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MEASUREMENTS OF RELATIVE BIOLOGICAL EFFECTIVENESS AND OXYGEN ENHANCEMENT RATIO OF FAST NEUTRONS OF DIFFERENT ENERGIES

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Abstract

MEASUREMENTS OF RELATIVE BIOLOGICAL EFFECTIVENESS AND OXYGEN ENHANCEMENT RATIO OF FAST NEUTRONS OF DIFFERENT ENERGIES. Impairment of the reproductive capacity of cultured cells of human kidney origin ($T\text{-}1_g$ cells) has been measured by the Puck cloning technique. From the dose-survival curves obtained in these experiments by irradiation of cells in equilibrium with air and nitrogen, respectively, the relative biological effectiveness (RBE) and the oxygen enhancement ratios (OER) were determined for different beams of fast neutrons. Monoenergetic neutrons of 3 and 15 MeV energy, fission spectrum fast neutrons (mean energy about 1.5 MeV), neutrons produced by bombarding Be with cyclotron-accelerated 16 MeV deuterons (mean energy about 6 MeV) and neutrons produced by bombarding Be with cyclotron-accelerated 20 MeV ^{3}He ions (mean energy about 10 MeV) have been compared with 250 kVp X-rays as a standard reference. The RBE for 50% cell survival varies from 4.7 for fission-spectrum fast neutrons to 2.7 for 15 MeV monoenergetic neutrons. The OER is not strongly dependent on the neutron energy for the various beams investigated. For the neutrons with the highest and lowest energies used OER values of 1.6 ± 0.2 and 1.5 ± 0.1 were measured.

An interpretation of these data on the basis of the shapes of the LET spectra is proposed and an approximate verification of this hypothesis is provided from measurements in which secondary particle equilibrium was either provided for or deliberately eliminated.

INTRODUCTION

In a study of the relation between the relative biological effectiveness (RBE) and the linear energy transfer (LET) of different ionizing radiations for impairment of the reproductive capacity of cultured cells of human origin, attention has initially been focussed on effects of monoenergetic heavy charged particles, employed in conditions whereby relatively small selected segments of the tracks of these particles traversed through the cells. This track segment method provides for irradiation conditions whereby the distribution of dose in LET is relatively small, Barendsen and Beusker [1]; Barendsen et al. [2]; Barendsen [3]; Barendsen et al. [4]; Barendsen [5]; Barendsen et al. [6].

For irradiation of objects with dimensions larger than about 1 mm the track segment method cannot be applied. To irradiate large objects with radiations which have an energy average LET in excess of about 10 keV/μm, fast neutrons of different energies have been employed.

The purpose of this paper is to present some results of experiments in which RBE values of different neutron beams were measured and to discuss the influence of the oxygenation condition of the cells on these RBE values.

Finally, the distribution of dose in LET of these neutron beams and the contributions to the total damage corresponding to two main dose components are discussed.

EXPERIMENTAL TECHNIQUES

(a) Biological materials

An established cell line derived from human kidney has been used in all experiments, van der Veen et al. [7]. An attempt has been made to obtain a homogeneous population by subcloning of the original cell line. The cell line used in the experiments reported here was designated T-1_g and has a modal chromosome number of 63, Barendsen [8]. The capacity of these cells for unlimited proliferation was determined by the clone technique developed by Puck et al.[9]. Survival of these cells was measured by their ability in a suitable culture medium to give rise to a clone containing at least 50 cells after an incubation period of 14 days. The doubling time of the number of cells per clone developed from an unirradiated cell is approximately 23 hours.

(b) Equilibration of cells with air or nitrogen

To control the gaseous environment of the cells during irradiation, the growth medium was removed from the dishes. Subsequently, air or pure nitrogen, equilibrated with water vapour by passage through washing bottles, was passed over the cells during eight to ten minutes prior to and during irradiation. Preliminary experiments had shown that after five minutes gassing sufficient equilibration of the cells with the nitrogen environment was attained to result in the maximum decrease in sensitivity of the cells to X-rays. In the experiments in which nitrogen was passed over the cells, the oxygen contamination was checked with a Hersch cell to be less than 100 ppm, Hersch [10].

(c) Fast neutron beams

Five different beams of fast neutrons were investigated.

Monoenergetic neutrons of 3.4 MeV energy were produced by bombarding deuterium, absorbed in a titanium layer on a copper backing, with 400 kV deuterons. A van de Graaff electrostatic accelerator was used, which provides deuterons at a current of 150 μA. The dose-rate determined with a tissue-equivalent ionization chamber at the position of the cells was about 1 rad/min, Broerse [11].

Monoenergetic neutrons of 15 MeV energy were produced by bombarding tritium, absorbed in a titanium layer on a copper backing, with 400 kV deuterons at a current of 150 μA from the van de Graaff electrostatic accelerator. The dose-rate at the position of the cells was about 15 rad/min.

Fission spectrum fast neutrons were produced by exposure of a ^{235}U converter plate to thermal neutrons from the Low Flux Reactor of the

Reactor Centre Netherlands at Petten.[1] The dose-rate at the position of the cells was about 12 rad/min. The neutron spectrum extends from 0 to about 18 MeV, with a maximum intensity at about 1.5 MeV. The γ-ray contamination of this beam contributed less than 10% to the total absorbed dose.

Fast neutrons with a spectrum extending from 0 to about 20 MeV, and with a maximum intensity at about 6 MeV, were obtained by bombarding a thick beryllium target with 16 MeV deuterons at a current of 50 μA from the Medical Research Council's 45-in. cyclotron at Hammersmith Hospital, London, United Kingdom. Details of this irradiation facility are described elsewhere, Barendsen et al. [6]; Bewley and Hornsey [12]. The dose-rate measured with a tissue-equivalent ionization chamber at the position of the cells was about 50 rad/min.

Fast neutrons with a spectrum extending from 0 to about 33 MeV were obtained by bombarding a thick beryllium target with 20 MeV ^{3}He ions at a current of 15 μA from the Philips cyclotron at Eindhoven, Netherlands.[2] The complex energy spectrum of the emitted neutrons is not known accurately. The mean neutron energy is assumed to be about 10 MeV. The dose-rate at the position of the cells at 17 cm from the Be-target was 12 rad/min.

(d) Secondary particle equilibrium

Fast neutrons dissipate energy in biological material mainly through interactions with the nuclei of H, C, O and N. In the distributions of dose in LET for energy deposition by fast neutrons, two important regions can be distinguished, corresponding respectively to the energy dissipated through protons and to the energy dissipated through interactions with nuclei of C, O and N. Protons set in motion by neutrons through elastic collisions dissipate energy with a distribution of dose in LET which extends from a low value, determined by the maximum proton energy, to a maximum value of about 96 keV/μm, which is equal to the maximum LET_∞ of protons at low energies corresponding to the Bragg peak. For 15 MeV neutrons, for instance, the minimum of the LET distribution is about 3 keV/μm. Energy dissipated by fast neutrons through interactions with C, O and N nuclei, is deposited mainly in the LET_∞ region between about 100 and 1000 keV/μm, Randolph [13]. To compare experimental data with respect to the RBE and OER of monoenergetic charged particles, employed in conditions whereby narrow LET distributions are obtained, with data on the RBE and OER of fast neutrons, it is of interest to study separately the RBE and OER of the components of the energy dissipated by these neutrons through interactions with hydrogen nuclei and through interactions with C, O and N nuclei, respectively. To a first approximation these differences can be investigated by irradiating cells in conditions with and without proton equilibrium. Protons produced by fast neutrons have ranges

[1] The authors are indebted to J.A.G. Davids of the Reactor Centre Netherlands at Petten for his co-operation in putting this facility at their disposal.

[2] The authors are indebted to P. Kramer of Philips-Geldrop for his co-operation in putting this facility at their disposal.

which, for the greater part, are large compared with the diameter of the cells studied. A proton of 1 MeV has a range in tissue of about 25 μm, which is approximately equal to the diameter of a T-1_g cell; a proton of 10 MeV has a range in tissue of about 1200 μm. In experiments in which the total effect of all interactions of neutrons with cellular constituents is to be assessed in thin objects, a layer of tissue-equivalent material is mounted in front of the object irradiated. This tissue-equivalent material should be at least equal in thickness to the range of the protons produced with maximum energy, which is equal to the maximum of the neutron energy spectrum. The result is that secondary particle equilibrium is obtained, i.e. the number and energy of protons set in motion outside the cell and entering it is equal to the number and energy of protons produced inside the cell which emerge from it.

In part of the experiments to be described this proton equilibrium was deliberately avoided by mounting a layer of pure carbon in front of the cells. Consequently, energy will only be dissipated through interactions of neutrons with nuclei of H, C, O and N present inside the cell. Furthermore, the protons with energies in excess of about 1 MeV, produced inside the cell, will dissipate most of their energy outside the cell. Thus the contribution of protons to the energy dissipation will be greatly reduced with a consequent reduction in the contribution of the component of dose corresponding to LET values below about 100 keV/μm. Most of the energy deposited in the cells irradiated in these conditions will result from interactions of neutrons with C, O and N nuclei. Since most of these interactions produce particles with ranges which are short compared to the dimensions of the cells, the material directly adjacent to the cell will not influence this component of the energy absorption. For instance, the range of an oxygen nucleus set in motion by a head-on collision of a 15 MeV neutron has a range of about 1 μm in tissue, and the energy dissipated corresponds to the LET region in excess of 100 keV/μm. In the experimental conditions employed in the authors' experiments cells are plated in dishes with a Melinex bottom (polyethylene terephthalate) which is only 6 μm thick. About four hours after plating these cells have attached firmly to the bottom and irradiation is carried out with the fast neutron beams entering approximately perpendicular through this thin bottom. Proton equilibrium was provided by mounting tissue-equivalent plastic discs, Shonka et al. [14], underneath the bottom of the dishes, and protons were eliminated by replacing these discs by discs of carbon.

EXPERIMENTAL RESULTS

In Fig. 1 survival curves are presented, obtained with the five neutron beams described. For comparison the curve obtained with 250 kVp X-rays is included. It is clear that for all neutron beams the survival curves show less curvature than the curve obtained with 250 kVp X-rays, although especially for 15 MeV monoenergetic neutrons and the "20 MeV ^{3}He on Be" neutrons some curvature is certainly present. From these curves it is possible to obtain RBE values of the neutron beams investigated. In Fig. 2

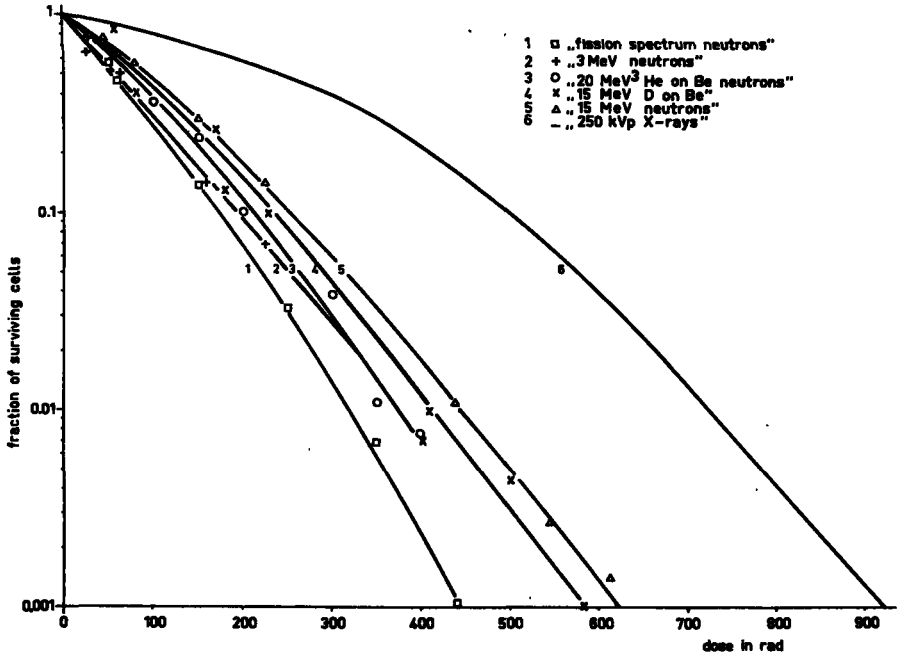

FIG.1. Survival curves obtained for T-1_g cells irradiated with different neutron beams and with 250 kVp X-rays

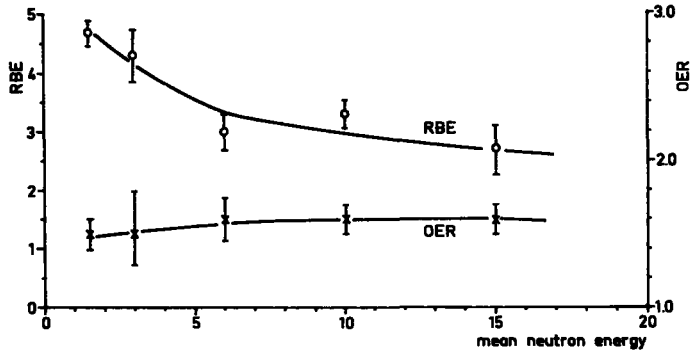

FIG.2. The RBE and OER of fast neutron beams as a function of the "mean" neutron energy, for impairment of the proliferative capacity of cultured cells of human origin (T-1_g cells). RBE values correspond to doses producing 50% cell killing. Specification of the beams is given in the text

these values, derived as the ratio of the doses of 250 kVp X-rays and the doses of fast neutrons, which produce 50% survival, are presented as a function of the mean neutron energy. For the neutrons produced by bombarding Be with 20 MeV ^{3}He ions, this value is taken as 10 MeV. Survival curves for cells equilibrated with nitrogen have been published elsewhere, Barendsen et al. [6], Barendsen and Broerse [15], for three of the neutron beams and a summary of the OER values is given in Fig. 2.

In Fig. 3 survival curves are presented for 15 MeV neutrons with cells irradiated with and without proton equilibrium for oxygenated conditions. In Fig. 4 data are presented for both oxygenated and anoxic conditions. It can be derived that the OER for cells irradiated in conditions where proton equilibrium is provided is 1.4, whereas if a large part of the protons is eliminated an OER of about 1.1 is obtained. The value of 1.4 does not differ significantly from the value of 1.6 reported earlier [15].

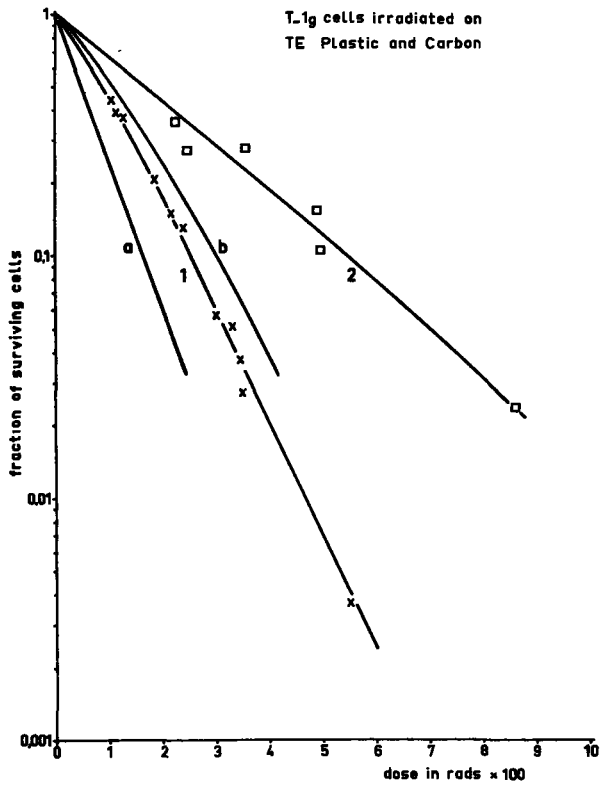

FIG.3. Survival curves obtained for T-1_g cells in equilibrium with air irradiated with 15 MeV neutrons whereby tissue-equivalent plastic and carbon discs were mounted under the bottoms of the dishes (curves 1 and 2 respectively). Curves a and b represent survival curves calculated from curves 1 and 2 for the "high LET" component and the "proton" component of the energy dissipation, respectively. The dose scale in rads refers to the absorbed dose. This is only correct for the curves 1, a and b. Curve 4 is obtained in conditions where the secondary particle equilibrium is not provided for and the proper unit is the kerma expressed in erg/g (kinetic energy released in matter). In conditions of particle equilibrium 1 rad is 100 erg/g. Thus, instead of "dose in rads X 100" the scale description should read "kerma in 10^4 erg/g"

DISCUSSION

From the data presented in Figs 1 and 2 it can be deduced that the RBE for 50% cell survival varies from 4.7 for fission spectrum fast neutrons to 2.7 for 15 MeV monoenergetic neutrons, i.e. by a factor of about 1.7. It

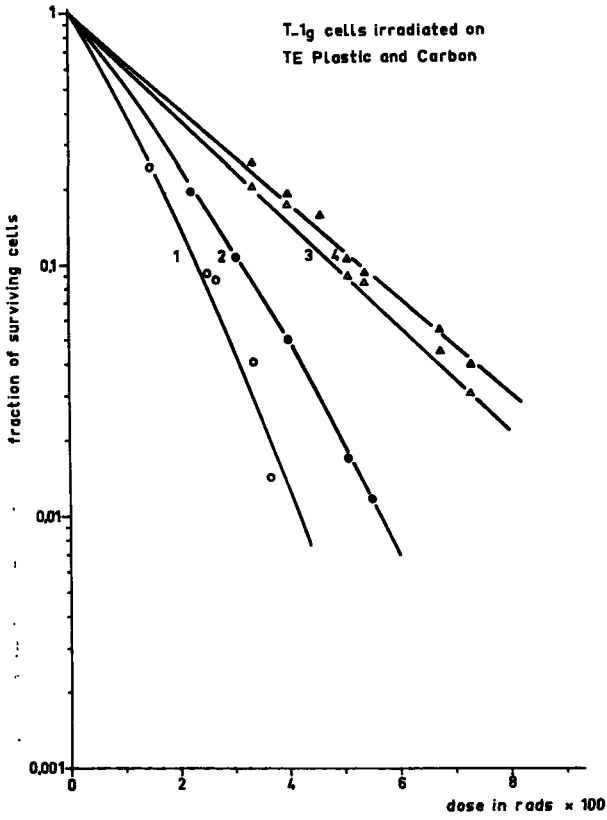

FIG.4. Survival curves obtained for T-1_g cells irradiated with 15 MeV neutrons. Curve 1, cells in equilibrium with air, proton equilibrium; Curve 2, cells in equilibrium with nitrogen, proton equilibrium; Curve 3, cells in equilibrium with air, no proton equilibrium; Curve 4, cells in equilibrium with nitrogen, no proton equilibrium. For curves 3 and 4 the units on the abscissa should be expressed in kerma in 10^4erg/g (compare caption of Fig.3)

is clear that the RBE is strongly dependent on the level of damage considered because the survival curves obtained with all fast neutron beams exhibit less curvature than the survival curve for 250 kVp X-rays, which is taken as the standard radiation. For a surviving fraction of 0.01, for instance, the RBE varies from 2.3 for fission spectrum fast neutrons to 1.6 for 15 MeV neutrons, i.e. by a factor of about 1.4.

From Fig.2 it can further be derived that the OER is not strongly dependent on the neutron energy for the various beams investigated. This is at first sight somewhat surprising, since the patterns of the energy deposition are different and this results in different RBE values. Randolph [13] has calculated that for 15 MeV neutrons about 70% of the energy is dissipated through interactions with hydrogen and 30% of the energy through interactions with heavier nuclei. For "fission spectrum" fast neutrons and 3 MeV neutrons more than 90% of the energy is dissipated through

interactions with hydrogen, [16]. However, the mean energy of the protons generated by 15 MeV neutrons is larger than that of the protons produced by "fission spectrum" neutrons and 3 MeV neutrons. The distributions of dose in LET are very complex and consequently the variation of the RBE as well as of the OER can only be correlated with the energy dissipation pattern of the various neutron beams if these are accurately known.

To distinguish to a first approximation between the effects of interactions of 15 MeV neutrons with hydrogen and other nuclei, respectively, the results presented in Fig. 3 can be used. From a comparison of curves 1 and 2 of this figure it can be deduced, for instance, that with a dose of 200 rad of 15 MeV neutrons, a fraction of 0.18 surviving cells is obtained in the case of proton equilibrium, whereas a fraction of 0.45 surviving cells is obtained if the same fluence is used but the contribution of protons is eliminated. This implies that the surviving fraction resulting from the protons alone is $0.18/0.45 = 0.40$. It is clear from this calculation that at a dose of 200 rad the contributions to the killing of cells of the two components of the LET distribution are about equal. If we assume Randolph's calculation to be correct, i.e. that 70% of the total dose is dissipated through protons, it can be concluded that, at a total dose of 200 rad, the partial dose of 140 rad of fast protons would cause a reduction of the fraction of surviving cells to 0.40. At 400 rad total dose the same type of calculation shows that the proton component equivalent to 280 rad would produce a surviving fraction of $0.021/0.19 = 0.11$. It is thus possible to derive survival curves for the two components and these are plotted in Fig. 3. Curve a represents the survival curve derived for the "high LET component" and curve b represents the survival curve derived for the "proton component".

The survival curves presented in Fig. 4 show that, for irradiation with 15 MeV neutrons, in conditions in which a large part of the proton component is eliminated, the OER is about 1.1. This agrees with the hypothesis that in these conditions the component with a LET in excess of 100 keV/μm produces most of the damage, Barendsen et al. [6]. An analysis of the effectiveness of the proton component in anoxic conditions compared with oxygenated conditions which can be made in a similar way, as discussed for the results obtained in oxygenated conditions, shows that the OER for this component is about 2.1.

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DISCUSSION

D.K. BEWLEY: With regard to the OER for fission neutrons, this represents the biggest discrepancy with the results of others, in particular Berry who found an OER of 1.2. I would like to ask what was the gamma contamination of the fission neutron beam and could this have any bearing on the observed result?

G.W. BARENDSEN: The γ-contamination of the "fission spectrum" neutron beam was less than 10% in terms of absorbed dose and taking into account the RBE value of at least about 4, it produced only a very small part of the biological effect of this beam. Consequently, this contribution cannot resolve a discrepancy between our results and a value obtained by Dr. Berry.

V. DRÁŠIL: If you express (in your experiment with combined X-ray and α-irradiation) the dose of α-particles in their number per cell area, how many particles are needed for D_{37} to go through the nucleus?

G.W. BARENDSEN: From the D_{37} value it is possible to calculate that this dose is equivalent to one particle passing per 35 μm^2 of the cell cross-section. The cross-section of the cell nucleus is, on average, about 70 μm^2 but this is only a rough estimate.

H.H. ROSSI: One of the worst ways to characterize a neutron spectrum is to say that it is a fission spectrum. I have personally done dosimetry in two different experiments in which the lethality of fission neutrons of mice was investigated. I used the same chambers, in both cases the contamination was about 10%, in one case the RBE was 4, in the other case it was 1.8, and these were both fission spectra. So, the word fission spectrum is very vague.