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The Effects of Single and Fractionated Doses of X-Rays and Neutrons on the Oesophagus

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Abstract—*Damage to the oesophagus of mice after irradiation with single doses, 2, 5 or 10 equal fractions of X-rays or fast neutrons was measured by estimating* LD_{50} *values for death between* 10 *and* 40 days *post-irradiation of the thorax. The slope of the isoeffect curve for X-irradiation was* 0.40 *and for neutrons was* 0.29. *RBE values obtained for oesophageal damage are close to those previously observed for intestinal injury and are among the highest observed with normal tissues. If, therefore, the RBE for oesophageal tumours follows that of the tissue of origin then oesophageal tumours would respond well to neutron therapy. However, the high RBE for the normal oesophageal damage may limit treatment at this site.*

Irradiation with single doses of X-rays of animals breathing oxygen or in normal air breathing conditions but after administration of the anoxic radiosensitizing drug misonidazole (Roche Products Ltd. Ro-07-0582) both gave enhancement of damage relative to air breathing anaesthetised mice, indicating that hypoxia in these anaesthetised animals affects the sensitivity of the oesophagus to large doses of radiation.

INTRODUCTION

THE TREATMENT of many cancers of the oesophagus presents serious clinical problems. Surgery causes permanent disabilities. Radiotherapy may be successful with small tumours and leave the patient with a functional oesophagus. However tumours often spread along the oesophagus and the resulting large field treatment seriously limits the dose which may be given, thus reducing the probability of control. Failure is often due to local recurrence, so that any treatment which would differentially increase tumour damage relative to normal tissue injury would be a great advance in the treatment of such tumours.

Neutron therapy may give a therapeutic gain in the treatment of some tumours. The primary rationale is that the sparing of hypoxic cells is less with neutron than with X- or γ-irradiation. However, the effectiveness of neutrons relative to X or γ-rays varies from tissue to tissue in, as yet, an unpredictable way, so it is important to establish the RBE for damage to the limiting tissues in radiotherapy. Whether or not RBE values obtained in animal systems are generally relevant to man is uncertain, but it has been shown to be the case for skin [1], and it might be so for oesophagus. Clearly the best condition for a possible therapeutic gain will be at sites where the RBE for the limiting normal tissue damage is low. Early reports on the effects of single and split doses with X-rays and neutrons had indicated that the RBE for oesophageal damage was relatively high [2, 3]. These results are consistent with the suggestion by Phillips and Margolis [4] that the oesophagus of the anaesthetised mouse might be somewhat hypoxic.

In this paper the RBE values for fast neutrons for oesophageal damage from 1 to 10 fractions are given. For single doses of X-rays it has also been shown by using the hypoxic cell sensitizer misonidazole or by irradiation with mice breathing warm oxygen, that hypoxia does influence the response at high doses.

MATERIALS AND METHODS

Two series of experiments are described. In the first, female TO mice were used when 12–15 weeks old for a comparison of the effects of single and fractionated doses of X-rays or fast neutrons. The animals were anaesthetised before each irradiation with sodium pentobar-

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bitone (60 mg/kg) and breathed air at room temperature throughout. The thorax only was irradiated with a collimated beam to give a field size 2.5 × 3.0 cm. The irradiation procedures have been described previously [5].

The X-rays used were 250 kVp filtered by 0.25 mm Cu and 1 mm Al to give an HVL of 1.3 mm Cu. The dose-rate was 170 rad/min and was measured in air using a Farmer-Baldwin ionization chamber. The dose-rate was checked by tubes of lithium fluoride placed in the oesophagus of a killed mouse. Six mice at a time, lying on their backs in individual Perspex holders on a rotating platform, were irradiated from above with a vertical beam. The whole-body X-ray dose was negligible. Eighteen mice were used at each dose.

The neutrons were from the MRC cyclotron at Hammersmith and were produced by bombarding a beryllium target with 16 MeV deuterons. The mean neutron energy was about 7.5 MeV [6]. The γ-ray contamination was about 3% and was not included in the quoted neutron dose. Details of the dosimetry have been described elsewhere [7]. The Hammersmith "neutron rad" was changed in 1975 to comply with international standardization [8] and the neutron doses quoted in this paper conform to this standard. The beam was horizontal and was collimated with paraffin wax and finally by steel to give identical field sizes to those used for X-irradiation. Some skin sparing was obtained by covering the thorax with a thin sheet of lead to stop secondary protons produced in the Perspex jig [9]. Only the dose to skin was reduced since full proton equilibrium is reached in 1 mm of tissue. The whole-body dose with neutrons was about 10% of that given to the thorax. Eight mice were irradiated at one time and 16 mice were used at each dose point.

After irradiation of the thorax mice may die between 10 and 40 days from damage to the oesophagus. Animals which survive this period may die later from radiation pneumonitis [4, 3]. The oesophageal endpoint (LD_{50} 10–40 days) was estimated for single doses, 2 fractions in 24 hr, 5 fractions in 4 days (5F/4d) and 10 fractions in 11 days (10F/11d) of X-rays and fast neutrons.

In the second series of experiments the possibility that the oesophagus was hypoxic was tested by giving the mice oxygen to breathe during X-irradiation and also by the administration of the hypoxic cell sensitizer misonidazole (Ro-07-0582, Roche Products Ltd.) The TO strain of mice was no longer available for these experiments, and female mice of the strain CFLP were used when 8–12 weeks old. All procedures were the same as those used with the TO mice. Misonidazole, 1 mg/g mouse, was given 30 min before irradiation and in these mice the anaesthetic dose was reduced by 25% to avoid lethalities. In the oxygen breathing experiments, mice were covered with a box and a flow of 4 l/min passed through for 20 min before and during irradiation, i.e., the box was flushed with approximately 20 times its volume before irradiation. Control animals were treated similarly except that the box was flushed with air.

RESULTS

The pattern of death over the first 40 days following irradiation of the thorax with single doses of either X-rays or neutrons is illustrated in Fig. 1. The pattern is similar for groups of animals irradiated with either X-rays or neutrons which show a similar survival level at 40 days. Histological examination has shown that, 8 days after single doses of X-rays or neutrons which give 20–30% killing at 40 days, the basal epithelial layer of the oesophagus is almost absent. Deaths after pairs of doses separated by 24 hr or 5 equal fractions given in 4 days followed a similar pattern to that after single doses, i.e., the majority of deaths occurring before 30 days post-irradiation, but after 10 equal fractions in 11 days the deaths occurred between 12 and 40 days post-irradiation, taking the first doses at zero time.

The percentage of TO mice surviving at 40 days after single doses, 2 or 5 equal fractions and after 10 equal fractions of neutrons are shown in Fig. 2 and after X-rays in Fig. 3. Each curve has marked upon it the LD_{50} value with 95% confidence limits estimated by probit analysis.

In Fig. 4 the LD_{50} values for both X-rays and neutrons are plotted on logarithmic scale against the number of fractions also on a logarithmic scale. The isoeffect curve after X-irradiation is shown as two broken lines between 1 and 2 fractions because of the uncertainty of the single dose value for fully oxygenated tissue. The slopes of these isoeffect curves between 2 and 10 fractions are 0.40 for X-rays and 0.29 for neutrons.

Figures 5 and 6 illustrate the results of experiments to test whether O_2 breathing during X-irradiation or pretreatment with the

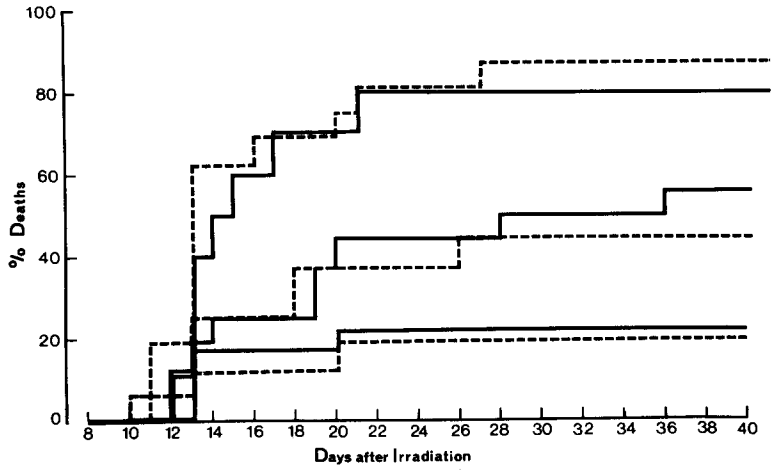

Fig. 1. Death of TO mice after irradiation of the thorax with X-rays ----- or neutrons ———. The doses used were: ~80% deaths, 4850 rad X-rays or 1900 rad neutrons in 5 fractions: ~40% deaths, single doses of 3200 rad X-rays or 1350 rad neutrons: ~20% deaths, single doses of 3600 rad X-rays or 1600 rad neutrons. The figure illustrates that the time course of death is independent of the quality of the radiation.

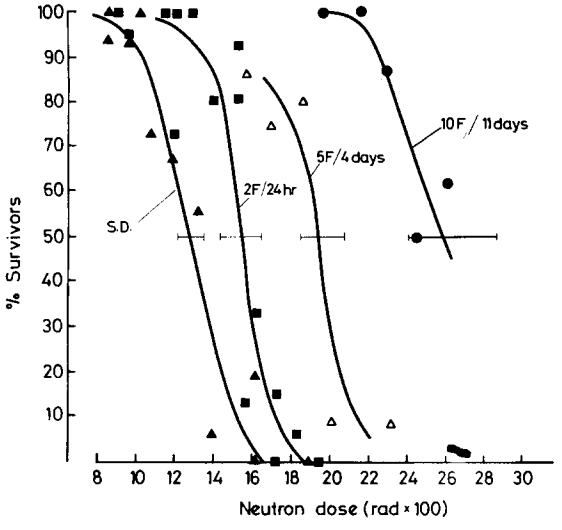

Fig. 2. Percentage of surviving mice (TO strain) at 40 days after irradiation with neutrons given as a single dose, 2 fractions in 24 hr, 5 fractions in 4 days or 10 fractions in 11 days. The errors shown are 95% confidence limits derived from probit analysis.

hypoxic cell sensitizer misonidazole [10] would increase the sensitivity of mice to oesophageal damage. As was pointed out CFLP mice were used in these experiments and TO mice for the RBE experiments. The two strains showed a similar time course of death, similar values for RBE with single doses, and a similar LD_{50} value for four fractions after X-irradiation (Fig. 4).

The results of the first experiment with the CFLP mice are shown in Fig. 5. Animals breathing air during irradiation were less sensitive than animals breathing oxygen or than animals given misonidazole before irradiation. Unfortunately the results are very scattered and at higher doses the sensitivity of all groups was less than expected. This is perhaps an indication that there is an insensitive, hypoxic tail. Anaesthesia has been shown to cause an increase in hypoxia in some tissues in animals breathing air and the depth of anaesthesia may affect the cell survival level at which an "hypoxic tail" can be detected [11, 12]. The animals given misonidazole were given 25% less anaesthetic than the other groups. Therefore, in a second experiment the anaesthetic dose was reduced by 5–10% for animals breathing air or oxygen and not given misonidazole and the higher doses repeated for animals breathing air or oxygen. The results are shown in Fig. 6. A 25% reduction in anaesthetic, as in mice given misonidazole, would not have anaesthetised these animals. The sensitivity at higher doses was greater than that seen in the first experiment. Dose/effect curves have been drawn through the low dose survival values from experiment 1 and the results from experiment 2. The LD_{50} for animals breathing air (3200 rad) is similar to that obtained with mice of the TO strain. The increased sensitivity obtained by animals breathing oxygen rather than air was 1.3. The enhancement of sensitivity obtained by giving animals misonidazole was 1.6 if the LD_{50} read from the misonidazole curve in Fig. 5 is compared with the air curve in Fig. 6 but 1.8 if compared with animals breathing air without the drug in the same experiment (Fig. 5).

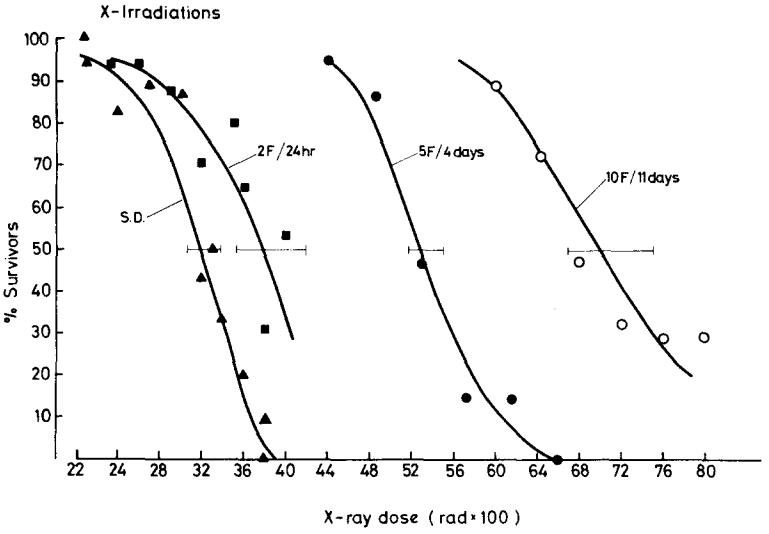

Fig. 3. Percentage of surviving mice (TO strain) at 40 days after irradiation with X-rays given as a single dose, 2 fractions in 24 hr, 5 fractions in 4 days or 10 fractions in 11 days. The errors shown are 95% confidence limits derived from probit analysis.

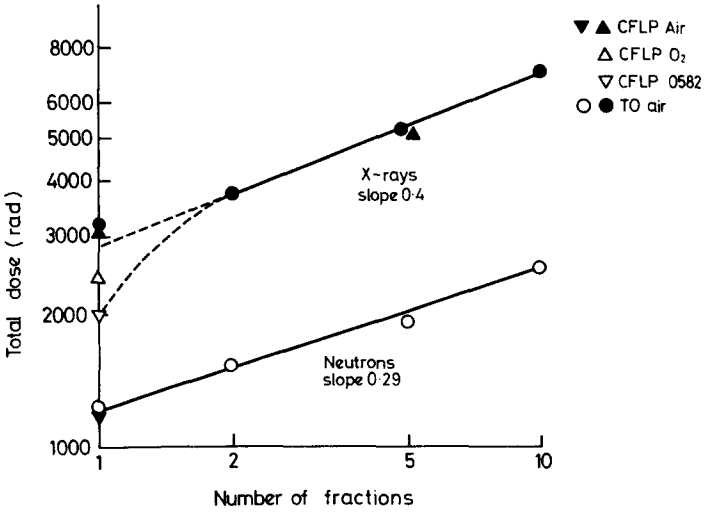

Fig. 4. Total dose of X-rays or fast neutrons, plotted as a function of a number of fractions, to produce an LD_{50} from damage to the oesophagus.

DISCUSSION

After irradiation of the thorax mice die between 10 and 40 days from damage to the oesophagus. This is primarily due to the loss of basal cells in the oesophageal epithelium leading progressively to a marked reduction of differentiating and keratinizing cells (4).

Kurohara and Casarett [13] observed similar damage in the rat and showed that death was associated with a marked reduction in food intake and loss in body weight. Phillips and Ross [14] suggested that round-cell inflammatory infiltration in the submucosa and occlusion of the oesophagus are a contributory factor leading to the death of the animal.

Phillips and Margolis (4) found that the oesophagus in mice could readily be protected by a slight reduction of the partial pressure of oxygen breathed, whilst other tissues such as the lungs were unaffected. Subsequently Phillips and Ross [14] showed a protective factor of 1.5 for oesophageal damage in mice breathing 5.5% oxygen compared with breathing air. In a preliminary report of RBE for

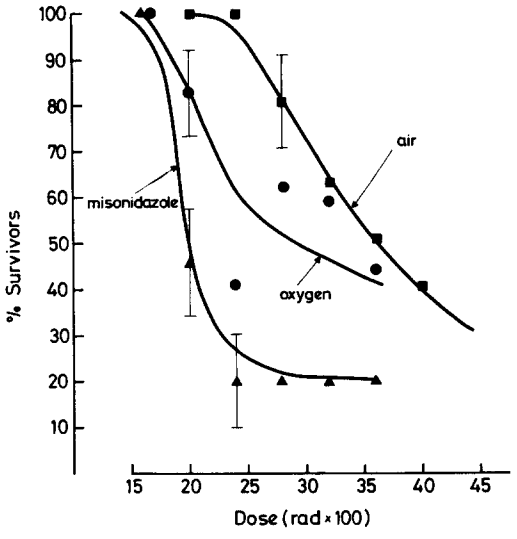

Fig. 5. *Percentage of surviving mice* (*CFLP strain*) *at* 30 days *after irradiation of the thorax with various doses of X-rays. Mice irradiated in normal conditions of air breathing* ■ *or breathing pure oxygen* ● *were anaesthetised with* 60 mg/kg *sodium pentobarbitone. Mice breathing air but preceded at* 20 min *by misonidazole at* 1 mg/g ▲ *were anaesthetised with* 45 mg/kg *sodium pentobarbitone.*

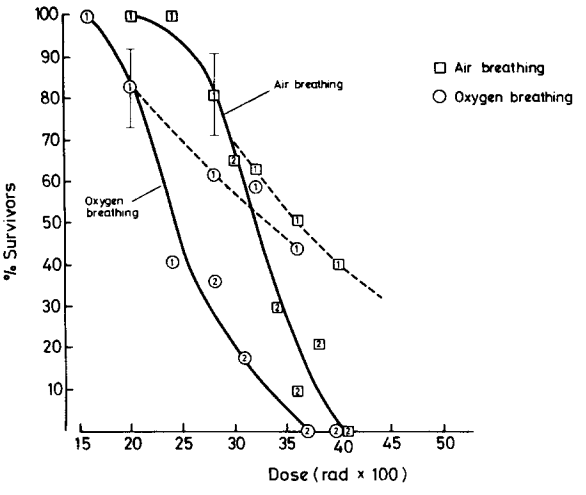

Fig. 6. *Percentage surviving mice* (*CFLP strain*) *as a function of dose of X-rays.* □ *denotes animals irradiated in normal conditions of air breathing, and* ○ *animals breathing oxygen during irradiation. No.* 1 *indicates the first experiment when animals were anaesthetised with* 60 mg/kg *sodium pentobarbitone and No.* 2 *the second, in which a reduced quantity of anaesthetic was administered, i.e.,* 54 mg/kg.

oesophageal damage [3] it was shown that the RBE was the same for single doses or two fractions of radiation and that the RBE's were higher than those observed for other tissues. It was suggested this might in part be due to some hypoxia in the oesophagus in animals under anaesthesia [15].

In the experiments reported in this paper, performed to test whether oxygen breathing or the hypoxic sensitizer misonidazole would increase the sensitivity of mice to oesophageal damage, the depth of anaesthesia greatly influenced sensitivity. In all cases oxygen breathing increased sensitivity although the results were subject to considerable scatter. It seems probable that anaesthesia is affecting sensitivity by the reduction in ventilation and the changed pattern of circulation caused by some peripheral vasodilation [16] which induces hypoxia in some tissues. The sensitivity of animals given misonidazole is greater than that of animals breathing oxygen. However, the animals given misonidazole had been given 25% less anaesthetic dose as this was sufficient to anaesthetise the animals when combined with the drug, and a normal anaesthetic dose combined with the drug was lethal. A 25% reduction in anaesthesia without the drug would not have anaesthetised the animals sufficiently to immobilize them throughout the irradiation and could not therefore be tested. At high doses the dose–effect curve for animals given misonidazole became flattened (Fig. 5) suggesting that an unsensitized hypoxic fraction still remained. It is not clear whether the increased sensitivity of animals given misonidazole compared with animals breathing oxygen is due to a better diffusion of the drug to the hypoxic tissues compared with oxygen, whether there is better oxygenation due to the reduction in anaesthesia dose, or whether there is some increased sensitization due to other factors associated with misonidazole such as reduction in repair capacity or direct cytotoxicity [17].

It seems probable that the sensitivity of the oesophagus in anaesthetized animals to large single doses of radiation is therefore strongly affected by hypoxia and that the level of this hypoxia will vary with anaesthetic conditions and with the strain of mice. Because of reoxygenation between dose fractions it is expected that the presence of hypoxic cells will have progressively less effect on sensitivity as the number of fractions is increased and the dose/fraction is decreased. As cells in the basal layer die or differentiate through the overall treatment time the oxygen diffusion pathway will be lengthened and fewer stem cells will be hypoxic. Whilst it is evident that hypoxia influences the sensitivity of the oesophagus of anaesthetized mice to large doses or doses/fraction this may have little relevance to radiotherapy. Relatively small doses/fraction are

normally used in radiotherapy treatment regimes and patients are not normally anaesthetized. Therefore it is unlikely that hypoxia will influence the sensitivity of human oesophagus in patients.

The oseophagus clearly has a very large capacity for repair of sublethal damage. The D_2–D_1 values obtained were 560 rad for X-rays and 235 rad for neutrons. These values would be even larger were the single doses not affected by hypoxia and if so would be larger than for other normal tissues [18]. The effect of any hypoxia would be expected to decrease with increase in fractionation of dose.

The large repair capacity is borne out by the relationships between total dose and number of fractions (Fig. 4). The slopes between 2 and 10 fractions are 0.40 for X-rays and 0.29 for neutrons. These slopes include both number of fractions and overall treatment time, as it is not possible to separate the components from these data.

The slope of the neutron isoeffect curve reported in this paper is much steeper than for X-rays and fast neutrons. However, we must point out that Phillips and Ross [14] demonstrated a very small effect of increasing the temporal separation of two doses of X-rays to the oesophagus in mice, for which we have no adequate explanation.

The RBE values for oesophageal damage obtained from a comparison of the curves in Figs. 2 and 3 are shown in Fig. 7 as a function of the neutron dose per fraction. The relationship to values for damage to skin, lung and intestine are shown for comparison. The RBE values obtained for single doses to the oesophagus, shown at 1250 rad per fraction of neutrons, are subject to some uncertainty due to the hypoxia. Values are shown as a range estimated from the LD_{50} values of animals breathing air or oxygen. The RBE obtained from the data of animals irradiated with X-rays after injection with the hypoxic radiosensitizer misonidazole is also shown as this may be the best estimate for a fully oxygenated oesophagus. The uncertainty may extend to the RBE values for the fractionated treat-

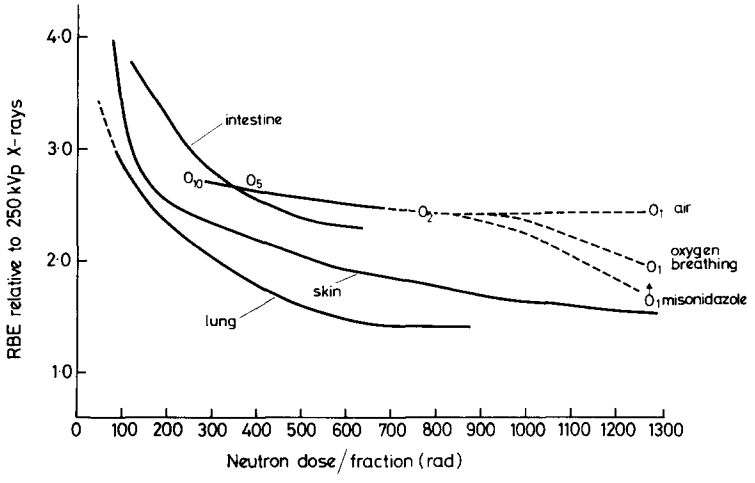

Fig. 7. The RBE for fast neutrons relative to 250 kV *X-rays as a function of dose per fraction of neutrons.* O, *refers to oesophagus* LD_{50} *and the subscript to the number of fractions. There are three values for* O_1 *according to whether the mice were breathing air with or without misonidazole, or oxygen.*

that seen for damage to any other tissue, except those with a high cell turnover, e.g., the intestine [19]. Oesophagus is a rapidly proliferating tissue. The cycle time for cells of the basal layer is about 2 days [20] when repopulation starts. This is consistent with death occurring soon after irradiation and also with the observation of a normally appearing oesophagus 21–28 days after a dose just below the lethal level. Thus the steep isoeffect curves shown in Fig. 4 may include a substantial component for overall treatment time, both

ments but because of reoxygenation between fractions and the smaller doses/fraction the probable influence of hypoxic cells will decrease with increasing fractionation. The RBE value will be a lower limit as the neutron irradiated animals did not receive misonidazole nor breathe oxygen and may therefore also be slightly protected from neutrons by hypoxia thus causing an increase in neutron LD_{50} and reduction in RBE.

The RBE curves obtained from a comparison of 250 kV X-rays and cyclotron pro-

duced fast neutrons for damage to various tissues have shown considerable variation from one tissue to another [3, 18]. That for oesophageal damage is closest to the RBE obtained for intestinal damage and is one of the highest, certainly higher than that for skin or for lung damage. It is pertinent that the irradiation geometry was identical for the treatments of lung and oesophagus.

At first sight it is somewhat surprising that the RBE's obtained for oesophageal damage are higher than those for skin. Both tissues are ectodermal in origin and have a similar structure. The stem cells differentiate in each case to a keratinized layer, covering surfaces exposed to the "outer environment". The result serves to highlight our lack of understanding of the factors which govern RBE.

With normal tissues we can measure the RBE's empirically in animals and cautiously apply these measured values to man. In a few tissues such as skin, lung, or the spinal cord, numerical factors describing effects of fractionation or RBE values have been shown to be similar for animals and man [1, 21–24].

The high RBE values obtained for oesophageal damage have two implications. Firstly, if the RBE for oesophageal tumours follows that of the tissue from which they arise then oesophageal tumours will respond well to neutron therapy. Secondly, the high RBE for the normal tissue damage in the oesophagus may limit the neutron dose to such an extent that no therapeutic gain is obtained, indicating that neutrons should be used with caution where oesophagitis is the limiting normal tissue response. The steepness of the neutron isoeffect curve, however, suggests that increasing the number of fractions and/or increasing the overall treatment time may be of significant benefit in sparing the oesophagus both to X-rays and to neutrons.

CONCLUSIONS

1. In anaesthetized mice the response of the oesophagus to large single doses of radiation is influenced by hypoxic cells.
2. The effect of these hypoxic cells is reduced with the mice breathing oxygen.
3. The effect of the hypoxic cells is further reduced by injection, prior to irradiation, with the hypoxic cell sensitizer, misonidazole. The enhancement factor with this drug for single doses is about 1.6.
4. The RBE for fast neutrons for oesophageal damage is especially high for large doses probably due to hypoxia, but even when this is allowed for or with fractionated treatments the RBE remains higher than for most other normal tissues.
5. The fractionation effect with X-rays or fast neutrons is greater than for most normal tissues implying a possible advantage of treating tumours, where oesophagus is a limiting normal tissue, with a large number of fractions or long overall treatment time, or both.

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