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THE IMPORTANCE OF SECONDARY PARTICLE EQUILIBRIUM FOR NEUTRON IRRADIATIONS OF CULTURED CELLS AND INTACT ORGANISMS

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ABSTRACT

At interfaces of tissues of different composition in intact organisms local perturbations of charged particle equilibrium occur, which may be different for irradiations with fast neutrons and electromagnetic radiation.

The variations in absorbed dose at the interface of bone and soft tissue have been investigated with a cell culture system. For X-irradiations and 15 MeV neutron irradiations survival curves have been determined for cells irradiated on muscle equivalent plastic and on bone equivalent plastic. These results have been related to the occurrence of the bone-marrow syndrome and the intestinal syndrome in mice after neutron- and X-irradiation.

Due to the various types of interactions of fast neutrons with the components of tissue, the distribution of the dose in LET is complex. It is therefore difficult to assess an average LET for the different types of fast neutrons. An exact knowledge of the relevant distributions of dose in LET is required. For a first approximation a distinction has been made between a "high-LET component" and a "proton component". In order to separate these components experimentally, irradiations where carried out in which perturbations of charged particle equilibrium were induced artificially. Survival curves have been determined for neutron irradiations with either layers of tissue equivalent plastic or layers of pure carbon mounted in front of the cells. These results will be discussed in relation to the OER and RBE values measured for fast neutrons of different energies.

I. INTRODUCTION

Over the past years we have studied the effects of fast neutrons of different energies for various biological systems (1, 2). For an adequate evaluation of these biological effects the energy deposition in the irradiated material has to be considered at different levels. In this respect three different areas of dosimetry can be distinguished. Measurements of the mean absorbed dose and of the inhomogeneity of the dose due to build up and absorption in the irradiated body can be designated as macrodosimetry. In irradiations of mammals discontinuities of the structure of the body, which are associated with the skeleton and the air-filled lung tissues, may produce serious inhomogeneities in the dose distribution on a smaller scale. Interface dosimetry is concerned with studies on the variations of the absorbed dose due to local perturbations of secondary charged particle equilibrium.

Furthermore, the energy dissipation by ionizing radiation in tissue is not a random phenomenon but occurs mainly along the tracks of charged particles. Investigations concerning the spatial distribution of the energy deposition on a cellular and subcellular scale are referred to as microdosimetry. For a first specification of the radiation quality the concept LET is generally used. Since neutrons dissipate their energy in tissue through different interactions with the various contituents of the material, a complex LET spectrum is obtained. It is now generally accepted that the concept of an average LET has limited significance for neutron radiation. Interpretation of the biological effects will require a spectral analysis of the distribution of the dose per LET interval (3) or exact knowledge on the spectra of event sizes and local energy densities in the irradiated material (4).

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In this paper our experimental results on interface dosimetry will be reported and the importance of secondary charged particle equilibrium for death of mice due to the bone-marrow and intestinal syndromes will be discussed. The survival of cultured cells after irradiation with various beams of fast neutrons was studied for different irradiation conditions. The resulting RBE values are higher than those of charged particles with a comparable track average LET, while the OER values for various neutron energies are fairly constant over a wide energy range. For a quantitative interpretation of these phenomena a distinction was made between the "low-LET component" of the neutron radiation due to recoil protons and the "high-LET component" due to interactions with carbon, oxygen and nitrogen nuclei. These components were separated by irradiating cultured cells in conditions with and without proton equilibrium. Survival curves will be reported for neutron irradiations with either layers of tissue equivalent plastic or layers of pure carbon mounted in front of the cells. From these results survival curves for the "high-LET component" and the "proton component" will be derived.

The survival curves for irradiations of cells on carbon and on tissue equivalent plastic present the maximum variation of absorbed dose, which can be found at an interface. In the irradiations of an intact animal the differences in absorbed dose due to local perturbations of secondary radiation equilibrium will be smaller. Survival curves for cells irradiated on muscle equivalent plastic and bone equivalent plastic will be presented and these results will be discussed in relation to the occurrence of the bone-marrow syndrome and the intestinal syndrome in mice after neutron- and X-irradiations.

II. RBE AND OER VALUES FOR NEUTRON IRRADIATIONS OF CULTURED CELLS AND MICE.

The experimental data on cell survival have been obtained with an

established line of cultured cells derived from human kidney. The cell culture technique has been described in detail elsewhere (1, 5). In all experiments culture dishes were used consisting of glass rings with a 6μ thick Melinex bottom. The cells were plated on the dishes; after several hours incubation at 37°C the cells adhered to the bottom of the dishes. For the investigations on the OER the medium was removed from the dishes after this incubation period and the dishes were flushed with air or pure nitrogen. For the irradiations of the cultured cells five different types of fast neutrons have been employed. A van de Graaff electrostatic accelerator was used for the production of mono-energetic neutrons of 3 MeV and 15 MeV energy, through the D-D and the D-T reaction, respectively. Fission spectrum fast neutrons with a maximum intensity at about 1.5 MeV were produced by exposure of a converter plate to thermal neutrons from the Low Flux Reactor of the Reactor Centre Netherlands at Petten. Fast neutrons with a maximum intensity at about 6 MeV were obtained by bombarding a thick beryllium target with 16 MeV deuterons from the MRC 45 inch cyclotron at the Hammersmith Hospital, London, England. The last type of fast neutrons was obtained by bombarding a thick beryllium target with 20 MeV helium-3 ions from the Philips AVF 51 inch cyclotron at Geldrop, the Netherlands. There is only scanty information on the energy spectrum of these neutrons. The mean energy is assumed to be about 10 MeV. A detailed description of the experimental conditions will be published in the near future (6). The macroscopic dosimetry for all experiments was carried out with tissue equivalent ionization chambers and sulphur activation detectors (7, 8).

The pattern of mortality of mice was only investigated for 15 MeV neutron irradiations. The (CBA/Rij x C57BL/Rij)F_1 hybrid male mice were irradiated at ages ranging from 10 to 12 weeks. The 30 day mortality and 5 day mortality were taken as criteria for the hematopoietic and intestinal syndrome respectively. The mice were irradiated in perforated Lusteroid centrifuge tubes. In order to compensate for the inhomogeneities

in the flux density distribution and to obtain muli-lateral exposure, the tubes with the mice were mounted on a Styrofoam irradiation cylinder, which was rotated around the target during the irradiations (1). A maximum dose rate of 7 rads/min was obtained for the centre-line absorbed dose in the animal. Consequently total irradiation times of about 2 hours were required to produce lethality of the animals.

The survival curves of the cultured cells obtained for standard irradiation conditions, i.e. secondary charged particle equilibrium and medium equilibrated with air, have been presented in figure 1. It can be seen from this figure that for all neutron beams the survival curves show less curvature than the curve obtained with 250 kVp X-rays which has been included for comparison. The RBE values, derived from these curves, decrease with the percentage survival, i.e. with increasing dose, which phenomenon is due to the different shapes of the survival curves. In further experiments survival curves were determined for cells equilibrated with air and nitrogen respectively (9). The OER values derived from these data vary only slightly with mean neutron energy. For neutrons with the highest and lowest energies OER values of 1.6 ± 0.2 and 1.5 ± 0.1 were found respectively.

Due to the limited output of the neutron generator a minimum irradiation time of 2 hours had to be accepted for the 15 MeV neutron irradiations of the mice. For these 2-hour irradiations it was found that doses of 735 rads of 250 kVp X-rays and 660 rads of 15 MeV neutrons produce 50 per cent mortality within 30 days. For the same irradiation conditions doses of 1350 rads of 250 kVp X-rays and 950 rads of 15 MeV neutrons were required to produce 50 per cent mortality within 5 days. On the basis of these LD_{50} values the RBE values for the bone-marrow syndrome and the intestinal syndrome were calculated at 1.1 and 1.4 respectively. In contrast with the results of other investigators (1) we have in our experiments not observed that 15 MeV neutrons exclusively produce the early intestinal death. It can be concluded, however, that the relative dose

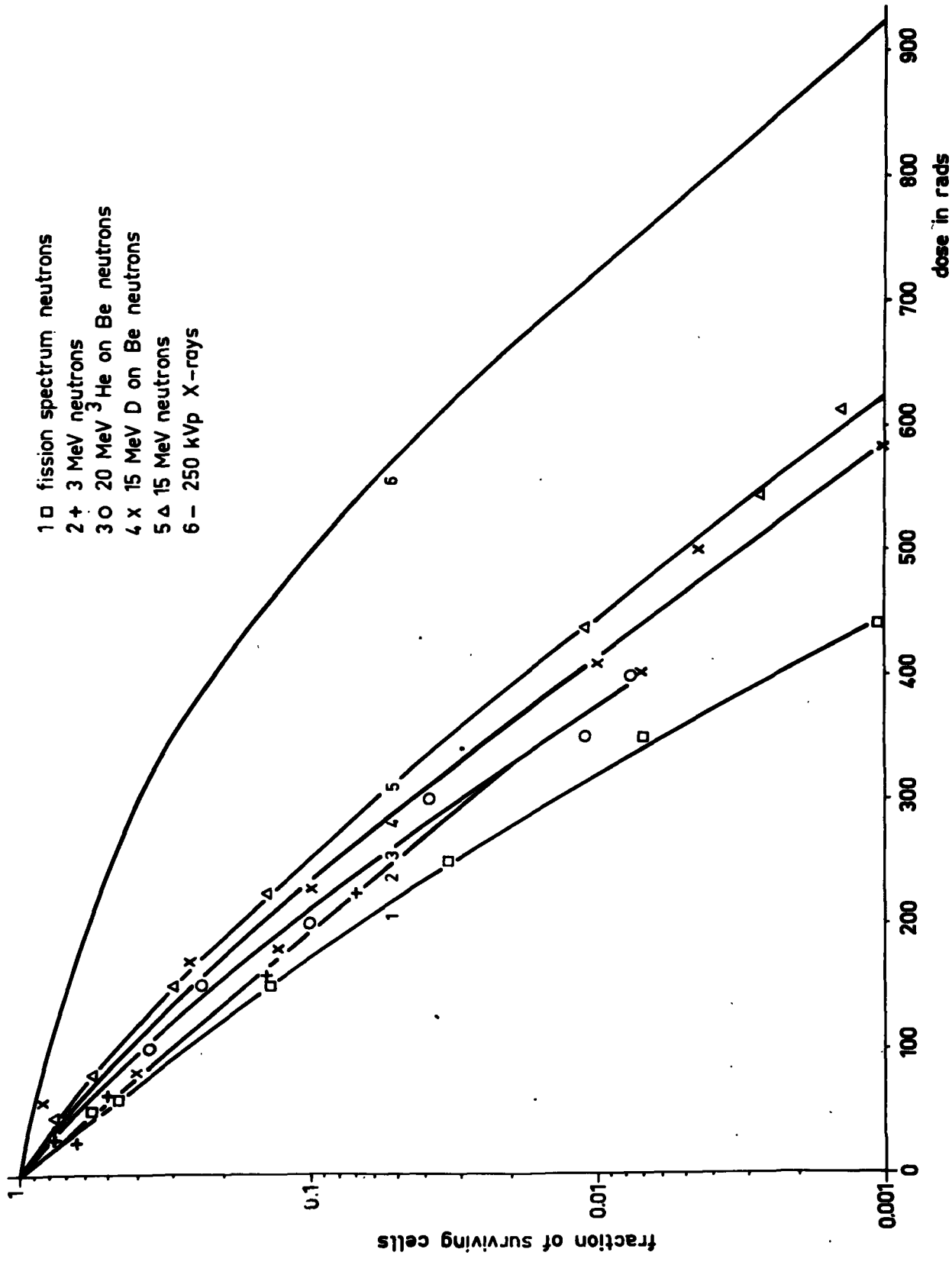

Figure 1. Survival curves obtained for cultured cells of human origin irradiated with different beams of fast neutrons and with 250 kVp X-rays.

interval between the LD_{50} for the intestinal syndrome and the LD_{50} for the bone-marrow syndrome is smaller for 15 MeV neutron irradiations than for X-irradiations. The ratio of the RBE values for the two radiation syndromes i.e. 1.4/1.1 = 1.27 is a measure for the variations of the relative intervals between the bone-marrow and intestinal syndromes occurring after X- or neutron-irradiations (1).

III. EXPERIMENTAL VARIATIONS OF SECONDARY PARTICLE EQUILIBRIUM IN IRRADIATIONS OF CULTURED CELLS.

An explanation for the observed independence of the OER from the neutron energy in the range investigated, can be given by considering the distribution of the dose over the LET range. Fast neutrons dissipate energy in tissue mainly by elastic collisions with hydrogen nuclei while, at energies in excess of about 10 MeV, interactions with other nuclei such as C and O contribute significantly to the absorbed energy (10). For the distribution in LET of the dose of fast neutrons, two important regions can be distinguished corresponding respectively to the energy dissipated through protons and to the energy dissipated through interactions with C, O and N. Protons set in motion by neutrons through elastic collisions dissipate energy with a distribution of the dose in LET which extends from low values of a few keV/μ to a maximum value of about 96 keV/μ at the Bragg peak. Energy dissipated by fast neutrons through interactions with C, O and N nuclei is deposited mainly in the LET_{∞} region between 100 and 1000 keV/μ (3, 10). The LET spectra for fission neutrons and neutrons of 3 MeV and 15 MeV energy, as calculated by Bewley (3), are presented in figure 2.

In order to distinguish experimentally between the biological damage produced by the protons and the heavier nuclei respectively, survival curves were determined in which secondary particle equilibrium was provided or deliberately avoided. This was accomplished by mounting under the 6μ thick Melinex bottoms of the culture dishes, 3 mm thick discs of

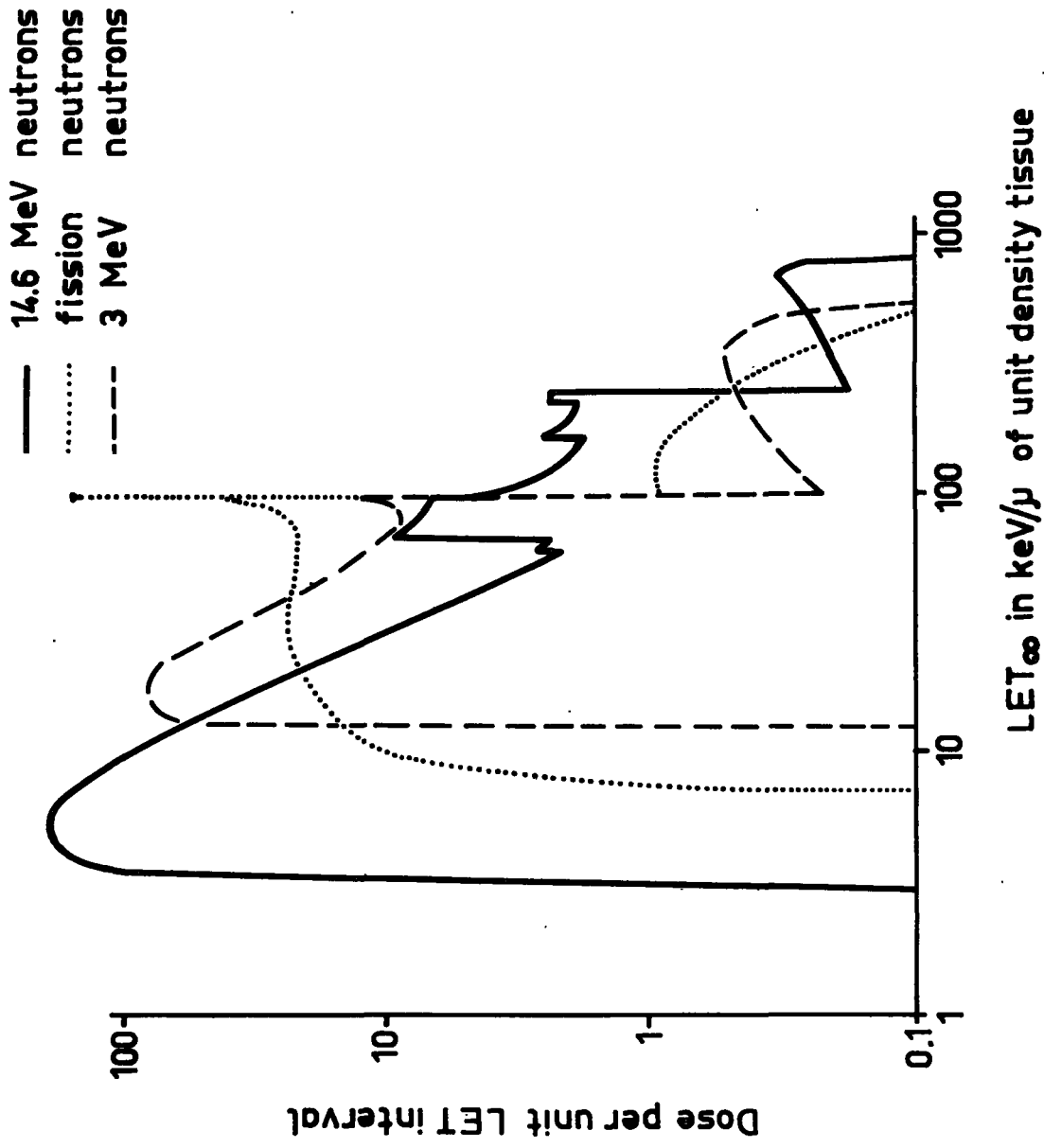

Figure 2. LET_{∞} spectra as calculated by Bewley (3) for three different types of fast neutrons. The curves have been normalized to equal areas.

either tissue equivalent plastic or pure carbon. For 15 MeV neutrons the recoil protons have ranges, which for the greater part are large compared to the diameters of the cells. A proton of 1 MeV energy has a range in tissue of about 25μ, which is approximately equal to the diameter of the cultured cells. However, a proton of 10 MeV energy has a range in tissue of about 1200μ. With tissue equivalent plastic adjacent to the bottom of the dishes, 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. For this case of secondary particle equilibrium Randolph (10) has calculated that with 15 MeV neutrons 70 per cent of energy is dissipated through interactions with hydrogen nuclei and 30 per cent of the energy through interactions with heavier nuclei. With pure carbon in front of the cells, the energy deposited in the cells only results from 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 for 15 MeV neutrons 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/μ. Most of the energy deposited in the cells, irradiated in these conditions, will result from interactions of neutrons with C, O and N nuclei, which produce particles with ranges which are short in comparison to the dimensions of the cells.

In figure 3 survival curves have been presented for 15 MeV neutrons with cells irradiated with and without proton equilibrium for oxygenated conditions. From these survival curves an approximate distinction can be made between the effects of interactions of 15 MeV neutrons with hydrogen and other nuclei respectively. From a comparison of the curves 1 and 2 of figure 3 it can be deduced for instance that with a dose of 200 rads of 15 MeV neutrons, a fraction of 0.18 surviving cells is obtained in the case

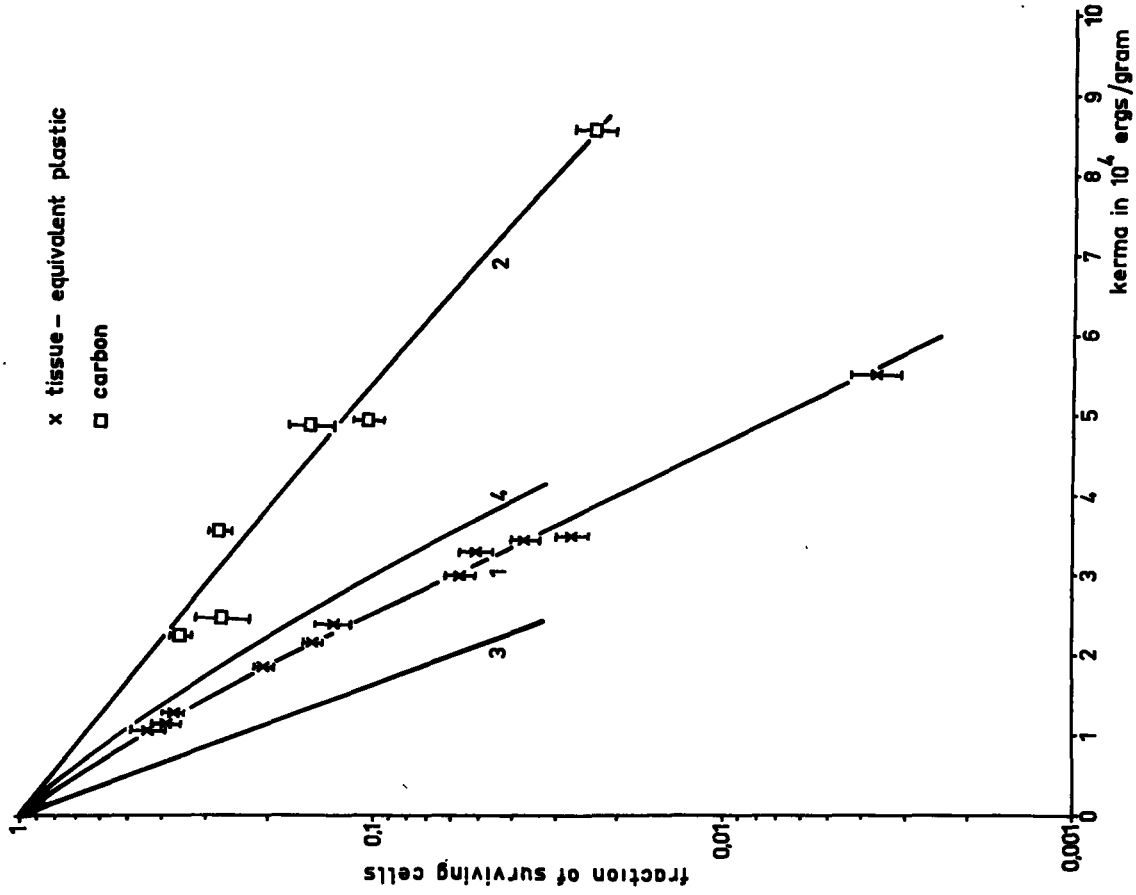

Figure 3. Survival curves obtained for cultured cells irradiated with 15 MeV neutrons whereby discs of tissue equivalent plastic or carbon were mounted under the bottoms of the dishes (curves 1 and 2 respectively). Curves 3 and 4 represent survival curves calculated for the "high LET component" and the "proton component" of the energy dissipation respectively. The dose scale is given in kerma units (kinetic energy released in matter). In conditions of secondary particle equilibrium, as present for curve 1, a kerma of 100 ergs per gram is equal to a dose of 1 rad. For curve 2 the dose in rads cannot be given because it is unknown which fraction of the secondary protons is eliminated by mounting carbon discs under the thin bottoms of the dishes. For a given kerma the exposure is the same, however, the absorbed doses for curves 1 and 2 are different.

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 rads the contribution to the killing of cells of the two components of the LET distribution is about equal. If we assume Randolph's calculation to be correct i.e. that 70 per cent of the total dose is dissipated through protons, it can be concluded that at a total dose of 200 rads, the partial dose of 140 rads of fast protons would cause a reduction of the fraction of surviving cells to 0.40. At 400 rads total dose the same type of calculations show that the proton component equivalent to 280 rads would produce a surviving fraction of 0.021/0.19 = 0.11. In this way survival curves for the two components can be derived. Curve 3 represents the survival curve derived for the "high-LET component" and curve 4 represents the survival curve for the "proton component". The RBE values for these two components can be calculated at 4.9 and 2.2 respectively for 50 per cent survival which values have to be compared with the RBE of 2.7 found for 15 MeV neutrons.

In addition to these different RBE values found for the two components, different OER values can be expected for the damage produced by recoil protons and heavy nuclei, respectively. In subsequent experiments the oxygen effect was investigated for irradiations with and without proton equilibrium. The results of the experiments with 15 MeV neutrons are presented in figure 4. It can be derived that the OER for cells irradiated in conditions where proton equilibrium is provided is equal to 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 mentioned earlier (9). An analysis of the effectiveness of the proton component in anoxic conditions compared with oxygenated conditions by a similar calculation as described earlier shows that the OER for this proton

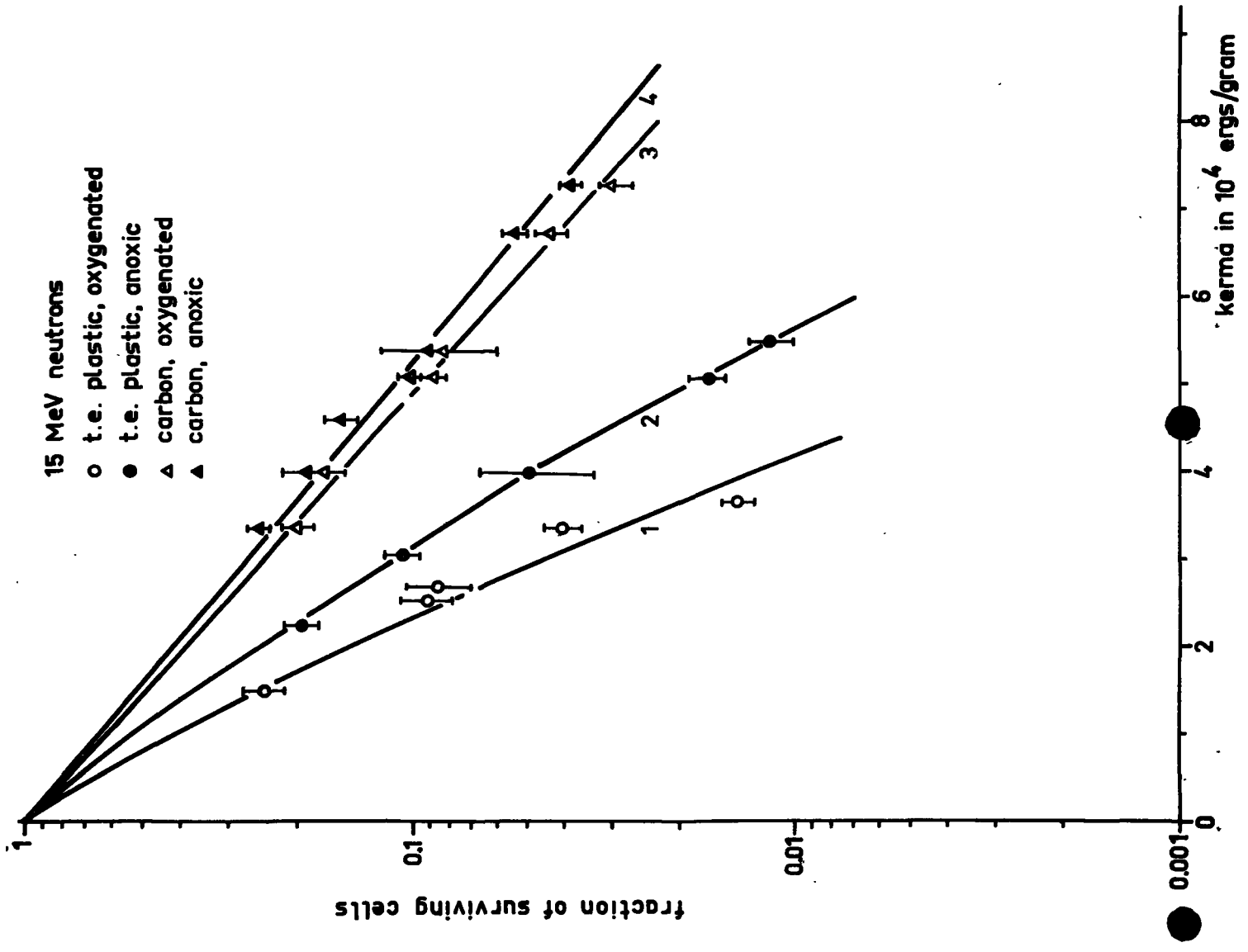

Figure 4. Survival curves obtained for cultured cells in equilibrium with air or nitrogen exposed to 15 MeV neutrons under conditions in which either secondary particle equilibrium was provided or part of the proton component was eliminated. For irradiations on tissue equivalent plastic curves 1 and 2 were obtained for cells equilibrated with air and nitrogen respectively. The OER in this experimental arrangement with secondary particle equilibrium is about 1.4. For irradiations on carbon curves 3 and 4 were found for oxygenated and anoxic conditions respectively. The OER in this experimental arrangement is equal to about 1.1.

component is equal to about 2.1. The high OER for the proton component and the low OER for the heavy recoils result in the OER of 1.6 for the 15 MeV neutrons. It can be concluded that the increasing contribution to the energy deposition of the heavy recoils with a high LET as a function of the increasing neutron energy, is compensated by the phenomenon that the protons exhibit a decreasing LET with increasing energy. In this way the independence of the OER of fast neutrons from the mean neutron energy of the various beams used might be explained.

For a confirmation of this interpretation the OER values for the "proton component" and the "high-LET component" should also be obtained for lower neutron energies. Therefore the oxygen effect was also investigated for irradiations with fission neutrons under conditions with and without proton equilibrium. In figure 5 the resulting survival curves are presented. It can be seen that the survival curves for irradiations on carbon for anoxic and oxygenated conditions show only a small shift to higher kerma values. No change in OER is found. This is in agreement with the fact that for fission spectrum fast neutrons only a small part of the energy is dissipated through protons with high energies. Furthermore it has to be realized that a proton of 1 MeV energy has a range which is comparable to the diameter of the cultured cells. Consequently for the irradiations with fission neutrons the contribution of the proton component to the biological damage can only be eliminated for a small part.

IV. PERTURBATIONS IN SECONDARY PARTICLE EQUILIBRIUM IN IRRADIATIONS OF MICE.

Studies on the intestinal and hematopoietic syndromes of mice after neutron- and X-irradiations resulted in different RBE values, which are directly related to the difference in sequence of the two syndromes for neutron- and X-irradiations respectively. Differences in the absorbed doses at interfaces, notably at those of bone and soft tissue, might be

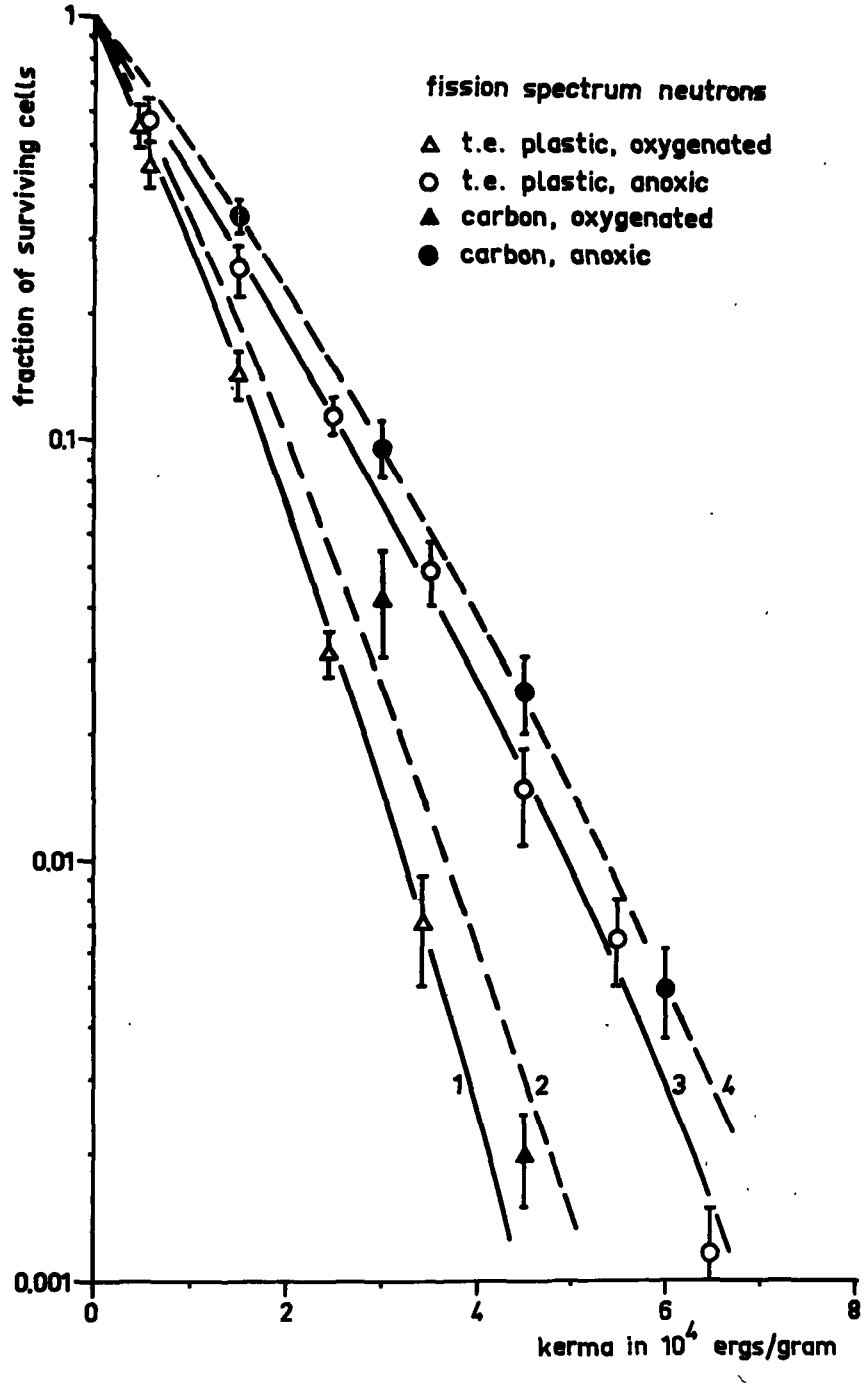

Figure 5. Survival curves obtained for cultured cells equilibrated with air or nitrogen irradiated with fission spectrum fast neutrons with discs of carbon and tissue equivalent plastic mounted under the dishes. For irradiations on tissue equivalent plastic curves 1 and 3 were obtained for cells equilibrated with air or nitrogen respectively. For irradiations on carbon curves 3 and 4 were found for oxygenated and anoxic conditions respectively. For both experimental arrangements the OER was found to be equal to 1.5.

responsible for the differences in the pattern of mortality of mice after neutron- and X-irradiations. Owing to the lower hydrogen content of the bone, the absorbed dose in the bone-marrow cells will be smaller than that in the intestinal cells for exposures to fast neutrons. On the other hand, in exposures of animals to 250 kVp X-rays the bone-marrow may receive a relatively high absorbed dose owing to the excess of secondary electrons produced by photo-electric absorption in the minerals of the bone.

For electromagnetic irradiations the inhomogeneities in the dose distribution at a bone- soft tissue interface were extensively investigated by both theoretical and experimental physical methods (11-14). However, for 15 MeV neutron irradiations this problem was only studied theoretically by Yamamoto (15, 16). All calculations are confined to plane parallel geometry of bone and soft tissue layers and therefore represent only a first crude approximation to the actual geometry of the bone-marrow cavities in the mouse. The average energy absorbed by the bone-marrow of the mouse can be estimated on basis of the distribution of the total marrow over all the different bone cavities of various shapes and sizes and the average absorbed dose in the bone-marrow in these different geometries (12).

The above mentioned theoretical data are subject to some limitations. As far as the neutron irradiations are concerned, Yamamoto (15) considered in his first calculations only the production of protons in the soft tissue or the bone and further assumed the energy of the protons to be independent on the scattering angle. Only recently calculations have been made for which the appropriate corrections were applied (16). In order to verify the theoretical data, the tissue culture system was employed as a biological dosimeter. Firstly the cultured cells were irradiated in monolayer with circular discs of bone-equivalent and muscle-equivalent plastic mounted under the bottoms of the dishes. Secondly cell suspensions were irradiated in either muscle- or bone-equivalent small tubes respectively simulating

the irradiation of the intestinal tract and the bone-marrow in the limb bones.

The experimental technique for the irradiations of the cells in monolayer has already been described in the sections II and III of this paper. The survival curves for cells adjacent to the various materials are shown in figure 6 for 15 MeV neutron irradiations. With respect to the curve for muscle-equivalent plastic the other curves show a shift to higher kerma values, which indicates that for the same exposure a smaller amount of energy was absorbed in the cells, adjacent to carbon and bone-equivalent plastic. A difference of 12 per cent was found between the kerma values required to produce equal effects in cells adjacent to bone-equivalent plastic and in cells adjacent to muscle-equivalent plastic. In figure 7 survival curves are presented for cells adjacent to bone-equivalent and muscle-equivalent plastic after irradiation with 250 kVp X-rays. The curve for bone-equivalent plastic shows a shift to lower kerma values with respect to the curve for muscle-equivalent plastic, corresponding to a difference of 17 per cent between the kerma values required to produce equal effects.

The factors 0.88 and 1.17 represent maximum variations in relative effectiveness which will be found in layers of cells directly adjacent to bone. In order to obtain data with respect to differences between mean absorbed doses in the total bone-marrow after neutron- and X-irradiations, the responses of cells should also be studied in other geometries. As demonstrated earlier (1), tissue culture cells contained in small tubes may be used as small-size biological dosimeters. In the experiments tubes were employed which were made of muscle-equivalent plastic and bone-equivalent plastic and which could be closed with small silicon rubber stoppers. Cell suspensions were brought into the tubes (with a length of 20 mm, an inside diameter of 1.5 mm and a wall thickness of 0.25 mm), which were placed in different positions in a mouse phantom. After irradiation the cell suspensions were removed from the tubes and plated after appropriate dilutions. The surviving

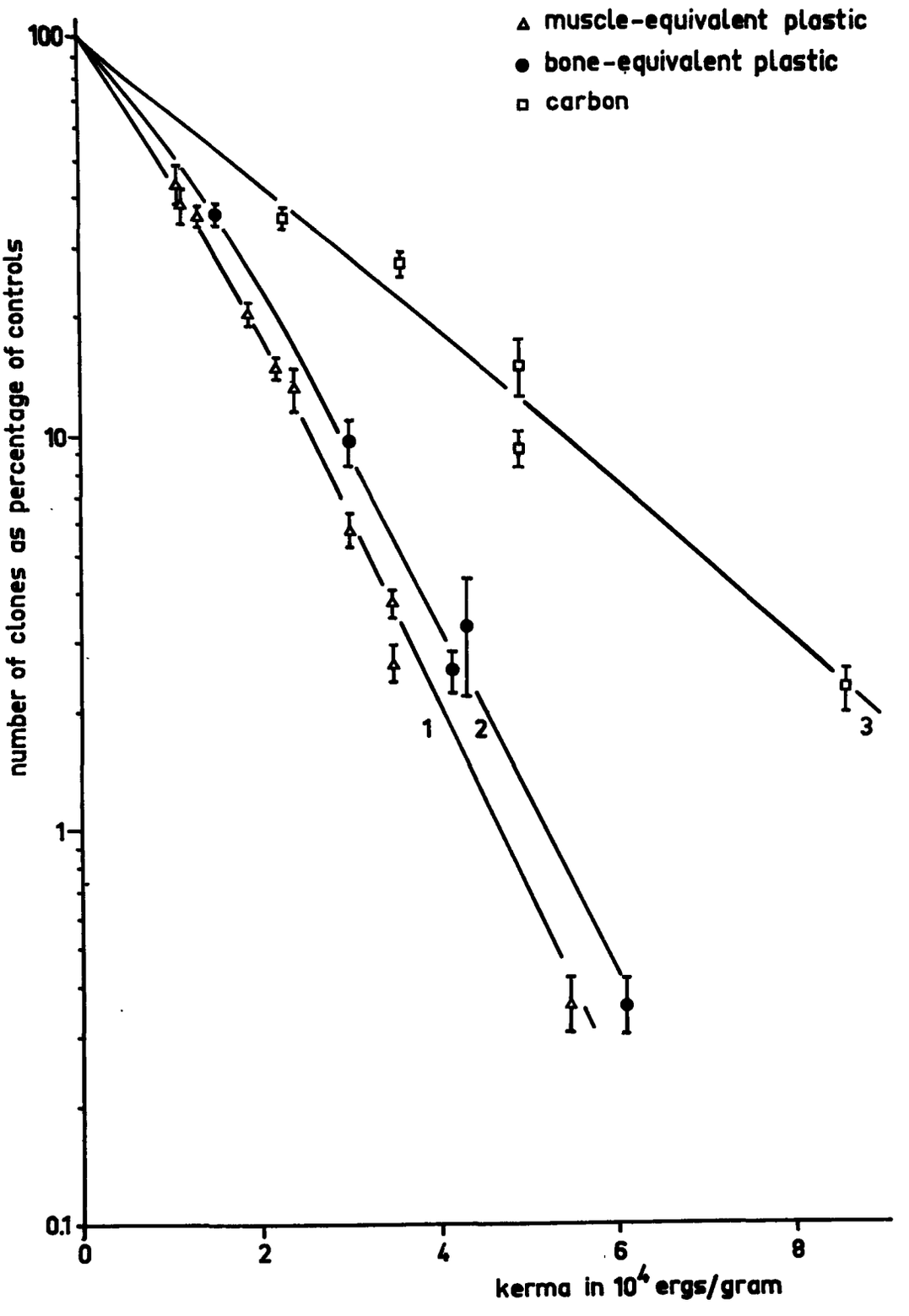

Figure 6. Survival curves for 15 MeV neutron irradiations of cells adjacent to muscle equivalent plastic (curve 1), bone equivalent plastic (curve 2) and carbon (curve 3).

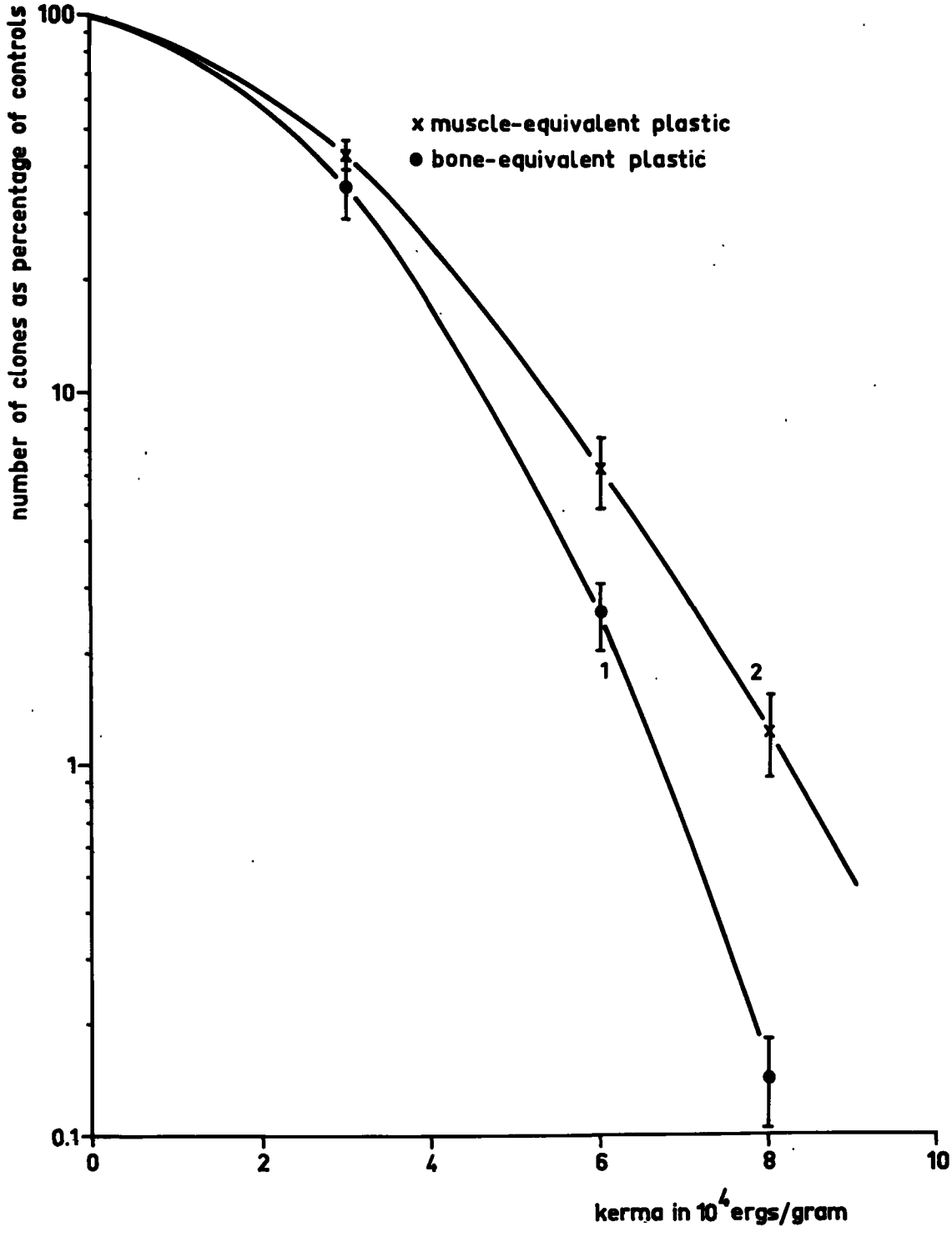

Figure 7. Survival curves for 250 kVp X-irradiations of cells adjacent to bone equivalent plastic (curve 1) and muscle equivalent plastic (curve 2).

fraction of cells was determined after 250 rads and 350 rads 15 MeV neutron irradiations and after 500 rads and 700 rads 250 kVp X-irradiations. The results are presented in figure 8 for neutron and X-irradiation. These experimental results show clearly a shift of the curves in the same direction as observed for cells in monolayer, but the absolute differences are much smaller.

In table I the results concerning the survival of the cultured cells are compared with the calculations of Epp et al. (12) and Yamamoto (16), carried out for the irradiation of a soft tissue layer adjacent to a quasi-infinite bone layer and the irradiation of the bone-marrow cells present in the femora. The human kidney cells, which themselves have a diameter between 10 and 30μ, are attached to the bottom of the culture dishes. The variations in absorbed doses found in the irradiation of the dishes are therefore comparable to the difference between the calculated average energy absorption in a 30μ thick bone-marrow layer adjacent to bone and the energy absorption in a bone-marrow layer remote from bone. The results obtained with irradiations of the cell suspensions in the tubes may be compared with the calculations concerning the irradiation of bone-marrow in the femora. From table I it can be seen that for the irradiations of the 30μ thick soft tissue layer the deviations are smaller than those calculated by Yamamoto (16) and Epp et al. (12). However, for the irradiations of the cells in the tubes a good agreement is found with the calculations for the cylinder of bone-marrow surrounded by bone.

From the data on two special geometries, an extrapolation can be made to the more complicated situation of the actual distribution of the bone-marrow stem cells over the various bone cavities according to a physical model proposed by Epp et al. (12). Since for the two extreme situations (i.e. cells in monolayer and cells in small tubes) a good agreement was found between calculations and experiments, it may be concluded that for 250 kVp X-irradiations the average effective dose in the total marrow of the mouse relative to the effective dose in soft tissue will not deviate more than 2% from the factor 1.09 calculated by Epp et al. (12). On the

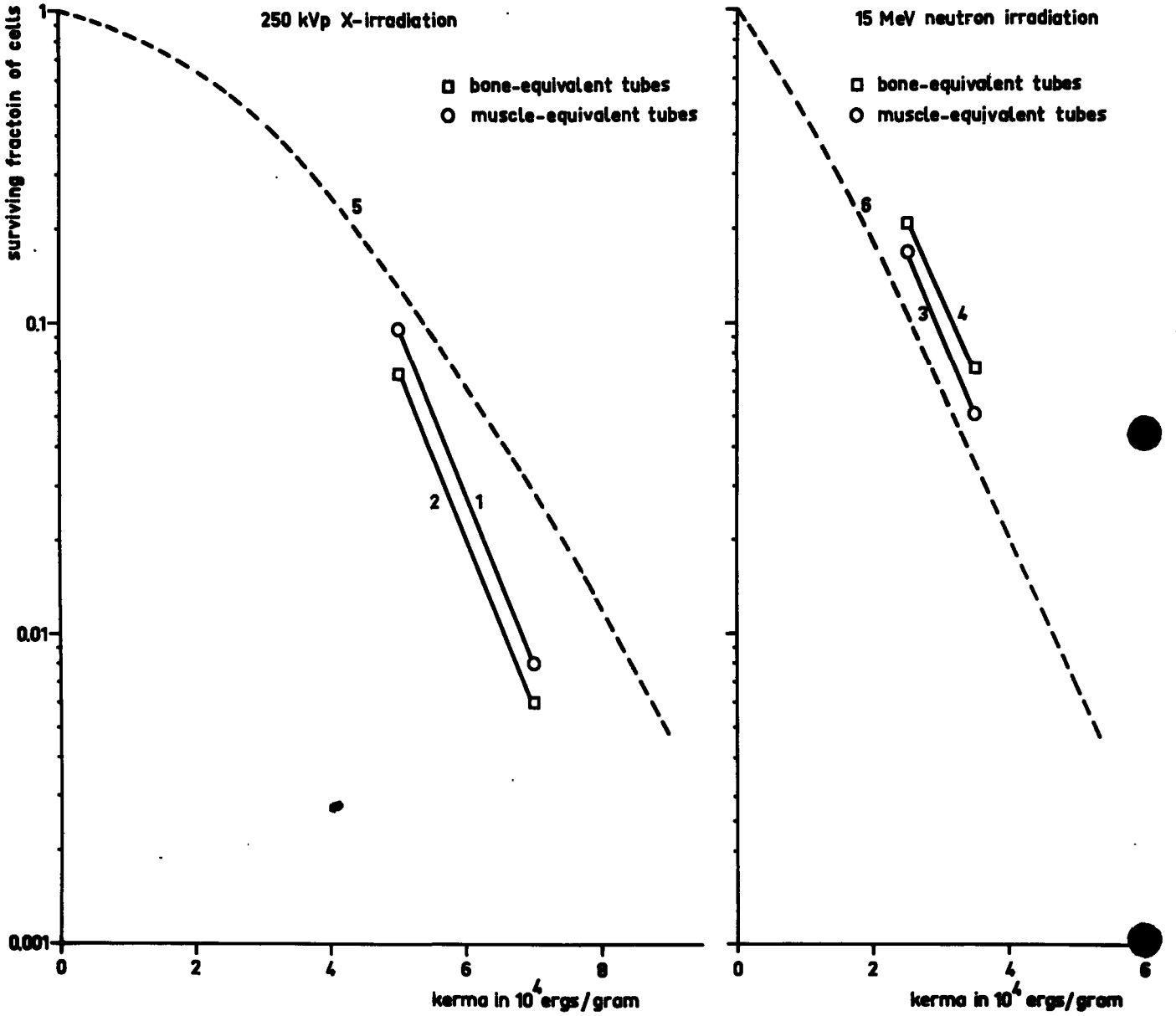

Figure 8. The surviving fraction of cells irradiated in muscle equivalent tubes (curves 1 and 3) and in bone equivalent tubes (curves 2 and 4) for 250 kVp X-irradiation (curves 1 and 2) and 15 MeV neutron irradiation (curves 3 and 4). The experimental points given are the average values of three different experiments. The dashed lines 5 and 6 represent the survival curves for cells adjacent to muscle equivalent plastic for 250 kVp X-irradiation and 15 MeV neutron irradiation respectively.

COMPARISON BETWEEN CALCULATED AND MEASURED EFFECTIVE DOSE IN BONE-MARROW RELATIVE TO EFFECTIVE DOSE IN SOFT TISSUE

Category	X-irradiation		Neutron irradiation	
	calculated (12)	present results	calculated (16)	present results
Layer of marrow 30 μ thick adjacent to quasi-infinite bone layer	1.20	1.17	0.78	0.88
Cylinder of marrow surrounded by bone	1.02	1.03	0.93	0.94
Average over total marrow of mouse	1.09	1.09	0.92	0.93

basis of our experimental data it was calculated that for 15 MeV neutron irradiations the average effective dose in the total marrow of the mouse relative to the effective dose in soft tissue is equal to 0.93. This value is in good agreement with the factor 0.92 derived by Yamamoto (16) from modified calculations.

As stated earlier the ratio of the RBE values 1.4/1.1 = 1.27 is a measure for the variations of the relative intervals between the bone-marrow and intestinal syndromes occurring after X- or neutron-irradiations . If only dosimetric factors would be responsible for this difference, this factor of 1.27 would imply a difference of 27 per cent between the effective dose absorbed in intestinal cells and that absorbed in bone-marrow cells for exposures to X-rays and fast neutrons. The factors 1.09 and 0.93 are the best estimates for the differences in effective dose between bone-marrow and soft tissue remote from bone for 250 kVp X-rays and 15 MeV neutrons, respectively. The ratio between these factors 1.09/0.93 = 1.17 is smaller than the ratio of the RBE values for intestinal syndrome and bone-marrow syndrome. Although the presence of dosimetric factors has been demonstrated clearly, the variations in the absorbed dose at the interface of bone and soft tissue are too small to account for the differences in RBE values for fast neutrons with regard to the bone-marrow syndrome and the intestinal syndrome. From our experiments on the protracted irradiation of mice with 15 MeV neutrons and gamma radiation it might be concluded that intrinsic differences between the radiosensitivities of bone-marrow cells and intestinal cells contribute to the differences in the patterns of mortality of mice after neutron- and X-irradiation (1).

V. CONCLUSION

An analysis is given of the importance of interface dosimetry for an interpretation of the effects of fast neutrons on cultured cells and mice. Perturbations of secondary particle equilibrium will always be present in the irradiations of intact organisms. The variations of the absorbed dose at

the interface of bone and soft tissue were investigated by employing a tissue culture system as a biological dosimeter. From the quantitative results it can be inferred that dosimetric factors are only partly responsible for the differences in the pattern of mortality of mice after neutron- and X-irradiations.

Investigations on the survival of cultured cells after irradiations with various beams of fast neutrons under different irradiation conditions have shown that the OER values are fairly constant over a wide range of neutron energies. For an interpretation of this phenomenon irradiations were carried out in which perturbations of charged particle equilibrium were induced artificially. Survival curves were determined for neutron irradiations with either layers of tissue equivalent plastic or layers of pure carbon mounted in front of the cells. From these results, survival curves for the "high-LET component" and the "proton component" of the neutron radiation could be derived. An explanation for the independence of the oxygen effect of fast neutrons from the mean neutron energy is suggested.

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DISCUSSION

Mr. POHLIT

Thank you, Dr. Broerse, for your very interesting paper. We have heard about two important facts of neutron irradiation. One is a clarification of the question of RBE of different effects in mice. The other is related to the concept of secondary particle equilibrium. From conventional X-rays we are used to having a secondary electron equilibrium. In this case the energy transported into a volume by electrons should equal the energy transported out of this volume. In the case of neutrons for secondary particle equilibrium it is not sufficient to achieve an equality of the total energy transported into and out of the volume. It is necessary here to have an equilibrium of each type of secondary particles due to their different reaction, as can be seen from your experiments.

Mr. SPIERS

I noticed that the same words tissue equivalent material have been used for both X-ray irradiations and the neutron radiations. Was one material tissue equivalent for X-rays and the other for neutrons? Or was it the same material used for both? Because I don't think you can have equivalence except by chemical identity for both neutrons and X-rays.

Mr. BROERSE

Tissue equivalence is a much more stringent requirement for neutron irradiations than for X-irradiations. To simulate the bone and soft tissue layers, we employed muscle-equivalent plastic and bone-equivalent plastic which were supplied by Dr. Shonka. As far as the muscle-equivalent plastic is concerned the hydrogen and nitrogen contents are identical to the corresponding contents in muscle. With respect to the composition of soft tissue, the majority of the oxygen was

replaced by carbon in the muscle-equivalent plastic. As far as the bone-equivalent plastic is concerned the hydrogen, nitrogen and calcium contents are fairly comparable to the corresponding contents in bone. Therefore we accepted the muscle- and bone-equivalence for both neutron- and X-irradiations.

Mr. ZANELLI

I would like to know what size your tubes were.

Mr. BROERSE

The tubes had a length of 20 mm, an inside diameter of 1,5 mm and a wall-thickness of 0.25 mm. These dimensions correspond with the sizes of the femora of the mouse.

Mr. ZANELLI

Yes, that is the width of the shaft of the femora.

Mr. BROERSE

Yes, of course, In the femora a variable geometry of the bone-marrow cavities is found, especially in the metaphyses. The irradiation of the cultured cells in the tubes simulated the irradiation of the bone-marrow in the largest bone shafts in the mouse. The tissue culture system was used as a biological dosimeter for the two extreme situations, i.e. cells in mono-layer and cells present in cylinders.

Mr. BOOZ

I have two questions; first, could you apply your mammalian cell system to much smaller diameters and second, could they perhaps also be used to measure the distribution in those cylinders?

Mr. BROERSE

In the experiments on the irradiation of cells in the cylinders, cell suspensions were brought into the tubes, irradiated, removed from the tubes and plated after appropriate dilution. Due to the homogeneity of the cell suspension, the energy distribution over the cylinder could not be measured. The experimental technique of handling the small tubes is rather complicated. Tubes of much smaller diameters cannot be applied. For the determination of the energy dissipation in these smaller cylinders experimental physical methods have to be employed.

Mr. FOWLER

May I ask whether you took account of the overall time of the exposure in this work? For example Dr. Hornsey has found a very rapid recovery in 4-day death of mice (intestinal death). She finds a measurable amount of recovery in 10 minutes. Now this probably does not apply in the same way to your cell survival experiments, but it surely does to the interpretation of any intestinal-death RBE values. You have to take exposure time into account both for your cell experiments and for the killing of animals.

Mr. BROERSE

The irradiations of the cells on bone-equivalent plastic and muscle-equivalent plastic were carried out for completely identical exposure periods. From these experiments the difference in effective dose at the interface can be deduced directly. The RBE values found for the two syndromes, namely 1.4 for the intestinal syndrome and 1.1 for the bone-marrow syndrome, pertain to 2-hour-irradiations. This is the minimum irradiation time which could be obtained in our experiments, due to the limited output of our neutron generator. As soon as our new machine will be operative, with an expected yield of 10^{12} neutrons/sec., the dose mortality studies will be repeated for smaller irradiation periods and fractionated exposures.

Mr. FOWLER

Were the total X-ray exposure times the same as those for neutrons?

Mr. BROERSE

For the determination of the RBE values for the mortality of the mice the irradiations with 15 MeV neutrons, gamma-rays and 250 kVp X-rays were protracted over a period of two hours. The ratio of the RBE values 1.4/1.1 = 1.27 is indeed only valid for these 2-hour-irradiations. For the 8-hour-irradiations a deviating value was found for this ratio. This discrepancy is another indication that dosimetric factors alone are not responsible for the differences in the pattern of mortality of mice after neutron- and X-irradiations.

Mr. WAMBERSIE

Nous avons déterminé les valeurs de l'EBR des électrons de 20 MeV par rapport aux photons de 20 MV, en étudiant la mort médullaire à 30 jours et la mort intestinale à 4 et 5 jours chez la souris. A notre grande surprise, nous avons observé une différence d'EBR en fonction du test considéré, alors que nous avions trouvé des valeurs voisines de l'unité pour d'autres tests biologiques comme la survie bactérienne ou la survie des levures diploïdes.

L'EBR des électrons de 20 MeV comparés aux photons de 20 MV était de 1,03 pour le syndrôme intestinal et de 0,96 pour le syndrôme médullaire, soit une différence en fonction du critère étudié de l'ordre de 7% nettement supérieure à la précision de chacune de ces expériences qui est de l'ordre de 3%.

Il ne pouvait s'agir d'une question d'étalonnage des dosimètres, puisque les mêmes dosimètres ont été utilisés pour l'étude du syndrôme intestinal et du syndrôme médullaire, et de plus, ces expériences ont été alternées.

D'autre part, pour des rayonnements aussi peu différents que les faisceaux de photons ou d électrons de 20 MeV, l'existence d'une différence d'EBR en fonction du critère biologique étudié, c'est-à-

dire en réalité "un effet différentiel" paraît peu vraisemblable.

Dans ces conditions, notre interprétation est la suivante. Les souris sont irradiées dans des "boîtes" en Plexiglas, dans lesquelles les cavités destinées à recevoir les animaux sont les plus petites possibles de manière à réduire les interstices d'air entre le Plexiglas et les animaux. Ces interstices d'air pourraient dans le cas des électrons, perturber le flux électronique et la distribution de la dose à cause des phénomènes de diffusion.

Compte tenu des dimensions de la cavité creusée dans les boîtes de Plexiglas, on pouvait admettre que l'abdomen de la souris est appliqué intimement contre les parois en Plexiglas. La dose au niveau de l'abdomen, qui conditionne l'apparition du syndrôme intestinal, est donc très voisine de celle qui aurait été calculée en milieu homogène pour les faisceaux d'électrons. Par contre, toujours pour les électrons, les interstices d'air sont plus importants au niveau de la tête et des membres de l'animal et il est vraisemblablement dangereux d'assimiler la dose à leur niveau à la dose calculée en milieu homogène. Il est possible qu'en raison des phénomènes de diffusion, la dose au niveau des extrémités soit légèrement réduite. Or, on connaît l'importance de la protection, ou d'un sous-dosage, d'une partie de la moelle osseuse dans l'apparition du syndrôme médullaire.

Cette hypothèse nous a été suggérée par des radiographies de souris effectuées au moyen d'électrons de haute énergie analysées au densitomètre.

D'autre part, dans le cas des photons de 20 MV, on peut admettre que la dose est homogène dans la totalité de l'animal irradié ou, en d'autres mots, que la dose absorbée au niveau des différents organes de la souris est semblable à celle calculée en milieu homogène.

Ceci expliquerait pourquoi nous avons observé une différence d'EBR en fonction du critère biologique étudié: syndrôme intestinal, conditionné par l'irradiation de l'abdomen, syndrôme médullaire, conditionné par l'irradiation de la totalité de la moelle osseuse et dont une partie peut-être a été légèrement sous-dosée dans le cas des électrons.

Pouvez-vous exclure une interprétation de ce type pour expliquer vos résultats obtenus au moyen de neutrons?

Mr. BROERSE

As far as dose variations at an interface are concerned the differences between neutrons and X-rays are much more important than the differences between the 20 MeV photons and the electron beams you are interested in.

I would agree that for 20 MeV electrons and photons differences between RBE values for the syndromes are unlikely, but this does not exclude this possibility for the neutrons.

For our dose mortality studies after 250 kVp X-irradiations and ^{137}Cs gamma-irradiations RBE values of 0.8 for the bone-marrow syndrome and 0.8 for the intestinal syndrome were derived.