culture, variations *within* an experiment are much smaller than those between experiments. Consequently, in order to exploit the precision of which the *in vitro* technique is capable, neutron facilities were intercompared in pairs within a given experiment, using cells from a common culture irradiated on the same

day. In each experiment appropriate numbers of cells were plated into Falcon tissue culture flasks and allowed to attach by overnight incubation at 37.5°C. The flasks were then filled brimful with medium, sealed and the temperature lowered to 15°C. Half of the flasks were transported to the Naval Research Laboratory (NRL) in Washington, D.C. and the other half to one of the facilities listed in Table 1. The cells were transported in insulated water-jacketed carriers to maintain the temperature at $15 \pm 2^\circ\text{C}$, which prevents cell division while maintaining viability for periods up to 24 hours. The irradiations at the two facilities were performed simultaneously, or as close as possible in real time.

A standardized treatment fixture was used at all facilities, constructed of lucite, with space for six tissue culture flasks, and provision for an ionization chamber to determine the dose received at the position occupied by the cells. Full build-up was ensured because the cells were overlaid with 2 cm of tissue culture medium. A substantial international effort to achieve compatible dosimetry has been mounted by the physicists at the various installations engaged in neutron therapy, as a consequence of which the world-wide agreement for dose measurements in air was within 5% by 1977¹ and better than 2% by 1981.² The use of the standard treatment fixture was an attempt to extend the compatible dosimetry to a practical set-up for the irradiation of cell cultures. In all cases the doses quoted are total absorbed dose, i.e. the sums of the neutron and gamma-ray doses. The dosimetry was the responsibility of the physicists at the respective neutron facilities.

Following the completion of all radiation exposures, the cells were incubated for 8 days to assess the proportion able to form macroscopic clones.

The data for the proportion of cells surviving graded doses of neutrons are shown in Figure 1. Two methods of analysis were used that are briefly described in the following.

Based on a talk given at the International Congress of Radiology, Brussels, July 1981.

This investigation was supported by Grant No. CA-18506 to the Radiological Research Laboratory/Department of Radiology, awarded by the National Cancer Institute, DHHS.

*Radiological Research Laboratory, College of Physicians and Surgeons of Columbia University, New York, N.Y. USA.

†Institut Für Med. Strahlenkunde de Universität Würzburg, West Germany.

Accepted for publication 21 May 1981.

Table 1. Neutron facilities intercomparison

	Facility	Location	Production process	Energy accelerated particle MeV	Mean neutron energy MeV
USA	U. Maryland	College Park, Maryland	$p^+ \rightarrow Be$	101	50
			$d^+ \rightarrow Be$	80	32
	Fermilab	Batavia, Ill.	$p^+ \rightarrow Be$	66	25
	TAMVEC	College Sta., Texas	$d^+ \rightarrow Be$	50	19.3
	NRL/MANTA	Washington, D.C.	$d^+ \rightarrow Be$	35	14.3
	NASA/GLANTA	Cleveland, Ohio	$d^+ \rightarrow Be$	25	10
UK	Univ./Wash.	Seattle, Washington	$d^+ \rightarrow Be$	22	8
	U. Chicago	Chicago, Ill.	$d^+ \rightarrow d$	7.5	8
	MRC Hammersmith	London	$d^+ \rightarrow Be$	16	7
Netherlands	MRC Edinburgh	Edinburgh	$d^+ \rightarrow Be$	15	6
	Antoni van Leeuwenhoek	Amsterdam	$d^+ \rightarrow T$	—	14
Japan	NIRS	CHIBA	$d^+ \rightarrow Be$	30	12

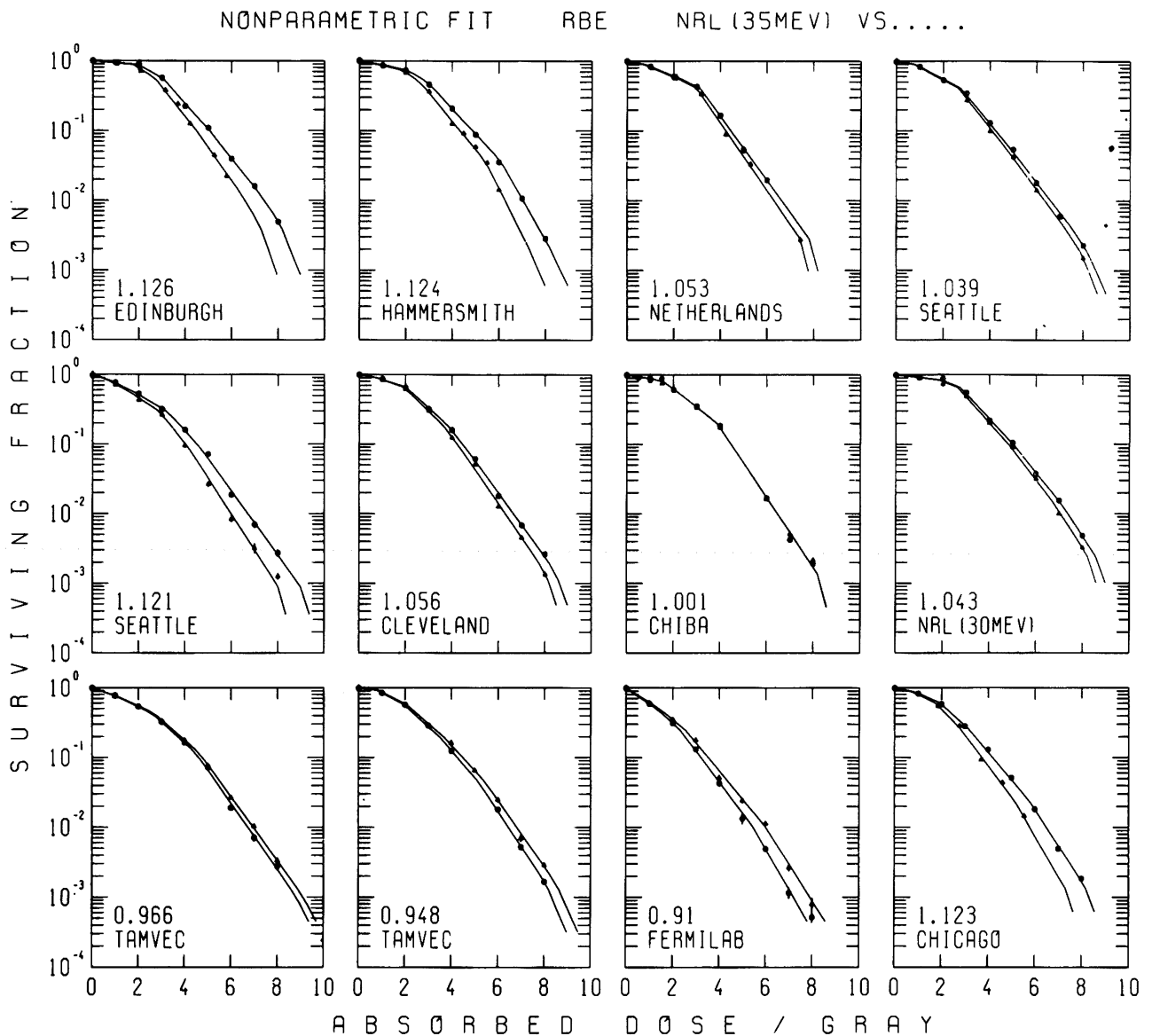


Fig. 1. Survival data for Chinese hamster V79 cells exposed to graded doses of neutrons. Each panel represents a self contained experiment in which data from the Naval Research Laboratory (closed circles) was compared with another neutron therapy facility (closed triangles). The curves were fitted to the data by a non-parametric method described in the text, which allows an estimate to be made of the RBE relative to NRL of each neutron beam. This quantity is shown in each panel.

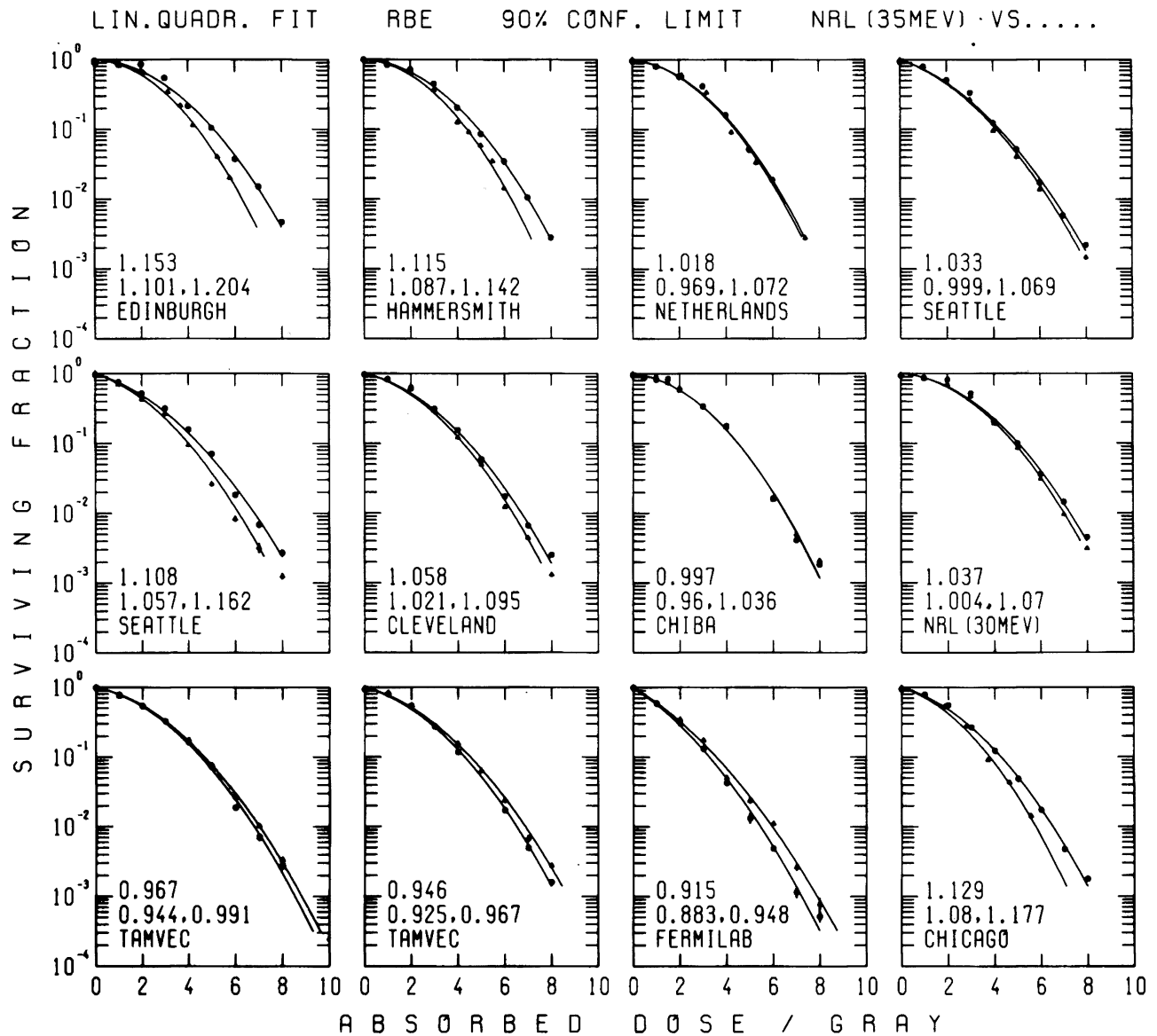


Fig. 2. Survival data for Chinese hamster V79 cells exposed to graded doses of neutrons. Each panel represents a self-contained experiment in which data from the Naval Research Laboratory are compared with another neutron therapy facility (close triangles). The data were fitted to a linear-quadratic expression as described in the text which allows an estimate to be made of the RBE relative to NRL of each neutron beam, together with confidence intervals. This quantity is shown in each panel.

Method of analysis

In order to avoid possible bias of the results due to the choice of particular equations for the dose-effect relations a non-parametric method has been applied that has been described earlier.¹³ However, this type of analysis does not permit the derivation of statistical uncertainties if pairs of curves are compared. For this reason, the analysis is also performed in terms of an assumed linear quadratic dose-dependence of the logarithm of survival, and standard deviations obtained by this method are utilized. The combination of the two methods is justified by the remarkable agreement of the estimates of RBE and OER from the non-parametric and the parametric method.

Non-parametric analysis. This method involves no

analytical equation for the dose-response relation. The survival data for the two neutron beams to be compared are fitted in terms of least squares by curves that have the same shape and differ only by a dose factor necessary to allow the common fit. The only constraint on the curves is that they are convex from below. The resulting dose factors are then the best estimates of the relative potency or RBE between the two beams, based on the data from all dose levels studied. All resulting curves are shown in Fig. 1 together with the raw data. The estimates of the RBE, always given relative to the simultaneous NRL-experiment, are inserted.

Parametric fit. In this additional analysis the data points were fitted in terms of least squares to an expres-

sion of the form

$$S = e^{-\alpha D - \beta D^2} \quad (1)$$

where S is the fraction of cells surviving a dose D while α and β are constants. In the comparison of two data sets the NRL-data are fitted to Eq. (1) while the second data set is fitted to the equation containing a dose modifying factor f :

$$S = e^{-\alpha f D - \beta (fD)^2} \quad (2)$$

Such a common fit requires the use of a comparatively simple iterative optimization routine, since only 4 parameters are to be optimized. Next to α, β and f the fourth parameter, not appearing in the equations, is a vertical shift factor that estimates the plating efficiencies, i.e. the curves are not forced through the control values at zero dose. Since a substantial number of control plates has been used in all experiments, it is nevertheless found that most fitted curves pass closely through the control points.

The computation in terms of an analytical expression with a specified number of free parameters permits (for a similar application see Kellerer and Brenot¹²) the derivation of standard errors or confidence intervals of the parameters α and β and of the dose modifying factors f . The 90%-error intervals for the dose modifying factors, i.e. the RBE values, are inserted in the graphs. The results of this analysis are shown in Fig. 2. It is evident that the fitted curves are largely equivalent to the curves obtained with the non-parametric fit. In some of the experiments there are observable differences, however, it is striking that even in these experiments the estimates of the RBE are very nearly identical.

The results of the analysis are plotted in Fig. 3 where the potency of each neutron beam relative to NRL is plotted as a function of a mean neutron energy.

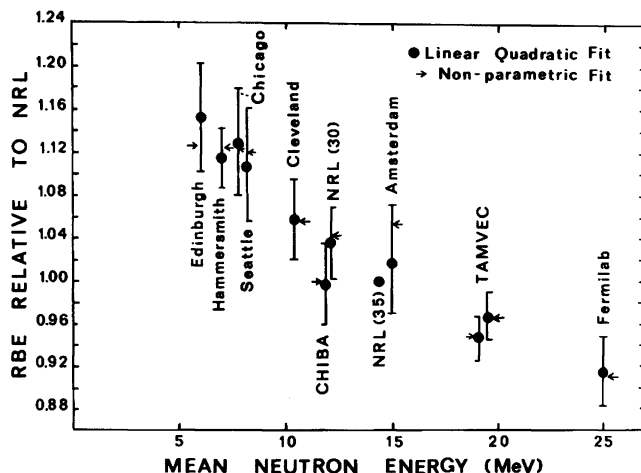


Fig. 3. The value of the Relative Biological Effectiveness (RBE) relative to the Naval Research Laboratory neutron beam, of each facility visited, plotted as a function of the mean neutron energy.

DETERMINATIONS OF THE OXYGEN ENHANCEMENT RATIOS

A standardized technique has been used to determine values for the oxygen enhancement ratio for neutrons generated by a range of energies. For these experiments V79 Chinese hamster cells were irradiated while sealed in ampules, which were shaken vigorously during irradiation to prevent the cells from settling onto the glass surface in order to minimize dosimetry errors. Hypoxia was achieved by using a high cell concentration (2×10^6 cells in 1 ml of medium), flushing the air from the ampule with a mixture of 5% CO_2 in nitrogen, and then incubating the sealed ampules for one hour at 37.5°C so that residual oxygen was scavenged from the system by cell respiration. A second series of ampules contained 10^4 cells and remained aerated. This technique has also been described in detail.⁷ After irradiation, the ampules were opened and aliquots of the cell suspension replated to test for colony formation. Survival curves under aerated and hypoxic conditions are shown in Fig. 4 for the various neutron facilities visited. The data were analyzed as described above for RBE. Again, very nearly the same estimates for the dose modifying factor, in this case the OER values, are obtained. However, the plots are given only for the linear-quadratic analysis that contains also the derivation of the 90% confidence intervals for the OER values. In Fig. 5 the OER values and the confidence intervals are plotted versus the mean neutron energy.

DISCUSSION

In general, the RBE of the various neutron beams decreases with increasing average neutron energy. All the data points fall around a common line which implies that within the accuracy with which RBE estimates can be made, mean neutron energy appears to be an adequate prediction of RBE. This is somewhat surprising in view of the fact that neutron beams were generated by three different processes involving protons on beryllium, deuterons on beryllium or deuterons on tritium with substantial differences in the shape of the neutron spectra resulting from these processes.

In the case of the oxygen enhancement ratio (OER) there is a tendency for its value to rise slowly with increasing neutron energy. However, the change is not significant for the range of neutron energies used in clinical practice. This confirms the early conclusion of Broerse and Barendsen based in much more limited data. From the neutrons generated by 5 MeV $d^+ \rightarrow \text{Be}$ at Hammersmith to the 66 MeV $p^+ \rightarrow \text{Be}$ neutrons at Fermilab, a value of about 1.6 applies.

For very high energy neutron beams our experience does not confirm the decreased OER previously reported by Harrison et al.,¹¹ but indicates an OER that is similar to, or possibly a little higher than, that characteristic of the presently used therapy beams. This is in agreement with the recently published report of Bewley and Cullen.⁴ Basic biophysical considerations have led to the conclusion that RBE is a function of absorbed dose and that,

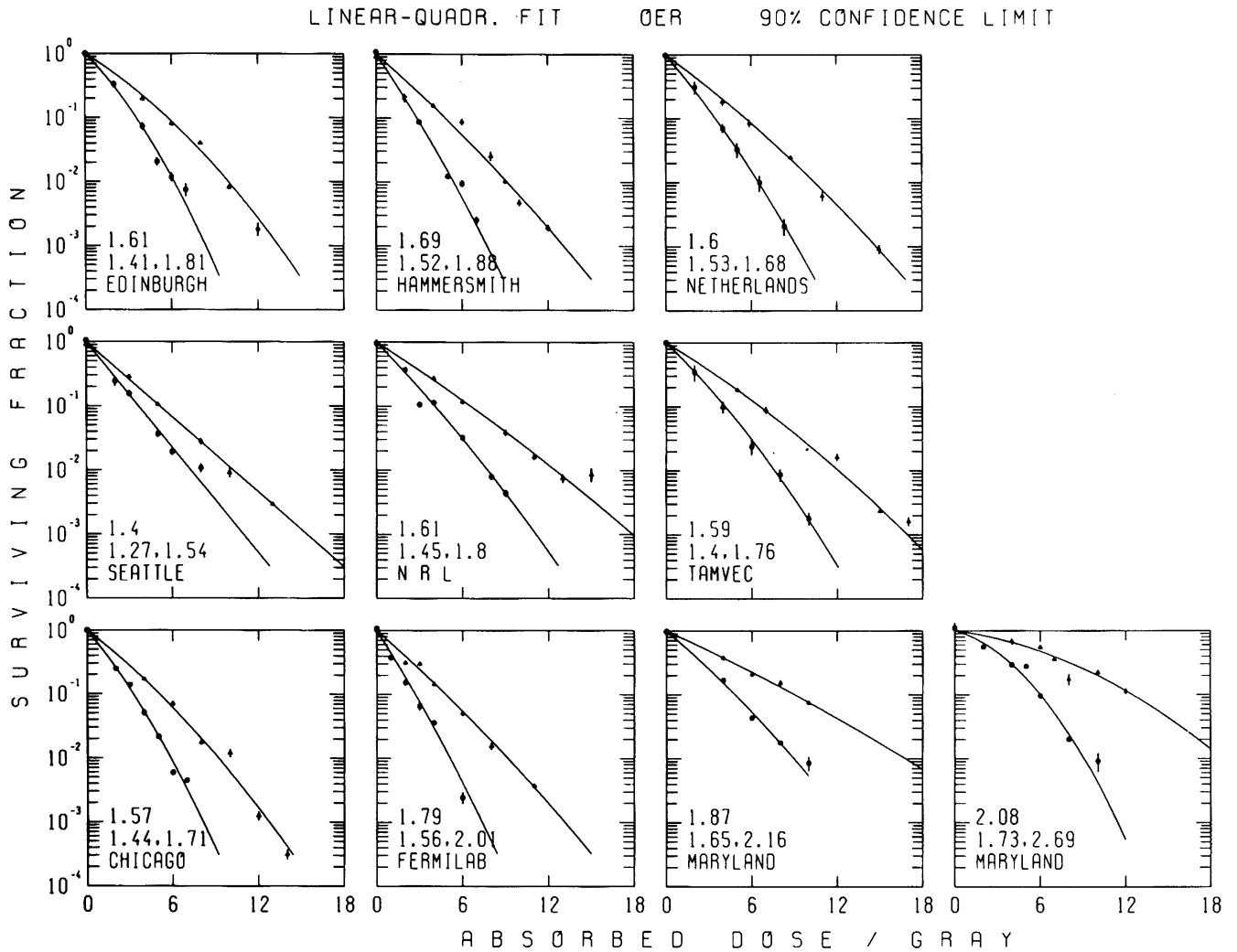


Fig. 4. Survival data for Chinese hamster V79 cells irradiated under aerobic and hypoxic conditions (circles and triangles respectively). Each panel represents a self-contained experiment at a different facility. The data were fitted to a linear quadratic model as described in the text which allows an estimate to be made of the oxygen enhancement ratio (OER). This quantity, together with confidence intervals, is shown in each panel.

specifically, the largest RBE values in the comparison of two radiations are obtained at the smallest doses.¹⁴ In the comparison of neutrons to sparsely ionizing radiations this dependence of RBE on absorbed dose is of fundamental importance and has been established in a number of

experimental systems. However, for two radiations that differ only slightly in their effectiveness, i.e. for RBE values close to 1 it is difficult to assess any variation of RBE with absorbed dose over a limited range of absorbed dose, such as in the survival experiments. For this reason the present analysis has been performed in terms of constant RBE values, and the results must, accordingly, be considered as representing typical RBE-values for a dose of a few gray. Far more data per intercomparison would be required if the dependence of RBE on absorbed dose were to be investigated. Similar statements apply to the analysis of OER, where constant values can also not be assumed but where a dose dependence has never been unequivocally established.

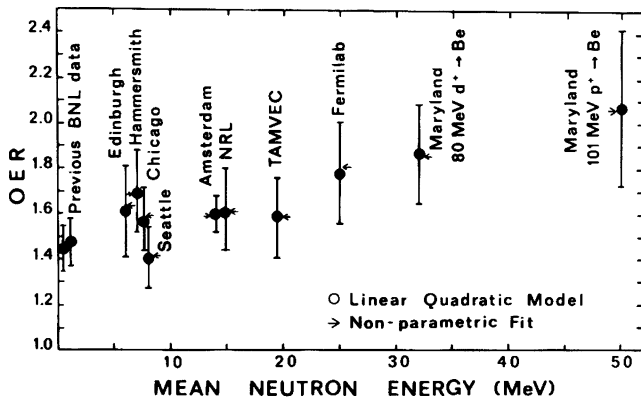


Fig. 5. The oxygen enhancement ratio (OER) for each facility visited, plotted as a function of the mean neutron energy.

REFERENCES

1. Almond, P.R., Smathers, J.B.: Physics intercomparisons for neutron radiation therapy. *Int. J. Oncol. Phys. Biol.* 3: 167-176, 1977.
2. Almond, P.R.: Personal communication. 1982.
3. Barendsen, G.W., Broerse, J.J. Breur, K.: High LET radia-

- tions in clinical radiotherapy. Proceedings of the 3rd Meeting on Fundamental and Practical Aspects of the Application of Fast Neutrons and Other High LET Particles in Clinical Radiotherapy. *Eur. J. Cancer* (Suppl.) 171-174, 1979.
4. Bewley, D.K., Cullen, D., Walker, H., Page, B.C.: Oxygen enhancement ratio for high energy neutrons. *Brit. J. Radiol.* **53**: 1204-1206, 1980.
 5. Geraci, J.P., Jackson, K.L., Christensen, G.M., Thrower, P.D., Weyer, B.J.: Mouse testes as a biological system for intercomparisons of fast neutron therapy beams. *Radiat. Res.* **71**: 377-386, 1977.
 6. Bragg, R.L., Humphrey, R.M., Meyn, R.: The response of Chinese hamster ovary cells to fast-neutron radiotherapy beams. I. Relative biological effectiveness and oxygen enhancement ratio. *Radiat. Res.* **65**: 71-82, 1976.
 7. Hall, E.J., Lehnert, S., Roizin-Towle, L.: Split dose experiments with hypoxic cells. *Radiology* **112**: 425-437, 1974.
 8. Hall, E.J.: The dependence on RBE and OER on neutron energy for damage to mammalian cells and plant systems. In *Radiation Research—Biomedical, Chemical and Physical Perspectives*, O.F. Nygaard, I. Adler and W.K. Sinclair (Eds.). NY, Academic Press. 1975, p. 1066.
 9. Hall, E.J.: Radiobiological intercomparison *in vivo* and *in vitro*. Proc. Particles and Radiation Therapy 2nd Int. Conf. *9. Int. J. Radiat. Oncol. Biol. Phys.* **3**: 195-201, 1977.
 10. Hall, E.J., Kellerer, A.M.: Review of RBE data for cells in culture. In High LET Radiations in Clinical Radiotherapy, G.W. Barendsen, J. Broerse, K. Breur (Eds.). *Europ. J. Cancer* (Suppl.), 171-174, 1979.
 11. Harrison, G.H., Kubiczek, E.B., Robinson, J.E.: OER of neutrons from 80 MeV deuterons on beryllium. *Brit. J. Radiol.* **48**: 409-410, 1976.
 12. Kellerer, A.M., Brenot, J.: On the statistical evaluation of dose-response functions. *Radiat. Environ. Biophys.* **11**: 1-13, 1974.
 13. Kellerer, A.M., Chmelesky, D., Hall, E.J.: Non-parametric representation of dose-effect relations. *Radiat. Res.* **84**: 173-188, 1980.
 14. Rossi, H.H.: The effects of small doses of radiation. *Phys. Med. Biol.* **15**: 255-262, 1970.
 15. Todd, P., Geraci, J.P., Furcinitti, P.S., Rossi, R.M., Mikage, F., Theus, P., Schroy, C.B.: Comparison of the effects of various cyclotron-produced fast neutrons on the reproductive capacity of cultured human kidney (T-1) cells. *Int. J. Radiat. Oncol. Biol. Phys.* **4**: 1015-1022, 1978.