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NEUTRON RADIOBIOLOGY REVISITED

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The present paper reviews the experimental results of normal tissue and tumour studies in animals. The dose per fraction dependence of the RBE in normal tissues has been long recognised, together with the steeper increase of RBE at low doses for late responding tissues compared with acute reactions. The dose dependence for tumours is more complex, because of hypoxia and reoxygenation, as well as differences in repair capability after high LET damage. A comparison of tumour and normal tissue RBE values shows that there is little experimental evidence for a therapeutic advantage at clinically relevant doses. In particular, the RBE for slow growing tumours is even lower than that for the faster growing mouse tumours. The reasons for the loss of expected neutron benefits in clinically relevant experiments are discussed. The disappointing prospects for neutrons are contrasted with the current multifactorial approaches to overcoming resistance to more conventional low LET radiations, including acceleration, hyperfractionation and several types of hypoxic cell radiosensitizers.

Radiobiologists have invested a great deal of effort into understanding the effects of neutrons relative to x-rays (for summary see ref. 1). This information was considered essential because of the clinical interest in neutron therapy following the installation of the first hospital based cyclotron at Hammersmith in the fifties. The warnings from the wartime Berkeley experience of Stone (2) and his colleagues were recognised but it was felt necessary to determine whether tumours could be more effectively treated with neutrons than with x-rays, using schedules that would not lead to excessive late morbidity.

It was already recognised by the early sixties that the survival curves of cells irradiated with neutrons were more nearly linear than after x-rays, that there was less recovery of the shoulder if two doses were used instead of one, and that the protective effect of oxygen was much reduced. Thus the RBE in the shoulder region was higher than that observed at higher doses. (RBE = Relative Biological Effectiveness obtained from the ratio of neutron and x-ray doses needed to achieve the same damage.) Much effort was put into developing functional assays of normal tissue injury in animals so that the results could be compared with clinical outcomes.

In the sixties and seventies a large body of data on skin reactions in mice, rats, pigs and humans was obtained. Dose–response curves were produced using graded doses of both x-rays and neutrons and the relative biological effectiveness was determined from the ratio of doses needed to produce an equal level of tissue injury. Scoring scales were developed to quantify the extent of erythema, moist desquamation and ulceration and the time course of this acute response to radiation was followed over the first few weeks. After healing occurred, the animals and patients were monitored for many months to see whether late fibrosis or other forms of late injury occurred. The RBE was then compared for acute and late reactions, e.g. Fowler & Morgan (3). It had been recognised that single dose studies were not sufficient and so fractionated schedules of 2, 5 or even 15 or 30 fractions were compared. Curiously the RBE was shown to be higher with the more fractionated schedules, where higher total doses were being compared, both for the acute and the delayed reactions. At first this seemed in conflict with the cell survival data where the RBE was highest in the low dose shoulder

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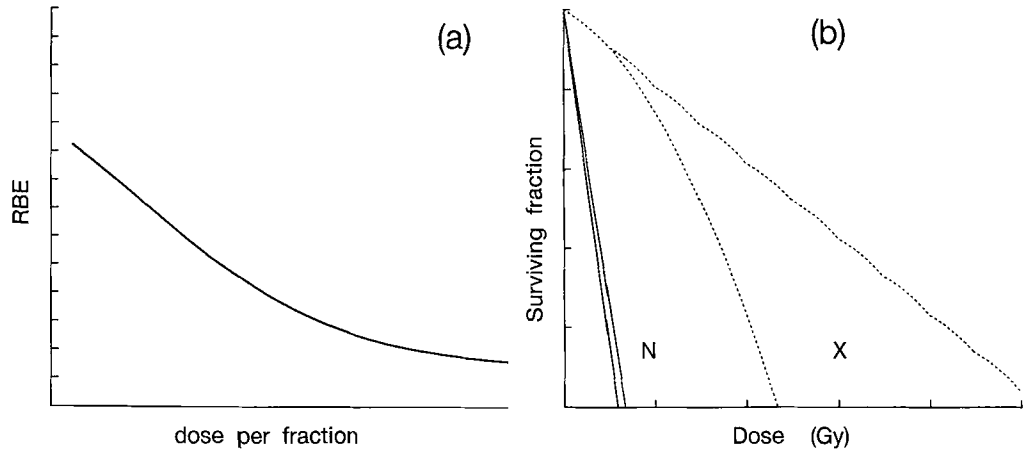

Fig. 1. Schematic representation of the RBE expressed as a function of dose per fraction and the survival curves that generate such RBE values. The response to single dose and fractionated neutrons is closely similar, but much higher doses have to be given to obtain the same level of damage with fractionated x-rays versus single dose treatments. The RBE curve can either be derived from comparing different effect levels on a single dose curve, or by comparing the same effect level, e.g. 10^{-1} or 10^{-2} survival using 1, 2, 5 or many fractions. This then gives a wide range of dose per fraction. For most normal tissues the RBE decreases with higher doses per fraction as illustrated in panel (a).

region. Field & Hornsey (4) made a major contribution when they recognised that the RBE should be considered, not in relation to the total dose, but rather to the dose per fraction in the fractionated schedules. They pointed out that fractionated schedules represented repeated irradiations in the 'shoulder' region of the dose response curve (Fig. 1). This now seems self-evident but at that time it allowed a previously unrecognised pattern to be detected in the wide spectrum of quoted RBE values for different cell types, endpoints and assays. It was also used to explain why Stone's disasters may have occurred. He had obtained radiobiological estimates of RBE before commencing his radiotherapy. However, these were obtained using one or a few large fractions and he then used those RBE values to devise a schedule of fractionated small dose treatments.

The experimental data for skin showed that, if expressed as dose per fraction, the RBE estimates were comparable for four species (mice, rats, pigs and humans) and that the acute and late skin RBE values were similar. This encouraged radiotherapists to take a renewed interest in neutron therapy. Radiobiologists went on to perform further work on other normal tissues and on experimental tumours in rats and mice. Most of the studies initially used single doses, partly for convenience and partly because of the limited availability of irradiation time on the neutron facilities. A spectrum of RBEs was obtained for different normal tissues but they all showed the now familiar tendency to higher RBEs at low doses per fraction than at high single doses (5, 6). This clearly reflected the differential sparing effect with fractionated schedules of x-rays compared with neutrons for all the normal tissues (except bone marrow, where sparing with both is negligible). No pattern emerged however between early responding and late responding tissues. The RBE curves were completely intermingled for acute responses seen over the first month, e.g. skin, intestine and bone marrow, and late responses seen after 3–6 months, e.g. lung and spinal cord. There seemed no support therefore for Stone's pessimism about late reactions, at least with the doses used, which were mainly above 2 Gy per fraction of neutrons.

At about the same time the RBEs derived from tumour data were showing encouragingly high values of 2–4 (7–13), especially those derived for local control, the endpoint of most interest to the clinicians. Little attention was paid to the fact that these were mainly from single dose studies, and used extremely high doses. Indeed, this emphasised the differentials in RBE, because at the high doses, the RBE for most normal tissues was only 1.5–2.0 (Fig. 2). It was

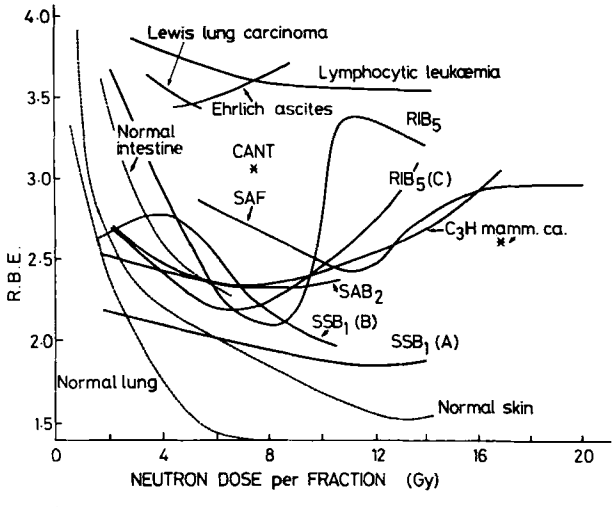

Fig. 2. Summary of RBE values compiled by S.B. Field (pers. comm.) for a range of normal tissues and tumours. The normal tissues showed the expected decrease in RBE at higher doses but the tumours showed more complex curves with a strikingly higher range of RBE values than for skin at doses above 10 Gy per fraction.

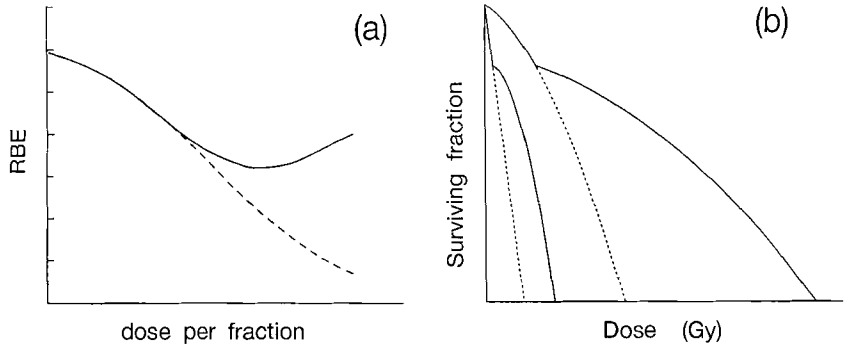

Fig. 3. Schematic illustration of the RBE that would be observed for a mixed population of oxic and hypoxic cells. Biphasic survival curves would be seen after x-rays, where hypoxia protects by a factor of ~3, but there is much less marked protection after neutrons (OER ~1.6). This would lead to increased RBE values at higher doses. The dotted line represents the response of completely euoxic cells, as in most normal tissues.

recognised that the high RBE at high individual doses resulted from the hypoxic tumour cell resistance to x-rays (Fig. 3). This gave rise to biphasic survival curves or dose response curves with x-rays, but there was less evidence of sensitive and resistant subpopulations with neutrons.

The data in Fig. 2 were of course a great stimulus to clinicians and were in harmony with the encouraging results beginning to be reported by Catterall et al. (14, 15) for head and neck cancer and other tumours at Hammersmith. This led to a wider interest in obtaining cyclotrons for radiotherapy and trials began in many centres throughout the world (see ref. 1 and 16 for summaries). By the early to mid seventies, the benefits of neutrons were described as resulting from a reduced hypoxic radioresistance, less ability to repair DNA lesions and differential sensitivity around the cell cycle. Thus all the modifiers that could be used to manipulate the response to x-rays or electrons were reduced with neutrons. It was argued therefore that tumour radioresistance should disappear with neutrons, and they should be as sensitive as normal tissues.

We must recognise that the complex patterns of RBE changes were due to the complexities and vagaries of tumour and tissue response to photons—not to neutrons. Neutrons were a more simple radiation in that very few modifiers could alter their biological effects. Unfortunately, however, clinicians had grown accustomed to the sparing of injury by fractionation with x-rays. Since the sparing effect of fractionation with neutrons was so small, it was argued that fewer neutron fractions might be used, perhaps in a shorter overall time.

Tremendous energy, enthusiasm and drive was now invested by radiotherapists, physicists, engineers and administrators in setting up clinical facilities worldwide. It was recognised that the Hammersmith neutrons were suboptimal in energy, giving poor physical depth dose distributions and that higher energy machines should be developed and installed. It took many more years before it was recognised that even such high energy machines would be less beneficial than the state of the art (80s and 90s) photon therapy if they did not have movable gantries, sophisticated couches and variable shaped fields. The original set-ups with fixed horizontal or vertical fields, with square or rectangular collimators were used to explain why some of the other clinical studies (e.g. (17)) were not supporting the optimistic view of the early Hammersmith trials. Severe toxicities were being reported. It was unclear whether this was an extension of Stone's early observations, or whether these could be attributed to unusually large volumes being included in the neutron treatment fields.

Meanwhile, the radiobiologists continued in their quest for understanding. They realized that the major contributions were coming from variations in photon response and therefore devoted more efforts to studying x-ray fractionation, cell cycle modifiers, oxygen mimetic radiosensitizers, radioprotectors, hyperthermia and manipulation of tumour vasculature. Studies within neutron radiobiology diminished and became more focused on determining the relative effectiveness of neutrons of different energies at the different clinical centres, and of combined neutron/photon schedules, since the limited availability of time on non-hospital-based machines made it difficult to give conventional multifraction neutron schedules. Some unpromising experimental results were reported at this time showing very small therapeutic gain factors, but the enthusiasm and impetus in the clinical trials was by now so great that they went unheeded (see ref. 1 for overview).

At Hammersmith Hospital, the radiobiology of neutrons was under investigation by scientists in 3 units: the MRC Cyclotron Unit, the MRC Experimental Radiation Research Unit and the Medical Physics Department of the Medical School. In 1970, Fowler moved to the Gray Laboratory, with a small team from the Hammersmith Medical Physics Department. This added a further complication to the collaborative studies using the Hammersmith Cyclotron since the two laboratories are about 30 km

apart. Gradually, attention at the Gray Laboratory became more focused on the chemical sensitizers being developed by Ged Adams and his team and the benefits of neutrons were compared with grains from altered fractionation with or without sensitizers. The early compounds were mostly too toxic or insoluble for practical application, but then in 1974 it was recognised that metronidazole, a compound already in clinical use, had the right structure to act as an electron affinic sensitizer and it was quickly tested in mice and in man. This was replaced by misonidazole which was widely tested in both animal tumours and in clinics (for review see (18, 19)). If this was an alternative means of overcoming tumour hypoxia, it represented serious competition for neutrons, with wider applicability since it could be used with the photon machines available in all radiotherapy departments.

A series of experiments were undertaken comparing x-rays alone (with various fractionation schedules), x-rays plus misonidazole, neutrons alone and neutrons plus miso (9, 10, 20). The object was to investigate the benefits that could be obtained by differential manipulation of hypoxia, repair capacity and repopulation. Within these series of experiments on four different tumour systems, the RBE of Hammersmith neutrons was compared for single doses and for fractionated schedules, and the benefits of neutrons were 'weighed' against the benefits of fractionation, with or without radiosensitizers. In three tumours only 2 and 5 fractions were used. Only in the more extensive studies of the C_3H mammary carcinoma were 9 and 15 fractions also included (20).

The data for RBE values obtained from the other three of these tumours are shown in Fig. 4. Some of these data (for slow sarcomas) have not been published in this form before, although a summary graph was provided for J. Rasey in her overview in 1977 (13). Each graph in Fig. 4 shows the tumour RBE values as a curve derived at various dose levels from pairs of curves of growth delay versus dose using single doses, 2 fractions/24 h, or 5 fractions/96 h. For comparison the RBE ranges are shown for two normal tissues, skin and lung. In each example the RBE values for tumours are higher than the normal tissue range for higher doses, i.e. longer growth delays, and fall at lower doses to within the range for normal tissues. The RBE for the slow sarcomas fell below the RBE values for normal tissues as the dose per fraction diminished. This pattern was attributed to the natural process of reoxygenation of hypoxic cells that begins as soon as cells respond to the first fraction of radiation.

It was also shown, using miso with neutrons, that even the beneficial ratio of RBEs (tumour vs skin), at high single doses where hypoxic cells are relevant, could be abolished if miso was used with both neutrons and x-rays (Fig. 5). These data suggested that the main therapeutic benefit with neutrons was related to hypoxia and that if this was abolished, either by natural reoxygenation or by

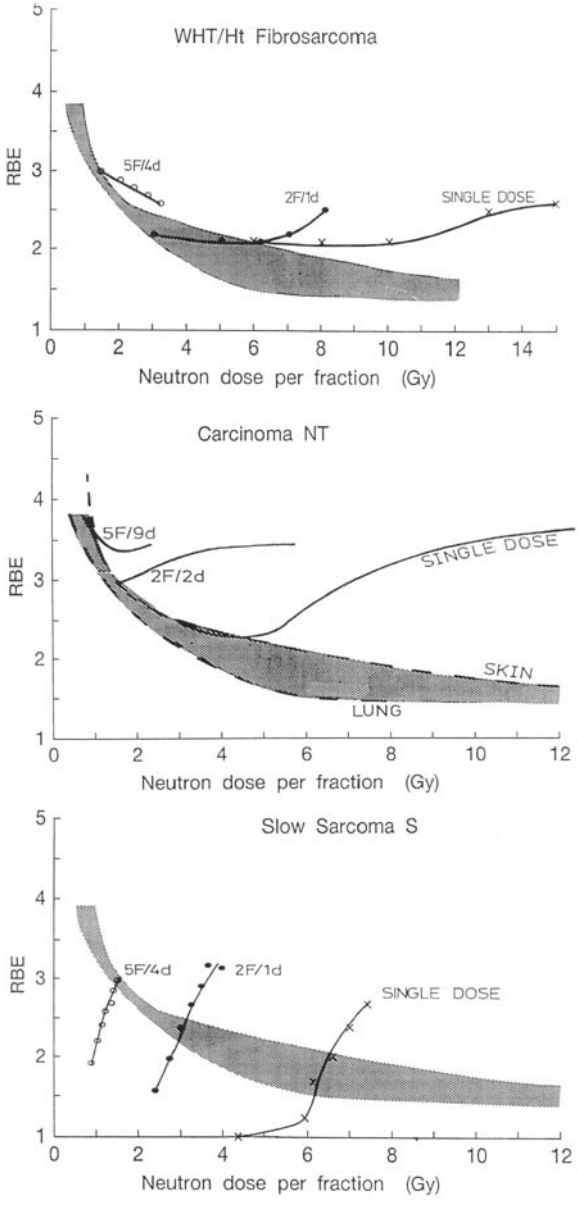

Fig. 4. RBE values derived from regrowth delay curves after irradiating 3 different tumour types with 1, 2 or 5 fractions, as indicated. The fibrosarcoma data were published in Denekamp (8) and the mammary carcinoma NT in Denekamp & Harris (9). Both those tumours had relatively short volume doubling times of 2–3 days at treatment size. The third panel shows previously unpublished data for a much slower growing fibrosarcoma, with a 14-day doubling time. This showed significantly lower RBE values at low regrowth delays (low doses on each curve) than for the normal tissues.

an oxygen mimetic chemical, there was no obvious residual benefit from differential repair or differential cell cycle sensitivity. In contrast with current clinical thinking that slow-growing tumours may benefit most from neutrons (16), the least promising results were obtained with the slow fibrosarcoma S, a tumour with a doubling time of 2 weeks, representing one of the slowest growing rodent

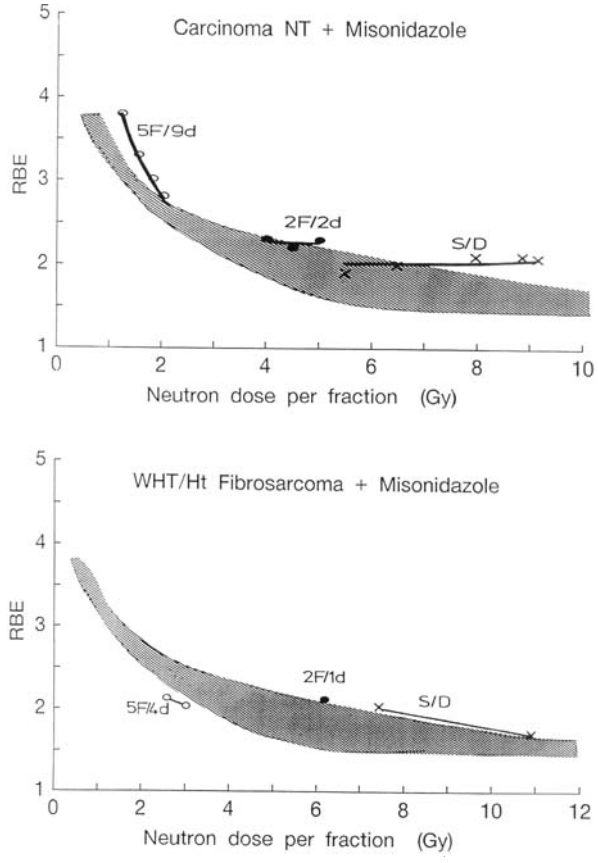

Fig. 5. RBE values for tumours treated with x-rays or neutrons 20 min after intraperitoneal injection of 0.67 mg/kg misonidazole. The tumour RBE values in the presence of the sensitizer fell close to the values seen in skin, and the upturn in RBE shown in Fig. 4 at higher doses has disappeared. This confirms that the therapeutic benefit with large neutron doses was due to hypoxia, since it is abolished by an oxygen-mimetic radiosensitizer (data derived from refs. 9, 10, 21).

tumours in experimental use. The data are combined in Fig. 6.

These results were clearly not encouraging for neutron therapy, and led the radiobiologists to divert even more of their attention to alternative methods of overwhelming hypoxic radioresistance, and to manipulating repair and repopulation characteristics during x-ray therapy to obtain therapeutic benefits. (Indeed, after presenting these data at several conferences, our interest in the studies declined and some of them were never published in detail.) Fig. 7 shows an example of the current multifactorial approach to photon therapy, which is entering phase I/II evaluation for four sites (head and neck, lung, bladder, brain) in EORTC multicentre trials (22, 23). Also, the sophisticated developments in imaging, in computer planning of dose distributions and in complex multileaf collimators, gantries and couches to enable conformal therapy with photons has changed the face of photon radiotherapy. It is against such novel approaches with photons that the current revival of interest in neutron therapy must be compared. Radiobiology studies with photons have given insight into how the variations in response can be used beneficially and physics has shown how the dose can be more accurately localised. Since almost all these biological effects are minimised with

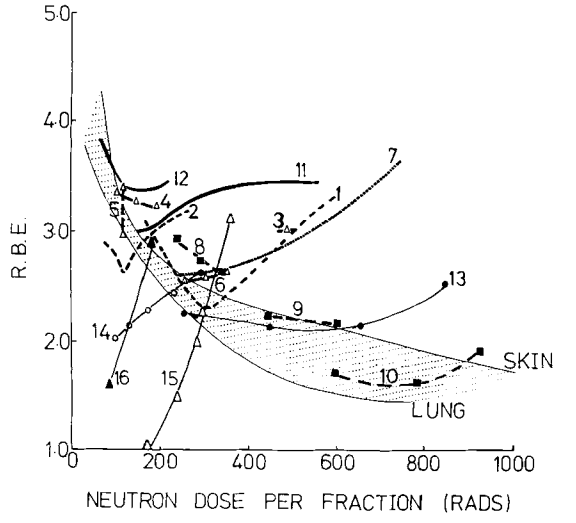

Fig. 6. Compilation of RBE data from fractionated studies of a spectrum of animal tumours, compared with the range of RBEs for skin and lung as examples of normal tissues. Update of data from Rasey (13) to include slow SaS.

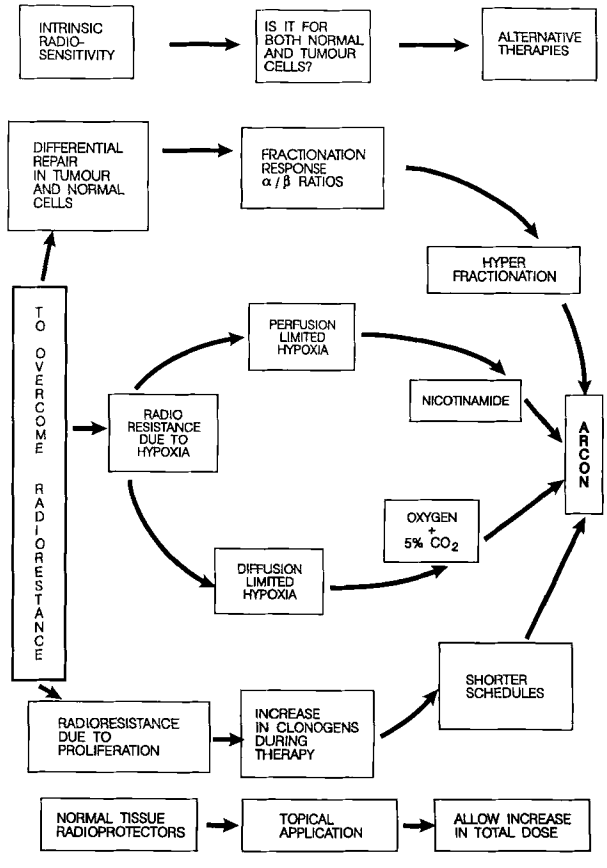

Fig. 7. Schematic representation of different methods of improving the therapeutic ratio of photons. Phase I/II studies have been initiated combining acceleration, using small doses per fraction with carbogen and nicotinamide (ARCON).

high LET radiations, the same philosophies would not apply.

Hyperfractionation. Detailed studies using very small x-ray dose fractions have shown a difference in the sparing effect of subfractionation in the clinically relevant dose range (1–4 Gy per fraction) for late-responding tissues, such as lung, kidney, spinal cord. Very little further sparing is seen, however, by hyperfractionation in acutely responding tissues, such as skin, intestine, etc. This fractionation dependence is expressed as the α/β ratio from the linear quadratic fit to such sets of fractionated data. If the LQ value is low, e.g. 3 Gy, then increasing dose above conventional 2 Gy fractions will be predictably more damaging and decreasing below 2 Gy fractions will spare such a tissue and allow more dose to be given. There is a spectrum of α/β ratios for a range of mouse tumours (mean value $\sim$15 Gy), acute reacting tissues ($\sim$10 Gy) and late reacting tissues ($\sim$3–4 Gy). A differential benefit, i.e. sparing of late reactions relative to tumour response, should therefore be expected from using more smaller fractions, and indeed a 15% benefit has recently been shown in an EORTC study (24). It is predicted from the radiobiology that acute reactions may be increased in such schedules, but will not be matched by greater late morbidity (25, 26).

Accelerated radiotherapy. It is generally accepted now that compensatory proliferation during radiotherapy only spares acutely reacting normal tissues and does not protect against late reactions. Fast proliferating tissues begin to express their injury during conventional irradiation and loss of organ function triggers cells to divide more rapidly to increase the differentiated mature cell pool. Late reacting tissues are not spared by protracting treatment times, e.g. to 8 or 10 weeks. The damage (by definition) is latent in these tissues for many months and therefore there is no biological signal of failing organ functions to trigger accelerated cell production. Thus accelerated (shortened) radiotherapy schedules should be no more damaging to late reacting tissues but would lead to more severe acute reactions unless the dose is reduced (26, 27).

The crucial question, of course, is what happens in tumours. If they have a cell production rate that is slow then there is no disadvantage to using protracted schedules which spare acute reactions, even if there is no sparing of late morbidity. External observations indicate a volume doubling time of several weeks to many months for most tumour types. This would suggest that very little proliferation should occur over a 6–7-week course of radiotherapy. However, it has been known for many years that the rate of cellular proliferation in human tumours is at least 10–20 times higher than the observed doubling time. Many cells are lost each day (often 90–95%), either as a result of differentiation, exfoliation or loss into the blood stream or lymphatics, or as a result of nutrient insufficiency leading to hypoxic cell death. If reoxygenation prevents this hypoxic cell loss then, in some tumours at least, the rate of cell production will be the relevant parameter, not the composite pre-treatment volume doubling time, once treatment has started.

A new technique became available in the eighties to measure T_{pot}, the reciprocal of the birth rate (28). This uses BUdR to label cells synthesising new DNA and a monoclonal antibody to detect these cells. If the BUdR (or IUdR) is given to a patient, and a biopsy is taken 5–6 h later, two important parameters can be determined simultaneously in a flow cytometer: LI, the fraction of labelled cells and T_s, the duration of DNA synthesis. These are the two parameters needed to measure the rate of cell production (T_{pot}). The labelled cells (at time zero) are distributed uniformly throughout the S-phase, but move towards higher DNA contents in the 5–6 h before biopsy. A simultaneous measure of DNA content and of BUdR labelling allows the T_s to be determined from the relative movement towards G_2. Such measurements have now been made on several thousand human tumours and have shown that T_{pot} is very short in many tumours with a median value of 4–5 days. Histological assessments in combination with flow cytometry indicate that even this may be an overestimate and that T_{pot} may be as short as 2–3 days (29, 30). Thus, there is a great deal of interest in shortening treatment schedules, e.g. by treating twice a day, or even 3 times a day including weekends as in CHART (31). (CHART = Continuous Hyperfractionated Accelerated Radiotherapy uses 36 fractions of 1.5 Gy in 12 days given 3 times each day.) Since some neutron schedules have been given over 3–4 weeks, it is possible that the observed benefits in some clinical trials have related to treatment acceleration rather than to the use of neutrons per se.

Hypoxic radiosensitizers. The chemical radiosensitizers, e.g. metronidazole, misonidazole, nimorazole, pimonidazole and etanidazole were welcomed by clinicians as simple methods of overcoming hypoxic radioresistance by the simple i.v. administration of a solution shortly before each fraction. These agents promised to be better than oxygen because they should diffuse through metabolising cells to the distant hypoxic cells without being consumed in cellular respiration. Many animal studies showed that with large single doses they could sensitize hypoxic cells by a factor of 2.0–2.2, virtually abolishing the problem of radioresistance (20). Clinical studies then commenced with great enthusiasm but were in the end disappointing (19). The experimental data had already shown that the SER would fall with fractionation, in all the models studied. This occurred for three reasons:

1. The drug showed cumulative toxicity and therefore the dose that could be used repeatedly was sub-optimal, giving lower SER values for the hypoxic cells.
2. The extent of cell kill at low x-ray doses is dominated by the radiosensitive well-oxygenated cells. Hypoxic

cells only dominate the response after most of the oxic cells have been killed.
3. Natural processes of reoxygenation reduced the level of hypoxic cells between the daily doses of x-rays.

In recent years it has been recognised that there are actually two forms of hypoxia, the classical diffusion-limited chronic version described by Thomlinson & Gray (32), and a transient acute hypoxia as individual vessels close down. These two versions of hypoxia are dealt with by using the combination of nicotinamide and carbogen (23, 33). Putting all these concepts together, i.e. hyperfractionation to spare late morbidity, acceleration to avoid tumour cell proliferation and the two sensitizers to overcome hypoxic resistance leads to a schedule like ARCON, illustrated in Fig. 7.

Clearly the way forward for neutron therapy must depend upon a critical reevaluation of the experimental and clinical data on fast neutron therapy. The potential benefits must be realistically compared with the best that can be obtained with photon therapy when all the radiomodifiers are simultaneously modified to maximise photon effectiveness. In very few animal models have the advantages of fractionation, sensitizers and acceleration with photons been directly compared with the benefits of neutrons. Where they have, as in the carcinoma NT, the therapeutic gain is much greater with a multifactorial photon approach like ARCON than with fast neutrons.

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